

INNOVATIVE APPLICATIONS AND RESEARCH METHODS IN HEALTH SCIENCES



EDITORS

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CHAPTER 1

Superior Vena Cava Syndrome

Muharrem Cakmak¹

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Etiology

Superior vena cava can be obstructed due to extrinsic or intrinsic factors. Intrinsic factors are causes that result in thrombosis, often related to venous catheterization. Adjacent to the superior vena cava are major vessels (ascending aorta, pulmonary artery) and branches, trachea, major bronchi (right main bronchus), lymph nodes, thymus, fat, and connective tissue within mediastinal structures. The benign or malignant mass lesions of these structures can create extrinsic compression on SVC, leading to obliteration of the flow (2, 3).

Currently, malignant diseases are the most significant cause of SVCS cases. The use of intravascular devices such as catheters, pacemakers, and the resulting venous thrombosis has also led to an increase in cases of benign SVCS. The prevalence of malignant diseases causing SVCS actually reflects the prevalence of cancers within the thorax. The most common cause of SVCS is lung cancer, followed by mediastinal malignant diseases. About 4% of lung cancer patients develop SVCS, and 80% of these cases are due to right lung cancers. Small cell lung cancers, due to their central localization, rapid growth potential, and high likelihood of metastasis to mediastinal lymph nodes, are more prone to causing SVCS. However, non-small cell lung cancers are more common, making up about 70% of lung cancers that cause SVCS. Non-Hodgkin lymphoma is the second most common cause of malignant SVCS, accounting for about 10% of cases. Hodgkin lymphoma, despite forming large masses, rarely causes SVCS due to its slow growth (4, 5).

Benign causes of superior vena cava syndrome have recently highlighted thrombosis related to intravenous catheter and similar device usage. Currently, in over half of benign SVCS cases, the cause is intravascular devices. Among vascular anomalies, true or false aneurysms of the aorta and brachiocephalic arteries, pulmonary artery aneurysms related to Behçet's disease, tuberculosis, histoplasmosis, and other infectious diseases are rare causes of SVCS (6).

Pathophysiology

After forming at the level of the 1st costal cartilage, the superior vena cava (SVC) progresses through the middle mediastinum and empties into the right atrium at the level of the 3rd intercostal space. It is short (6 cm in length), wide (2 cm in diameter), thin-walled, lacks valves, and has low pressure and slow flow, making it susceptible to compression. Blockage of venous blood flow leads to increased pressure within the veins drained by the SVC. Pressure in cervical veins can rise to 20-40 mmHg (normally 2-8 mmHg). The rate of obstruction development and its size determine the degree of venous pressure elevation and the severity of clinical presentation. Although a common belief is that increased venous pressure can lead to brain edema, there is no evidence indicating that SVC obstruction causes brain damage (7, 8).

When the flow of the superior vena cava is obstructed, a wide collateral venous system develops. Collateral venous structures facilitate continued venous drainage and decrease in venous pressure. In cases of slow-obstructing conditions, collateral venous structures can develop over a span of several weeks (9).

Numerous collateral venous pathways that facilitate the return of blood to the heart have been identified in both the thorax and abdomen. Major collateral venous structures include (10, 11, 12, 13 14):

Azygos venous system

Paravertebral venous system

Lateral thoracic venous system

Internal mammary venous system

Azygos venous system: This system consists of the azygos vein, hemiazygos vein, and accessory hemiazygos vein. The azygos vein is the continuation of the right ascending lumbar vein. Before entering the thorax, it receives some branches from segmental lumbar veins and drains into the inferior vena cava (IVC) through some branches, known as the azygos veins, without entering the thorax. After entering the thorax, the azygos vein drains the posterior intercostal veins and connects to the SVC at the level of the vein's entry into the pericardium.

The hemiazygos vein is the continuation of the left ascending lumbar vein and receives segmental lumbar veins. Before entering the thorax, similar to the azygos vein, it drains into the IVC through some branches. After entering the thorax, the hemiazygos vein receives contributions from several lower posterior intercostal veins and empties into the azygos vein anterior to the Th8 vertebra.

The accessory hemiazygos vein collects blood from the left upper posterior intercostal veins and drains into the brachiocephalic vein. It can also drain into the azygos or hemiazygos veins.

These three veins form interconnected pathways for the retrograde drainage of blood from the SVC, functioning as a single system.

Paravertebral venous system: Within the vertebral column, there are two venous plexuses at each vertebral level-one inside the vertebral canal and the other around the vertebral bodies. These plexuses are interconnected. The drainage of these veins is directed upward toward the posterior intercostal veins, then to the azygos vein and SVC through the lateral thoracic veins or downward toward the lumbar and iliac veins, eventually reaching the inferior vena cava (IVC). These venous plexuses lack valves, allowing reverse flow.

Lateral thoracic venous system: The lateral thoracic vein drains the venous blood of the serratus anterior and pectoralis major muscles. It empties into the

axillary veins, which drain into the SVC. This vein also forms collateral connections with the posterior intercostal veins.

Internal mammary venous system: The internal mammary veins arise as epigastric veins in the abdomen. These veins drain into the external iliac veins before entering the thorax. After entering the thorax, they receive the anterior intercostal veins and then drain into the brachiocephalic veins.

In addition to these systems, the jugular venous system is also useful in cases of proximal SVC obstruction. The right and left anterior jugular veins, which drain into the internal jugular veins, are interconnected. When there is an obstruction at the level of the brachiocephalic veins, blood flowing into the internal jugular veins can reach the SVC through the interconnected anterior jugular veins and contralateral brachiocephalic vein.

Clinical findings:

The occlusion of the superior vena cava's blood flow leads to an increase in venous pressure and congestion in the areas drained by this vein. This results in various symptoms and signs. The severity of symptoms and signs depends on the location of the obstruction (above or below the azygos vein), the rate of development, and the degree of obstruction. The most frequently encountered symptom is swelling in the face, neck, arms, and upper chest region. These symptoms usually become evident within a few weeks. In the initial days, the patient might notice swelling only in the face or eyelids in the mornings, which decreases when they are standing. However, after a few weeks, more widespread swelling may occur in the face, neck, and arms. Increased venous pressure in the head area can cause symptoms such as headaches, a strong pressure sensation upon bending forward, dizziness, and ringing in the ears. Slowed venous flow can lead to venous thrombosis, resulting in loss of vision due to thrombosis in the retinal vessels. Furthermore, specific symptoms related to the underlying cause of the superior vena cava syndrome can also manifest. Since the most common underlying condition is lung cancer, respiratory symptoms are frequently encountered. Shortness of breath is the most common symptom in these patients. Due to the effect of gravity, venous return is somewhat improved in an upright position, so patients often find relief in a seated position and may avoid lying down. Therefore, patients often assume a sitting position known as the "orthopneic position." Additionally, various symptoms related to the intrathoracic structures affected by the underlying disease can be observed (15, 16).

Diagnosis:

Superior vena cava syndrome is a clinical diagnosis. The diagnostic process begins with the patient's history. Besides the symptoms and findings, the patient's medical history can shed light on the diagnosis. The diagnostic process generally starts with a chest X-ray. Computed Tomography (CT) of the thorax with

contrast, if performed, provides highly valuable information. CT can reveal both the underlying disease and the occlusion in superior vena cava, as well as the collateral vessels that have developed due to this occlusion. Thoracic Magnetic Resonance Imaging (MRI) can provide useful information. The advantages of this test include more detailed evaluation of certain intrathoracic masses and the ability to evaluate the flow in superior vena cava in cases where contrast cannot be administered, unlike in CT scans. Ultrasonography can be used to examine structures in the supraclavicular area and assess the presence of lymph nodes. While ultrasonography may not be highly effective in examining intrathoracic structures, it can be used to evaluate extrathoracic veins such as jugular, subclavian, and axillary veins (17).

If an intervention (stent or surgery) is planned for the superior vena cava, contrast venacavography is recommended. This helps determine the location and degree of occlusion and also shows the developed collateral venous structures. Despite concerns about the risk of bleeding due to elevated venous pressure during this procedure, the risk is minimal. Obtaining a tissue diagnosis related to the underlying cause of superior vena cava syndrome is crucial for guiding treatment. Even though these procedures were traditionally considered high-risk, recent studies have not shown a significant risk associated with them. Therefore, obtaining a tissue diagnosis before starting treatment is recommended. The procedures for this purpose can vary based on the disease and may involve different imaging techniques guided by radiology. A simple sputum cytology might be diagnostic for a localized lung cancer in the hilar region. Bronchoscopy can provide more successful diagnostic material in patients suspected of lung cancer. Depending on the suspected condition, other methods used for diagnosing thoracic diseases might be necessary. In cases where obtaining a tissue diagnosis through less invasive methods is not possible, surgical procedures might be necessary for diagnosis. Supraclavicular lymph node biopsy can be highly diagnostic with low morbidity when performed carefully. Mediastinoscopy, mediastinotomy, video-assisted thoracoscopic biopsy, or thoracotomy might be required in some cases. Mediastinoscopy and mediastinotomy are highly successful in obtaining samples from masses compressing the superior vena cava. There are a few studies suggesting that mediastinoscopy might be risky in patients with superior vena cava syndrome. However, in large series, the morbidity rate is low, and the diagnostic yield is around 100%. In some cases, thoracotomy might be necessary for definitive diagnosis (18, 19).

Treatment:

Superior vena cava syndrome (SVCS) is a clinical condition that can arise from various underlying diseases. Therefore, there is no single standardized treatment method. Due to this variability, there are limited studies that investigate

and compare the effectiveness of different treatment methods. Much of the information on this topic relies heavily on case series and expert opinions.

The main aim of treatment is to manage the underlying disease causing the syndrome. While doing so, supportive care to alleviate obstructive symptoms should also be maintained. Supportive care can be provided during the diagnostic process. Additionally, in cases where the underlying disease cannot be effectively treated, supportive care may be the only treatment option.

Supportive Care:

Elevating the head can help alleviate symptoms by reducing venous pressure. Patients often benefit from conservative treatment methods such as head elevation and oxygen administration. Glucocorticoids are commonly used agents for symptomatic improvement. They are used to reduce presumed laryngeal and cerebral edema. However, the benefit of glucocorticoids has only been established at the level of case presentations in the literature. Moreover, this benefit is usually limited to cases of SVCS obstruction caused by diseases that respond to steroids, such as lymphoma or thymoma. The reason glucocorticoids might be beneficial in these diseases is not primarily due to reducing laryngeal and brain edema, but rather due to shrinking the mass causing the SVCS obstruction, thus mitigating its clinical effects. In fact, it has not been proven that SVCS itself causes cerebral edema, and the central nervous system findings are generally attributed to brain metastases from underlying malignancies. Diuretics are also widely used to reduce laryngeal and brain edema. However, there is no evidence of symptomatic improvement with diuretics either.

Treatment of Underlying Disease:

Benign Conditions:

Antibiotherapy, anticoagulation and thrombolytic treatment are given. Angioplasty and endovascular stent applications are other treatment methods.

Surgery: The indications for surgical treatment in superior vena cava syndrome are not well defined. Generally, patients with benign conditions causing the syndrome are considered candidates for surgery. Surgical treatment is clearly indicated in some benign diseases that cause SVCS. For example, in the presence of mediastinal goiter compressing the SVCS, thyroidectomy can be curative. Surgical treatment might also be preferred in cases of aneurysmal conditions. Various interventions have been described to surgically treat SVCS patients when the disease causing compression on the superior vena cava cannot be eliminated without damaging the SVCS. These interventions can be categorized as grafting methods and bypass methods. Autografts, homografts, or various artificial grafts can be used as grafts. Different techniques have been described to divert SVCS blood to other veins for bypass procedures (20).

Malignant Conditions:

Radiation Therapy: Radiation therapy is the most commonly used treatment method for SVCS cases, including all etiologies. This is mainly due to malignancies being the most common causes of SVCS, and patients usually tolerate this treatment well. Radiation therapy can also be effective in treating most malignancies causing SVCS, including small-cell and non-small-cell lung cancer, non-Hodgkin lymphoma, germ cell tumors, thymic tumors, and metastatic tumors. In adults, high-dose radiation therapy (300-400 cGy per day in 4 fractions, followed by 150-200 cGy per day, total radiation of 3000-5000 cGy) has been found to be safe and effective in alleviating SVCS symptoms and findings. However, the specific radiation therapy plan can vary depending on the underlying disease (21).

Chemotherapy: In cases of SVCS caused by tumors sensitive to chemotherapy, such as lung cancer, lymphoma, and germ cell tumors, appropriate chemotherapy can result in tumor shrinkage and symptom relief (23).

Endovascular Stenting: There are reports that stenting procedures can be beneficial in some cases of malignant SVCS. Stenting can be considered in cases where chemotherapy or radiation therapy has been ineffective in treating malignant SVCS. The one-year patency rate for stents is approximately 25% (20).

Surgery: Patients with malignant SVCS obstruction are often in an inoperable stage of the disease. Since the expected lifespan is short in patients with malignancies, surgery is not a preferred option in this group. However, in cases where life expectancy is longer and appropriate, reconstruction can be performed using grafting or bypass techniques. Surgery with SVCS reconstruction might also be successful in cases of thymoma resistant to chemotherapy and radiation therapy (21, 22).

Prognosis:

The presence of superior vena cava syndrome itself does not influence survival. Survival is determined by the underlying disease. For malignant diseases, if the disease responds to treatment, the presence of SVCS may not significantly impact survival. However, the prognosis is generally poor in cases of treatment-resistant malignancies.

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CHAPTER 2

Bronchiectasis

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Bronchiectasis is a chronic and progressive lung disease characterized by abnormal and irreversible dilatation of the medium-sized airways. This condition typically results from inflammation, infection, and structural destruction of the bronchial wall. The clinical spectrum of bronchiectasis is quite broad, ranging from mild symptoms to life-threatening respiratory failure.

The term *bronchiectasis* was first introduced in the early 19th century by René Laennec, who described the disease in 1819 using the stethoscope he had developed. He established the correlation between clinical signs and pathological findings (1). However, the pathophysiological basis and true scope of the disease were better understood through autopsy studies conducted in the 20th century. Until the mid-20th century, bronchiectasis was often considered a sequela of severe childhood bacterial infections, such as pertussis, measles, or pneumonia. The widespread use of high-resolution computed tomography (HRCT) has since led to the identification of previously unrecognized forms of bronchiectasis. These advancements have revealed that the disease is not limited to post-infectious etiologies however, it may occur in the context of diverse underlying conditions, including immunodeficiencies, autoimmune diseases, and congenital disorders such as primary ciliary dyskinesia.

In recent years, interest in bronchiectasis has been revitalized for several key reasons. First, diagnostic rates have significantly increased facilitated by the more frequent incorporation of advanced cross-sectional imaging like HRCT. Second, recent investigations have revealed that the burden of bronchiectasis is higher than previously assumed, across both resource-limited settings and high-income countries alike. Third, there has been a shift toward phenotyping non-cystic fibrosis bronchiectasis based on clinical, microbiological, and radiological characteristics, which has supported the development of personalized treatment strategies. As a result, the traditional management approach has increasingly given way to more targeted therapeutic interventions (2,3).

Etiology:

Bronchiectasis is a heterogeneous disease that may arise secondary to diverse underlying etiologies. Permanent bronchial dilatation occurs due to a combination of bronchial wall damage, impaired mucociliary clearance, and recurrent infections triggering chronic inflammatory processes. Identifying the etiological factors is of critical importance for appropriate disease management and prognostic assessment. In approximately 40–50% of patients, an underlying cause can be determined, whereas the remaining cases are classified as idiopathic bronchiectasis (2).

In developed healthcare settings, cystic fibrosis is the predominant cause of bronchiectasis diagnosed in children (4). In patients with immunodeficiencies, bronchiectasis is typically diffuse and presents at an early age (5). Chronic microaspiration has been demonstrated to correlate with bronchiectasis localized

particularly in the right lower lobe. Bronchial inflammation due to gastroesophageal reflux is a silent yet significant pathway of bronchial injury (6,7).

Allergic bronchopulmonary aspergillosis (ABPA) is an immunologic hypersensitivity response to *Aspergillus fumigatus*, typically developing in individuals with asthma or cystic fibrosis. It is characterized by central bronchiectasis with a "gloved finger" appearance on imaging. Elevated serum IgE levels and the presence of specific antibodies support the diagnosis (8).

Despite comprehensive diagnostic testing, some patients remain without an identified cause and are labeled as having idiopathic bronchiectasis. Detailed evaluation is still essential in these patients, as some may have subtle forms of primary ciliary dyskinesia (PCD) or latent immunodeficiencies presenting later in life (9). Additional etiological factors associated with bronchiectasis are listed in Table 1.

Table 1.Etiology of Bronchiectasis

Infections	Bacterial - Bordetella pertussis - Pseudomonas aeruginosa - Haemophilus influenzae - Mycoplasma pneumoniae - Mycobacterium tuberculosis - Mycobacterium avium complex (MAC) - Pneumonia Viral - Measles - Pertussis - Human Immunodeficiency Virus (HIV) - Epstein-Barr Virus (EBV) - Influenza - Human T-Lymphotropic Virus type 1 (HTLV-1) - Herpes Simplex Virus (HSV) - Adenovirus types 7 and 21 Fungal - Aspergillosis - Histoplasmosis - Coccidioidomycosis
Immunodeficiencies	- Complement deficiencies - Waldenström’s macroglobulinemia - Hypogammaglobulinemia - Chronic granulomatous disease - Selective IgA deficiency

Hereditary Disorders	- Cystic fibrosis - Alpha-1 antitrypsin deficiency - Williams-Campbell syndrome - Swyer-James syndrome - Mounier-Kuhn syndrome
Mucociliary Clearance Defects	- Primary ciliary dyskinesia - Kartagener syndrome - Young syndrome
Systemic Diseases	- Rheumatoid arthritis - Sjögren's syndrome - Ulcerative colitis - Crohn's disease - Yellow nail syndrome - Celiac disease - Systemic lupus erythematosus - Relapsing polychondritis - Ankylosing spondylitis - Marfan syndrome
Mechanical and Obstructive Causes	- Foreign body aspiration - Tumor compression - Lymph node compression - Middle lobe syndrome
Other Causes	- Exposure to toxic chemicals - Aspiration of gastric contents - Heroin use - Pulmonary fibrosis - Bronchial tree malformations - Gastroesophageal reflux disease (GERD)

Epidemiology:

For many years, bronchiectasis was considered a rare condition and primarily regarded as a sequela of childhood infections. Nevertheless, the widespread implementation of high-resolution computed tomography (HRCT) together with heightened clinical recognition has demonstrated that the true prevalence of the disease is substantially greater than formerly appreciated. Although prevalence varies between countries, an increasing trend has been observed even in developed nations.

Studies conducted in Europe have reported the prevalence of bronchiectasis to be approximately 67 per 100,000 individuals (3). In the United States, the prevalence has been estimated at approximately 52 cases per 100,000 individuals (4). The prevalence of bronchiectasis increases markedly with age. While the disease is rare among individuals under the age of 20, it shows a dramatic rise in

populations over 60 years. Of note, a disproportionately elevated rate of diagnosis has been observed in females above the age of 65 (6).

The prevalence of bronchiectasis in childhood shows significant regional variation. In developing countries, post-infectious sequelae from childhood infections still predominate, whereas in developed countries, underlying genetic, immunological, and structural disorders are more commonly reported. Within the indigenous pediatric populations of Australia and New Zealand, the prevalence of bronchiectasis has been found to be remarkably high; which is largely ascribed to inadequate healthcare accessibility and densely populated living environments (7).

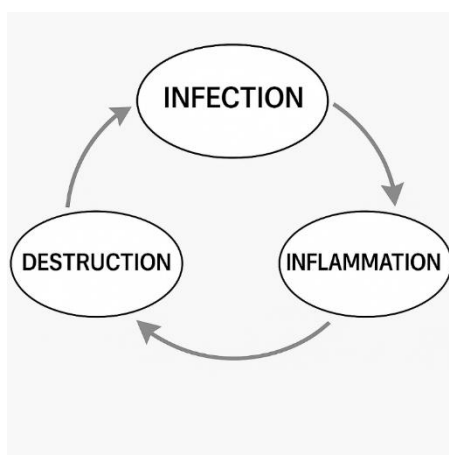
Bronchiectasis not only leads to chronic symptoms but also increases healthcare utilization and economic burden due to frequent exacerbations. One study demonstrated that patients with bronchiectasis experience an average of 1.5 to 3 exacerbations annually, with 20–30% of these episodes resulting in hospitalization (8).

Pathophysiology:

Bronchiectasis is a chronic pathologic condition defined by irreversible dilatation of the bronchial tree, accompanied by concomitant structural and functional abnormalities. The underlying pathophysiology typically involves damage to the bronchial wall caused by recurrent infections and the associated inflammatory response. Over time, this process leads to impaired mucociliary clearance, accumulation of secretions, and a self-perpetuating cycle of infection.

The most widely accepted model describing the development of bronchiectasis is the "infection-inflammation-destruction cycle" (Figure 1 demonstrating the inherently progressive course of the disorder.

Figure 1. The vicious cycle of bronchiectasis.



The initial triggering factor is most often an infection. Infections damage the bronchial epithelium and disrupt the mucociliary transport system. This leads to mucus accumulation, impaired clearance of secretions, and a predisposition to secondary infections. Persistent infections stimulate an inflammatory response. Neutrophil-mediated inflammation culminates in the discharge of reactive oxygen species along with proteases, especially elastase, as well as matrix metalloproteinases (MMPs). This enzymatic activity causes degradation of collagen and elastin within the bronchial wall. The loss of structural support in the bronchi forms the pathological basis for permanent dilatation (4).

The ciliated epithelial cells of the bronchial mucosa are responsible for transporting the mucus layer upward. Damage to these structures or genetic disorders such as primary ciliary dyskinesia impairs this function, leading to mucus accumulation in the lungs. The retained mucus facilitates bacterial colonization and initiates or perpetuates the inflammatory cycle.

Chronic inflammation and subsequent healing responses lead to bronchial wall thickening, glandular hyperplasia, fibrosis, and loss of elastic tissue. These changes cause the bronchi to become both dilated and rigid. As a result, the airways fail to expand adequately during inspiration and cannot fully collapse during expiration. This leads to air trapping, secondary alveolar hypoventilation, and chronic hypoxemia (5).

Bronchiectasis is not merely a localized airway disease; it is also associated with systemic inflammation. Elevated concentrations of systemic inflammatory markers, including plasma C-reactive protein (CRP) and fibrinogen, indicate that the condition exerts effects beyond the lungs, involving the organism systemically. This systemic inflammatory response is hypothesized to be linked with comorbid conditions such as cardiovascular disease and osteoporosis (7).

Morphology: Bronchiectasis is morphologically classified into three types: cylindrical (tubular), varicose (saccular), and cystic. High-resolution computed tomography (HRCT) constitutes a key modality for differentiating between these subtypes (3) (Table 2).

Cylindrical Bronchiectasis (Tubular Type): This is the earliest and mildest form of bronchiectasis, characterized by uniform, mildly dilated bronchi that retain a cylindrical shape. The clinical course may be relatively mild. Although irreversible, the disease may progress slowly, and symptoms can be well-controlled with appropriate therapy. It has the best prognosis among the bronchiectasis types. However, if left untreated, it may progress to saccular or cystic forms.

HRCT typically demonstrates a bronchial diameter that is comparable to, or slightly larger than, the accompanying pulmonary artery (bronchus-to-artery ratio > 1). The bronchi fail to taper at their peripheral ends, instead ending abruptly

(normally, bronchi should taper conically). The bronchial wall may be mildly thickened. A characteristic “tram-track” appearance can often be observed.

Varicose Bronchiectasis (Varicoid Type): This type is characterized by irregular dilatation, narrowing, and ballooning of the bronchi. Chronic cough, expectoration, and repeated respiratory infections constitute the characteristic clinical manifestations. It is frequently observed in post-infectious bronchiectasis and carries a worse prognosis compared to the cylindrical type. Exacerbations tend to occur more frequently.

On HRCT, the bronchi appear as a “string of beads” or “sausage-shaped” structures. Alternating areas of dilatation and narrowing can be visualized sequentially. Irregular bronchial wall thickening and intraluminal secretions may also be present

Cystic Bronchiectasis: This is the most advanced form of bronchiectasis, in which the bronchi form balloon-like dilatations and cystic cavities. Mucus retention, secondary infections, and hemoptysis are more frequently observed. It is frequently linked to underlying genetic conditions, most notably cystic fibrosis. Among the morphological types, it carries the poorest prognosis. Patients may experience significant dyspnea and reduced quality of life. In some cases, surgical resection may be necessary.

On HRCT, the bronchi appear as thin-walled cysts, often containing air-fluid levels. The dilated airways resemble clusters of bubbles within the lung parenchyma. Care must be taken not to confuse this appearance with “honeycombing,” particularly in cases with coexisting fibrosis.

Diagnosis:

The diagnosis of bronchiectasis requires a multidisciplinary approach. Therefore, clinical, radiological, laboratory, and functional data must be integrated for a comprehensive evaluation.

Typical clinical manifestations of bronchiectasis include chronic cough, sputum production, recurrent lower respiratory tract infections, and hemoptysis. However, since these symptoms are non-specific, high-resolution computed tomography (HRCT) remains the cornerstone of diagnosis. In addition to confirming structural abnormalities, HRCT is crucial for identifying the underlying etiology and determining appropriate treatment and prognosis. Furthermore, extensive laboratory evaluations and functional tests are required to elucidate the etiologic factors.

Individuals with bronchiectasis typically exhibit a prolonged history of persistent cough accompanied by sputum production. The sputum is typically mucopurulent or purulent, tends to be more abundant in the morning, and may

display a characteristic three-layered appearance. During exacerbations, symptoms such as fever, increased sputum volume, and dyspnea are common. Hemoptysis is a frequently encountered complication, especially in developing countries, and can occasionally be life-threatening. On physical examination, inspiratory crackles (particularly in the lower lobes) are commonly heard. Digital clubbing may occur in the later or more advanced stages of the disease.

High-resolution computed tomography (HRCT) is regarded as the definitive imaging modality for the diagnosis of bronchiectasis. Radiologic hallmarks that substantiate the diagnosis include an increased broncho-arterial ratio (>1), with the bronchial diameter surpassing that of the paired pulmonary artery ('signet-ring' sign), absence of normal bronchial tapering toward the lung periphery ('tramline' or 'railway track' pattern), bronchial wall thickening, presence of mucoid impaction, and a central distribution of bronchiectatic changes.

Most patients exhibit an obstructive pattern with a low FEV₁/FVC ratio. In extensive disease, restrictive defects may also be observed. A positive bronchodilator response may suggest the presence of coexisting asthma or allergic bronchopulmonary aspergillosis (ABPA). Pulmonary function tests are essential for assessing disease severity and monitoring therapeutic response (4). During exacerbations or in cases of chronic colonization, sputum culture results guide antibiotic selection. The pathogens most commonly isolated in patients with bronchiectasis are *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Staphylococcus aureus*. Non-tuberculous mycobacteria (NTM) can also be significant pathogens in certain cases, particularly among elderly women with middle lobe and lingula involvement—a pattern characteristic of "Lady Windermere syndrome" (5).

A range of investigations may be undertaken to identify underlying causes of bronchiectasis. An immunological panel can assess levels of IgG, IgA, IgM, IgE, and specific antibody responses. CFTR gene analysis and the sweat chloride test are used to exclude cystic fibrosis. In suspected primary ciliary dyskinesia, nasal nitric oxide measurement may be helpful. If a connective tissue disease is suspected, an autoimmune panel (including ANA, RF, ENA, and ANCA) can be evaluated. Additionally, tests for ABPA (total IgE, *Aspergillus*-specific IgE/IgG, skin prick test) are essential components of the etiological workup. Although chest radiography is less sensitive than HRCT, it may still show dilated airways and air-fluid levels in advanced bronchiectasis. Bronchoscopy is indicated in cases of suspected obstructive lesions, and it is also utilized for tissue sampling and for bronchial toilette in cases of mucus retention.

Table 2: Radiological and Clinical Features of Bronchiectasis Types

Feature	Cylindrical	Varicose	Cystic
HRCT Appearance	Uniform dilation	Beaded appearance	Thin-walled cysts
Bronchus-to-artery ratio	Mildly increased (<2)	Variable	Markedly increased (>2)
Wall Thickness	Mildly thickened	Irregular, thick	Thin, fragile walls
Bronchial Termination	Straight, not tapered	Irregular	Cystic appearance
Severity	Mild	Moderate	Severe
Clinical Course	Slowly progressive	Prone to exacerbations	Persistent, symptomatic
Prognosis	Best	Intermediate	Worst
Associated Disease	Nonspecific/early-stage	Post-infectious	Cystic fibrosis
Risk of Complications	Low	Moderate	High (e.g., hemoptysis)
Lung Function	Mildly impaired	Moderately impaired	Severely impaired
Response to Treatment	Good	Variable	Poor, prone to recurrence

Treatment:

The primary goals of bronchiectasis treatment are to alleviate symptoms, prevent exacerbations, improve quality of life, and slow disease progression. Management is multidisciplinary and is based on four fundamental principles: infection control, airway clearance, reduction of inflammation, and treatment of the underlying cause.

Exacerbations are typically recognized by increased sputum volume and purulence, worsening cough, dyspnea, or systemic symptoms. Antibiotic therapy guided by sputum culture is essential. *H. influenzae*, followed by *P. aeruginosa*, *S. pneumoniae*, and *S. aureus*, are among the pathogens most commonly recovered on culture. While oral antibiotics may be sufficient for mild cases, intravenous therapy may be required for severe exacerbations (3). Colonization by *Pseudomonas aeruginosa* has been linked to an unfavorable clinical prognosis. In such cases, inhaled antibiotics (e.g., tobramycin, colistin, aztreonam) can be beneficial. Cyclic regimens (e.g., 28 days on, 28 days off) are commonly employed. Long-term suppressive therapy may reduce the frequency of exacerbations (4).

Effective airway clearance in patients with bronchiectasis reduces mucus retention and thereby the risk of infection. Recommended methods include chest

physiotherapy (postural drainage, percussion, vibration), active breathing techniques (autogenic drainage, huff coughing), positive expiratory pressure (PEP) devices (Flutter, Acapella), and inhalation of hypertonic saline or mannitol to decrease mucus viscosity. These techniques should be tailored to individual patients and, when possible, taught under the supervision of a physiotherapist (5).

Prolonged, low-dose administration of macrolides such as azithromycin and erythromycin has been demonstrated to decrease the frequency of exacerbations, attributable to their anti-inflammatory and immunomodulatory properties. The therapeutic effect is most pronounced in individuals with recurrent exacerbations and colonization by non-*Pseudomonas aeruginosa* pathogens (6).

The use of systemic corticosteroids in bronchiectasis is generally limited. However, in patients with coexisting asthma, COPD, or allergic bronchopulmonary aspergillosis (ABPA), systemic or inhaled corticosteroids are indicated.

Identifying and treating the underlying cause is essential for the successful management of bronchiectasis. In ABPA, oral corticosteroids and antifungal agents (e.g., itraconazole, voriconazole) are used. In cases of immunodeficiency, immunoglobulin replacement therapy is recommended. For gastroesophageal reflux, proton pump inhibitors (PPIs) and aspiration prevention strategies should be employed. In patients with cystic fibrosis, specific CFTR modulators such as ivacaftor or tezacaftor/ivacaftor combinations help slow disease progression and improve control.

Surgical intervention may be warranted in carefully selected patients. In individuals with localized disease refractory to medical management, segmentectomy or lobectomy can constitute appropriate therapeutic options. In cases of frequent and severe hemoptysis, bronchial artery embolization is the preferred intervention (7).

Additionally, supportive measures such as influenza and pneumococcal vaccinations, nutritional support, regular exercise, and smoking cessation are important components of bronchiectasis management.

Prognosis: Bronchiectasis is a chronic and progressive disease with a variable course, depending on etiological factors, disease extent, microbiological profile, and the patient's response to treatment. While some patients may remain stable over long periods, many experience a gradual decline in pulmonary function, increased frequency of exacerbations, and impaired quality of life. As such, bronchiectasis is now regarded not merely as a morphological diagnosis but as a systemic disease with functional consequences (2).

In non-cystic fibrosis bronchiectasis, overall mortality rates range between 10% and 30% over a five-year follow-up period. Mortality is typically associated with severe exacerbations, chronic hypoxemia, pulmonary hypertension, massive

hemoptysis, or the presence of comorbidities. Chronic colonization with *Pseudomonas aeruginosa* is one of the strongest predictors of mortality (3).

In cystic fibrosis–related bronchiectasis, respiratory failure is the most common cause of death, especially among individuals reaching adulthood. However, the introduction of CFTR modulators into clinical practice has significantly improved life expectancy (3).

Factors influencing prognosis in bronchiectasis include the extent of clinical and radiological involvement, frequency of exacerbations, microbiological profile, degree of systemic inflammation, presence of comorbidities, nutritional status, and physical activity level (7, 8).

Several validated indices exist for the assessment of bronchiectasis severity and for prognostic stratification. The most widely used tools are the **Bronchiectasis Severity Index (BSI)** -which includes parameters such as age, FEV₁, number of exacerbations, *Pseudomonas aeruginosa* colonization, and body mass index- and the **FACED score**, which is based on FEV₁, age, presence of *P. aeruginosa*, number of affected lobes, and degree of dyspnea. These scoring systems guide clinicians in determining follow-up intervals and in individualizing treatment strategies (9).

The **2023 ERS guidelines** emphasize the importance of etiological investigation, early use of inhaled antibiotics prior to exacerbations, and personalized treatment approaches in the diagnosis and management of bronchiectasis. Additionally, artificial intelligence–based analysis of HRCT images is being explored as a tool for disease classification. In response to rising antibiotic resistance, bacteriophage therapy is also emerging as a potential therapeutic option (10).

Recent advances in bronchiectasis research include microbiota-targeted therapies, gene expression profiling, and the investigation of novel biomarkers for disease monitoring and stratification (11).

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CHAPTER 3

Thymoma

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Thymoma represents a neoplastic process arising from the epithelial elements of the thymic gland, typically encapsulated, slow-growing, and predominantly located in the anterior mediastinum, with a tendency for local invasion. Although thymoma predominantly arises in the anterior mediastinum, it may infrequently be encountered in ectopic sites such as the cervical region, hilar area, lung parenchyma, or middle mediastinum. Despite its potential occurrence in all age groups, thymoma is most commonly diagnosed around the fifth decade.

Thymoma constitutes nearly 20–30% of neoplasms localized in the anterior mediastinum and represents the most prevalent tumor arising from thymic tissue. While thymomas are often detected incidentally, they tend to grow slowly and remain asymptomatic for an extended period. Nevertheless, when the tumor is large or exhibits substantial local invasion, it may exert pressure on neighboring structures, resulting in manifestations such as dyspnea, chest discomfort, or cough.

Thymoma is commonly linked to autoimmune disorders, with myasthenia gravis (MG) being the most frequently observed associated condition. A thymoma is detected in nearly 10–15% of patients presenting with myasthenia gravis, while conversely, myasthenia gravis develops in approximately 30–50% of individuals diagnosed with thymoma (1). Furthermore, a relationship has been reported between thymomas as well as disorders such as pure red cell aplasia, hypogammaglobulinemia, alongside other paraneoplastic syndromes (Table 1).

Histologically, thymomas are classified into low-risk (Type A and AB) and high-risk (Type B1, B2, and B3) subtypes. The severity and spread of thymoma are commonly classified according to the Masaoka–Koga staging scheme and the TNM system. The treatment strategy depends on the tumor stage and histological subtype and may involve surgical resection, radiotherapy, and chemotherapy. In thymomas detected at an early stage and amenable to complete surgical excision, the overall prognosis is typically good; conversely, outcomes are less favorable in the presence of invasive or metastatic disease.

Histopathological Classification And Staging:

Thymomas are categorized into distinct subtypes according to their histopathological features and clinical behavior, and staging systems are used to determine disease extent. There are multiple staging systems for thymoma, including the Masaoka-Koga staging system, TNM (Tumor, Node, Metastasis), Groupe d'Etude des Tumeurs Thymiques (GETT), WHO (World Health Organization) classification, Müller-Hermelink (1989), Suster & Morran (1997), and Kuo (2001). Among these, the TNM system is considered the most suitable staging method according to modern oncological standards, while the Masaoka-Koga system remains the most widely adopted in clinical practice. The WHO classification is also used for clinical evaluation and prognostic purposes.

According to the World Health Organization (WHO) histological scheme, thymomas are grouped into five principal categories based on their cellular architecture:

- **Type A:** Composed of spindle- or oval-shaped epithelial cells with a low lymphocyte ratio. It typically exhibits a biologically indolent and benign clinical course.
- **Type AB:** A mixed form containing areas similar to Type A and lymphocyte-rich regions. It is generally correlated with a favorable clinical course and overall prognosis.
- **Type B1:** Characterized by a high proportion of lymphocytes, resembling normal thymic tissue. Prognosis is generally favorable.
- **Type B2:** Contains a greater number of epithelial cells and exhibits significant interaction with immune cells. It has an increased risk of invasion.
- **Type B3:** Composed of round or polygonal epithelial cells. It has a high malignant potential and may progress aggressively.

Staging Systems

The Masaoka–Koga staging system is the most commonly utilized framework for assessing the extent of thymoma:

- **Stage I:** Tumor confined within the capsule, no invasion.
- **Stage II:** Microscopic capsular invasion or macroscopic minimal extension into adjacent tissues.
- **Stage III:** The tumor exhibits direct invasion into contiguous structures, including the pericardium, major vascular structures, or lung parenchyma.
- **Stage IVa:** Tumor spreads to the pleura or pericardium.
- **Stage IVb:** Presence of hematogenous or lymph node metastasis.

The TNM system is used to stage both thymomas and thymic carcinomas:

T (Tumor Stage)

- **T1:** Tumor confined to the thymus
 - **T1a:** No macroscopic capsular invasion
 - **T1b:** Presence of capsular invasion
- **T2:** Tumor infiltrates neighboring structures, including the pericardium, mediastinal pleura, or surrounding fibrous tissues.

- **T3:** Tumor invades major vessels, lung, diaphragm, or deep layers of the pericardium
- **T4:** Tumor invades the aorta, pulmonary artery, myocardium, or trachea

N (Lymph Node Metastasis)

- **N0:** Regional lymph node involvement is absent
- **N1:** Metastatic spread is confined to anterior mediastinal (perithymic) lymph nodes
- **N2:** Metastases are present in deep intrathoracic or cervical lymph node stations

M (Distant Metastasis)

- **M0:** No distant metastasis
- **M1a:** Metastasis to the pleura or pericardium
- **M1b:** Distant organ metastasis (e.g., lung, liver)

Clinical Findings:

Thymoma is a slow-growing tumor located in the anterior mediastinum and remains asymptomatic in most patients. In many cases, it is incidentally detected on thoracic radiographs or computed tomography (CT) scans performed for unrelated reasons. Symptoms of thymoma vary depending on tumor size, compression of adjacent structures, and paraneoplastic effects.

By compressing neighboring anatomical structures such as the trachea, bronchi, or great vessels, thymomas may produce symptoms including shortness of breath, chronic cough, thoracic pain, stridor, or wheeze. In some cases, compression of mediastinal structures may result in dysphagia or superior vena cava syndrome (SVCS). SVCS presents with facial and neck swelling, headache, dizziness, and jugular venous distension.

Thymoma demonstrates a strong association with autoimmune diseases. Among the paraneoplastic syndromes related to thymoma, myasthenia gravis (MG) is the most commonly observed condition. Patients with MG may present with muscle weakness, particularly affecting the ocular and facial muscles, as well as ptosis, diplopia, dysarthria, and dysphagia.

In advanced or invasive thymomas, particularly when the tumor compresses major vessels or demonstrates direct invasion, patients may experience palpitations, chest pain, tachycardia, hypotension, syncope, or pericardial effusion. Additionally, neuromuscular and musculoskeletal symptoms such as muscle weakness, early fatigue, involuntary muscle fasciculations, cramps, gait disturbances, and balance impairment may also be observed.

Thymoma is among the tumors most frequently associated with paraneoplastic syndromes, occurring in 30–50% of patients (Table 1).

Table 1: Paraneoplastic Diseases Associated with Thymoma

Hematologic Syndromes • Pure red cell aplasia • Erythrocytosis • Pancytopenia • Megakaryocytopenia • T-cell lymphocytosis • Acute leukemia • Multiple myeloma • Immune Deficiency Syndromes ◦ Hypogammaglobulinemia ◦ T-cell disorder syndrome Neuromuscular Syndromes • Myasthenia gravis • Myotonic dystrophy • Eaton-Lambert syndrome • Myositis Collagen Diseases and Autoimmune Disorders • Systemic lupus erythematosus • Rheumatoid arthritis • Polymyositis • Myocarditis • Sjögren’s syndrome • Scleroderma • Ulcerative colitis	Skin Diseases • Pemphigus • Chronic mucocutaneous candidiasis Endocrine Disorders • Hyperparathyroidism • Hashimoto’s thyroiditis • Addison’s disease • Chemodektoma Renal Diseases • Nephrotic syndrome • Minimal change nephropathy Bone Diseases • Hypertrophic osteoarthropathy Malignancies • Hodgkin’s and Non-Hodgkin’s lymphoma • Carcinomas (colon, lung, etc.) • Kaposi’s sarcoma

Diagnosis:

As most patients are asymptomatic, thymomas are often detected incidentally on imaging studies obtained for reasons unrelated to their underlying pathology. For a definitive diagnosis, **imaging modalities, biochemical tests, and histopathological evaluations** should be assessed collectively. Thymomas may be mistaken for other mediastinal lesions; therefore, the differential diagnosis should encompass lymphoma, thymic carcinoma, germ cell tumors, and masses originating from the thyroid or parathyroid glands.

1. Imaging Modalities

Radiography

Although chest radiographs may suggest the presence of a mediastinal soft-tissue mass, their sensitivity is limited and its location in the anterior mediastinum on lateral views, they are insufficient for determining tumor margins,

characteristics, or invasion status. Additionally, small lesions may be missed on conventional radiography.

Computed Tomography (CT)

CT confirms the presence of an anterior mediastinal mass and helps differentiate solid from cystic lesions, assess the presence of calcifications, and determine the tumor's relationship with adjacent structures. Features suggestive of malignancy include vascular invasion, capsular invasion, pleural involvement, and, in later stages, pleural effusion confirmed by cytology.

Magnetic Resonance Imaging (MRI)

Although MRI is costlier compared with CT, and conventional radiography, it provides superior preoperative evaluation of neurovascular structures and potential vascular invasion.

Positron Emission Tomography (PET)

PET imaging has been investigated for its potential role in **differentiating** thymic hyperplasia from neoplasia and distinguishing thymoma from thymic carcinoma. Additionally, FDG-PET is frequently utilized for tumor staging, detecting distant metastases, and monitoring recurrence during follow-up.

In recent years, artificial intelligence (AI)-assisted imaging techniques have demonstrated high accuracy in distinguishing thymoma from other mediastinal pathologies, providing detailed guidance on diagnostic and therapeutic approaches (2).

2. Biochemical and Serological Tests

Although no specific biomarker has been identified for the diagnosis of thymoma, certain laboratory tests may provide supportive findings. Acetylcholine receptor (AChR) antibodies are elevated in patients with myasthenia gravis. Furthermore, in all young patients found to have a mediastinal mass, serum beta-human chorionic gonadotropin (β -HCG) and alpha-fetoprotein (AFP) levels should be assessed to exclude malignant germ cell tumors.

3. Histopathological Diagnosis (Biopsy and Examination Methods)

In localized and resectable thymoma cases, tissue biopsy is generally not recommended due to the risk of capsular disruption and tumor spread. However, in cases with suspected invasive thymoma or metastasis, and when a histopathological diagnosis is required, transthoracic fine-needle aspiration biopsy (TTFNA) and Tru-Cut biopsy may be considered.

- **Transthoracic Fine-Needle Aspiration Biopsy (TTFNA):** Useful for diagnosis but may be insufficient in determining histological subtypes.

- Tru-Cut Biopsy: Provides larger tissue samples, increasing sensitivity and specificity, enabling definitive diagnosis and aiding in subtype classification.
- Video-Assisted Thoracoscopic Surgery (VATS), Mediastinotomy, Mediastinoscopic Biopsy: These surgical sampling techniques should be pursued when a conclusive diagnosis cannot be achieved through less invasive modalities.

Treatment:

Surgical Treatment

Surgical resection constitutes the principal therapeutic approach for thymoma, with the indication determined by tumor stage and the degree of local invasion (3,4). The procedure requires en bloc dissection and complete resection of the entire thymus gland along with the surrounding mediastinal adipose tissue. In early-stage thymomas (Stage I and II), surgery offers a curative treatment option, whereas in advanced-stage thymomas (Stage III and IV), surgery is typically combined with adjuvant therapies such as radiotherapy and chemotherapy. In Stage IVb thymomas (with distant organ metastases), surgery is generally not recommended, and systemic therapy is preferred. In patients with myasthenia gravis, thymectomy may be indicated regardless of tumor presence.

Surgical Approaches

Selection of the surgical technique is determined by the tumor's stage, dimensions, and degree of local invasion. The terminology outlined below is routinely utilized in the context of thymic surgical procedures:

- Extended Thymectomy: En bloc resection of the thymoma, thymic gland, and mediastinal adipose tissue, including the region between the bilateral phrenic nerves, innominate vein, and diaphragm.
- Thymothymectomy: Complete resection of the thymoma and the entire thymic gland.
- Thymomectomy: Removal of the thymoma with preservation of residual thymic tissue, ensuring safe margins.

The main surgical techniques used in thymoma treatment include (5,6):

- Median Sternotomy
- Video-Assisted Thoracoscopic Surgery (VATS) - Minimally Invasive Thymectomy
- Robotic-Assisted Thymectomy (RATS)
- Partial Thymectomy

- **Extended Thymectomy**

Until recently, complete and/or partial sternotomy was the most commonly used approach for thymectomy. Alternative thoracotomy and (hemi)clamshell incisions have also been utilized in thymoma surgery. With advancements in minimally invasive surgery, VATS has emerged as a safe and effective approach for thymomas and other selected mediastinal masses.

In cases where unilateral phrenic nerve invasion occurs, the phrenic nerve may be excised along with the tumor, depending on the patient's pulmonary function. However, in patients with myasthenia gravis, preserving the phrenic nerves is a priority. In cases of bilateral phrenic nerve invasion, debulking surgery is performed while attempting to preserve phrenic nerve function. Similarly, if the aorta, trachea, or major vascular structures are involved, and resection is not feasible, debulking surgery may be the only viable option (7). Following debulking surgery, residual tumor sites may be marked with metal clips to guide postoperative radiotherapy.

Regardless of the surgical incision type, the primary goal of thymoma surgery is complete removal of the tumor while preserving capsular integrity (no-touch technique) along with mediastinal adipose tissue.

Radiotherapy

Although no prospective studies have thoroughly investigated the efficacy of radiotherapy in thymic tumors, it is commonly utilized as part of postoperative management and in the treatment of unresectable malignancies. Adjuvant radiotherapy administered after resection of invasive thymoma has been shown to enhance local disease control and improve overall survival (8).

Stage I tumors are completely encapsulated and fully resected; thus, no additional treatment is required. In Stage II thymomas, due to macroscopic capsular invasion, extension into the surrounding mediastinal adipose tissue, or the presence of dense adhesions in the mediastinal pleura, the risk of recurrence increases. Therefore, adjuvant radiotherapy is recommended, particularly in histological subtypes B2, B3, and C. In Stage III and IV thymomas, postoperative radiotherapy is mandatory to achieve optimal local disease control.

Neoadjuvant radiotherapy (RT) is rarely used but may be considered in locally invasive cases to achieve R0 resection (9).

In patients with Stage IVb disease (distant metastases), severe cardiopulmonary comorbidities, or extensive locally invasive tumors where surgical margins cannot be clearly defined, chemotherapy and radiotherapy are considered first-line treatments. Neoadjuvant chemotherapy is particularly utilized to reduce tumor size and increase resectability before surgery.

Chemotherapy

An evidence-based standard chemotherapy protocol for thymoma and thymic carcinoma has not been firmly established, largely owing to the absence of randomized controlled trials in this population. Chemotherapy may be considered in cases with R1–R2 resections, unresectable disease, or distant metastatic spread. The primary agents used in chemotherapy regimens include anthracyclines and cisplatin (10).

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CHAPTER 4

The Role of Pediatric Nurses in Holistic Health Care for Children During Earthquakes

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What is an Earthquake?

An earthquake, ground tremor, seismic event, or earthquake is a seismic event that occurs as a result of energy released unexpectedly in the Earth's crust, causing the Earth to shake. Seismic activity refers to the frequency, type, and magnitude of an earthquake in the area where it occurs. Earthquakes are measured with seismography. The branch of science that studies these events is called seismology. The magnitude of an earthquake is determined by the Moment Magnitude Scale, or the formerly used Richter Scale. According to this scale, earthquakes of magnitude 3 and below are generally not felt, while earthquakes of magnitude 7 and above can be devastating. The intensity of the tremor is measured by the Mercalli Intensity Scale. The depth of the earthquake also affects the destructive force; therefore, earthquakes occurring closer to the surface of the Earth cause more damage (Edemen et.al., 2023; Karakuş, 2013).

Turkey is located on the Alpine-Himalayan earthquake zone, one of the world's most active earthquake zones (Duman et al., 2018; Gül and Bayram, 2014). Twenty earthquakes with magnitudes greater than 7 have occurred in Turkey since the 1900s. This places Turkey among the countries most affected by earthquakes. Between 1900 and 2023, 269 earthquakes have occurred in Turkey, causing loss of life or damage. The largest earthquakes in terms of loss of life and severe damage were, respectively, the 2023 Kahramanmaraş earthquake, the 1999 Gölcük-centered Marmara earthquake, and the 1939 Erzincan earthquake. (Kahramanmaraş and Hatay Earthquakes 2023 Report, 2023).

Holistic Health Services

Holistic paradigm is an approach that accepts that the nature of the subject can be grasped by evaluating interrelated and articulated units together (Ventegodt et.al., 2016).

The Concept of Holistic Health is based on the principle that the individual is considered as a whole in physical, spiritual, mental and social areas, that each individual is different from each other and that health and illness cannot be considered separately (Smedberg, 2009).

Children and Earthquakes

An earthquake is a natural event that can cause loss of life and property, unpredictable in terms of its impact area and intensity. Recent years have led to numerous natural events that have left their mark on society. Children, as members of society, are among the most innocent individuals affected by natural disasters. Every year, millions of children and young people are exposed to many natural disasters and are affected in different ways. These disaster experiences, which are not normal during childhood, can cause

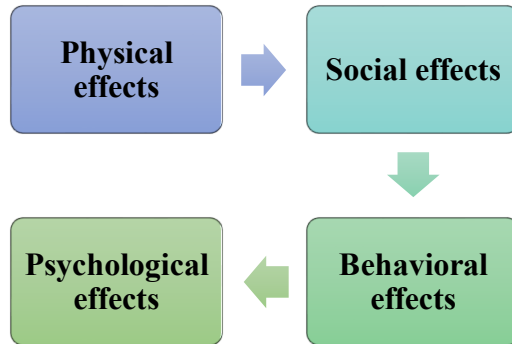
various physical and mental disorders in children (Gürbüz and Koyunca, 2023; Karabulut and Bekler, 2019).

Among the disasters our country has faced throughout history, the February 6 earthquake, known as the disaster of the century, is the largest natural disaster we have ever experienced. All living things were harmed in this disaster, and our children are among the most vulnerable members of this group (Gürbüz and Koyunca, 2023; Karabulut and Bekler, 2019).

It can result in emotional abuse for earthquake survivors, especially children, and psychological impacts for children and adults who did not directly experience the earthquake. Children, in particular, are at greater risk in this context (Aral, 2023).

The Effects of The Earthquake on Children

Effects of earthquakes on children are shown in figure 1 (Gürbüz and Koyunca, 2023).



Physical effects: When examining the health problems of children affected by the earthquake, it was stated that they experienced musculoskeletal problems, were at risk for developing respiratory problems, had digestive system disorders, and experienced sleep problems. Furthermore, inadequate post-earthquake hygiene conditions, difficulties accessing food and water, inadequate access to healthcare, housing problems, crowded living, delays in the burial of those who lost their lives in the earthquake, and consequently, the risk of infectious diseases due to all these unsanitary conditions (Felix et.al. 2016; Ekşi, 2016).

Social effects: Children and adolescents forced to leave their homes may experience difficulties adapting to their new living spaces due to factors such as language, religion, and culture. Children and adolescents forced to work at a young age due to poor socioeconomic conditions may experience disruptions in their education and declines in academic achievement. All of these difficulties can also lead to psychological problems (Kaştan, 2015).

Psychological effects: Most studies examining the psychological effects of natural disasters on children and adolescents have focused on symptoms of depression (Cheng et al. 2018; Felix et al. 2011). However, it would be a misconception to assume that every child will experience the same psychological problems after a natural disaster. Studies have concluded that depression, generalized anxiety, and pathological grief reactions are the most common psychological effects in children after natural disasters (Karabulut and Bekler, 2019; Hansel et al. 2015).

Behavioral effects: Children's reactions to earthquakes can vary depending on their developmental stages. For example, during childhood, they may experience adjustment difficulties in response to changing living conditions and disrupted routines. School-age children are now aware of events and can approach them more realistically. For adolescents, experiencing a loss of friends during this period can lead to health problems such as depression and post-traumatic stress disorder (Sönmez, 2023; Karabulut and Bekler, 2019).

Earthquake and Holistic Health Services

When the effects of earthquakes on individual and public health are evaluated, food and hygiene problems, the urgency of chronic diseases, psychological processes, the possibility of the spread of infectious diseases, and the health needs of special groups such as women, the elderly, the disabled, and children emerge as important topics (Gürbüz and Koyuncu, 2023; Fatema et al., 2019; Nakajima, 2012;). Disasters, which can be considered a global health problem, cause significant burdens on the healthcare system. The burden of post-earthquake healthcare services and public access to the system are among the issues that must be evaluated to ensure continuity of living conditions (Manirambo et al., 2023). According to the World Health Organization (WHO) (2019), the risks associated with health emergencies and disasters worldwide are very diverse. Holistic health services are vital for managing these risks, ensuring national and global health security, and strengthening access to universal health services to build the resilience of societies, countries, and health systems.

The Role of Nurses in Holistic Health Care For Children During Earthquakes

In post-disaster emergency health interventions, the capacity to provide individualized care and the ability to intervene quickly in times of crisis are crucial. Despite the challenges faced by nurses in the disaster environment, their ability to identify children's medical and psychological needs and provide personalized care plays a vital role in crisis management (Topal, 2025; Kurt et al., 2024; Akbaba et al., 2024). Additionally, social workers, through their roles as organizers and coordinators in crisis situations, can create an environment of effective communication and collaboration between local health units, non-governmental organizations, and families. In particular, home health care teams

providing psychosocial support while continuing the medical care of children after a disaster contributes to the acceleration of post-traumatic recovery (Topal, 2025; Karaağaç, 2023).

In large-scale disasters such as earthquakes, multidisciplinary approaches are essential in emergency response and long-term rehabilitation processes. Collaboration among experts from the fields of nursing, social work, psychology, rehabilitation, and education is critical to overcoming the unique challenges faced by young children (Topal, 2025; Topal et al., 2024; Dağ et al., 2024). Disaster-related health policies should be restructured with an approach that prioritizes the needs of these children. Emergency plans, education programs, and crisis communication strategies should be designed to include specific approaches for children with special needs (Topal, 2025; Kurt et al., 2024; Bilik, 2023).

With a multidisciplinary approach, nurses can improve children's psychosocial adjustment and reduce problems such as anxiety and depression (Topal, 2025; Çakır and Kıziler, 2022; Çakır and Altay, 2021). This support can reduce parents' stress and help them overcome the challenges they face in caring for their children. Community-based social support networks play a critical role in protecting the physical health of these children and strengthening their psychological resilience. Nursing interventions increase the functionality of these networks and facilitate caregiving processes (Topal, 2025; Çakır and Kıziler, 2022; Çırlak and Törüner, 2021).

Required equipment for a pediatric nurse who will work in natural disasters and earthquakes:

Integrity: recognizes the multisystemic nature of changes occurring at the organismic and personality levels, including the central nervous system.

Structuralism: Explains the natural unity of structural and functional changes at all levels of the systemic organization of the child's body and personality.

Causality: Examines the causality of the development and characteristics of pathological disorders in children's bodies during emergencies, the stages of these changes, and the stages of development.

Dynamism: Understands the complex structure of the child's body systems and the system of self-regulation in emergencies, their reliability and stability, and the dynamic nature of normal and pathological conditions. In short, recognizes the body's ability to "develop itself."

Hierarchy: Integrates the complex relationships between physiological and mental processes in children, psychological and social patterns, and establishes the interaction between these processes (Manav and Nazik, 2023; Shapovalov et al., 2022).

Roles of the pediatric nurse:

- Participates in national and local disaster preparedness efforts and actively participates in the care of children after a disaster.
- Must ensure that medical supplies, disaster management teams, pediatric disaster teams, and local hospitals are staffed and have appropriate equipment.
- Must believe in the need to include child-specific disaster preparedness content in pediatric nursing education programs and continuing education.
- Must engage in local and national educational and advocacy efforts to ensure the availability of pediatric healthcare providers in disaster response and mitigation of child impacts.
- Must engage in local and national advocacy efforts to ensure adequate resources and funding for necessary disaster relief services, recovery efforts, and disaster management, and to ensure adequate resources and funding are available to communities.
- Displaced children should be quickly identified and reunited with their families or referred to appropriate care if their parents do not survive the disaster.
- Special attention should be paid to the mental health and psychological needs of children and families after a disaster, and pediatric nurse training should be encouraged to provide psychological first aid.
- Special policies and processes should be supported and implemented to support and protect breastfeeding mothers and their infants before, during, and after a disaster. A safe environment for breastfeeding should be provided, as adequate nutrition and the immunological properties of breast milk are critical to the supply.
- Disaster preparedness screening and proactive guidance should be integrated into primary care, including vaccination to prevent disease.
- All families and communities should be encouraged to have a post-disaster reunification plan (Manav and Nazik, 2023; NAPNAP, 2011).

Conclusion

Regardless of the hospital setting, nurses play a crucial role in disaster planning and management that extends beyond providing immediate emergency care. Pediatric nurses possess a comprehensive understanding of the

vulnerabilities that put children at increased risk during disasters. In addition, pediatric nurses must regularly facilitate communication among team members and coordinate care under stressful circumstances. Consequently, pediatric nurses must work within their local communities, including schools, daycare centers, and local governments, as well as within hospitals or acute care settings, to ensure disaster preparedness.

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CHAPTER 5

Oncolytic Potential of Morbilliviruses

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Introduction

Morbilliviruses are enveloped, negative-strand RNA viruses that cause respiratory diseases (Lin, 2020; Arruda et al., 2021). Advances in reverse genetics and molecular virology have enabled the redesign of attenuated morbilliviruses both as vaccine platforms and as oncolytic virotherapy agents (Lin, 2020; Zhao et al., 2020). Oncolytic virotherapy is an innovative treatment approach that aims to eliminate specific cancer cells by infecting them with viruses that retain their replication ability. This therapeutic method exploits defects in antiviral defense mechanisms within cancer cells—particularly deficiencies in apoptosis, translational suppression, and interferon response—to therapeutic advantage. According to Russell and colleagues (2012), the mechanisms by which viruses trigger cell death are not limited to direct cytolytic effects but also include indirect effects such as the destruction of tumor vessels via the immune system and the enhancement of the anti-tumor immune response. Therefore, classical cell death definitions (apoptosis, necrosis, and autophagy) are insufficient to fully explain the multifaceted biological effects of oncolytic virotherapy.

General Characteristics of Morbilliviruses

Morbilliviruses are enveloped viruses belonging to the Paramyxoviridae family, which cause systemic infections in humans and various mammalian species through respiratory transmission. This virus group includes species such as Measles virus (MV), Canine distemper virus (CDV), Rinderpest virus (RPV), Peste des petits ruminants virus (PPRV), Phocine distemper virus (PDV), and the recently identified Porcine morbillivirus (PMV) (Lin, 2020; Arruda et al., 2021). Viruses belonging to the Paramyxoviridae family are generally 150–200 nm in diameter and exhibit distinct pleomorphism, appearing in both spherical and filamentous forms under electron microscopy. The nucleocapsid of these viruses has helical symmetry and contains a segmented, linear, negative-sense single-stranded RNA genome. The genome length varies between approximately 14.6 and 20.1 kb and carries the virus's essential genetic information. Morbillivirus genomes encode six structural proteins: nucleocapsid (N) protein, phosphoprotein (P), matrix (M) protein, hemagglutinin (H) protein, fusion (F) protein, and large polymerase (L) protein. In addition, two non-structural proteins, C and V, are expressed from the P open reading frame and are thought to suppress innate immune responses in at least one subgroup of members of the Paramyxoviridae family (Lin, 2020; Arruda et al., 2021).

The selective binding of morbilliviruses to host cells is determined by the cell receptors utilized by the virus. Natural measles virus strains target the SLAMF1/CD150 (SLAMF1) receptor found on immune cells and also bind to the nectin-4 (PVRL4) receptor on epithelial cells. This receptor plays a critical role in the processes of spreading throughout the respiratory epithelium and exiting the host (Laksono et al., 2020; Noyce et al., 2011; Noyce and Richardson, 2012).

In contrast, the primary receptor for laboratory-adapted/vaccine-derived strains derived from the Edmonston lineage is CD46. CD46 is found in many nucleated cells in humans and is also highly expressed in many tumor cells. Therefore, Edmonston-based oncolytic vectors derived from the natural measles virus exhibit strong tropism for tumors that highly express CD46 (Anderson et al., 2004; Dhiman, 2004). The clinical and translational oncolytic measles virus literature documents this receptor-based tropism in detail (Msaouel et al., 2018; Russell et al., 2019).

After the virus enters the cell, the genome is released into the cytoplasm, and mRNA synthesis is initiated by the viral RNA polymerase complex. Subsequently, viral protein production accelerates, new genomes are copied, and envelope proteins are transported to the cell membrane, resulting in the budding of mature virus particles (Lin, 2020). One of the typical cytopathic effects of morbillivirus infections is the formation of giant cells (syncytia) resulting from cell-cell fusion mediated by H and F glycoproteins. This facilitates the spread of the virus between cells. In oncolytic measles virus applications, syncytia formation may be associated with virus-induced cytotoxicity or immunogenic cell death in tumor cells; however, this effect may vary depending on the type of tumor and context (Aref et al. 2016; Burton and Barteo, 2019; Pidelaserra and Engeland, 2020; Russell et al. 2019; Engeland and Unge-rechts, 2021).

Advances in modern genetic technology have made it possible to resynthesize morbilliviruses and genetically engineer them as desired. This development allows for increased target specificity in oncolytic virotherapy studies by incorporating therapeutic genes, immunomodulatory cytokines (e.g., IL-12, IL-15) or imaging markers (e.g., NIS) into the viral genome; the immune system can be strengthened, and the infection can be monitored in vivo (Backhaus et al., 2019; Lin, 2020; Zhao et al., 2020).

Mechanisms of Oncolytic Action

The antitumor effect of oncolytic morbilliviruses consists of complex processes involving both direct cellular destruction (oncolysis) and indirect tumor clearance mechanisms mediated by the host immune system (Russell et al., 2012; Engeland and Ungerechts, 2021).

The direct oncolytic effect of morbilliviruses is particularly related to their ability to selectively enter tumor cells and their high replication capacity within these cells. The source of this selectivity is the use of specific cell receptors by different viral strains, such as CD46 (attenuated strains), SLAM/CD150 (immune cells), and Nectin-4/PVRL4 (epithelial and tumor cells) (Dörig et al., 1993; Tatsuo et al., 2000; Noyce et al., 2011). Different receptors play a role in viral entry; for example, MV enters host cells via CD46, SLAM (CD150) or Nectin-4, while CDV specifically enters human breast cancer cells (MCF-7) via Nectin-4 (PVRL4). Following infection, viral replication increases, leading to cytopathic

effects; cell proliferation is also suppressed. CDV has been reported to activate intrinsic apoptotic pathways, suppress MCL-1 and PIK3R1 gene expression, and trigger cell death by increasing DNMT3A levels (Jhala et al., 2024).

Canine distemper virus (CDV) has the potential to enter human tumor cells via the Nectin-4 (PVRL4) receptor. A study by Jhala et al. (2024) revealed that CDV causes a significant cytopathic effect in MCF-7 breast cancer cells, restricting cellular proliferation by disrupting cellular morphology and triggering apoptosis. Molecular-level analyses showed that MCL-1, SP1, and PIK3R1 gene expression decreased in infected cells, while DNMT3A levels increased. These changes were reported to be associated with the activation of intrinsic apoptotic processes. Furthermore, it has been demonstrated that CDV inhibits tumor cell growth by suppressing the PI3K/AKT signaling pathway, and it has been shown that these properties may contribute to its oncolytic potential. These findings indicate that viruses such as attenuated MV and CDV, which belong to the Morbillivirus family, have the capacity to target cancer cells using common receptors such as Nectin-4 and could serve as important models for developing interspecies translational oncolytic vectors (Jhala et al., 2024).

Oncolytic MV infection not only directly lyses tumor cells but also triggers immunogenic cell death (ICD) mechanisms, thereby eliciting a potent anti-tumor immune response. Pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) released during tumor cell lysis activate dendritic cells, enhancing antigen presentation. This process allows CD8⁺ T lymphocytes to infiltrate the tumor microenvironment intensively and supports the strengthening of systemic anti-tumor immunity. Thus, oncolytic MVs enable tumor elimination via the immune system not only in the primary tumor site but also in distant metastases (England & Ungerechts, 2021).

Oncolytic morbillivirus infections have been shown to have the potential to reprogram the tumor microenvironment. These infections can result in the conversion of immunosuppressive M2-phenotype macrophages to the pro-inflammatory M1 phenotype, increased NK cell activation, and elevated IFN- γ production. Experimental studies have demonstrated that genetically modified morbillivirus strains expressing IL-12 or IL-15 enhance antitumor efficacy by increasing T and NK cell infiltration (Backhaus et al., 2019).

Additionally, oncolytic morbilliviruses can create a potent immunovirotherapeutic synergy when used in combination with immune checkpoint inhibitors (anti-PD-1, anti-CTLA-4). This approach increases T cell infiltration by converting immunosuppressed ('cold') tumor microenvironments into more immunogenic ('hot') tumors, significantly enhancing the overall efficacy of immunotherapy (England and Ungerechts, 2021).

Furthermore, certain metabolic stress conditions, such as short-term fasting or serum/glucose restriction, can increase susceptibility to morbilliviruses; in such

cases, tumor cells become more open to infection (Scheubeck et al., 2019). One of the most important factors determining the oncolytic efficacy of morbilliviruses is the metabolic state of tumor cells. A study by Scheubeck et al. (2019) revealed that colorectal cancer cells become more susceptible to morbillivirus infection under metabolic stress conditions (starvation or serum restriction). This study reported increased viral replication, enhanced cytopathic effect, and a more pronounced oncolytic response in cells maintained in low glucose and low serum conditions. This increased susceptibility was particularly observed in the HT-29 cell line, while no significant cytotoxic effect was detected in normal colon epithelial cells. The findings indicate that metabolic stress supports the tumor cell tropism of morbilliviruses and that nutrient restriction may enhance therapeutic efficacy (Scheubeck et al., 2019).

On the other hand, various experimental models have demonstrated the potent oncolytic effects of morbilliviruses. In melanoma models, the Leningrad-16 strain was found to exhibit marked cytolytic activity (Ammour et al., 2020). Ammour and colleagues (2020) determined that the attenuated Leningrad-16 strain caused effective cytolytic activity in human melanoma cells. The study observed that the L-16 strain replicates in melanoma cells, leading to cell lysis, whereas infection is limited in normal fibroblast cells. Widespread syncytia formation and apoptotic cell death were recorded as a result of viral infection; it was also stated that high expression of the CD46 receptor is associated with cell sensitivity. In studies based on in vivo xenograft mouse models, a reduction in tumor volume of approximately 78% was achieved in groups treated with L-16. These findings indicate that the Leningrad-16 strain is a promising candidate for morbillivirus-based oncolytic virotherapy due to its selective tropism and potent cytolytic potential (Ammour et al., 2020). In colorectal carcinoma models, measles virus replication and associated cytopathic effects increased under nutrient starvation conditions; this was found to render tumor cells more susceptible to infection (Scheubeck et al., 2019).

In a phase I study conducted by Dispenzieri et al. (2017), systemic administration of the oncolytic MV-NIS to refractory multiple myeloma patients was generally well tolerated, and transient reductions in tumor burden were observed in some patients. A complete hematological response was achieved in only one patient, and this response persisted for 9 months. These findings suggest that morbilliviruses can be considered a potent viroimmunotherapeutic agent, not only due to their direct oncolytic effects but also because of their ability to re-regulate the immune system.

Genetic Engineering and Molecular Manipulations

With the development of reverse genetics systems, morbilliviruses have become vectors that can be easily manipulated both in fundamental virology studies and in oncolytic virotherapy/vaccine platforms. Systems initiated with

full-length cDNA clones and helper plasmids enable targeted insertion/deletion and modifications on the genome, thus allowing immunomodulatory genes, imaging markers, or targeting ligands to be stably integrated into the virus (Radecke et al., 1995; Lin, 2020; Zhao et al., 2020).

In tropism retargeting, the ability of the H protein to bind to its natural receptor is functionally restricted, and tumor-specific scFv or ligand fusions are added. This enables selective infection of specific targets such as HER2, CD38, or EphA2 (Radecke et al., 1995; Nakamura et al., 2005; Miest et al., 2013).

Immunostimulation strategies enhance the anti-tumor immune response by increasing T/NK cell infiltration through the expression of cytokines such as IL-12 and IL-15 (Backhaus et al., 2019; Engeland and Ungerechts, 2021). The addition of the NIS (sodium/iodine scavenger) gene to provide visualization enables the monitoring of in vivo viral replication using radioiodine-mediated PET/SPECT methods and, when necessary, its combination with radioiodine therapy (Dispenzieri et al., 2017). By combining reverse genetics, immunogenicity, and imaging modules, morbilliviruses are transformed into highly target-specific, trackable, and immunogenic oncolytic platforms. These combinations have been shown to provide a strong foundation for rapid tumor elimination in preclinical studies and safe clinical applicability (Dispenzieri et al., 2017; Lin, 2020; Zhao et al., 2020).

One notable example of the clinical effects of oncolytic virotherapy is the phase I clinical trial conducted by Dispenzieri et al. (2017). This study investigated the systemic applicability and safety of the attenuated Edmontons-derived measles virus (MV-NIS) in cases of relapsed or treatment-resistant multiple myeloma. The virus used was characterized as a morbillivirus that recognizes the CD46 receptor on the cell surface and was engineered to express the human sodium-iodide symporter (NIS) gene. This enabled infected tumor cells to be visualized with radioiodine, thereby increasing the monitorability of the treatment and ensuring targeting accuracy (Dispenzieri et al., 2017).

Advances in the clinical use of oncolytic morbilliviruses have reached a significant milestone with the pioneering human study conducted by Russell and colleagues (2014). In this study, an Edmonston strain attenuated measles virus (MV-NIS), modified by adding the sodium/iodine scavenger (NIS) gene, was administered systemically to multiple myeloma patients. Following treatment, complete remission was observed in one patient, while another patient experienced a marked but temporary tumor reduction. No serious adverse events were encountered during the study; only mild-to-moderate side effects such as fever, headache, and transient hematological changes were observed. The researchers also directly observed MV-NIS-induced viral replication in tumor regions using radioiodine (^{123}I) SPECT/CT imaging, demonstrating that the in vivo distribution of the virus can be monitored non-invasively. The findings

revealed that morbillivirus-based oncolytic agents are suitable for clinical applications due to their safety and visualizable properties (Russell et al., 2014).

One of the main problems encountered in the intravenous administration of oncolytic morbilliviruses is that preformed anti-MV antibodies in the circulation rapidly neutralize the virus, limiting its systemic distribution. This significantly reduces the efficacy of treatment, particularly in individuals with natural measles immunity. Peng et al. (2013) demonstrated that this obstacle can be overcome by using approved cyclophosphamide (CPA) protocols in clinical practice to suppress the antiviral antibody response.

Conclusions and Future Perspectives

Although morbilliviruses are classically known as lethal respiratory pathogens, they exhibit extraordinary therapeutic potential when engineered using reverse genetics technologies. The potent fusion (F) and hemagglutinin (H) glycoproteins of these viruses can effectively trigger selective infection and syncytium-mediated cell death processes in cancer cells. In particular, attenuated MV and CDV strains have gained a natural tumor tropism by targeting receptors such as CD46 and Nectin-4, which are highly expressed in tumors. Furthermore, immunomodulatory effects observed in vitro and in vivo models, such as immunogenic cell death, dendritic cell activation, and T/NK cell infiltration, transform morbilliviruses from mere cytolytic agents into potent viroimmunotherapeutic agents.

Genetic engineering applications enabling the addition of cytokines such as IL-12 and IL-15, in vivo visualization using NIS, and the re-targeting of the H-protein with tumor-specific ligands allow these platforms to be made clinically personalizable and safe. Clinical studies conducted by Russell et al. (2014) and Dispenzieri et al. (2017) have demonstrated that MV-NIS is tolerable during systemic administration, resulting in significant reductions in tumor burden in some patients and even complete remission in rare cases. However, the limitation of antiviral immunity to systemic effects, neutralization processes during circulation, and the presence of a resistant immunosuppressive microenvironment remain fundamental challenges for the clinical translation process.

Future research is expected to focus on combination with immune checkpoint inhibitors, metabolic stress-based sensitivity enhancement, and systemic distribution strategies using carrier cells (Scheubeck et al., 2019). With these approaches, morbillivirus-based oncolytic vectors are considered strong candidates for integrated cancer immunotherapies that could enable not only local tumor destruction but also systemic immune reprogramming, thereby transforming ‘cold’ tumors into ‘hot’ ones. Current data clearly demonstrate that morbilliviruses possess a critical platform potential beyond serving as a model system in the field of clinical oncolytic virotherapy, capable of forming the basis for future multimodal, trackable, and immunogenic cancer therapies.

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CHAPTER 6

Comparison of Complications in Screw- and Cement-Retained Implant-Supported Fixed Protheses: A Review

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Introduction

Nowadays, the treatment of tooth loss with implant-supported prostheses is an effective and acceptable option in terms of function and esthetics. However, as with every treatment option, various complications, failures, and limitations may occur (Pita et al., 2011). There are two types of retention for fixed implant-supported prostheses: screw-retained and cement-retained. Each type of retention has its own advantages and disadvantages (Millen, Brägger, & Wittneben, 2015). Screw-retained prostheses offer several advantages, including their ability to be removed by the clinician, their suitability for use in cases with limited interocclusal space, and the absence of residual cement. This absence of residual cement contributes to the preservation of peri-implant tissue health. However, there are also some disadvantages, such as esthetic limitations caused by the screw-access hole, the potential structural weakening of the restoration, and the technical difficulty of achieving passive fit. These factors may lead to marginal discrepancies or stress concentration at the implant–abutment interface and related components (García-Minguillán, Del Río, Preciado, Lynch, & Castillo-Oyagüe, 2020). In contrast, the fabrication of cement-retained restorations, being similar to tooth-supported fixed restorations, offers advantages in achieving esthetics, balanced load distribution, and ease of production. The cement layer between the abutment and the superstructure can compensate for minor misfits, contributing to improved passive fit (Hebel & Gajjar, 1997; Heckmann et al., 2004). However, residual cement has been associated with inflammation in peri-implant soft tissues and is considered a significant biological disadvantage. In the literature, studies have reported varying results regarding the incidence of mechanical and biological complications between screw- and cement-retained implant restorations. Furthermore, with the advancements in technology, recent innovations in implant-supported prosthetic systems are thought to influence these outcomes. Therefore, the aim of the present review is to compare the complications associated with both retention systems by analyzing recent clinical studies published between 2015 and 2025.

Materials and Methods

Literature Search Strategy

A literature search was performed in the PubMed database utilizing the Boolean query ((implant-supported OR implant-retained) AND (prosthesis OR crown OR bridge) AND (screw OR cement OR screw-retained OR cement-retained) AND (complication OR failure OR screw loosening OR peri-implantitis) AND ("2015/01/01"[Date -Publication]: "2025/10/31"[Date -Publication])). The article types were restricted to Clinical Trial, Controlled Clinical Trial, Comparative Study, and Randomized Controlled Trial, in English, yielding 103 articles from this query.

Selection Criteria

The initial PubMed search yielded 103 records. After screening titles and abstracts, 21 full-text articles were reviewed, and eight studies met the inclusion criteria and were incorporated into the present review. Clinical studies with a follow-up duration of at least 12 months that investigated the complications associated with cement and screw systems were included. Publications including in-vitro studies, finite element analyses, laboratory or animal experiments, reviews, meta-analyses, case reports, and research pertaining to removable or overdenture prostheses were excluded.

Data Extraction

Data were systematically extracted from each included article regarding the author(s), year of publication, journal, study design (randomized, prospective, or retrospective), retention type (screw- or cement-retained), follow-up duration (in months or years), and type and rate (%) of complications. Duplicate records were verified and eliminated.

Data Analysis

Mechanical complications (screw loosening, porcelain fracture, prosthesis fracture, etc.) and biological complications (peri-implantitis, mucositis, implant loss, etc.) associated with screw and cement-retained systems were systematically summarized and compared under distinct headings.

Ethical Considerations

As this study was conducted using data from published literature, no ethical approval was necessary.

Results

Eight clinical studies (Amorfini, Storelli, Mosca, Scanferla, & Romeo, 2018; Cacaci et al., 2017; Kronsteiner, & Rammelsberg, 2022; Kraus et al., 2024; Lv et al., 2021; Naumann, Happe, Holtkamp, & Blender, 2025; Naumann et al., 2023; Weigl et al., 2019; Wolfart et al., 2021) published between 2015 and 2025 that evaluated mechanical and biological complications in screw- and cement-retained prostheses were included in this review. Mechanical and biological complications were observed in both retention systems. In seven of the eight studies, no statistically significant difference was found between the two retention types, whereas only one study (Kraus et al., 2024) reported a significantly higher total complication rate in cement-retained restorations compared to screw-retained restorations ($p = 0.0041$).

Among the mechanical complications, the most frequently reported were screw loosening, abutment fracture, ceramic fracture (chipping), and decementation. Screw loosening was observed in both systems but was reported

more frequently in screw-retained prostheses. Chipping and decementation were more commonly seen in cement-retained restorations.

Regarding biological complications, mucositis was the most frequently reported event, while peri-implantitis and implant loss were observed only rarely. The main characteristics of the included studies are summarized in Table 1, and the mechanical and biological complication rates for screw- and cement-retained systems are presented in Table 2.

Table 1. Overview of the included clinical studies

Author (Year)	Journal	Study Type	Patients (n)	Follow-up Period (years)	Objective
C. Cacaci et al. (2017)	Clin Oral Investig	Prospective Clinical Study	58	3	To compare screw-retained and cemented implant-supported zirconia single crowns.
L. Amorfini et al. (2018)	Int J Prosthodont	Randomized Prospective Study	30	10	To compare screw-retained and cement-retained single crowns supported by customized zirconia abutments.
P. Weigl et al. (2019)	Clin Oral Investig	Prospective Clinical Study	22	1	To evaluate the clinical performance of screw-retained monolithic zirconia and cemented porcelain-fused-to-metal implant crowns.
S. Wolfart et al. (2019)	Clin Oral Implants Res	Randomized Controlled Clinical Study	41	2	To compare cemented and screw-retained posterior implant-supported single crowns.
X.L. Lv et al. (2021)	Clin Implant Dent Relat Res	Randomized Controlled Clinical Trial	60	1	To compare screw-retained and cemented single-implant crowns in the esthetic region.
M. Naumann et al. (2023)	Clin Oral Implants Res	Randomized Controlled Clinical Trial	20	3	To compare monolithic hybrid abutment crowns (screw-retained) with monolithic hybrid abutments with

					adhesively cemented monolithic crowns.
M. Naumann et al. (2024)	J Esthet Restor Dent	Prospective Clinical Study	10	3	To compare screw-retained and cemented monolithic hybrid abutment crowns.
R.D. Kraus et al. (2024)	Clin Oral Implants Res	Randomized Controlled Clinical Study	31	7.5	To compare cemented and screw-retained one-piece zirconia-based implant-supported single crowns.

FDP: Fixed Dental Prosthesis

Table 2. Reported overall complication rates in screw- and cement-retained implant-supported fixed prostheses (2015–2025)

Author (Year)	Screw-Retained Complication Rate (%)	Cement-Retained Complication Rate (%)	p-value	Outcome (S vs C)
C. Cacaci et al. (2017)	Mechanical: No veneering chipping (0%), Contact wear on restoration (4.6%), Contact wear on antagonistic teeth (10.7%) Biological: None	Mechanical: Veneering chipping (3.3%), Contact wear on restoration (1.8%), Contact wear on antagonistic teeth (16.5%) Biological: None	$p = 0.518$ (chipping) $p = 0.318$ (wear)	ns
L. Amorfini et al. (2018)	Mechanical: Ceramic chipping (3%), Screw loosening (6%) Biological: Mucositis (3%)	Mechanical: Screw loosening (6%), Crown decementation (3%), Abutment fracture (3%) Biological: Mucositis (3%)	$p = 0.65$ (all)	ns
P. Weigl et al. (2019)	Mechanical: Screw loosening (4.5%), Filling defect (9.1%) Biological: None	Mechanical: Ceramic chipping (9.1%), Decementation (9.1%) Biological: None	$p > 0.05$ (all)	ns
S. Wolfart et al. (2019)	Mechanical: Screw loosening (3%), No chipping (0%), Loss of occlusal contacts (32%),	Mechanical: No chipping (0%), Loss of occlusal contacts (32%), Loss of proximal contacts (18%),	$p > 0.05$ (all)	ns

	Loss of proximal contacts (18%) Biological: Mucositis (14.2%)	Cement residue (6.9%) Biological: Mucositis (17.9%)		
X.L. Lv et al. (2021)	Mechanical: Filling material loss (3.45%) Biological: None	Mechanical: None Biological: None	p = 0.330 (all)	ns
M. Naumann et al. (2023)	Mechanical: None Biological: None	Mechanical: Ti-base debonding (10%) early failure, repaired and functional until 36 months Biological: None	p > 0.05 (all)	ns
M. Naumann et al. (2024)	Mechanical: None Biological: None	Mechanical: None Biological: None	p > 0.05 (all)	ns
R.D. Kraus, et al. (2024)	Mechanical: Abutment fracture (19.1%), Minor chipping (4.8%), Screw loosening (0%) Biological: Peri-implant mucositis (9.5%), Peri-implantitis (4.8%), Implant loss (0%)	Mechanical: Abutment fracture (15.8%), Minor chipping (15.8%), Screw loosening (10.5%) Biological: Peri-implant mucositis (31.6%), Peri-implantitis (0%), Implant loss (10.5%)	p = 0.0041 (Total) p = 0.314 (Mechanical) p = 0.0775 (Biological)	S < C (Cemented crowns showed significantly more total complications)

ns: no significant difference, S: Screw-Retained Prosthesis, C: Cement-Retained Prosthesis

Mechanical complications include screw loosening, abutment fracture, chipping, decementation, filling defect, and Ti-base debonding.

Biological complications include mucositis, peri-implantitis, and implant loss.

Discussion

This review assessed eight clinical studies published from 2015 to 2025. The follow-up durations for the studies span from 1 to 10 years. Upon reviewing all studies, it was determined that complication rates were minimal in both screw-retained and cement-retained prostheses, with no statistically significant difference observed between the two systems. Survival rates for both retention types are typically reported to exceed 90%.

Upon evaluation of the included studies, mechanical complications were observed to be more prevalent than biological complications. The prevalent mechanical complications include screw loosening, ceramic fracture (chipping), abutment or substructure fracture, and decementation in cement-retained restorations. Screw loosening is typically linked to screw-retained prostheses; however, its occurrence in cement-retained systems, as reported in certain studies (Amorfini et al., 2018; Kraus et al., 2024), warrants further examination. Screw loosening, a prevalent concern in screw-retained restorations, can be rectified through a straightforward retightening procedure and does not compromise the long-term efficacy of the prosthesis. The loosening of the screw that secures the implant-abutment connection in cement-retained restorations may result in more severe complications. The prosthesis remains clinically stable, allowing the patient to use it without noticing. During this period, microscopic movements or occlusal discrepancies could end up in complications such as screw fracture or joint issues. Decementation in cement-retained restorations is believed to be associated with stress accumulation at the bonding interface and subsequent adhesive failure. Moreover, the research conducted by Naumann et al. illustrates that the application of Ti-base debonding in screw-retained prostheses demonstrates the technical precision necessary in clinical practice. Mechanical complication rates reported to be under 10% in most studies indicate that both retention systems are appropriate for clinical application. In the investigated studies, chipping was reported to differing extents (Cacaci et al., 2017; Kraus et al., 2024; Weigl et al., 2019). Chipping is believed to be associated with stress accumulation at the porcelain-core interface, insufficient ceramic support, or inadequate occlusal design. Although these fractures are clinically repairable, they underscore the significance of porcelain application methodology and occlusal modification. In the study by Wolfart et al., the comparable rates of occlusal and proximal contact loss observed in prostheses utilizing both retention methods can be regarded as technical complications associated with functional adaptation, stemming from occlusal wear, microscopic movements, or alterations in periodontal tissues over time. This situation does not compromise the structural integrity of the prosthesis clinically; however, it may necessitate the restoration of occlusal harmony and proximal contacts in the long term.

Biological complications were infrequently observed in the studies analyzed. Mucositis emerged as the most prevalent complication, with comparable incidence rates observed in both groups (Amorfini et al., 2018; Wolfart et al., 2021). Kraus et al. reported a peri-implant mucositis rate of 31.6% in cement-retained restorations, which is 9.5% higher than that of screw-retained crowns. This is believed to result from inadequately cleaned cement, leading to local irritation, which fosters plaque accumulation and inflammation. With proper cementation protocols and radiographic monitoring, the biological results of both retention systems seem to be equivalent.

The ability of screw-retained restorations to be easily removed by the clinician when necessary for maintenance or adjustment, along with their lower incidence of biological complications, can be considered among their advantages. However, it should be noted that precise technical execution is required during fabrication, and the position of the screw-access hole may influence the esthetic outcome of the restoration. In contrast, achieving passive fit and fulfilling esthetic requirements are generally easier with cement-retained restorations. Nevertheless, certain critical factors—such as interocclusal space—must be carefully considered during their fabrication. Therefore, the choice of retention type should be based on individual clinical and esthetic considerations rather than complication risk alone.

This review possesses specific limitations. The studies varied in sample size, follow-up duration, implant systems employed, and types of restorative materials, resulting in heterogeneity among the findings. The absence of standardized reporting criteria for complications complicates direct comparisons between studies. The restricted patient cohort and the lack of distinct analyses for technical and biological complications constitute significant limitations. Future long-term randomized controlled clinical trials with larger sample sizes are necessary to elucidate the potential effects of retention type on implant survival and peri-implant tissue stability.

Conclusion

Within the limitations of this study, screw- and cement-retained implant-supported fixed prostheses showed similar rates of mechanical and biological complications. In screw-retained restorations, fewer biological complications were observed, and mechanical complications such as screw loosening were easier to manage. In cement-retained restorations, biological complications associated with residual cement were observed more frequently. It is considered that both systems provide predictable long-term outcomes when appropriate clinical protocols are followed.

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CHAPTER 7

The Histopathological Effects of Juglone: From Cellular Mechanisms to Organ-Level Manifestations

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1. Introduction

Juglone (5-hydroxy-1,4-naphthoquinone) is a secondary metabolite synthesized primarily as its glycoside form, hydrojuglone, in the leaves, bark, and green husk (pericarp) of plants belonging to the *Juglandaceae* family, particularly the walnut tree (*Juglans regia*). This compound, which is converted to its active form, juglone, upon tissue damage or oxidation, is well-known in nature as a potent allelopathic agent, suppressing the growth of surrounding vegetation (Meraati et al., 2025). However, the biological effects of juglone are not limited to plants. Extensive research over the last two decades has revealed that this naphthoquinone also possesses potent pharmacological and toxicological effects on mammalian cells. Its antimicrobial, antifungal, anti-parasitic, and especially its cytotoxic/anticancer properties have made juglone a focus of scientific interest, both as an environmental toxin and as a potential therapeutic agent (Bayram et al., 2019; Shah et al., 2023; Deng et al., 2025).

The aim of this chapter is to examine the histological and histopathological effects of juglone on mammalian tissues with a holistic approach. In this context, we will first detail the core molecular mechanisms of juglone's action, such as the generation of reactive oxygen species, induction of apoptosis, and modulation of the cell cycle. Subsequently, the microscopic manifestations of these cellular events in different organ systems—namely the histopathological lesions in the liver, kidney, lungs, and nervous system—will be discussed in light of their characteristic findings. Finally, the dose-response relationship and the delicate balance between juglone's toxicology and its pharmacological potential will be debated.

2. Core Cellular and Molecular Mechanisms of Action

The pathological changes induced by juglone in tissues are the result of multi-layered and interconnected cellular mechanisms.

Oxidative Stress: Redox Cycling and the Collapse of Antioxidant Defenses At the heart of juglone's cytotoxicity lies the redox cycling capability afforded by its quinone structure. Intracellularly, enzymes such as NADPH-cytochrome P450 reductase, located primarily in the endoplasmic reticulum and mitochondria, reduce juglone via a single-electron transfer to a highly reactive semiquinone radical. This radical rapidly reacts with molecular oxygen (O_2) to generate the superoxide anion radical ($O_2^{\cdot-}$), while being re-oxidized back to its original quinone form (Seshadri et al., 2011; Jha et al., 2015; Erkoç et al., 2024). This reaction chain creates a continuous and self-perpetuating cycle of superoxide production. Superoxide is subsequently converted to hydrogen peroxide (H_2O_2) by the enzyme superoxide dismutase (SOD). In the presence of cellular iron, H_2O_2 can be converted via the Fenton reaction into the hydroxyl radical (OH), the most

reactive of the oxygen species. This deluge of reactive oxygen species (ROS) depletes the cell's endogenous antioxidant defense mechanisms. Specifically, glutathione (GSH), the cell's most crucial reducing molecule, is rapidly consumed either through direct conjugation with juglone or by being oxidized to its disulfide form (GSSG) while neutralizing ROS. This dramatic drop in the GSH/GSSG ratio disrupts cellular redox homeostasis and leads to oxidative stress. The consequences of oxidative stress form the molecular basis for the histologically observable damage:

- **Lipid Peroxidation:** The oxidation of polyunsaturated fatty acids in cellular and organellar membranes leads to impaired membrane fluidity, dysfunction of ion channels, and ultimately, cell lysis.
- **Protein Oxidation:** The modification of proteins with carbonyl groups results in the inactivation of enzymes and the denaturation of structural proteins.
- **DNA Damage:** The formation of oxidative DNA lesions, such as 8-hydroxy-2'-deoxyguanosine (8-OHdG), can lead to mutations and genomic instability.

Induction of Apoptosis (Programmed Cell Death) Juglone is an effective inducer of apoptosis, particularly in cancer cells. This process occurs predominantly through two main pathways:

- **The Intrinsic (Mitochondrial) Pathway:** Oxidative stress induced by juglone and its direct interactions with mitochondria disrupt the balance between pro-apoptotic (e.g., Bax, Bak) and anti-apoptotic (e.g., Bcl-2, Bcl-xL) proteins on the outer mitochondrial membrane. Activation of proteins like Bax promotes Mitochondrial Outer Membrane Permeabilization. This leads to the collapse of the mitochondrial membrane potential and the release of apoptotic factors, such as cytochrome c, from the intermembrane space into the cytosol. In the cytosol, cytochrome c binds with Apaf-1 and pro-caspase-9 to form the "apoptosome" complex, which activates caspase-9. Activated caspase-9 then activates the effector caspases, caspase-3 and -7, initiating the cleavage of cellular substrates (Du et al., 2024).
- **The Extrinsic (Death Receptor) Pathway:** Some studies suggest that juglone may also trigger this pathway by upregulating the expression of death receptors like Fas or TNF receptors or by sensitizing cells to their ligands, though this mechanism is less prominent.

Cell Cycle Arrest Another significant anticancer mechanism of juglone is its ability to halt the cell cycle at specific checkpoints. It has been reported to cause an arrest predominantly at the G2/M phase transition. This effect is associated with the inhibition of cyclin-dependent kinases (CDKs), particularly the

Cdk1/Cyclin B1 complex, which is critical for mitosis. Halting the cell cycle provides the cell with an opportunity to repair DNA damage or, if the damage is too severe to be repaired, directs it towards apoptosis (Wang et al., 2025).

3. Histopathological Findings in Specific Organ Systems

Liver (Hepatotoxicity) As the central hub of xenobiotic metabolism, the liver is a primary target of juglone toxicity. Histopathological findings are consistent with a profile of acute chemical injury:

- **Centrilobular Necrosis:** The most prominent lesion is the necrosis of hepatocytes surrounding the central vein (Zone 3 of the hepatic lobule). This region has the highest concentration of cytochrome P450 enzymes and is therefore most exposed to juglone's redox cycling. Necrotic hepatocytes appear with ill-defined borders, intensely eosinophilic (pink) cytoplasm, and pyknotic (shrunken, dark), karyorrhectic (fragmented), or karyolytic (dissolved) nuclei.
- **Hydropic Degeneration:** In areas adjacent to necrosis or in less affected zones, sublethally injured hepatocytes exhibit vacuolization (ballooning degeneration) due to intracellular water accumulation.
- **Sinusoidal Congestion and Hemorrhage:** Excessive filling of blood vessels (sinusoids) with blood and occasional extravasation of blood into the parenchyma.
- **Inflammatory Infiltration:** Focal accumulation of inflammatory cells, predominantly neutrophils and macrophages, around necrotic areas, mobilized to clear cellular debris (Şenol et al., 2023a).

Kidney (Nephrotoxicity) The renal excretion of juglone and its metabolites makes the kidneys another major target.

- **Acute Tubular Necrosis (ATN):** The primary lesion is observed in the epithelial cells of the renal tubules, particularly the proximal convoluted tubules. The cells become swollen, lose their brush border, their cytoplasm appears granular and eosinophilic, and their nuclei show necrotic changes. Sloughed necrotic cells accumulate in the tubular lumina, forming eosinophilic "cellular casts," which can lead to tubular obstruction (Şenol et al., 2023b).
- **Interstitial Edema and Nephritis:** Fluid accumulation in the connective tissue between the tubules (interstitium) and focal infiltration of mononuclear inflammatory cells, such as lymphocytes and plasma cells.

Nervous System (Neurotoxicity) Juglone has the potential to cross the blood-brain barrier and can exert toxic effects on the central nervous system. Findings in experimental models include:

- **Neuronal Apoptosis and Necrosis:** Cell death of neurons, especially in vulnerable regions like the hippocampus and cerebral cortex. Apoptotic neurons are identified by their shrunken and hyperchromatic (darkly stained) nuclei (pyknosis).

- **Gliosis:** A reactive activation and proliferation of astrocytes and microglia in response to neuronal damage. This can be demonstrated immunohistochemically with markers such as GFAP (for astrocytes) and Iba1 (for microglia).

- **Spongiform Changes:** The formation of small, round vacuoles in the neuropil (the network of neuronal and glial processes) (Şenol et al., 2023c; Bektaş et al., 2024).

Lungs Following systemic exposure or inhalation, an acute injury pattern can develop in the lungs.

- **Alveolar and Interstitial Edema:** The filling of alveolar spaces and the interstitial tissue of alveolar walls with protein-rich fluid.

- **Hyaline Membrane Formation:** Eosinophilic membranes composed of plasma proteins and necrotic cellular debris lining the alveolar walls. This is a characteristic finding of Acute Respiratory Distress Syndrome (ARDS).

- **Inflammatory Cell Infiltration:** A dense influx of neutrophils and macrophages into the alveolar spaces and interstitium (Şenol et al. 2023d; Zhong et al., 2024).

4. Dose-Response Relationship and the Apoptosis-Necrosis Continuum

The morphology of cell death induced by juglone exhibits a distinct transition depending on the concentration of exposure. This phenomenon is described as the "apoptosis-necrosis continuum."

- **Low Concentrations:** At low doses where the cell can maintain its ATP production capacity, **apoptosis**—an energy-dependent, programmed, and controlled process—is dominant. Histologically, this is observed as the death of single cells with minimal inflammation.

- **High Concentrations:** Exposure to very high doses rapidly impairs mitochondrial function and brings ATP production to a halt. As the cell lacks the energy to execute apoptosis, it undergoes **necrosis**, a passive and uncontrolled death process. Histologically, this is characterized by lesions affecting large areas of tissue, where cells swell and lyse, accompanied by a prominent inflammatory response .

5. The Balance Between Toxicological Risk and Therapeutic Potential

The histopathological profile of juglone reveals it to be a "double-edged sword." On one hand, its ability to trigger apoptosis and cell cycle arrest, particularly in cancer cells, makes it a promising anticancer agent candidate. Cancer cells, with their higher metabolic rates and often compromised antioxidant defenses, may be more susceptible to juglone's pro-oxidant effects than normal cells. On the other hand, its potential to cause severe necrotic damage in vital organs like the liver and kidneys at high doses or with uncontrolled systemic exposure constitutes a significant toxicological risk. Therefore, strategies to harness juglone's therapeutic potential must focus on increasing its selectivity and minimizing systemic toxicity. Approaches such as nanoparticle-based targeted drug delivery systems or the development of less toxic juglone analogs could play a key role in achieving this balance.

6. Conclusion and Future Perspectives

Juglone is a biologically highly active naphthoquinone that generates intense oxidative stress through redox cycling and induces apoptosis and cell cycle arrest. These molecular mechanisms translate at the organ level into dose-dependent, predictable, and characteristic histopathological lesions, most notably centrilobular necrosis in the liver and acute tubular necrosis in the kidney. A detailed understanding of these effects is a fundamental prerequisite for both assessing the potential toxicity risks associated with walnut products or environmental exposure and for exploiting its cytotoxic properties in the treatment of diseases like cancer. Future research should further elucidate its effects on specific signaling pathways and focus on developing pharmaceutical strategies to widen its therapeutic window.

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CHAPTER 8

Bruxism and Treatment Methods

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Bruxism is a repetitive chewing-muscle activity that may involve teeth clenching or grinding, or strong mandibular movements (1). It is a pathophysiological condition generally associated with transient arousals during light non-REM sleep, in which excitatory neurotransmitters temporarily overcome inhibitory influences, leading to jaw-muscle activation (2).

Classifications of Bruxism

Classification by Time of Occurrence

Category	Description
Awake (Diurnal) Bruxism	Occurs during wakefulness; mostly characterized by teeth clenching rather than grinding (3,4).
Sleep (Nocturnal) Bruxism	Occurs during sleep; involves rhythmic masticatory muscle activity (5,6).
Combined Bruxism	Occurs both while awake and during sleep.

Awake bruxism is observed as conscious or unconscious teeth clenching or, less commonly, grinding in awake individuals (3). In awake bruxism, involuntary contraction of the jaw muscles and teeth clenching predominate, whereas grinding is less frequent (4).

Sleep bruxism is characterized by rhythmic masticatory muscle activity detectable through electromyographic recordings during sleep (5). Unlike awake bruxism, sleep bruxism involves both grinding-type and clenching-type activity (6).

Sleep bruxism may cause significant damage to the teeth and dental restorations and can lead to morning jaw pain or fatigue, temporal headaches, and temporomandibular joint mobility limitations. Although diagnosis is primarily clinical, polysomnography is required in cases with suspected sleep disorders such as obstructive sleep apnea.

Restless legs syndrome, periodic limb movements during sleep, sleep-related gastroesophageal reflux disease, REM sleep behavior disorder, and sleep-related epilepsy are associated with sleep bruxism. Except for REM sleep behavior

disorder and Parkinson’s disease, sleep-related arousal appears to be the common factor linking all these conditions (7).

Awake bruxism is typically associated with stress and increased alertness, which may lead to enhanced autonomic cardiac activity. It occurs as masticatory muscle activity during wakefulness and is associated with repetitive or continuous tooth contact, mandibular bracing, or thrusting (8).

According to Period of Occurrence

Classification by Period

Category	Description
Past Bruxism	Previously observed but not currently active.
Current Bruxism	Presently occurring bruxism behaviors.

According to Severity

Severity Level	Characteristics
Mild	Rare nighttime episodes; no dental or psychosocial impairment.
Moderate	Occurs at night; mild psychosocial dysfunction (9).
Severe	Nightly episodes; dental damage, TMJ disorders, psychosocial difficulties (10).

According to Etiology

Type	Description
Idiopathic (Primary)	Not associated with medical, psychiatric, or neurological disorders.
Iatrogenic (Secondary)	Associated with systemic diseases, psychiatric/neurological conditions, sleep disorders, or medication (11).

According to Motor Activity Type

Type	Description
Tonic Bruxism	Continuous muscle contraction > 2 seconds.
Phasic Bruxism	0.25–2 seconds episodes with ≥ 3 EMG bursts.
Mixed Bruxism	Combination of tonic and phasic patterns.

Clinical Symptoms and Findings

Symptom	Notes
TMJ pain	Common complaint
Masticatory & cervical muscle pain	Often due to hyperactivity
Temporal headaches	Especially in morning
Tooth hypersensitivity	Due to wear and microtrauma

Symptom	Notes
Tooth mobility	Secondary to excessive forces
Poor sleep quality	Fatigue, unrestful mornings
Recurrent migraines	Frequent in severe cases

Clinical Findings

Clinical Sign	Explanation
Abnormal tooth wear	Attrition on occlusal/incisal surfaces
Tongue indentations	“Scalloped tongue”
Linea alba	Buccal mucosa keratinization line
Torus maxillaris/mandibularis	Common anatomical findings
Masseter hypertrophy	Due to chronic overactivity
Reduced salivary flow	Often secondary to muscle tension
Fractured teeth/restorations	Due to excessive force
Limited mouth opening	TMJ or muscle involvement
Tooth sensitivity	Especially to cold stimuli

Various factors may contribute to bruxism. These include missing teeth, over-erupted teeth, faulty restorations, steep cusp inclines, other occlusal disorders, antidepressant use, genetic predisposition (12), stress and psychosocial factors, recreational drug use, smoking, caffeine and alcohol intake, anxiety, and medications such as benzodiazepines, neuroleptics, amphetamines, tricyclic antidepressants, and selective serotonin reuptake inhibitors (13–16). However, the most common causes are considered to be sleep apnea and anxiety (15).

Epidemiology of Bruxism

Sleep bruxism is a common sleep disorder, with a prevalence of approximately 8% in the general adult population (17). It is more common in children (14–18%), and its prevalence decreases with age (18). There is no significant sex difference (19).

Diagnosis of Bruxism

Questionnaires, clinical examination, electromyography, and polysomnography are commonly used in diagnosis. Polysomnography (PSG) is the ideal method for diagnosing sleep bruxism (20).

Treatment Methods

The primary goal of treatment is to prevent dental damage and alleviate associated symptoms (21). Since bruxism has no single definitive etiological cause, there is no definitive cure. Current treatment approaches focus on preventing hyperactivity of the masticatory muscles and excessive loading of the dentition and TMJ. These include restorative procedures, occlusal splints, psychological therapy, correction of sleep disturbances, pharmacological therapy, hydroxyzine use, botulinum toxin injections, physiotherapy, acupuncture, hypnosis, and orthodontic interventions (22–25).

Occlusal Splints

These are interocclusal devices made of hard acrylic, silicone, or soft acrylic, used to protect teeth, reduce excessive muscle activity, and decrease joint loading.

Types of occlusal splints (26):

Splint Type	Description
Soft Splint	Flexible material; used mainly for mild cases
Stabilization Splint	Hard acrylic; most widely used
Reconstruction Splint	For full-arch repositioning therapy
Anterior Splint	Provides anterior guidance, reduces posterior contact
Palatal Splint	Covers palatal surfaces

Goals of occlusal splint therapy:

- Achieving ideal mandibular positioning during occlusion
- Muscle relaxation and improved coordination
- Elimination of occlusal interferences
- Relief of symptoms caused by occlusal disharmony
- Positioning the condyle and disc into centric relation in TMJ disorders
- Reducing bruxism and parafunctional activities (27, 28)
- Providing a placebo effect (27)

Cognitive and Behavioral Approaches

- Stress management, relaxation exercises, sleep hygiene
- Habit awareness and conscious relaxation of masticatory muscles (especially for awake bruxism)
- Cognitive-behavioral therapy (particularly in cases associated with stress, anxiety, or sleep disorders) (29)

Pharmacological Treatment

Benzodiazepine-like drugs and muscle relaxants are used to reduce nocturnal bruxism. Recently, serotonergic and dopaminergic drugs and acetylcholine inhibitors such as botulinum toxin have gained popularity (30).

Botulinum Toxin (BoNT-A)

Low-dose injections into the masseter, temporalis (and if necessary, pterygoid) muscles can reduce contraction strength.

- Systematic reviews report significant reductions in pain, muscle activity, and bite force; however, the methodological quality of studies varies and long-term data are limited (31).
- Requires expertise of a dentist, maxillofacial surgeon, or physical medicine specialist; generally considered in resistant cases that do not respond to splints or behavioral therapy.

Physical Therapy and Musculoskeletal Approaches

- Stretching exercises, manual therapy, dry needling, and trigger-point treatments may help alleviate myalgia and movement restriction related to bruxism.
- Particularly beneficial in patients with accompanying TMJ dysfunction and predominant muscle pain (32).

General Therapeutic Approach to Bruxism

Step	Approach
1st Line	Education, stress management, lifestyle modification, night guard
2nd Line	Medication, physiotherapy, psychological counseling
3rd Line (Resistant cases)	Botulinum toxin, multidisciplinary management
Always	Protection of teeth, TMJ, soft tissues; management of underlying factors

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CHAPTER 9

The Role of Iron in Oxidative Stress and Neurodegenerative Diseases

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1. Introduction

The human body's weight is largely composed of essential elements such as oxygen, carbon, hydrogen, and nitrogen. While these four elements account for approximately 96% of the total mass, calcium and phosphorus make up about 2–3%. The remaining approximately 1–1.5% consists of trace elements that play critical roles in various biochemical processes. These include elements such as potassium, sodium, magnesium, sulfur, fluorine, copper, iron, zinc, manganese, cobalt, selenium, iodine, chromium, molybdenum, vanadium, arsenic, and bromine.

Trace elements are involved in numerous physiological processes, ranging from enzymatic reactions to cellular signaling, from maintaining redox balance to regulating neurological functions. However, imbalances in the intracellular levels of these elements may disrupt metabolic functioning and lead to the emergence of toxic effects. In particular, disturbances in the homeostasis of metal ions contribute to the pathogenesis of many chronic diseases due to increased oxidative stress.

It is known that in neurodegenerative diseases such as Alzheimer's Disease (AD) and Parkinson's Disease (PD), copper and iron ions accumulate excessively in brain tissue. This accumulation increases the formation of reactive oxygen species (ROS), leading to elevated oxidative stress levels and the progression of neuronal damage (1-5).

2. Iron Metabolism

Iron is an essential element for sustaining life; however, due to its redox properties, it can exert toxic effects when it accumulates uncontrollably. In an average adult male weighing 70 kg, approximately 4 g of iron is found, while this amount is generally lower in females. Most of the iron in the body is functionally present in hemoglobin, myoglobin, cytochromes, and various enzymes that use iron as a cofactor. This functional pool accounts for approximately 70–90% of the total iron.

The remaining iron is stored in the liver, spleen, bone marrow, and muscle tissue in the form of ferritin and hemosiderin. In the plasma, there is a circulating pool of approximately 3 mg of iron bound to transferrin. The disruption of balance in iron metabolism is a significant risk factor particularly for the central nervous system, because increased iron accumulation can trigger oxidative stress mechanisms and pave the way for neuronal damage (2).

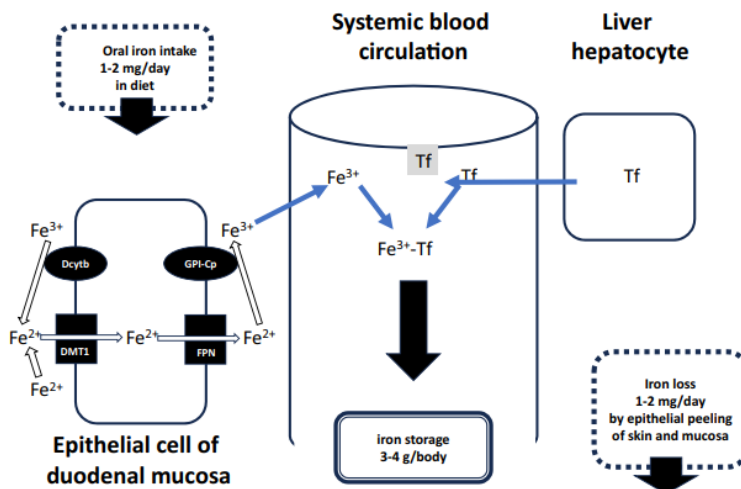


Figure 1. Iron Metabolism

2.1. Iron Absorption and Transport

Most of the inorganic iron in the diet is in the ferric (Fe^{3+}) form, and in order to be absorbed by enterocytes, it must be reduced to the ferrous (Fe^{2+}) form. This conversion occurs through an ascorbate-dependent reductase enzyme located on the brush border of the small intestine; ascorbic acid (vitamin C) is an important cofactor that enhances the efficiency of this reaction.

Following absorption, iron is transported to tissues where it is needed via transferrin. The bone marrow is one of the main target sites in this process. When the transferrin-iron complex binds to receptors on the surface of erythroblasts, iron is taken into the cell and participates in heme synthesis in the mitochondria by combining with the protoporphyrin ring. Iron that is not used for heme production is stored in the form of ferritin by binding with apoferritin.

Ferritin is the primary protein complex that stores iron in a form that does not exhibit toxic properties, and a single ferritin molecule can safely hold 3,000–4,500 iron atoms. When needed, iron is released from ferritin through the reduction of Fe^{3+} ions to Fe^{2+} and becomes available for cellular metabolism(2,6).

2.2. Iron Metabolism in the Brain

Iron is one of the most abundant transition metals in the central nervous system and plays a critical role in maintaining normal neurophysiological processes. Due to its high oxygen consumption and rich lipid content, brain tissue is considered one of the most vulnerable organs in the body to oxidative stress. Neuronal membranes, which are particularly rich in polyunsaturated fatty acids, are highly susceptible to oxidative attack.

Most of the iron in the brain is present in a ferritin-bound form. However, components such as ascorbate and molecular oxygen can accelerate the redox cycling of iron, triggering the formation of hydroxyl radicals ($\bullet\text{OH}$) through the Fenton and Haber–Weiss reactions. Hydroxyl radicals are highly reactive oxidants and can increase lipid peroxidation, thereby disrupting the integrity of neuronal structures. Excessive activation of these mechanisms may contribute to the increased oxidative stress burden, which is a characteristic feature of neurodegenerative disorders such as AD.

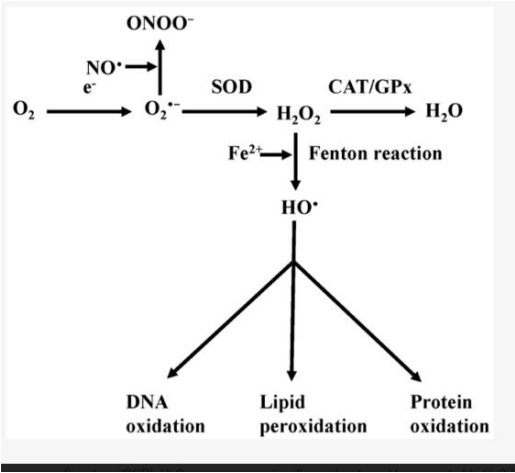


Figure 2. Fenton Reaction

Oxidative stress can activate programmed cell death pathways in neurons, damage essential proteins and lipids, and ultimately lead to synaptic dysfunction and neuronal cell death. Current research highlights the importance of iron in regulating tau phosphorylation and aggregation, which may contribute to the formation of neurofibrillary tangles in neurodegenerative diseases such as AD. There appears to be a significant interaction between iron and tau that influences disease progression and symptoms. Interestingly, tau also binds to iron, which promotes its aggregation and accumulation in the brains of Alzheimer’s patients as iron-rich tangles. Moreover, elevated iron levels can increase tau phosphorylation in cultured neurons, suggesting a possible link between iron elevation and pathological tau in AD. Findings showing decreased total tau levels in the cortex of Alzheimer’s patients and the requirement of tau for the trafficking of amyloid precursor protein (APP) to the neuronal membrane support the idea that disruptions in tau and APP levels may lead to iron retention in neurons, which is a feature of AD (2).

3. Oxidative Stress Mechanisms and Iron

It has been shown in previous studies that iron-induced oxidative stress may lead to neurodegeneration and the development of AD, and that there is an association between serum iron levels and brain iron levels. In another study, the severity of dementia was found to be positively associated with serum iron levels and negatively associated with serum ferritin levels. Low serum ferritin levels and high serum iron levels may lead to a relative reduction in iron storage and a relative increase in the passage of iron into the brain. This is one of the mechanisms contributing to disease progression. Additionally, these results also support the oxygen radical hypothesis in the formation of cellular damage in the brain.

During normal metabolic pathways within the cell, free radicals are continuously formed as intermediates in enzymatic reactions at the active sites of enzymes. Sometimes these free radical intermediates leak from the active sites of enzymes, accidentally interacting with molecular oxygen and generating reactive oxygen radicals. Transition metals, especially iron and copper, take part in oxidation-reduction reactions under physiological conditions in the form of electron exchange. Because of these properties, transition metals act as catalysts that accelerate free radical reactions. Fe(II) and Cu(II) catalyze the formation of thiyl radicals from thiols (Figure 3).

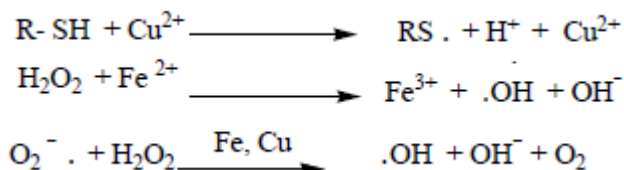


Figure 3. Reaction mechanism of $\text{OH}\cdot$ formation from H_2O_2 and $\text{O}_2^{\cdot-}$ (2,6)

In the body, iron is transported in the plasma by binding to transferrin in the form of Fe^{3+} , and transferrin-bound iron does not participate in free radical formation.

3.1. Reactive Oxygen Species

Free radicals are atoms or molecules that contain one or more unpaired electrons in their outer orbitals, emerge during reactions in cellular metabolism, and tend to react with other biological materials. The unpaired electrons are highly reactive toward adjacent lipids, carbohydrates, proteins, and DNA. Phospholipids, glycolipids, unsaturated fatty acids, and membrane proteins located in membrane structures targeted by free radicals are exposed to oxidative

stress in situations where oxidant agents increase and antioxidant agents decrease or become insufficient. As a result, free radicals cause cellular damage.

Reactive oxygen species (ROS) formed within the cell are eliminated by mechanisms known as “antioxidant defense systems,” or simply “antioxidants.” However, sometimes more reactive oxygen species (ROS) may be produced than can be removed by the cellular defense mechanism. The condition in which the amount of reactive oxygen species (ROS) generated in the organism exceeds the amount eliminated by the cellular defense mechanisms is defined as oxidative stress (Figure 4).

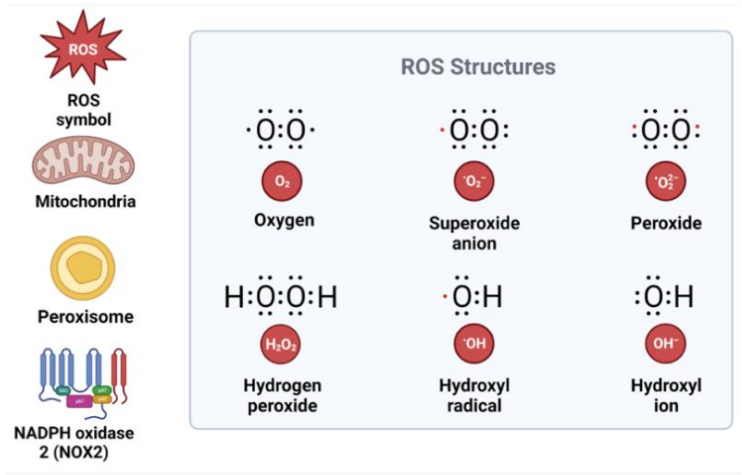


Figure 4. Structure and sources of reactive oxygen species (10).

3.2.Brain

Iron plays a role in many essential biological processes in the brain. In addition to oxygen transport, it is involved in DNA synthesis, myelination, neurotransmitter synthesis, and mitochondrial respiratory metabolism. To maintain normal brain function, iron homeostasis is required. Homeostatic mechanisms ensure the necessary conditions for cellular function by maintaining the balance of existing iron concentrations between cellular compartments and preventing the toxic effects caused by excess free iron.

However, disruption of iron homeostasis leads to neurotoxicity through different mechanisms. When iron concentration exceeds the iron-binding capacity of storage proteins or other cellular molecules, the concentration of unstable iron within the labile iron pool can increase. This results in oxidative damage and cell death (9).

4. Iron Dysregulation in Neurodegenerative Diseases (NDs)

NDs are characterized by the irreversible loss of nerve cells over time and the resulting impairment in various brain functions (memory, movement, cognition).

Ferroptosis is a form of cell death dependent on iron and lipid peroxidation, and emerging evidence shows that ferroptosis has a strong explanatory potential for neuronal loss and associated central nervous system dysfunction in various neurodegenerative diseases (such as Alzheimer's, Parkinson's, and Huntington's diseases).

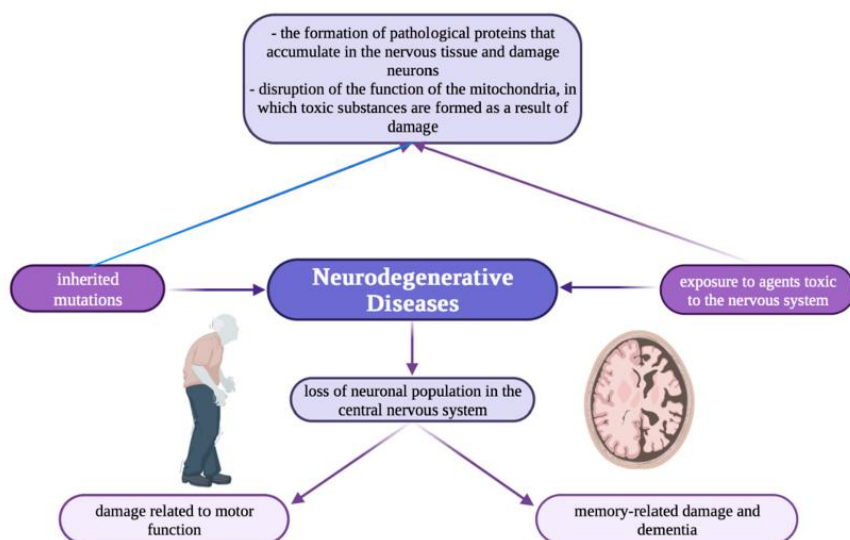


Figure 5. Schematic illustration showing the mechanism of neurodegenerative diseases. (11)

4.1. Alzheimer's Disease

AD is a disease first identified in 1906 by the German neurologist Alois Alzheimer, characterized by the degeneration of nerve cells in the brain and subsequent brain atrophy. The brain is a target for oxidative damage caused by environmental pollutants. Amyloid precursor protein (APP) is thought to have a direct interaction with Zn, Cu, and Fe(III) in AD. The β -amyloid peptide ($A\beta$) has a high affinity for metal ions. The accumulation of Cu(II) and Fe(III) metals is believed to be influenced by the levels of $A\beta$ -metalloenzymes, contributing to their toxic effects. On the other hand, the levels of these metals have been found to be elevated in the brains of individuals with AD, and they are thought to induce ROS formation. These findings support the link between $A\beta$ and metal ions.

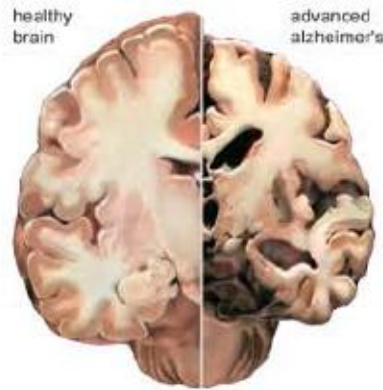


Figure 6. Brain damage caused by Alzheimer's Disease (6).

4.2. Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disease after AD. In PD, postural instability, impaired posture, freezing of gait, reduced facial expressions and movements, hypophonic speech, resting tremor in arms and legs, bradykinesia, and loss of dexterity are observed (12).

PD results from the neurodegeneration of dopaminergic neurons located in the substantia nigra pars compacta of the brainstem. Dopamine is a neurotransmitter essential for many vital brain functions in advanced organisms. Unlike neurons containing other neurotransmitters, dopamine renders dopaminergic neurons susceptible to oxidative stress. After synthesis, dopamine is almost entirely stored inside synaptic vesicles, where it reaches high concentrations and is stabilized by the low pH of the vesicle lumen. Dopamine may then undergo an auto-oxidation process, leading to the formation of both ROS and dopamine quinones (DAQ), which also possess reactive properties. As a result, selective degeneration of dopaminergic neurons occurs. Additionally, in Parkinson's disease, Lewy bodies (accumulation of proteins mainly composed of α -synuclein) disrupt communication between nerve cells by preventing neurotransmitter transfer. Consequently, the motor symptoms observed in Parkinson's disease emerge (13).

4.3. Amyotrophic Lateral Sclerosis (ALS) Disease

Amyotrophic Lateral Sclerosis (ALS) is a progressive and fatal neurodegenerative disease characterized by the death of upper motor neurons in the cortex and lower motor neurons in the spinal cord and brainstem. The disease affects 2 to 3 out of every 100,000 people worldwide. Although most ALS cases are sporadic, approximately 5–10% have a genetic inheritance history. Iron accumulation has been detected in the spinal cord and motor cortex along with

motor neuron degeneration in ALS. This accumulation is associated with SOD1 gene mutations (superoxide dismutase 1, one of the most commonly observed mutations); the SOD1 enzyme may misfold and form toxic aggregates by interacting with iron (14).

There are many causes of motor neuron degeneration in ALS. However, the oxidative stress theory in ALS is particularly important due to cumulative damage and disease progression. Experiments conducted using the transgenic G93A Sod1 model, the most widely used animal model of ALS, by Liu et al. (2002) showed that increased production of free oxygen radicals in the spinal cords is associated with ALS-like disease onset and progression. Oxidation markers such as protein carbonyls and protein nitration have been found to be elevated both in human ALS patients and in ALS-like transgenic mice. It has been suggested that an imbalance between oxidant and antioxidant mechanisms contributes to neuronal damage (2).

4. Huntington's Disease

In Huntington's disease, increased iron accumulation in the striatum and cortex has been reported. Impairments in ferritin gene expression lead to the loss of cellular iron balance. In animal models, iron chelators (e.g., deferiprone) have been shown to improve motor functions.

5. Alternative Therapeutic Approaches

Considering the role of iron-induced oxidative stress in neurodegenerative diseases, iron-binding chelators and antioxidant support gain importance in treatment strategies.

5.1. Iron Chelators

Agents such as deferoxamine (DFO), deferasirox (ICL-670), and deferiprone (L1) reduce free iron levels by binding iron ions (chelation) (Figure 7).

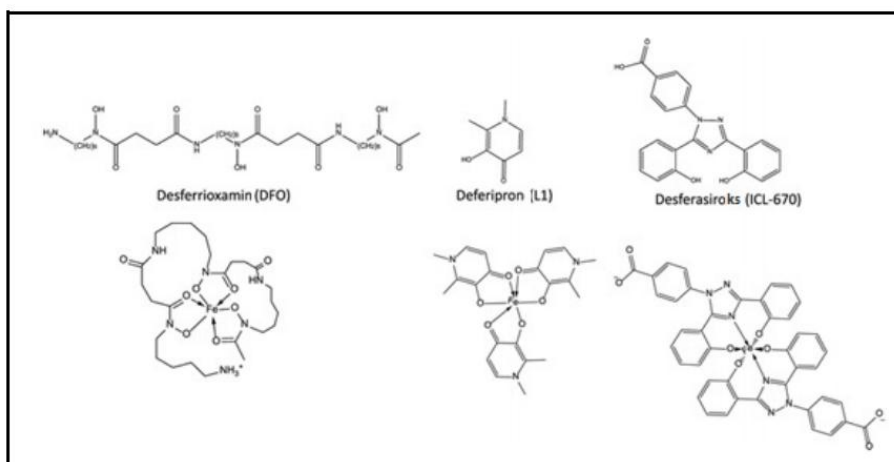


Figure 7. Chelators used in chelation therapy (15).

Clinical studies have shown that especially deferiprone reduces iron accumulation in patients with Alzheimer’s and Parkinson’s diseases and provides partial improvement in cognitive functions. However, due to the risk of anemia and neurotoxicity with long-term use, careful dose adjustment is required (16). Dosage regimens are shown in Table 1.

Table 1. Iron chelators currently used in clinical practice

Iron chelator	Administration protocol	Advantages	Disadvantages	Crossing the blood-brain barrier	Special regulations by country/region
Deferasirox (DFX)	Unique dose of 20–30 mg/kg/day, oral administration	Once daily administration	Important side effects: potentially fatal gastrointestinal hemorrhage, renal and hepatic toxicity High costs	Via nanocarrier	FDA and EMA approved.Adults with chronic iron overload (in Europe also in children 6 years and older)
Deferiprone (DFP)	75 mg/kg/day (divided into 2–3 doses/day), oral administration	Most effective in cardiac iron excretion	Important side effects: gastrointestinal symptoms, hepatic toxicity, neutropenia, and agranulocytosis Frequent (weekly) blood count monitoring Important side effects	Yes	Available in the United States, Canada, and other countries Second-line therapy in adult patients with thalassemia major
Deferoxamine (DFO)	25–50 mg/kg/day, subcutaneous or intravenous infusion over 8–12 h 5 days/week	More than 30 years of experience	visual and auditory neurotoxicity, gastrointestinal symptoms, increased risk of infections (mucormycosis) Decreased compliance because of the administration route	Yes	FDA and EMA approved.First-line therapy for hemochromatosis

5.2. Antioxidant Therapies

Compounds such as coenzyme Q10, resveratrol, curcumin, and N-acetylcysteine (NAC) support mitochondrial functions by reducing ROS production. The combination of antioxidants with iron chelators may produce a synergistic effect. Nrf2 activators are also considered among promising therapeutic targets for the future (10).

6. Conclusion

Although iron is an essential element for life, it becomes one of the most potent triggers of oxidative stress when its homeostasis is disrupted. Moreover, accumulated iron in brain tissue accelerates neuronal dysfunction and directly contributes to the pathogenesis of neurodegenerative diseases such as Alzheimer’s, Parkinson’s, ALS, and Huntington’s diseases.

Recent studies indicate that the regulation of iron metabolism may form the basis of neuroprotective strategies. Therefore, the importance of treatments such as iron chelators, antioxidant agents, and Nrf2 activators is gradually increasing, and in the coming years, combination therapies targeting iron metabolism may potentially slow the progression of neurodegenerative diseases

CHAPTER 10

Clinical Management of Perianal Fistula Disease

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1. INTRODUCTION

A perianal fistula is an abnormal connection between an epithelialized duct in the anorectal region and the perianal skin, from the rectum or anal canal. The condition is often associated with chronic inflammation, infection, or systemic conditions such as Crohn's disease [1]. Perianal fistulas cause significant pain, discharge, irritation, and a decreased quality of life. Clinical management is based on the anatomic classification of the disease, the underlying cause, and the risk of complications [2].

The importance of perianal fistulas lies not only in their local symptoms but also in their potential impact on social life, psychological well-being, and functionality. Chronic discharge and perianal discomfort can lead to sleep disturbances, the need for constant cleaning, and limitations in daily activities. Furthermore, untreated or mismanaged fistulas can lead to infection, abscesses, and complex fistulas. Therefore, a multidisciplinary approach is crucial in the evaluation and treatment planning of the disease. This section will provide a detailed overview of the definition, epidemiological characteristics, pathophysiology, and clinical significance of perianal fistulas.

2. EPIDEMIOLOGY

Perianal fistulas are more common in men in the adult population (male:female ratio approximately 2:1) and usually occur between the ages of 30 and 40 [3]. Fistula development is observed in 30–50% of patients with a history of anal abscess [4]. Conditions such as Crohn's disease, tuberculosis, and post-radiation significantly increase the risk of fistula formation [5].

3. PATHOPHYSIOLOGY

Fistula development usually begins with infection and obstruction in the anal crypts. The cryptoglandular theory explains the formation of an abscess as a result of obstruction in the anal glands and the formation of an abnormal fistula between the skin and the duct during drainage [6]. Initially, a small focus of infection creates local inflammation and edema in the perianal tissue. As the abscess expands and extends to the skin surface, the inflammatory response triggers fibrous tissue proliferation, resulting in the formation of a chronic duct.

Chronic inflammation, particularly in systemic inflammatory conditions such as Crohn's disease, facilitates the epithelialization of fistula tracts and the formation of complex fistulas [7]. Granulation tissue and fibroblast proliferation play a role in tract stabilization during this process. Furthermore, inadequate or excessive immune responses lead to ongoing inflammation, microbial colonization, and local tissue necrosis in the perianal tissue. This can lead to the

formation of perianal fistulas complicated by sinuses, multiple fistula tracts, and abscesses [8].

Fistula tracts are classified according to the anatomic location of the internal and external openings, which determines the surgical approach. Intersphincteric fistulas generally consist of a single tract, while transsphincteric, suprasphincteric, and extrasphincteric fistulas create complex tracts that cross the sphincter muscle. Perianal vascularization, microenvironmental pH, local immune cell responses, and the role of microorganisms are also considered important factors in pathophysiology. These mechanisms influence fistula chronicity, risk of recurrence, and response to treatment.

4. CLINICAL FINDINGS

Patients typically present with perianal discharge, pain, itching, and sometimes perianal swelling. In the acute abscess phase, severe pain, local edema, and fever may occur [9]. In the chronic phase, the discharge may increase, and skin irritation, eczema, or dermatitis may develop. Many patients feel the need for constant cleansing and experience restrictions in their social life.

On examination, a small hole or discharge from the perianal skin, firmness along the fistula tract, tenderness to palpation, and sometimes pus drainage are observed. Complex fistulas may exhibit multiple external openings, perianal skin changes, sinuses, and sometimes connections between fistula orifices [10]. The route of the fistula tracts, the relationship between the internal and external openings, the distance to the sphincter muscle, and the presence of an abscess are important in clinical evaluation.

This section also details the classification of fistulas, signs of active infection, signs of chronic inflammation, associated anal fissures, and skin complications. Patients' quality of life is assessed in terms of chronic pain, sleep disturbance, and psychological distress.

5. DIAGNOSTIC METHODS

Accurate anatomic assessment of the fistula is critical for surgical management. Diagnostic methods include:

1. **Clinical examination:** Simple fistulas are most often diagnosed by physical examination [11].
2. **Proctoscopy and anoscopy:** Used to detect internal opening [12].
3. **Imaging methods:**
 - **Endoanal ultrasonography (EAUS):** Provides high sensitivity in monitoring fistula tracts [13].

- **Magnetic resonance imaging (MRI):** It is the gold standard in complex fistulas, showing in detail the presence of multiple channels and abscesses [14].

6. FISTULA CLASSIFICATION

Fistulas are classified according to their anatomical features:

- **Simple fistulas:** Intersphincteric or transsphincteric, with a single channel and a single external opening.
- **Complex fistulas:** High transsphincteric, suprasphincteric, multiple external opening, Crohn's disease-associated or post-radiation fistulas [15].

7. TREATMENT APPROACHES

The treatment chosen depends on the type of fistula, whether it is complicated or not, and the general condition of the patient.

7.1. Surgical Management

- **Fistulotomy:** It is preferred for simple, low transsphincteric fistulas; healing is achieved by cutting and opening the channel [16].
- **Seton placement:** Used to provide drainage while preserving the sphincter in complex and high transsphincteric fistulas. Setons are used for infection control and channel reduction [17].
- **Fibrin glue and plug applications:** These are minimally invasive methods and provide sphincter-protecting and recurrence-reducing effects [18].

7.2. Medical Treatment

- **Antibiotics:** Used as a supplement in the presence of active infection or peri-fistular abscess [19].
- **Immunomodulators and biologic agents:** In Crohn's disease-associated fistulas, treatment with infliximab or adalimumab reduces recurrence [20].

7.3. Postoperative Care

- Perianal hygiene and regular dressing are important.
- Pain control and infection monitoring are performed during the healing process [21].
- Regular check-ups are needed for early diagnosis of fistula recurrence.

8. COMPLICATIONS

- Anal incontinence: The risk is particularly high in high transsphincteric fistulas [22].
- Recurrence: It is seen in 5-10% of simple fistulas and 20-50% of complex fistulas [23].
- Infection and abscess recurrence: Occurs in case of inadequate drainage or immune system disorder [24].

9. CONCLUSION

Perianal fistula disease has a high success rate when managed with accurate classification and appropriate treatment. Surgery is often the most effective treatment for simple fistulas. For complex fistulas, a combination of setons, biologic agents, and minimally invasive techniques reduces the risk of complications and recurrence.

Chronic and recurrent fistulas can significantly impact patients' quality of life and cause psychosocial stress. Therefore, early diagnosis, accurate classification, and a multidisciplinary approach are crucial to improving both physiological and psychological outcomes.

Additionally, the effectiveness of biologic agents, optimization of minimally invasive techniques, and patient-specific surgical planning continue to be investigated in developing future treatment strategies. The balanced use of both surgical and medical treatment options in the management of perianal fistulas is fundamental to reducing complications and preventing recurrence [25].

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CHAPTER 11

Preservation of Pulmonary Functions After Surgery

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1. Introduction

Surgical procedures can have temporary or permanent complications affecting respiratory function. The likelihood of these complications occurring is particularly high in surgeries performed under general anesthesia and in surgical procedures involving the thorax or upper abdomen. Postoperative pulmonary complications can include atelectasis, pneumonia, hypoxemia, secretion accumulation, and loss of respiratory muscle strength. Maintaining and improving respiratory function after surgery is possible with teamwork of healthcare professionals from different disciplines. In this collaborative effort, nurses and physical therapists aim to enhance the patient's respiratory capacity, mitigate complications, and expedite recovery.

This section covers structured respiratory physiotherapy and nursing care for patients undergoing surgery. Pulmonary physiotherapy techniques, such as breathing exercises, inspiratory muscle training, airway clearance methods, and early mobilization, play a key role in maintaining ventilation and preventing complications. Similarly, comprehensive nursing assessment, patient education, and timely interventions support the patient's recovery. Prevention and treatment of surgery-related respiratory complications in patients should be based on an integrated, multidisciplinary approach combining pulmonary physiotherapy, nursing care, and careful postoperative follow-up.

2. The Importance of Postoperative Respiratory Functions

Surgery, the most commonly used treatment method after medical treatments, poses various risks to physiological systems in the postoperative period due to anesthesia and surgical stress. Impairment of respiratory function is common and can affect other physiological systems. Respiratory complications, a significant cause of postoperative morbidity and mortality, generally occur within the first 12-24 hours. Therefore, preservation of respiratory function is crucial for patient safety and recovery. Factors such as general anesthesia's inhibition of mucociliary activity and suppression of surfactant production, sedatives' impairment of respiratory muscle function, and opioids' depressant effect on the respiratory center and decreased respiratory rate all contribute to the development of early postoperative pulmonary complications. This is followed by complications related to ineffective breathing and ineffective coughing. It is clearly stated in the literature that postoperative pulmonary complications prolong hospital stays, increase treatment costs, reduce patient satisfaction and comfort, and, most importantly, increase morbidity and mortality rates (Hinkle & Cheever, 2018; Gürel & Koçalışlı, 2022; Sigona & Richman 2023).

The significant deterioration in pulmonary function that occurs after major surgeries, especially abdominal and thoracic surgeries, requires nursing care to focus on this area (Boden et al., 2024). In surgical nursing, close monitoring of postoperative respiratory functions and their protection with appropriate

interventions form the basis of surgical care. In the event of pulmonary complications, the nurse assumes various independent and collaborative roles as a member of the healthcare team.

3. Classification of Postoperative Pulmonary Complications

Postoperative pulmonary complications, one of the most common postoperative complications, refer to clinical conditions that arise after surgery and negatively affect the respiratory system. These complications can lead to various and increasingly severe outcomes, ranging from prolonged hospital stay to mortality. The primary goal of surgical care is to identify and prevent complications early, rather than resorting to treatment and care after they arise (Dikmen & Yılmaz, 2019). The main postoperative complications are atelectasis, pneumonia, acute respiratory failure, pulmonary embolism.

Atelectasis is the loss of functionality due to partial or complete collapse or contraction of the lungs. Postoperative complications often occur due to factors such as retained respiratory secretions, inadequate coughing, and shallow breathing, and can predispose to pneumonia. Correct and regular breathing exercises using incentive spirometry to prevent atelectasis reduce the risk of atelectasis.

Acute respiratory failure is an inflammatory lung disease, is a respiratory complication that can be fatal if left untreated. Inadequate airway clearance and atelectasis predispose to pneumonia.

Pneumonia is a respiratory complication characterized by hypoxemia/hypercapnia, which occurs when the alveoli fail to perform their physiological functions due to factors such as side effects of anesthesia and the accumulation of respiratory secretions. This sudden complication also leads to cardiovascular and metabolic problems.

Pulmonary embolism is a life-threatening complication, if not promptly identified and treated, involves a thrombus in the circulation obstructing the pulmonary vessels. Deep vein thrombosis, which develops in the lower extremities, particularly in the postoperative period due to decreased patient mobility, can lead to pulmonary embolism.

Early diagnosis and prevention of these complications are the most critical components of physiotherapy services and nursing care (Gürel & Koçuşlı, 2022; Sweity et al., 2021; Wang et al., 2025).

4. Respiratory Physiotherapy Interventions Following Surgical Procedures

Postoperative respiratory physiotherapy interventions are the subject of this section. However, it is essential that a program initiated before surgery continues afterward. Respiratory physiotherapy interventions aim to preserve the patient's

lung function and prevent or alleviate potential physiological and/or functional problems that may arise in the postoperative period. A review of evidence-based approaches to respiratory physiotherapy has reported that it can prevent pulmonary complications, reduce hospital stay, and reduce mortality, morbidity, and healthcare costs (Dhillon et al., 2023). An individualized intervention plan is developed based on the patient's needs based on the assessments made. The fundamental respiratory parameters encompassed in this evaluation are and physiotherapy techniques enumerated below.

Accurate monitoring of respiratory activity can enable early diagnosis and treatment of potential respiratory failure. However, spontaneous breathing can vary significantly (Sola-Soler et al., 2023). The respiratory pattern involves changes in an individual's tidal volume and respiratory rate, assessed by data tracing lung or chest wall volume over time (LoMauro et al., 2022). Observation, palpation, high-resolution techniques such as optoelectronic plethysmography, motion sensors, circumferential belt or optical surface imaging systems, and thoracic and abdominal breathing patterns can be used for respiratory pattern analysis. An incentive spirometer is a mechanical device commonly used to measure the volume of air drawn into the lungs during inhalation (Ulubay et al., 2019).

Chest wall range of motion is important for both respiratory depth and detecting potential respiratory problems. The chest wall is defined as all the structures on the exterior of the body that surround the lungs and move as the lungs breathe in and out. It includes both the chest wall (ribs) and the abdominal wall. Chest wall range of motion is important for both respiratory depth and for detecting potential respiratory problems. Chest wall range of motion can be measured in a variety of ways. The first involves physical devices placed on the chest wall, which measure the linear displacement of the chest wall along its anterior-posterior axis using a magnetometer or accelerometer. The second involves physically measuring changes in chest wall circumference using strain gauges or impedance pneumography, which measures changes in chest wall cross-sectional area using respiratory inductive plethysmography or changes in rib cage volume using electromagnetic inductive plethysmography. There are also methods using external imaging devices such as radiology and optical techniques (Seddon, 2015).

Respiratory muscle strength is an important indicator of respiratory function in postoperative patients. Various methods are available for assessing respiratory muscle strength. These methods are divided into voluntary tests (which require the patient's understanding and cooperation) and involuntary tests. Voluntary tests, which measure maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP), are the most commonly used because they are readily available. However, they require patient cooperation. Involuntary tests include

magnetic stimulation of the phrenic nerve accompanied by measurement of inspiratory mouth pressure, inspiratory esophageal pressure, or inspiratory transdiaphragmatic pressure. Another commonly used method is diaphragm ultrasonography (Caruso et al., 2015). Additionally, methods to determine the presence of secretions and the necessity of airway clearance techniques should be determined. A patient's functional capacity, defined as the ability to perform activities of daily living that require sustained aerobic metabolism, should be assessed routinely for postoperative patients. Its primary application is in the preoperative assessment of surgical risk. The term principally refers to the maximum oxygen volume that is measured during exercise tests. However, there are self-report or performance-oriented functional capacity measurement methods that have been developed, including stair climbing, Activities of Daily Living, and the Duke Activity Status Index (Daza et al., 2025).

4.1. Breathing Exercises

Breathing exercises involve breathing, either in conjunction with or independent of upper extremity and trunk movements. In some cases, rib cage maneuvers may also be integrated. The purpose of breathing exercises varies depending on the type of breathing exercise and the method used. These goals may include improving the patient's breathing pattern and lung expansion, increasing respiratory muscle strength, and increasing functional residual capacity and inspiratory reserve volume (Hussein & Taha, 2018). Depending on the patient's respiratory pattern goals, diaphragmatic breathing exercises, segmental breathing exercises, and pursed-lip breathing exercises can be selected. Respiratory muscle coordination and efficiency are enhanced, and the chest wall is mobilized, through the implementation of breathing exercises.

4.2. Inspiratory Muscle Training

The appropriate weight for the patient is determined by a method such as 'Delorme Watkins' Inspiratory muscle training (IMT), which supports the respiratory muscles, improves respiratory muscle strength and diaphragm movement and reduces the incidence of postoperative pulmonary complications (Huang et al., 2022). A study comparing individuals undergoing IMT with those not exercising showed that IMT can improve dyspnea, physical fitness, and quality of life (Ammous et al., 2023). Another study reported that IMT was effective in improving inspiratory and expiratory muscle strength, tidal volume, peak expiratory flow, and reducing hospital stay (Cordeiro et al., 2023). Caution is required when performing inspiratory muscle training in the presence of respiratory tract infection, hemoptysis of unknown origin, pneumothorax, uncontrolled hypertension, aneurysm, recent thoracic, abdominal or eye surgery, nausea, vomiting, pain, confusion or dementia (Franklin & Anjum, 2025).

4.3. Airway Clearance Techniques

Airway clearance techniques include techniques that facilitate the expulsion of excess lung secretions from the airways. These techniques include percussion, vibration therapy, postural drainage techniques, cough training, and manually assisted coughing (Tripathi & Sankari, 2025). Percussion is a technique that involves applying rhythmic wrist flexion and extension movements to the rib cage using cupped palms. The mechanical oscillations generated by percussion are transmitted through the chest wall, aiding in secretion mobilization. During percussion, the patient's intrathoracic pressure increases. Vibration is oscillation transmitted through the chest wall. These oscillations can increase expiratory airflow, helping to clear the airway and stimulate coughing. Postural drainage involves positioning the patient's body so that gravity can clear mucus from specific lung areas, thereby improving ventilation and perfusion. Postoperative cough training can help the patient expel secretions. If the patient is unable to cough effectively, manual-assisted cough techniques are used. This technique involves applying external manual pressure to the rib cage or epigastric region during forced expiration. All of these interventions are effective methods for facilitating secretion clearance, and the physiotherapist can select and implement the appropriate intervention based on the patient's needs.

4.4. Device Supported Breathing Exercises

Intensive spirometry and resistive breathing exercises using devices such as Flutter/Acapella can be part of a physiotherapy intervention plan. Numerous studies report that these complementary devices increase the effectiveness of conventional physiotherapy (Jage & Thakur, 2022; Majeed et al., 2025; Sheware & Ruchika, 2023). These devices are widely used in clinics because they provide feedback to the patient during exercise and are easy to use.

4.5. Continuous Positive Airway Pressure

In the presence of respiratory failure, continuous positive airway pressure (CPAP) can be used in conjunction with mechanical ventilation, before mechanical ventilation, or independently of mechanical ventilation, depending on the patient's condition. CPAP has been shown to increase alveolar stability, improve functional residual capacity, and optimize oxygenation by facilitating ventilation-perfusion compatibility (Pinto et al., 2025). It has also been shown to play a significant role in preventing atelectasis and increasing lung capacity (Gharib, 2023). Contraindications to continuous positive airway pressure include hemodynamic instability and impaired consciousness.

4.6. Mobilization and Exercise Program

Mobilization practices, including activities such as sitting at the edge of the bed, standing with support, and walking, are part of postsurgical physiotherapy interventions. Mobilization indirectly affects respiratory function. Indeed,

surgery causes surgical stress and immobility. Therefore, mobilization plays a crucial role in improved postoperative recovery, offsetting the physiological consequences of surgery. Early mobilization reduces the risk of postoperative complications, accelerates recovery of functional walking capacity, and reduces costs by reducing hospital stay. Recent advances in mobilization include real-time feedback on mobilization rates using wearable technology (Tazrean et al., 2022). While mobilization protocols have been developed for various surgical procedures, these protocols should be planned and implemented according to each patient's needs (Castelino et al., 2016). Based on the patient assessment, strengthening, restoring joint motion, correcting posture, or relaxation exercises tailored to the patient's needs can be added to the physiotherapy intervention program. These exercises can also contribute to respiratory function. In fact, one study showed that lower extremity strengthening exercises reduced the risk of respiratory tract infection and postoperative hospital stay in patients who underwent upper abdominal surgery, and also reduced subjective shortness of breath (Bhatt et al., 2017).

5. Nursing Care for the Preservation of Respiratory Functions after Surgery

Preservation of respiratory function after surgery involves a holistic approach encompassing the preoperative, intraoperative, and postoperative phases of the surgical care process. Nurses assume a variety of roles, both independent and collaborative, during this process. To prevent complications, it is crucial to support the patient in coping with stressors that disrupt the patient physically and psychologically. When it comes to surgery, stressors range from the fear of postoperative suture separation to poorly managed pain. While preservation of respiratory function may seem like a physiological goal, accurately defining the extent of the problem through comprehensive patient assessment, and not neglecting patient education to foster patient motivation and participation in care, demonstrate a multifaceted approach that supports all other nursing care interventions. The nursing diagnoses described in Table 1 and the evidence-based interventions listed in Table 2 can significantly reduce postoperative complications (Dhillon et al., 2023; Abdeljalil et al., 2021).

5.1. Patient Risk Assessment

High-risk patient groups for respiratory complications include obesity, advanced age, chronic respiratory diseases, smoking, and immobility. Nurses plan the care of high-risk patients by identifying risk factors such as smoking, chronic diseases, and obesity in the preoperative period. Aging is a significant risk factor for respiratory complications due to decreased physiological reserves and weakened organ functions. In addition to taking a health history, conducting a physical examination, and evaluating current laboratory findings, a meticulous

psychological and mental assessment is also performed (Smetana et al., 2024; Fiddes & McCaffrey, 2022).

5.2. Education and Motivation

Teaching surgical patients breathing exercises and coughing techniques plays an important role in ensuring compliance with these practices in the postoperative period. The optimal time for education is 1-2 days before surgery. If training is delayed until after surgery, success rates drop significantly due to factors such as pain and the effects of anesthesia (Candaş & Yeşilyurt, 2024).

5.3. Care intervention

Evidence-based nursing interventions, such as encouraging early mobilization, optimal pain management, correct positioning, adequate fluid intake, and oxygen administration, should be planned and implemented (Odhah et al., 2015; Balvardi et al., 2021; Cheung et al., 2022; Pettenuzzo et al., 2024). If these interventions are implemented using nursing diagnoses in conjunction with other nursing interventions, more effective care can be provided compared to the task-oriented approach. Nursing interventions related to potential nursing diagnoses for postoperative respiratory problems are listed in Table 1 (Kapucu et al., 2018; Karadağ & Bulut, 2022). Because some interventions are common to the nursing diagnoses listed in the table, the diagnoses and interventions are discussed together.

Table 1. Nursing Diagnoses and Interventions for Postoperative Respiratory Problems

Nursing Diagnosis	Nursing Intervention
Ineffective Breathing Pattern: Associated with anxiety, fatigue, anesthesia, neurological and neuromuscular impairments, pain, ineffective cough, and airway obstruction. The goal is to develop an effective breathing pattern.	• The head of the bed is elevated 15-30 cm, unless contraindicated. • Oxygen therapy is administered as ordered by the physician. • Deep breathing exercises are taught; the patient is encouraged and monitored. • Cough exercises are taught; the patient is encouraged and monitored.
Ineffective Airway Clearance: Associated with smoking, anesthesia, surgical position, inadequate mobilization and continuous bed rest, excessive respiratory secretions, and thickened sputum. The goal is to clear the airway and prevent aspiration.	• The use of an incentive spirometer is taught; the patient is encouraged and monitored. • The importance of mobilization is explained, and the patient is mobilized appropriately.
Impaired Gas Exchange: Associated with ventilation-perfusion imbalance	• The patient is positioned appropriately to facilitate comfortable chest expansion. The

and hypoventilation. The goal is to reestablish the disturbed gas balance.	position is changed at least every 2 hours.
Aspiration Risk: Ineffective coughing, the effects of anesthesia, and short preoperative fasting time increase the risk of aspiration. The goal is to prevent aspiration and maintain functional swallowing. A swallowing test can be used to assess the risk of aspiration.	• Optimal pain control is provided. • Adequate hydration is ensured, and the patient is encouraged to consume water and monitored. • Tapotment is administered, unless contraindicated. • If the patient's airway cannot be cleared, oropharyngeal suctioning is performed. • Treatment to facilitate the removal of respiratory secretions is administered as ordered by the physician.

In summary, the nurse should implement a comprehensive and continuous care process aimed at minimizing the incidence of postoperative pulmonary complications by identifying and meeting the individual's needs to maintain respiratory function (Hinkle & Cheever, 2018). Table 2 lists the main interventions for preventing postoperative respiratory complications (Kara Kaşıkçı & Akın 2021).

Table 2. Care Interventions for Preventing Postoperative Respiratory Complications

Maintenance Initiative	Description	Points to Consider
Early Mobilization	The patient should begin moving as soon as possible. This may include in-bed mobilization (turning, active and passive exercises) and walking, depending on the patient's condition. If the patient remains immobile despite mobility, they will experience difficulties affecting respiratory function, such as secretion accumulation, decreased circulation, and delayed pain relief.	• Necessary precautions should be taken to prevent the risk of falls due to orthostatic hypotension. • It should be noted that the concept of "early mobilization" is a relative definition that varies depending on the surgical technique and type. • The decision on how mobilization will be performed (assisted or unassisted) should be evaluated based on the available data.

Smoking Cessation	Quitting smoking as early as possible before surgery reduces postoperative respiratory complications.	• It should be kept in mind that addicted patients may attempt to smoke even in the surgical clinic.
Breathing and Cough Exercises - Use of Incentive Spirometry	Deep breathing exercises, pursed-lip breathing exercises, diaphragmatic breathing exercises, and incentive spirometry exercises are taught to the patient in the preoperative period. These practices support the preservation of lung capacity, improved ventilation and gas exchange, and reduce the risk of atelectasis. The incentive spirometer used in breathing exercises can come in different shapes and types (Figure 1).	• During these exercises, supporting the chest and abdomen with a soft pillow can help alleviate any pain. • Timing the exercises, especially after analgesic administration, can help alleviate any pain. • Due to the risk of dizziness and vertigo, patients are advised not to stand for 10-15 minutes after the exercises. • Incentive spirometry should be used individually due to the risk of infection transmission.
Postural Drainage	Appropriate positions should be used to evacuate lung secretions through tapping, vibration, percussion, and coughing. Postural drainage adequacy should be assessed postoperatively. It promotes the passage and removal of bronchial secretions into the upper respiratory tract.	• This procedure may not be performed in some surgeries because it may create additional postoperative risks. • The physician's medical instructions for the lungs should be reviewed before the procedure.
Pain Management	The goal is to optimize the patient's pain level. This involves a combination of pharmacological and non-pharmacological methods, along with a meticulous pain assessment.	• It should be known that problems in the evaluation of pain, which is a subjective sensation and feeling, and analgesic application that does not comply with correct

Noninvasive Ventilation Practices	Especially after thoracic and upper abdominal surgeries, noninvasive ventilation methods such as CPAP and BiPAP can reduce the risk of atelectasis by optimizing gas exchange in the postoperative period.	principles directly negatively affect pain management. • The patient should be evaluated for mask compliance and skin integrity.
Implementation of Ordered Medical Treatment	For example, medications for medical purposes such as mucolytics, bronchodilators, and antibiotics should be administered as ordered.	• Side effects as well as the effects of medical treatment should be observed and evaluated.



Volume-focused



Airflow-focused

Source: <https://kocmedical.com>

Figure 1. Incentive spirometry

6. The Importance of Interdisciplinary Collaboration

The efficacy of postoperative rehabilitation in achieving successful surgical outcomes is an increasingly recognized phenomenon. Postoperative rehabilitation has been demonstrated to impact patients' overall health, functionality, and quality of life (Polastri, 2025). As surgical techniques undergo advancements, patient populations become increasingly heterogeneous. Groups that are particularly susceptible to adverse outcomes, such as the elderly and those with comorbidities, necessitate a coordinated and comprehensive healthcare approach.

Preserving respiratory function after surgery requires the coordinated work of physicians, nurses, and physiotherapists. Systematic reviews have shown that physiotherapy interventions significantly reduce postoperative complications (Boden et al., 2024). Nurses are the healthcare professionals who spend the most time with patients throughout the entire perioperative process. They are the primary providers, ensuring the coordination and continuity of all interventions, and play a critical role in reducing complications by identifying early symptoms (Dhillon et al., 2023).

A multidisciplinary team comprises healthcare professionals from various specialties, with each professional contributing their expertise to facilitate optimal patient recovery. The collaborative efforts of the nursing and physical therapist team have been identified as a pivotal factor in the reduction of postsurgical respiratory complications. Collaboration encompasses a range of components, including joint assessment, integrated exercise training programs, ongoing communication and monitoring, early recognition of complication risks, and a holistic approach to patient and family education. This model has been demonstrated to enhance patient satisfaction while concomitantly reducing hospital stays.

7. Conclusion

Breathing is a vital function. Maintaining and improving respiratory function after surgery is crucial for preventing respiratory complications. During this process, nursing care and physiotherapy should be implemented holistically and with coordination, based on scientific evidence. An interdisciplinary approach has both short- and long-term benefits. Among these, the most important are improving patient outcomes and increasing the effectiveness of healthcare services. Preventing postoperative respiratory complications relies on patient participation, effective pain control, and early mobilization. All of these directly impact respiratory quality and airway clearance. Ensuring patient participation in breathing exercises, proper positioning, and monitoring breathing patterns allow for the early detection of systemic deterioration. Consistent and collaborative implementation of these strategies within the healthcare team not only preserves lung function but also accelerates functional recovery, reduces hospital stay, and improves overall patient well-being.

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CHAPTER 12

Surgical Techniques in Hemorrhoid Disease

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1. INTRODUCTION

Hemorrhoidal disease is a common anorectal disorder characterized by the dilation, prolapse, and symptomatic development of normal vascular structures (hemorrhoidal cushions) in the anal canal. It is known that up to 40% of the population experiences hemorrhoidal complaints at some point in their lives [1].

Physiologically, hemorrhoids are vascular engorgements that contribute to the continence mechanism of the anal canal. Therefore, hemorrhoidal tissue is not entirely pathological anatomically; what is pathological is the increased blood supply to this tissue, the weakening of the connective tissue, and the prolapse of the tissue.

Risk factors:

- Chronic constipation
- Working while standing or sitting for long periods
- Pregnancy
- A low-fiber diet
- A sedentary lifestyle
- Genetic connective tissue weakness [2].

Patients often present with anal bleeding, pain, itching, hygiene difficulties, prolapse, and a palpable mass. While medical treatment, diet, and lifestyle changes can help manage the condition in the early stages, surgical options become available in advanced cases.

Currently, there are many procedures available for hemorrhoid treatment, including:

- Classical excisional surgical methods
- Stapled hemorrhoidopexy
- Doppler-guided arterial ligation
- Laser hemorrhoidoplasty
- Sclerotherapy and rubber band ligation.

This section provides an expanded overview of surgical techniques, assessing their advantages, disadvantages, indications, and postoperative procedures.

2. SURGICAL INDICATIONS

For surgical treatment to be considered, patients must meet one or more of the following conditions:

- Grade 3 or 4 internal hemorrhoids

- Persistent bleeding despite minimally invasive treatments
- Large or frequently thrombosed external hemorrhoids
- Severe deterioration in quality of life due to prolapse
- Chronic pain and hygiene problems

The grade of the hemorrhoid is the basis of clinical evaluation. The classification is as follows:

Grade 1: Only bleeding, no prolapse

Grade 2: Prolapse present but spontaneously returns to its place

Grade 3: Prolapse can be manually pushed back into place

Grade 4: Persistent prolapse

The decision to proceed with surgery is determined not only by the grade but also by the patient's age, comorbidities, perianal anatomy, previous procedures, and the surgeon's experience [3].

3. SURGICAL TECHNIQUES

3.1. Excisional Hemorrhoidectomy

Excisional hemorrhoidectomy is the classic method of surgically removing hemorrhoidal cushions and has been performed for over a century. The two most well-known techniques are the Milligan–Morgan (open) and Ferguson (closed) methods.

3.1.1. Milligan–Morgan Method (Open)

In this method, the hemorrhoidal tissue is dissected away, leaving the wound open. Healing occurs secondary to surgery. Its advantage is a low recurrence rate. However, postoperative pain is more pronounced, and the recovery period is longer [4].

3.1.2. Ferguson Method (Closed)

After hemorrhoidal tissue is removed, the wound is closed primarily. The closed technique has been reported to:

- Reduce pain and discharge
- Provide faster healing
- However, the risk of infection is slightly higher than the open method.

Both methods can present complications such as postoperative urinary retention, bleeding, anal stenosis, and difficulty defecating. However, it is one of the fastest and most effective permanent solutions for patients with severe prolapse.

3.2. Stapled Hemorrhoidopexy (Longo Method)

Described by Longo in 1998, this method has been considered revolutionary, particularly for prolapsed internal hemorrhoids [1]. The basic principle is to reduce the size of the hemorrhoidal tissue by removing a circular mucosal ring from the proximal anal canal and:

- Moving it higher
- Reducing arterial blood flow.

Technical Summary:

1. An anoscope is inserted into the anal canal.
2. A circular stapler is applied.
3. The mucosal ring is removed, creating the staple line.

Advantages:

- Significantly less postoperative pain
- Short hospital stay
- Rapid return to daily life

Limitations:

- Does not treat external hemorrhoids
- High device cost
- Rarely, serious complications such as pelvic sepsis have been reported.

3.3. Doppler-Guided Hemorrhoidal Artery Ligation (HAL-RAR)

In this technique, the hemorrhoidal arteries are located using Doppler ultrasound using a probe inserted into the anal canal and ligated with sutures. If prolapse is present, mucopexy (HAL-RAR) is added.

Advantages:

- Reduced vascular flow without tissue loss
- Minimal pain
- Rapid return to work
- Hygiene and anal anatomy are preserved

Disadvantages:

- Recurrence rate may be higher in grade 3-4 hemorrhoids
- Success depends on the surgeon's experience [5]

3.4. Laser Hemorrhoidoplasty

This is a procedure that has become increasingly popular in recent years. The laser probe is inserted into the hemorrhoidal lump, and energy is applied to thermally destroy the vascular structure. This:

- Reduces blood flow
- Shrinks the cushion
- No tissue loss

The most significant advantage of this method is its minimal pain and short surgical time. However, it is not sufficient alone for patients with advanced prolapse.

3.5. Minimally Invasive Clinical Applications

3.5.1. Band Ligation

This is the most commonly used method for first- and second-degree hemorrhoids. The base of the hemorrhoid is compressed with a rubber band, which then collapses through necrosis within 7–10 days.

3.5.2. Sclerotherapy

Fibrosis and shrinkage are achieved by injecting a sclerosing agent into the hemorrhoidal tissue. It can be performed on an outpatient basis and has a low complication rate. However, it is insufficient for high-grade hemorrhoids.

4. POSTOPERATIVE MANAGEMENT

Postoperative care is critical to the success of treatment. Patients are advised to:

- Consume plenty of water
- Follow a high-fiber diet
- Avoid straining during defecation
- Use analgesics
- Warm sitz baths for 10–15 minutes
- Short-term laxative support if necessary

Early complications:

- Bleeding
- Urinary retention
- Infection

- Pain

Late complications:

- Anal stenosis
- Recurrence
- Very rarely, fecal incontinence

Regardless of the surgical technique, the risk of recurrence increases if bowel hygiene and lifestyle changes are not implemented postoperatively [2].

5. CONCLUSION

Surgical treatment for hemorrhoidal disease should be tailored to the individual, based on the severity of the hemorrhoid, the severity of symptoms, the patient's expectations, and the surgeon's experience. While excisional methods still have the lowest recurrence rate, minimally invasive methods offer the advantages of less pain and a faster recovery.

Stapled hemorrhoidopexy provides particularly good results for prolapsed internal hemorrhoids, while methods such as HAL-RAR and laser are more modern approaches that preserve the anatomy.

The postoperative course and healthy lifestyle habits are as important as the surgical technique for successful treatment.

6. RESOURCES

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CHAPTER 13

An Analysis of Turkish Social Media Users' Communication Regarding Antibiotic Use

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Introduction

Since their introduction, antibiotics have been crucial in public health, making many previously fatal infections treatable and saving countless lives. Nevertheless, the irresponsible use of these drugs inevitably leads to a reduction in their effectiveness, resulting in antimicrobial resistance (AMR).

The escalating issue of AMR represents a major global health threat, especially as the most powerful existing drugs begin to fail (Antimicrobial Resistance Collaborators, 2022). If not managed effectively, this rise in resistance is projected to cause substantial economic, societal, and health crises for both present and future populations (Broom et al., 2023).

In response to this global crisis, Türkiye has actively participated in international efforts against AMR. The country developed and launched its first National Action Plan (NAP), which ran from 2014 to 2017. The primary goal of this plan was to establish rational antibiotic use across the country and successfully lower AMR rates (Aksoy et al., 2021). However, a common obstacle globally is not the creation of such a plan, but the difficulty in ensuring its evidence-based implementation and demonstrating consistent, measurable progress.

Türkiye's NAP focused on several key areas: raising public awareness, encouraging judicious antibiotic consumption, and positively influencing the attitudes and behavioural patterns of healthcare professionals concerning antibiotic use and AMR. Despite these objectives, there is a lack of clarity regarding how Turkish policymakers evaluate and monitor the progress made toward these goals, and whether these initiatives have led to tangible and significant changes in the public and professionals' behaviour regarding antibiotic usage.

Therefore, this study aims to analyse social media communications to gain insight into how AMR is presented in online sources and what Turkish social media users are discussing about antibiotic use. This analysis will help assess the efficacy of national behavioural change efforts related to antibiotic use and pinpoint new, actionable strategies for future development of the program.

Background

An increasing number of people now use the internet to obtain information about diseases and treatment options. Social media (SM) also enables users to exchange support, share experiences, and discuss health-related issues (Yan et al., 2019). However, these platforms can also contribute to the spread of misunderstandings and misinformation (Wang et al., 2019).

Due to their rapid and low-cost reach, SM channels have become valuable tools for public health researchers seeking to access large or specific audiences and understand public opinions (Capurro et al., 2014). Previous studies have examined public misconceptions about antimicrobial resistance (AMR) (Essack et al., 2023), the spread of information on antimicrobials (Scanfeld et al., 2010), and factors shaping online discussions about antibiotic use (Dyar et al., 2014), particularly on X (formerly Twitter).

In this context, SM analysis was considered an appropriate method to identify themes related to antimicrobial use practices and perceptions among people in Türkiye. Since SM reflects cultural and social trends, examining online content can help clarify the motivations, expectations, and experiences associated with antimicrobial use and therapy (Rachel Henzell et al., 2014). Moreover, many adults and parents may consult SM platforms for health advice in line with prevailing social norms.

Therefore, this study was planned as a qualitative analysis of communication on widely used SM platforms in Türkiye. Its aim was to understand how Turkish users communicate and seek information regarding AMR and antibiotic use.

The research question guiding this study was:

How is AMR represented on social media, and what topics do Turkish users discuss concerning antibiotic use?

In this chapter, *social media platforms* refer to Facebook, Instagram, X (formerly Twitter), LinkedIn, and similar channels. A *social media post* denotes a brief message or content shared on these platforms.

Study Design

This research utilized the Qualitative E-Research Framework (Salmons, 2017) to address the complexities of collecting and analysing social media (SM) data, which is an emerging interdisciplinary research area.

The research design integrated knowledge from multiple disciplines through:

- Consultation with a social media expert and a computer engineer.
- Adopting the '*SM as an instrument*' approach, positioning the researcher as an 'outsider' to passively collect data on user behaviour and communication patterns (Zeller, 2017; Zhang et al., 2018).
- Employing a '*multiplatform approach*' to ensure a wide variety of data was collected.
- Utilizing content analysis to quantify qualitative data, supplemented by textual analysis.

- Incorporating SM theories to enrich the discussion of the findings.

Methods

Data Collection

The data collection strategy was defined as '*extant*' (Salmons, 2017), focusing on obtaining a substantial amount of communication where users freely expressed their views on antibiotic use and AMR without researcher influence.

A multiplatform approach was chosen to leverage the unique characteristics and content types of various social media platforms (McCay-Peet & Quan-Haase, 2017). Following a review and expert consultation, four platforms were selected for analysis:

- Two popular global platforms: X and YouTube.
- Two nationally prominent Turkish platforms: Sour Dictionary and Onedio.

Data was retrieved using an iteratively refined set of Turkish keywords, word groups, and hashtags related to AMR and antibiotic use (e.g., #antibiotic, #antibioticresistance, #flu, #TITCK, #SGK). Boolean logic was applied to ensure accuracy, acknowledging Turkish as an agglutinative language.

Data was gathered from X via Application Programming Interface (API) search (with a computer engineer's assistance) and manual search (using X's advanced search).

For other platforms, historical data between 2015 and 2020 was retrieved, assuming that public interest in AMR increased following the implementation of Türkiye's NAP.

Data Analysis

The study primarily focused on topic-related metrics and the content of social media posts to understand Turkish users' interest and the concepts discussed regarding antibiotic use (Robinson et al., 2019). Thus, content analysis is found to be the most commonly employed technique for analysing various forms of social media data (Wang et al., 2019).

Coding Procedure: A deductive approach was used to establish the coding frames, drawing on prior research (Scanfeld et al., 2010; Chew & Eysenbach, 2010).

First set of codes: Initial categorization of posts into general types (e.g., resource-related, expressing personal opinion/interest, jokes/parody, marketing-related, spam).

Second set of codes: Focused on antibiotic-specific content, excluding certain categories from the first set. Categories included: general use, advice and information, side effects/negative reactions, diagnosis, resistance, misunderstanding/misuse, positive reactions, animals, wanting/needing, cost, and others.

Intercoder Reliability (ICR): To ensure research quality, ICR was established by having a second human coder independently code a 20% randomly selected subsample of the data from X and Sour Dictionary. The Kappa coefficient was used to calculate reliability (Cohen, 2008). The agreement level (Cohen's Kappa) was estimated at 0.84, indicating almost perfect agreement. Following the establishment of satisfactory reliability, the researcher proceeded to code the remaining data alone (Campbell et al., 2013).

Ethical Considerations

As the research involved analysing publicly available SM posts from open websites without an identifiable gatekeeper, formal ethical approval was not sought. However, the study strictly adhered to ethical research guidelines proposed for health research on social media (Benton et al., 2017), carefully considering principles such as anonymity, confidentiality, and avoiding harm to participants.

Findings

Data for the social media communication analysis was collected from four platforms: X, YouTube, Onedio, and Sour Dictionary, starting on September 8, 2020.

Descriptive Findings

Findings from X

Data were collected from X via a three-part strategy, as detailed in the methodology. The initial step involved sampling tweets that contained specific Turkish keywords related to antibiotic use through the platform's accessible API. This API search was executed by a computer engineer using Python software for a seven-day period, spanning September 8 to September 16, 2020.

Tweets collected from all keywords and hashtags were merged and subjected to pre-processing to eliminate duplicates and irrelevant content. Following this cleaning phase, the resulting dataset for the specified week comprised 906 tweets, 21,397 retweets, and 5,127 likes.

Subsequently, a manual search utilizing the same keywords was conducted to identify additional tweets posted within the same timeframe that may have been

missed due to API limitations. Duplications were removed, and these newly found tweets (n=251) were categorized and integrated into the dataset.

In the final retrieval step, the URLs contained within the API-obtained tweets were cross-referenced. This process was extended to include X threads—sequences of interconnected tweets from a single user. This technique provided crucial additional context to the full X dataset, significantly aiding the cohesive understanding of complex discussion points. The subsequent sections of the findings elaborate on the specific categories and themes that emerged from this X data.

Findings from YouTube

The YouTube search was conducted manually using relevant keywords. To ensure impartiality and prevent any potential influence from the researcher's viewing history, a new, dedicated YouTube account was established for this study. All searches utilized YouTube's default relevancy filter. The first 20 results for each keyword were meticulously recorded, based on the assumption that users typically do not browse beyond the initial pages.

Videos were subjected to strict exclusion criteria: any content exceeding the ten-minute duration threshold was eliminated, as previous literature indicates this as the optimal length for YouTube engagement (Steinberg et al., 2010). Furthermore, irrelevant videos, live streams, online educational material, duplicates, and any videos uploaded more than five years prior were excluded. Ultimately, 139 YouTube videos meeting all criteria were included in the analysis. Although these videos generated 1,533 comments in total, the majority consisted of non-substantive interactions (e.g., compliments or casual exchanges). After excluding these irrelevant comments, the qualitative analysis focused on 94 text comments containing substantive user discussions.

Findings from Onedio and Sour Dictionary (Eksisözlük)

The data retrieved from Onedio identified eight pieces of social content shared regarding antimicrobial use over the preceding five years. The corresponding reader comments and reactions to this content were collected.

The search strategy for the Sour Dictionary involved performing two rounds of searches for relevant headings through the platform's recommendation box. This process yielded 19 topics in the first round and 17 topics in the second, totaling approximately 378 pages of comments (with roughly ten comments per page). To manage this extensive volume and maintain focus, the analysis was restricted to comments posted between 2015-2020, and all irrelevant posts were

excluded from the qualitative analysis to ensure the content directly addressed the study objectives.

Overview of Themes and Presentation of Social Media Communication

To ensure clarity and provide necessary context for the analysis, specific conventions were used when presenting social media data:

Quotations: All direct quotes from social media posts were abbreviated using sequential tags (e.g., Q1, Q2, and Q3). Each quotation is accompanied by its date and the platform from which the data was retrieved.

Dialogues: Relevant conversations or dialogues involving multiple users were quoted to capture the full context of the exchange.

Users: Individual participants within these conversations were abbreviated (e.g., U1, U2, U3, etc.).

Contextual Usernames: Usernames that included professional titles (e.g., "dr," "pharm," or "nurse") were retained in the quotes. This inclusion is intended to provide important context regarding the perspective within the conversation, although it is explicitly noted that these titles may not always guarantee real-life professional identification.

Details of the Findings from SM Communication regarding AMR

RQ1: How is AMR portrayed in the SM and what are the topics debated by Turkish users regarding antimicrobial use?

The content analysis was instrumental in determining the prominence and significance of AMR and antibiotic-related subjects within the social platforms frequently accessed by the Turkish population. Consequently, Theme 1 was developed by synthesizing these discussions observed in Türkiye. This theme ultimately illuminates the various ways in which the Turkish public depicts and engages with the issue of AMR on social media.

Theme 1: Qualitative Insights from Numbers

The main SM channels extensively used in Türkiye, including X, Sour Dictionary, Onedio, and YouTube, were found productive in terms of the number of shares and comments related to antibiotics.

Table 1

Findings from First Coding of Content Categories According to SM platforms

	X	YouTube	Sour D.	Onedio	Total
Resource	140	-	86	6	232
Personal Experience	94	43	84	38	259
Personal Opinion and Interest	110	51	159	82	402
Jokes/ Parody	93	27	360	8	488
Marketing	150	-	1	-	151
Spam	570	36	13	12	631
Total	1157	157	703	146	2163

A significant initial observation was the abundance of AMR-related posts categorized under jokes/parody, marketing, and spam, particularly on platforms like X and Sour Dictionary.

Once these non-substantive posts (jokes, spam, and marketing) were removed, a high volume of the remaining content, especially on X, fell into the '*resources*' category. This indicated that the majority of social media posts contained news or factual information disseminated by governmental health accounts or professional organizations. Consequently, the online conversations surrounding AMR and antibiotic use were, to some extent, informative and factual, a finding that aligns with observations in other studies (Andersen et al., 2019; Scanfeld et al., 2010).

However, the accuracy and presentation of information appeared to vary significantly across platforms. For example, while AMR-related YouTube video content frequently originated from credible sources or recognizable channels, none of the associated user comments under these videos contained external resource links. This highlights a clear distinction in how information is presented

versus how it is discussed and shared across different social media platforms regarding AMR and antibiotic use.

The remaining dataset provided a sufficient volume of posts to analyse Turkish users’ personal opinions and experiences regarding antimicrobial use. Therefore, posts categorized under '*resource*,' '*personal experience*,' and '*personal opinion and interests*' were subsequently re-categorized using the second, more detailed coding frame, as outlined below:

Table 2

<i>The Findings from Second Coding of Content Categories According to SM Platforms</i>					
	X	YouTube	Sour D.	Onedio	Total
General Use	102	15	17	26	160
Advice and Information	81	31	110	44	266
Side Effects/Negative Reactions	34	7	47	10	98
Diagnosis	23	17	6	16	62
Resistance	18	13	19	14	64
Misunderstanding or Misuse	32	8	65	13	118
Positive Reactions	10	-	24	-	34
Animals	28	3	2	3	36
Other	-	-	24	-	24
Wanting and Need	16	-	9	-	25
Cost	-	-	6	-	6
Total	344	94	329	126	893

As illustrated in Table 2, the most frequently observed category across the entire dataset was '*advice and information*'.

Shares within this dominant category primarily consisted of comments classified under '*personal experiences/opinion*' and '*interests*', with a smaller segment originating from the '*resource*' category. For example, some updates

offered simple personal advice, such as *"You should seek a doctor for having antibiotics."* In contrast, other posts referred to external news articles about antibiotics and included a link, such as the example quoting Prof. Dr. X stating, *"A spoon of honey is more effective than antibiotics."*

Another category meriting detailed discussion was *'misunderstanding/misuse,'* which was notably common within the X and Sour Dictionary datasets. X users predominantly shared narratives detailing their own antibiotic misuse behaviors, such as intentionally skipping a dose or prematurely discontinuing treatment for a minor reason. Conversely, Sour Dictionary users often shared narratives that served to criticize the misunderstandings or misuse behaviors of others regarding antibiotic consumption.

The content demonstrated the dual role of social media: certain comments referenced information from well-known, credible accounts, while others highlighted the platforms as spaces for the informal exchange of advice and information related to AMR. This finding is consistent with results from prior research (Dyar et al., 2014; Scanfeld et al., 2010). The informal 'personal advice and information' subcategory included user exchanges on various non-specific health issues, such as influenza symptoms, toothache, or acne. Notably, some users even cited specific antibiotic brand names, with *'Augmentin'* being the most frequently mentioned.

RQ2: What are the influential factors that appear to impact on public thinking in Türkiye towards AMR and antibiotic use?

Theme 2: Online Information and Advice Seeking

The contemporary information era is fundamentally characterized by a vast abundance of data and knowledge circulating rapidly through the internet and SM platforms.

The following public conversations, sourced from X, illustrate how SM actively functions as a key platform for the exchange of information and advice regarding antibiotics among Turkish users:

Q1: Sep 12, 2020 -X

U1: *How can I get rid of this persistent dry cough? I haven't been able to sleep for days.*

U2: *You could try herbal teas.*

U3: *You might need a 1000 mg antibiotic.*

U3: Lower dose antibiotics don't work for me. I believe I have antibiotic resistance, but the 1000 mg antibiotic is a miracle. It suddenly kills all the symptoms. Of course, it should be under the doctor's supervision.

Q2: Sep 13, 2020 - X

U1: What's good for a toothache?

U2: Try Dexametoprolfen; it helps relieve pain (a nonsteroidal anti-inflammatory drug).

U3: It won't be cured without an antibiotic.

U4: Place smashed garlic on your tooth; then, you won't need an antibiotic or painkiller.

Social media (SM) communication concerning antibiotics among Turkish users primarily appears to center on seeking advice for common health issues, such as toothache, colds, and flu. The examples observed on X (formerly Twitter) suggest that the Turkish public often turns to SM for guidance on acute symptoms rather than immediately consulting a physician. The motivations underlying this online health information-seeking behaviour are complex, encompassing cognitive, practical, and social constructivist factors.

In practical terms, the behaviour of seeking health information online has become widespread as internet access and usage rates have increased. Users continually generate and share health-related information through information communication technologies and SM (Zimmerman, & Shaw, 2020).

This behaviour is supported by Wilson's Model of Information Seeking Behaviour, which hypothesizes that "*individuals with high information needs and low barriers will be more likely to seek information*" (Das, 2013, p. 27). Applied to the Turkish context, this suggests that individuals who require information about their health condition and experience barriers to accessing healthcare professionals may prefer seeking advice from SM platforms, given that social media presents minimal hurdles to readily available information (Del Vicario et al., 2016).

A subsequent iteration of Wilson's Model introduces the concepts of self-efficacy and Risk and Reward Theory (Wilson, 1999) to account for individual variations in the motivation and urgency with which information needs are pursued. Within this framework:

- The Turkish patient's perception of risk related to their condition could act as a catalyst, prompting the recognition of their information requirements concerning antimicrobial use.

- Self-efficacy, conversely, impacts their confidence and belief in their own capability to successfully engage in information-seeking activities online.

Conversely, although this was a less frequent occurrence in the analysed dataset, X emerged as a social platform through which healthcare professionals disseminated crucial Antimicrobial Resistance (AMR)-related information to the public, occasionally initiating collaborative discussions on the topic.

Q4: X- 2020 September (87 Retweets 2 Quote Tweets 572 Likes)

Dr U1: Antibiotic resistance will return us to medieval medicine! Read this article...

U1: Did it happen because doctors prescribe unnecessary antibiotics without diagnostic tests, and people buy randomly non-prescription antibiotic from pharmacies in our country after the 1980s?

U2: I wonder if this is an irreversible issue. Could abstaining from certain antibiotics for 10 years restore their effectiveness against infections?

U3: What about those who responsibly take antibiotics? Does antibiotic resistance impact them as well?

U4: What do you mean when you say we won't find antibiotics? Is this indicative of a scarcity of antibiotics for the global population or their diminishing efficacy against infections?

The analysis revealed that information concerning antibiotic usage and Antimicrobial Resistance (AMR) on social media (SM) is collaboratively generated by multiple Turkish stakeholders. This diverse group includes healthcare professionals (such as doctors and pharmacists) and laypeople (comprising the general population and patients).

Consequently, this finding suggests a significant opportunity: the deliberate inclusion and promotion of co-constructed dialogues involving healthcare professionals could serve as an effective mechanism. Such engagement has the potential to facilitate the wider dissemination of evidence-based knowledge while simultaneously mitigating the spread of health-related misconceptions and inaccurate information across these digital platforms.

RQ3: Are there synergies between the messaging on social media and the objectives of the Turkish National Action Plan (NAP) for AMR?

The analysis of social media communication among Turkish users has provided valuable insight into the factors that both encourage and impede rational antibiotic use in Türkiye. Within this context, several prominent themes have emerged, illuminating prevalent public misconceptions regarding antibiotics, as

well as the dynamics of professional-patient communication and the critical role of trust. Collectively, these insights offer a comprehensive view of how these multifaceted factors have significantly shaped the perspectives of healthcare professionals and subsequently influenced their prescription practices, ultimately impacting overall antimicrobial use in Türkiye.

Theme 3: No cure without antibiotics

The primary data driving this theme originated from professional accounts, specifically those complaining about patients who express the sentiment, *"I cannot recover without antibiotics,"* during clinical consultations.

A selection of example quotations from X (formerly Twitter) users is presented below. Most of these narratives are likely shared by doctors detailing their consultation experiences, often focusing on interactions with parents who insist on antibiotic therapy for their child.

Q1: Sept 10, 2020, X

Dr User: -Your patient does not need an antibiotic; she will recover by herself within a few days.

-Even so, prescribe one, she cannot recover without antibiotics.

Q2: 18 Likes - 4:17 PM · Sept 12, 2020, X

Dr User: "This morning, a father came with his child due to fever and said his child cannot recover without antibiotic. He was told no necessary for antibiotic and they left. He came back in the evening for could not control their child fever. All diagnostic tests has done and found antibiotic was still unnecessary for the child's condition. However, we had to do prescribe antibiotic for this child, as child's father was impatient and not wanted to wait to control fever. And, we do know that the father will always say 'my child cannot recover without antibiotics'.

U2: In fact, the only thing cannot heal is their obsession with antibiotic use

Q3: 19 Retweets 525 Likes – 9:32 AM, Sept 15, 2020, X

Dr User 1: The first patient of the day... While I am prescribing medications, the mother asked, "You prescribed an antibiotic, did not you? My child's fever does not go down without antibiotics." I explained why no antibiotic is needed. She was not convinced, felt unsatisfied, and left.

Dr User 2: I respond them as if I already prescribe antibiotic. I don't care if they realise the truth in the pharmacy.

U1: Yes, we all believe that we cannot get better without antibiotic therapy by reason of doctors or pharmacist who overdo in giving antibiotic for many years.

Q4: X -16 September 2020

U1: If there are swollen parts around the tooth and feel pain in every motion of your mouth, it is probably a dental abscess. It cannot be cured without antibiotics.

Q5: X -15 September 2020

Dr User: Maybe the flu is not be cured with antibiotics. Why not it is the real reason of that the doctor did not prescribe an antibiotic?

U1: How could throat infection be cured without antibiotic?

The narratives from healthcare professionals on social media revealed that physicians frequently feel compelled to prescribe antibiotics due to intense patient demand, particularly originating from parents. While quantifying the overall antibiotic demand using the social media dataset was not feasible, the examples provided demonstrated that users recognized the inclination to seek antibiotics, sometimes attributing these patient expectations to prior instances of physician over-prescription (Q3- U1).

Despite parental insistence on antibiotics in the context of scenario Q3, the physician's reluctance to prescribe resulted in patient dissatisfaction. However, it is important to note that primary care patients who request antibiotics are rarely denied them (McNulty et al., 2013), suggesting the aforementioned example might represent an exception.

The social pressure to request antibiotics is a common occurrence in Türkiye. Patients often visit doctors specifically to request certain antibiotics previously recommended by acquaintances, such as neighbors or relatives, for their condition (Hosoglu et al., 2021). This findings suggest that social media communication may contribute another element to this pressure, amplifying misbeliefs through user interactions.

In Türkiye, physicians often feel a sense of security when prescribing antibiotics due to the large patient volume and time constraints that inhibit thorough examinations or waiting for laboratory results (Hosoglu et al., 2021). Furthermore, they often feel obligated to protect themselves against patient accusations that might arise if an antibiotic is withheld (Hosoglu et al., 2021). Clinically inappropriate antibiotic expectations and requests have been associated with perceived social norms and the anticipated regret of leaving a consultation "empty-handed" (Thorpe et al., 2021).

Given that the largest single driver of unnecessary antibiotic prescription among physicians is the perception of high patient demand, the most effective

solution likely lies in addressing the root causes of this elevated public demand and implementing strategies to reduce it in Türkiye.

Therefore, educational campaigns must move beyond simply providing factual knowledge (e.g., viral etiology, antibiotic efficacy, usage, and resistance). They must also integrate an understanding of the cognitive and motivational mechanisms (such as illness representations, antibiotic beliefs, and the influence of social media) that underlie inappropriate expectations and requests for antibiotics (Thorpe et al., 2021).

Such targeted interventions can lead to patients being less likely to pressure physicians for antibiotics (Cabral et al., 2016) and significantly enhance the effectiveness of initiatives aimed at appropriate antibiotic prescribing. In addition to broad public education, increasing public knowledge can also be achieved through improved communication between patients/family members and providers during the consultation itself.

Theme 4: Poor Professional-Patient Communication

Numerous social media users shared their personal experiences as patients during clinical consultations. A pervasive theme in these accounts was the complaint regarding a limited opportunity to ask questions of the physician or the inadequate attention given to their health concerns, thereby highlighting deficiencies in professional-patient communication within Türkiye.

Q1: 2019- YouTube- When you should take antibiotics?

U1: Doctors do not allow asking a question, not a chance for getting information from them. When you ask some questions, they remind you patients waiting and give you the gate. I wonder if other people waiting are patients, am I not?

Q2: X- 2020-September

U1: What kind of health system is this? I caught flu and felt seriously ill. The FP said 'don't exaggerate' and did not prescribe antibiotics. I am coughing; I feel so much pain on my throat. No antibiotic, no painkiller. Even worse, no one is taking an action for it even if I file a complaint. I am informed that it is the doctor's right. Where is my right as a patient?

In the context illustrated by Q1, a central factor contributing to this suboptimal communication quality appears to be the constrained consultation time available to doctors. Professionals may find themselves unable to dedicate sufficient time to offer comprehensive explanations regarding treatment options, which results in a lack of patient involvement in shared decision-making. However, the

dynamic between patient and doctor encompasses complexities beyond mere time limitations; it constitutes an interpersonal exchange requiring a deep understanding of the expectations, obstacles, and emotional state of both parties (Kee et al., 2018).

For patients, the act of asking questions of healthcare professionals is a crucial way to articulate their concerns and foster a sense of control over their own health (Medina-Perucha et al., 2020). While patients desire this control over their overall health, the previously noted quote underscores a feeling of being marginalized from the core of the consultation experience. Consequently, the user seemed to feel unheard in a situation where they were unsure how to manage their health or effectively demand a solution from the doctor. While obtaining a prescription, such as an antibiotic, might superficially instill a sense of control (Medina-Perucha et al., 2020), the user's expectations were fundamentally centered on active listening and interactions rooted in empathy during the consultation.

Q2 further showcased the user's experience of feeling that patients could not receive respect from the physician for their subjective health concerns. For both cases, the issue transcended mere respect or empathy; it underscored the need to recognize that patients are often in a vulnerable position due to a convergence of factors, including low health literacy, paternalistic communication styles, and an inherent power imbalance.

In this context, initiatives focused on supporting health literacy and fostering a more balanced power dynamic between patients and professionals could significantly contribute to more rational antibiotic use by positively modifying patient expectations and increasing their satisfaction with care (Morgan et al., 2011; Medina-Perucha et al., 2020).

Theme 5: Non-standard practices

Social media communication has served to bring to light indications of variations in physicians' prescribing practices and discrepancies in pharmacists' adherence to antibiotic sales regulations.

Consequently, this theme has emerged through two distinct subthemes, both of which delve into these non-standard professional practices. These subthemes are crucial not only for highlighting these deviations from established standards but also for exploring the subsequent implications they have on public perceptions and behaviours regarding the use of antimicrobials.

Subtheme 1- Inconsistent prescribing and lack of trust in health system

The data from SM suggests that inconsistent prescribing practices among physicians appear to erode patients' trust in the medical profession.

Q1: 2019- Sour Dictionary- Doctors give advice, not antibiotics

U1: I visited two different doctors for the same infection, and one did not prescribe antibiotics, the other said that you cannot recover without antibiotics.

Q2: 2019-YouTube- The harm of Excessive Antibiotic Use

U1: It tells us the harm of antibiotic use, but if doctors are prescribing antibiotics, what should we do? Should we not take them?

Q1: One out of every three prescriptions is for antibiotic treatment in Türkiye- Onedio-2016

U1: As a biology student, I warn you that you do not use antibiotics even if the doctor prescribes it.

U2: Doctors prescribe antibiotics for every infection. Why? They have monthly quota for getting incentives from pharmaceutical companies.

U3: Not only doctors are guilty of high consumption of antibiotics. Even my family could barely understand that they do not need antibiotics for flu and cold.

U4: How we expect that people use antibiotics wisely? No education is provided for the public. These uneducated people get antibiotics and stop therapy after taking 2 days. Bacteria get the resistance after these all inappropriate use.

U5: It is the issue that needs to be put on the agenda 10 years ago. Too late for Türkiye

Q3: Türkiye has one of the highest rates of the total consumption of antibiotics- Onedio-2016

U1: While the time for the per patient in the consultation is 3-4 mins, if a few additional urgent cases come for examination, doctors have to allocate 2 mins per patient. Doctor prescribe antibiotics necessary or not because they do not want to argue with patients. Even so, no one asks why they are examined in a rush.

U2: Go to see a doctor, tell your complaints, take antibiotics, and come back

U3: It is normal because doctors and pharmaceutical firms have an agreement. Our health is beyond caring

Q4: 40 per cent of prescriptions include antibiotics in Türkiye- Onedio-2019

U1: The reason is that pharmaceutical agencies manipulate doctors.

U2: Doctors have to prescribe such amount of antibiotics because Syrian immigrants brought new infections to our country. Antibiotics are not as effective as the past for infections because they spoiled our health.

U3: But we do not heal without antibiotics.

U4: Then, let doctors not prescribe antibiotics.

U5: When some doctors did not prescribe antibiotics, people are asking another doctor "we went to a doctor, but he/she did not prescribe antibiotics for us, can you do that?" When they get refused, they are bullying to doctor.

U6: I think doctors take the easy way for treatment. When I go to the doctor to ask for advice to make my children feel better, they always prescribe antibiotics.

U7: Antibiotics are the fast, cheap, and unhealthier solution for sick people who live in a developing country like Türkiye. People are uneducated, malnourished or under bad conditions here. Doctors have to choose that way.

Another example was a discussion thread between doctor users and general users from X:

Q5: 214 Retweets 16 Quote Tweets 2.7K Likes

Dr U1: Half of the public believes doctors make money from pharmaceutical firms, and thus, they unnecessarily prescribe antibiotics; the other half believe they cannot heal without antibiotics but say Turkish doctors deliberately do not prescribe antibiotics for them.

Dr U2: I am a doctor who rarely prescribes antibiotics. When I prescribe antibiotics, it runs into the group who asks, 'Is that really necessary?' When I do not, I coincide with the other group who say, 'But I cannot get better without antibiotics'.

U1: Then, the half who cannot get antibiotic prescriptions want to access antibiotics from pharmacies secretly.

U2: I agree with the first half. We know the relationship between doctors and pharmaceutical representatives based on mutual interests. So I don't trust doctors.

Dr U3: I wonder which pharmaceutical firms they are. I did never coincide with such representatives as a doctor.

U3: The ultimate dilemma

As demonstrated in the communications, patients expressed confusion stemming from physicians' inconsistent treatment decisions. This lack of clarity, compounded by the observed divergence in prescribing practices, has led to a diminished level of patient trust.

Low patient trust has been shown to impede patients' adherence to prescribed treatment regimens and obstruct the adoption of appropriate health behaviours (Murray & McCrone, 2015). Consequently, it is apparent that Turkish patients have, at times, opposed treatment decisions—irrespective of whether antibiotics were prescribed or not—due to underlying suspicions concerning the medical practices of their physicians.

Subtheme-2 Noncompliance with the New Legislation for Antibiotic Sales

Since 2014, antibiotic sales without a prescription have been prohibited in Türkiye under new national legislation. This regulation signals Türkiye's ongoing transition toward a new regulatory framework designed to foster responsible antibiotic usage nationwide.

However, limited information was available concerning the experiences of both professionals and patients during this transitional phase. While interviews conducted in preceding chapters offered minimal insight into their perspectives on this new regulation, historical data retrieved from social media communication provided a more comprehensive understanding of the public acceptance, adherence, and implementation of the new rule across community pharmacies.

Significantly, numerous social media users expressed support for the new regulation, viewing it as a necessary measure to curb irrational antibiotic usage in Türkiye.

Q1: 2016- Onedio- The calm before storm: Antibiotic Resistance

U1: Antibiotic sales without prescription must urgently be prohibited. Parents are giving antibiotics to their young children even for self-recovering infections.

Q2: 2016-Sour Dictionary-Antibiotic

U1: The only intervention that I appreciated is that the change in legislation on antibiotic sales 9 months ago. If you did not aware of it, I would like to inform you that you are no longer able to buy any of the antibiotics without a prescription. The next intervention must be changing the mentality of doctors who want to prescribe antibiotics for every infection.

However, a large group of users held differing opinions from its supporters of the necessity of this regulation. One reason for their dissatisfaction with the regulation was the requirement to pay examination fees following doctor visits in Türkiye.

Q3: 2019- Sour Dictionary- No sales of antibiotics without a prescription

U1: A meaningless regulation. Let's say I will buy an antibiotic for 10 Turkish Lira but now I should pay an examination fee for 60 Turkish Lira, time loss and

pecuniary loss in my business. That's bullshit. Whoever wants to buy antibiotics should make payment for it and get it. People who use antibiotics unconsciously are mostly low-educated and low-income, so they cannot buy this without prescription to not pay for by themselves. The reason of high consumption of antibiotics is not caused by non-prescription antibiotics. Stop doctors to prescribe antibiotics unnecessarily.

Q4: Sour Dictionary- 2019- No sales of antibiotics without a prescription

U1: I suffer from cystitis from time to time. After this regulation, I could not buy Monurol (an antibiotic primarily used to treat lower urinary tract infections) just in case. The family practice service is closed on Saturday. It is not a case requiring going to the public hospital emergency service. I do not want to pay money to the private hospital for this situation.

Another reason for disliking the regulation, which restricted easy access to antibiotics, was the waiting times for doctor appointments.

Q5: 2018- YouTube-Rational Antibiotic Use Public Service Ads

U1: Thanks for prohibition of antibiotic sales. I could not get rid of the flu for a month. I have to wait in a long queue, yellow area, red area in the hospitals, thanks for all bullshit!

U2: You even do not know that no need for antibiotics when you catch flu and you are complaining as if that was not enough. Antibiotic sales are prohibited so that people like you cannot use them irresponsibly.

Q6: 2019- Sour Dictionary- No sales of antibiotics without a prescription.

U1: The most stupid regulation in the health sector. We are not antibiotic-lovers, but I have tooth abscess right now and the soonest appointment that I have is after 15 days!

A notable characteristic of the modern world is the aversion to waiting for health services. Consequently, patients may anticipate direct access to antibiotics from pharmacies to initiate treatment promptly. While over-the-counter antibiotic sales might temporarily satisfy patients, the severe national consequences, such as the alarming rise of Antimicrobial Resistance (AMR), and substantial risks at the individual level are often overlooked. Immediate hazards for patients include unnecessary antibiotic exposure and the possibility that the requested antibiotic may not effectively treat their specific infection (Guinovart et al., 2018).

As evidenced by the social media posts shared previously, the health issues for which the Turkish public historically resorted to antibiotics mostly involve conditions manageable with non-antibiotic treatments, such as painkillers (e.g.,

flu and toothache). Even in cases that genuinely necessitate antibiotic therapy, pharmacists lack both the patients' medical histories and the requisite diagnostic training (Guinovart et al., 2018). Therefore, advising patients to consult a doctor is the most prudent course of action for pharmacists.

Owing to these critical factors, the regulation prohibiting non-prescription antibiotic sales represents an important step toward reducing unnecessary antibiotic use by restricting ease of access in Türkiye.

However, there appears to be a deficiency in public awareness and understanding concerning the rationale behind this regulation. This lack of comprehension can lead to conflicts, potentially prompting individuals to resort to inappropriate methods to obtain antibiotics or causing professionals to implement the regulation with varying approaches. Illustrating this point, a few example quotations are presented below. In the first example, the user—who is likely a pharmacist or pharmacy technician—shared an experience involving a patient seeking an antibiotic:

Q7: 2020- Sour Dictionary- No sales of antibiotics without a prescription

U1: This night a taxi driver came to our pharmacy and wanted Augmentin [an antibiotic medication used for the treatment of a number of bacterial infections] for his abscessed tooth. When I refused to give antibiotics without a prescription, he was shocked and claimed that other pharmacies do that. He tried to find another way of obtaining antibiotics because there was no dental clinic around to get a prescription. He desperately bought a painkiller and left. A few days later, when he came back with a prescription, told me that doctor said to him "you waited unnecessarily, most pharmacies are giving antibiotics in that way, and you could get one." I hope that the doctor indeed did not give such information as he told, but the taxi driver was angry and scolded on me. This happened a few times more. So, if you do not have a prescription and cannot get an antibiotic from a few pharmacies, don't worry, you can get it from 8 out of 10 pharmacies around.

Q8: X- 2020 Sept

Pharmacist U1: The year is 2020, still, there are someone wants non-prescription antibiotics.

Pharmacist U2: We remained 100 years ago. Someone just asked for it too here.

U2: But another pharmacy gives antibiotics in that way!

Q9: September 2020-X

U1: I have a toothache but it is forbidden to get antibiotics from the pharmacy. I went to the dental clinic but no dentist had been there. We all blow our mind during the pandemic.

U2: Bro, get better soon. I can find antibiotics for the tooth if you want.

U1: I sorted it out. Thanks.

Q10: 15 Likes- 9:07 PM · Sept 16, 2020, X

U1: Next door neighbour came to ask antibiotics. Okey, neighbours help each other, but it is too much, isn't it?

Q11: 2016-Sour Dictionary

U1: Not surprising. Today I filled a patient's tooth, and he asked me "should I have finished the course of antibiotic?" He had already started to take antibiotics by himself because of the pain in the decayed tooth. Who did prescribe the antibiotic? If no one, how did he get antibiotic? And why did he take an antibiotic for reducing pain?

Concerning the implementation of the new antibiotic regulations, quotation Q7 revealed a notable disparity: while some pharmacists adhere to the rule prohibiting the sale of antibiotics without prescriptions, most appear not to comply. Similar practices involving non-prescription dispensing have been documented across various Middle Eastern nations, including Iran, the United Arab Emirates, Kuwait, Oman, Yemen, Jordan, Syria, and Lebanon (Alhomoud et al., 2017; Sakeena et al., 2018).

The primary evidence regarding non-prescription antibiotic dispensing in Türkiye stems from a 2017 study (Okuyan et al.) that used the simulated patient approach. This research found that non-prescribed antibiotics were dispensed in 45% of community pharmacies in Türkiye (Okuyan et al., 2017). Although this rate is considered moderate compared to some other countries—where rates can be significantly higher, such as Indonesia (91%), Syria (87%), Saudi Arabia (85%), and Ethiopia (85%) as per a global study (Auta et al., 2019)—recent global data indicates that dispensing antibiotics without prescriptions frequently exceeds 60.0% in many developing countries, despite regulatory frameworks (Batista et al., 2020). These findings collectively confirm that the unauthorized supply of antibiotics is a common occurrence in Türkiye and numerous other nations.

As a result, this theme underscores the urgent need for the rigorous enforcement of existing policies and regulatory frameworks governing antibiotic distribution. Additionally, there is a necessity for introducing new policies aimed

at providing comprehensive training and educational resources for all pharmacy staff, including pharmacists and pharmacy assistants.

First, given the pressure patients exert on pharmacists to dispense antibiotics without a prescription, it is essential to focus on changing Turkish patients' expectations regarding antibiotics and improving their awareness of AMR risks (Alrasheedy et al., 2020). Second, professional education provided to pharmacists within the context of the Turkish National Action Plan (NAP) must go beyond rational antibiotic use and the risks of illegal sales. It explicitly needs to address and focus on the external factors (e.g., financial motivations and social norms) that influence pharmacy personnel's attitude towards antibiotic dispensing in their daily practice (Guinovart et al., 2018).

Conclusion

The data from social media communication confirmed Turkish patients' misconceptions and inappropriate use of antimicrobials. Additionally, both poor professional-patient communication and non-standard practices in antimicrobial prescribing and sales were validated through social media narratives.

Secondly, the role and importance of social media communication were evident in influencing Turkish social media users' understanding and attitudes regarding antimicrobial use. Social media was identified as a tool for online information related to antimicrobial use and for seeking advice among Turkish people. However, it also seemed to function as a public sphere in which misinformation has been spread. Therefore, policymakers should develop strategic actions to utilise social media as a space for correcting users' misconceptions and as a source providing reliable information related to antimicrobials to improve their knowledge and awareness of AMR.

Strengths and Limitations

This study, while offering unique insights derived from a complex social media environment, is subject to inherent limitations related to data retrieval and sampling. Nonetheless, several methodological decisions were implemented to mitigate these risks, which concurrently form the core strengths of the research design.

The most notable concern in the data retrieval process relates to collection and sampling bias. Common sources of bias in this context include limitations in platform access, inherent biases within the SM population and potential sampling errors.

SM platforms do not accurately represent the general population of Türkiye, as different platforms cater to distinct user demographics and serve varying

purposes. This introduces a specific bias as the dataset may not reflect the full spectrum of Turkish citizens.

The absence of precise demographic information (such as gender, age, location, or education level) across the SM platforms analysed prevents fine-grained representativeness assessment and may introduce inherent biases that cannot be fully accounted for.

Lastly, access restrictions imposed by platform APIs can lead to missing data, and the scope of these restrictions is not always fully transparent. For instance, questions have been raised regarding whether the data provided by the X (formerly Twitter) API accurately represents the platform's overall activity.

The study employed several strategic measures designed to enhance the robustness of the data collection and analysis, thereby addressing the aforementioned limitations.

To directly counteract the potential bias stemming from a single, unrepresentative SM population, a multiplatform approach was adopted. By analysing four distinct platforms X (Twitter), YouTube, Sour Dictionary, and Onedio, the study was able to capture a broader variety of user demographics and communication styles relevant to the Turkish public.

To ensure a more representative understanding of X activity, the data collection went beyond the limitations of the advanced API search. This study strategically utilized both manual search and the cross-referencing of URLs. This comprehensive approach allowed to better grasp the context of each tweet and, critically, access the entirety of user conversations and reciprocal debates via the embedded URLs.

To minimize the risk of sampling bias, the keyword-based data collection methodology was meticulously documented. This included detailed records of the selection of primary search terms, various synonymous keywords, and potential alternatives. This rigorous documentation ensures a high degree of transparency and replicability for the data collection process, promoting confidence in the comprehensive nature of the sampled data.

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CHAPTER 14

Ultrasound-Supported Triage Applications in the Emergency Department

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1. INTRODUCTION

Triage in the emergency department (ED) is a critical process aimed at rapidly and accurately determining the urgency of patients' conditions to optimize resource allocation and reduce delays in care. However, traditional triage systems—primarily based on vital signs, patient-reported symptoms, and physical examination—have well-documented limitations. Several studies demonstrate that 15–30% of patients with clinically significant or time-sensitive conditions are initially misclassified into lower-acuity categories, a phenomenon that increases the risk of delayed diagnosis and treatment (Platts-Mills et al., 2010; Newgard et al., 2016). Mis-triage is especially common among patients presenting with non-specific complaints, occult shock, or subtle early signs of physiological deterioration.

Point-of-care ultrasound (POCUS) has emerged as a transformative tool in emergency medicine owing to its rapid, portable, repeatable, and radiation-free nature. Beyond its established diagnostic applications, POCUS enhances early clinical decision-making by enabling the emergency physician to obtain real-time imaging findings at the bedside, thereby reducing dependence on delayed radiologic studies and facilitating more accurate early risk stratification (Moore & Copel, 2011). Integration of POCUS into the triage phase may identify high-risk conditions—such as pneumothorax, pericardial tamponade, hemoperitoneum, or severe heart failure—before they become clinically obvious, ultimately improving patient flow and outcomes.

Recent advancements in handheld ultrasound technologies, artificial intelligence–supported image interpretation, and standardized educational frameworks have accelerated the adoption of ultrasound-enhanced triage. Emerging evidence suggests that these developments may enable POCUS to become an integral component of ED triage workflows in the near future, improving accuracy, consistency, and efficiency during the earliest phase of patient assessment (Kim et al., 2022; Shokoohi et al., 2019).

2. EVIDENCE BASE FOR ULTRASOUND-SUPPORTED TRIAGE

The incorporation of point-of-care ultrasound (POCUS) into emergency department (ED) triage is supported by a large and growing body of evidence highlighting the shortcomings of traditional triage approaches. Conventional triage tools may underestimate severity when vital signs are initially normal or minimally abnormal, particularly in patients with early shock, occult bleeding, pneumothorax, or other time-sensitive conditions. Delays in physician assessment caused by ED crowding further compound this problem. POCUS, which can be performed within 1–2 minutes and provides immediate, actionable

diagnostic information, has demonstrated particular value in high-risk presentations such as trauma, undifferentiated hypotension, dyspnea, and acute abdominal pain (Atkinson et al., 2018; Shokoohi et al., 2019).

International professional societies increasingly endorse the use of bedside ultrasonography during early emergency evaluation. The European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) and the World Interactive Network Focused on Critical Ultrasound (WINFOCUS) both identify POCUS as an essential component of frontline assessment, emphasizing its role in rapid risk stratification and early detection of life-threatening pathology (EFSUMB, 2015; WINFOCUS, 2010). Similarly, the American College of Emergency Physicians (ACEP) describes emergency ultrasound as a primary, immediately available diagnostic modality capable of influencing critical decisions at the point of care, supporting its use at the earliest stage of patient evaluation, including triage (ACEP, 2017).

Collectively, these recommendations and clinical data establish a robust scientific rationale for integrating POCUS into triage workflows, where it has the potential to enhance diagnostic accuracy, reduce delays in care, and improve patient safety—particularly in settings of high ED volume and resource strain.

3. ULTRASOUND PROTOCOLS USED IN EMERGENCY DEPARTMENT TRIAGE

Using structured point-of-care ultrasound (POCUS) protocols standardizes bedside assessments, enhances diagnostic accuracy, and facilitates effective communication among emergency clinicians. Protocols such as FAST/E-FAST, RUSH, ACES, BLUE, and FALLS are the most widely validated and commonly applied frameworks in ED presentations relevant to triage—including trauma, undifferentiated shock, hypotension, and acute respiratory failure. These protocols enable rapid identification of time-critical conditions and support early clinical prioritization.

3.1. FAST and E-FAST Protocols

The Focused Assessment with Sonography in Trauma (FAST) examination was developed to rapidly identify intra-abdominal free fluid in injured patients and has since become fully integrated into the Advanced Trauma Life Support (ATLS) algorithm as a core imaging modality during initial trauma evaluation (Scalea et al., 1999; Kirkpatrick et al., 2004).

The classic FAST examination includes four primary windows:

1. **Pericardial (subcostal/subxiphoid):** Detecting pericardial effusion or cardiac tamponade

2. **Right upper quadrant (hepatorenal/Morrison's pouch):** Common site of early hemoperitoneum
3. **Left upper quadrant (splenorenal):** Evaluation for splenic injury and free fluid
4. **Pelvic/suprapubic (Douglas pouch):** Free fluid surrounding the bladder

In a hemodynamically unstable trauma patient, identification of free fluid strongly correlates with the need for emergent operative or interventional radiologic management.

The Extended FAST (E-FAST) incorporates thoracic imaging to detect pneumothorax and hemothorax. E-FAST has demonstrated excellent sensitivity and specificity for clinically significant hemoperitoneum and pneumothorax, especially when CT imaging is delayed or unavailable (Nandipati et al., 2016; Alrajhi et al., 2012).

These findings support the role of ultrasound as the first-line imaging modality in trauma triage, allowing rapid categorization and immediate routing of high-risk patients to resuscitation bays, the operating room, or interventional radiology.

3.2. RUSH Protocol (Rapid Ultrasound in Shock)

The Rapid Ultrasound in Shock (RUSH) protocol provides a systematic approach to evaluating undifferentiated shock using the “**Pump–Tank–Pipes**” framework (Perera et al., 2010):

- **Pump:** Cardiac contractility, pericardial effusion, ventricular size
- **Tank:** IVC collapsibility, intraperitoneal fluid, pleural fluid
- **Pipes:** Abdominal aortic aneurysm/dissection, deep venous thrombosis

The original RUSH literature and subsequent studies show that the protocol significantly improves early diagnostic accuracy and guides resuscitation priorities in patients with hypotension (Perera et al., 2010; Jones et al., 2004).

In the triage environment, RUSH is especially valuable for early identification of life-threatening conditions such as cardiac tamponade, massive pulmonary embolism, or ruptured abdominal aortic aneurysm.

3.3. ACES Protocol (Abdominal and Cardiac Evaluation with Sonography in Shock)

The ACES protocol offers another structured approach for evaluating undifferentiated hypotension, emphasizing abdominal and cardiac causes of shock. Standard ACES components include:

- Cardiac function and pericardial effusion
- Inferior vena cava (IVC) volume status
- Abdominal aorta assessment
- RUQ and LUQ free fluid
- Pelvic free fluid
- Thoracic evaluation when indicated

Atkinson et al. (2009) demonstrated that ACES provides diagnostic information not obtainable through physical examination alone, expediting identification of the underlying cause of shock. Additional studies—including case series from Turkish EDs—have supported ACES as a practical and effective method for managing non-traumatic shock (Atkinson et al., 2012).

In triage, ACES assists clinicians in rapidly identifying unstable patients and making informed decisions regarding placement in monitored or resuscitation areas.

3.4. BLUE Protocol (Bedside Lung USG in Emergency)

The BLUE protocol is a highly influential ultrasound algorithm for evaluating acute respiratory failure. Introduced by Lichtenstein & Mezière (2008), it uses lung artifacts (A-profile, B-profile, A'-profile), sliding pleura, consolidations, and effusions to differentiate among:

- Acute heart failure
- Pneumonia
- Pneumothorax
- COPD/asthma exacerbation
- Pulmonary embolism

The original study reported diagnostic accuracy exceeding 90%, and follow-up prospective research confirmed the protocol's robustness across diverse patient populations (Lichtenstein et al., 2009). Studies conducted in Turkish ED

settings have also demonstrated high sensitivity and specificity for identifying causes of acute dyspnea (Gül et al., 2020).

In triage, BLUE assists clinicians in rapidly distinguishing high-risk respiratory conditions and appropriately allocating patients to red, yellow, or green priority zones.

3.5. FALLS Protocol (Fluid Administration Limited by Lung Sonography)

The FALLS protocol provides a physiologic approach to guiding fluid resuscitation in shock. Developed by Lichtenstein (2012), it uses dynamic lung ultrasound to monitor for evolving B-lines and avoid iatrogenic pulmonary edema.

Key steps include:

1. Excluding tamponade and severe cardiogenic shock via cardiac views
2. Performing lung assessment (similar to BLUE)
3. Excluding obstructive shock (massive PE, tension pneumothorax)
4. Initiating fluid therapy with serial monitoring for B-lines

FALLS helps clinicians determine which patients will benefit from aggressive fluid resuscitation versus early vasopressor therapy or advanced hemodynamic monitoring. This has direct triage implications for selecting patients requiring early ICU admission or mechanical ventilation.

4. CLINICAL BENEFITS OF ULTRASOUND-SUPPORTED TRIAGE

Ultrasound-supported triage in the emergency department (ED) extends far beyond its traditional diagnostic role. By integrating point-of-care ultrasound (POCUS) into the earliest phase of ED assessment, clinicians gain rapid access to actionable information that improves patient flow, enhances decision-making, optimizes resource allocation, and increases patient safety. A substantial body of evidence shows that POCUS meaningfully improves triage accuracy and accelerates critical interventions, particularly for patients with trauma, shock, respiratory distress, abdominal pain, or non-specific presentations (Atkinson et al., 2018; Moore & Copel, 2011).

4.1. Improvement in Diagnostic Accuracy

A major clinical advantage of POCUS is its ability to uncover life-threatening pathology even when vital signs and physical examination findings appear unremarkable. Lung ultrasound, for example, demonstrates a diagnostic sensitivity of approximately **90–95% for pneumothorax**, significantly

outperforming auscultation and chest radiography (Alrajhi et al., 2012; Staub et al., 2019).

Focused ultrasound protocols allow rapid identification of conditions such as hemoperitoneum, hemothorax, pericardial tamponade, free intraperitoneal fluid, and acute heart failure—often within minutes (Kirkpatrick et al., 2015; Volpicelli et al., 2012). This capability reduces the likelihood of missing critically ill patients who present with deceptively normal vital signs.

Studies evaluating the Emergency Severity Index (ESI), Canadian Triage and Acuity Scale (CTAS), and Manchester Triage System (MTS) show that **15–30% of patients with serious illness are initially assigned to low-acuity categories** (Platts-Mills et al., 2010; Newgard et al., 2016). By directly visualizing underlying pathophysiology, POCUS enables early reclassification of these patients into higher-acuity categories, contributing to a transition from traditional physiology-based triage toward more pathophysiology-informed decision-making.

4.2. Acceleration of Clinical Decision-Making

Amid increasing ED crowding, the ability to make rapid, accurate triage decisions is crucial. Bedside ultrasonography provides immediate imaging and interpretation performed by the treating clinician, integrating seamlessly into the diagnostic workflow and eliminating delays inherent in traditional imaging pathways (Moore & Copel, 2011).

In high-acuity presentations—trauma, dyspnea, shock, acute abdomen, or cardiac arrest—POCUS reduces diagnostic uncertainty and shortens time to definitive intervention. Studies demonstrate that performing POCUS within the first hour of ED presentation improves diagnostic accuracy and reduces ED length of stay (Moss et al., 2023; Manson et al., 2009).

POCUS frequently prompts changes in clinical management, including:

- initiation or adjustment of fluid resuscitation,
- early vasopressor administration,
- urgent drainage procedures,
- escalation to ICU care,
- expedited transfer to the operating room.

These early decisions accelerate the entire clinical trajectory (Weile et al., 2020; Shokoohi et al., 2015).

4.3. Enhancement of Patient Safety

In conditions such as trauma, shock, dyspnea, and hemorrhage, **every minute counts**. By shortening diagnostic and therapeutic delays, POCUS directly improves safety for critically ill patients.

Structured protocols (FAST/E-FAST, RUSH, BLUE, FALLS) help reduce unnecessary diagnostics and minimize radiation exposure. FAST examinations may eliminate the need for CT scanning in selected trauma patients, while lung ultrasound reliably distinguishes cardiogenic pulmonary edema from pneumonia without immediate CT (Kirkpatrick et al., 2015; Volpicelli et al., 2012).

These benefits are especially relevant for:

- pediatric patients,
- pregnant patients,
- radiation-sensitive or radiation-averse populations.

4.4. Improvement of ED Flow and Resource Optimization

ED overcrowding is a global challenge, and improving throughput is essential. Ultrasound-supported triage enhances ED flow by accelerating early evaluation and enabling rapid prioritization of resource-intensive interventions. Early POCUS has been associated with:

- decreased waiting times,
- fewer unnecessary imaging studies,
- shorter consultation and procedure delays,
- more efficient allocation of monitored beds (Moss et al., 2023; Andersen et al., 2019).

Studies show that early POCUS may reduce chest radiography, CT imaging, and laboratory testing by **15–40%**, depending on population and clinical protocol (Andersen et al., 2019; Shokoohi et al., 2015).

By reducing misprioritization—preventing low-acuity patients from occupying high-acuity areas and ensuring immediate recognition of critically ill patients—POCUS improves both clinical efficiency and resource stewardship.

4.5. Objectification and Consistency of Assessment

Traditional triage is inherently subjective and varies across clinicians. POCUS introduces **image-based, reproducible findings**, reducing variability and establishing a more consistent standard of care. Standardized ultrasound protocols provide a shared diagnostic language that enhances team

communication and reduces human error (Atkinson et al., 2018; Volpicelli et al., 2012).

Studies indicate that even novice clinicians, following brief focused ultrasound training, can make high-accuracy diagnostic and management decisions comparable to more experienced providers (Killu et al., 2014; Shokoohi et al., 2015).

This is particularly beneficial in EDs where experience levels vary across staff and shifts.

4.7. Patient Satisfaction and Clinical Communication

POCUS enhances patient understanding, engagement, and trust. Patients can view real-time images during evaluation and receive immediate explanations, increasing transparency and satisfaction with care.

Shorter waiting times and faster diagnostic pathways further improve patient experience. EDs that use POCUS routinely report higher satisfaction metrics compared with departments relying exclusively on traditional assessment pathways (Kim et al., 2014; Andersen et al., 2019).

5. EDUCATIONAL, STANDARDIZATION, AND COMPETENCY FOUNDATIONS OF ULTRASOUND-SUPPORTED TRIAGE

For ultrasound-supported triage to function safely, efficiently, and consistently, robust educational pathways, standardized protocols, and well-defined competency frameworks are essential. The diagnostic accuracy and clinical impact of point-of-care ultrasound (POCUS) depend heavily on the operator's training, experience, and adherence to established guidelines. Consequently, major international organizations—including the American College of Emergency Physicians (ACEP), the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB), WINFOCUS, and the American Institute of Ultrasound in Medicine (AIUM)—classify emergency ultrasound as a core component of emergency medicine practice (ACEP, 2017; EFSUMB, 2012). Similarly, the Turkish Emergency Medicine Association (TATD) identifies ultrasound as a fundamental element of specialty training (TATD, 2020).

To ensure standardized implementation, structured educational models, competency-based assessment tools, and continuous quality assurance systems must be incorporated into emergency medicine training and clinical operations.

5.1. Fundamental Requirements in Ultrasound Education

The foundation of ultrasound-supported triage training begins with structured theoretical education. Insufficient theoretical knowledge increases the likelihood of misinterpreting artifacts, producing false-positive results, and overlooking life-threatening conditions (Shokoohi et al., 2015; Killu et al., 2014). ACEP and WINFOCUS recommend at least *4–6 hours of formal didactics* covering ultrasound physics, instrumentation (knobology), common artifacts, and emergency-specific applications (ACEP, 2017; Via et al., 2014).

Hands-on training is indispensable for developing practical competency. Simulation-based education using ultrasound phantoms, high-fidelity manikins, and curated video case libraries enhances skill acquisition in cardiac, trauma, and lung ultrasound (Blehar et al., 2014). Simulation accelerates progression along the learning curve, reduces error rates, and prepares learners for supervised bedside practice. In emergency medicine education, direct bedside instruction from expert faculty remains the most powerful means of skill development, particularly during the early stages of training.

Following the COVID-19 pandemic, blended and remote educational formats have expanded rapidly. Video-based modules, interactive online platforms, and virtual ultrasound simulators hosted by ACEP, WINFOCUS, and other professional societies now serve as widely used adjuncts for reinforcing foundational skills and supporting lifelong learning (Via et al., 2014).

5.2. Competency Criteria and Assessment

Competency evaluation in ultrasound-supported triage ensures that clinicians can perform, interpret, and apply ultrasound findings accurately and safely within a time-critical environment. Competency frameworks emphasize repeated assessments of both theoretical knowledge and practical skills, ideally using standardized evaluation tools.

A) Core Technical Competencies

Operators must demonstrate the ability to:

- Correctly grip and manipulate the transducer
- Optimize image parameters (depth, gain, focal zones)
- Acquire diagnostic views relevant to the clinical scenario

International standards typically recommend **25–50 supervised scans per ultrasound application** (e.g., lung, cardiac, abdominal), representing the minimum exposure required to achieve baseline proficiency (AIUM, 2014; ACEP, 2017).

B) Clinical, Context-Specific Competencies

Given the rapid decision-making required in triage, clinicians must correctly interpret ultrasound findings and integrate them into appropriate triage categories. This includes the ability to recognize:

- Free fluid consistent with hemoperitoneum
- Presence/absence of cardiac activity and global ventricular function
- Pneumothorax indicators (lung sliding, lung point, M-mode “barcode/sign”)
- Shock markers such as IVC collapsibility or severely impaired contractility

These competencies are essential to protocols such as FAST/E-FAST, RUSH, BLUE, and FALLS (Perera et al., 2010; Volpicelli et al., 2012).

C) Speed and Safety of Application

Triage environments demand rapid acquisition of essential views. Guidelines recommend obtaining key cardiac and lung windows in **60–90 seconds** for unstable patients while maintaining patient comfort and adhering to infection control standards (EFSUMB, 2012; WINFOCUS, 2010).

D) Educational and Assessment Standards

Most international recommendations assert that clinicians must complete:

- **25–50 supervised POCUS examinations** per core application
- **10–20 triage-focused clinical scenarios**
- Successful performance in an **OSCE-style structured skills assessment**
- A theoretical examination with **≥70–80% passing threshold**
- Ongoing documentation in a **POCUS logbook**, reviewed periodically by faculty

EFSUMB and WINFOCUS emphasize logbook documentation and continuous quality improvement as mandatory components of POCUS credentialing (EFSUMB, 2012; WINFOCUS, 2010).

6. AI-ASSISTED ULTRASOUND AND FUTURE PERSPECTIVES

The rapid advancement of artificial intelligence (AI) and deep learning is reshaping the use of point-of-care ultrasound (POCUS) in emergency medicine and is poised to significantly influence triage systems. AI-driven image interpretation tools can reduce longstanding limitations of ultrasound—such as

operator dependence, interobserver variability, and lack of standardization—by providing automated, reproducible analyses of key sonographic findings (Evans et al., 2021; Roy et al., 2020). Integrating AI with POCUS (“AI-POCUS”) has the potential to enhance diagnostic accuracy and accelerate clinical decision-making during emergency department (ED) triage, especially for less-experienced users.

Emerging AI systems can synthesize ultrasound images with vital signs, electrocardiography, and laboratory parameters to generate early risk stratification scores or automated alerts for high-acuity patients. Several studies have demonstrated the feasibility of AI-enhanced models to identify conditions such as pneumothorax, pulmonary edema, cardiac dysfunction, and deep venous thrombosis with accuracy comparable to expert interpreters (Jang et al., 2020; Annarumma et al., 2019). Future integration of these systems into ED information platforms may enable automated flagging of critically ill patients, prediction of resuscitation needs, and real-time clinical decision support—features especially valuable in overcrowded EDs.

Next-generation portable ultrasound devices increasingly incorporate real-time guidance features that use embedded sensors, augmented reality overlays, and AI algorithms to instruct users on optimal probe positioning and acquisition of diagnostic views (Kim et al., 2020; Cao et al., 2022). These systems are particularly beneficial for trainees, paramedics, and clinicians working in high-pressure triage settings, where missed views or protocol deviations can compromise diagnostic accuracy. By standardizing image acquisition for protocols such as FAST, RUSH, or BLUE, AI-guided scanning reduces user variability and enhances reproducibility.

AI-enabled tele-ultrasound is another transformative application. In rural, resource-limited, or disaster environments, remote experts can review ultrasound images in real time while AI algorithms support view recognition, quality assessment, and preliminary triage classification (Uschnig et al., 2022; Smith et al., 2021). This hybrid human-AI model holds particular promise for mass-casualty incidents and low-resource settings where trained ultrasound operators may be scarce.

However, several challenges accompany these innovations. Current AI models often show limited generalizability across diverse patient populations, and algorithmic bias remains a major concern (Gichoya et al., 2022). Additional risks include data privacy vulnerabilities, overreliance on automated outputs, and lack of transparency in deep learning decision pathways (“black-box” problem) (Topol, 2019). Importantly, most AI applications in ultrasound remain in proof-of-concept or retrospective phases, with limited validation in real-world clinical environments (Mawri et al., 2022).

Accordingly, consensus statements emphasize that AI should serve as a decision-support adjunct, not a replacement for clinician judgment. Recent ACEP guidance reiterates that clinicians retain ultimate responsibility for image acquisition, interpretation, and management decisions, even when AI tools are used (ACEP, 2023; Blaivas, 2024).

As POCUS becomes increasingly integrated into triage workflows, AI and deep learning are expected to play an expanding role in enhancing speed, accuracy, and standardization. In the near future, many triage applications are likely to rely on handheld ultrasound devices equipped with embedded AI algorithms, improving access and reliability across diverse clinical settings.

7. CHALLENGES, LIMITATIONS, AND DEBATES IN ULTRASOUND-SUPPORTED TRIAGE

Although ultrasound-supported triage has become a powerful tool in emergency medicine, its implementation faces several logistical, educational, methodological, and system-level challenges. While numerous studies support the clinical effectiveness of point-of-care ultrasound (POCUS), real-world outcomes vary widely depending on institutional resources, operator experience, and workflow constraints (Maw, 2020; Shokoohi et al., 2019). Consequently, understanding these limitations is essential to contextualize the benefits of ultrasound in ED triage settings.

7.1. User Dependence and Operator Variability

Ultrasound is fundamentally operator-dependent, and the accuracy of any examination relies on sequential steps of image acquisition, optimization, interpretation, and synthesis with clinical data. Errors in probe handling, choice of acoustic windows, or misinterpretation of artifacts may produce false-positive or false-negative findings (Gillman et al., 2017; Moore & Copel, 2011).

While standardized protocols such as FAST/E-FAST, BLUE, RUSH, and FALLS reduce cognitive variability, they still require different levels of training and practice. Short, focused training may be sufficient to master protocols such as FAST or lung ultrasound, whereas advanced cardiac assessment and hemodynamic ultrasound require prolonged training, mentored practice, and competency validation (Atkinson et al., 2018; Volpicelli et al., 2012).

Operator variability remains one of the most frequently cited limitations in POCUS literature and can significantly influence triage accuracy, especially in high-pressure environments where rapid decisions must be made (Moore & Copel, 2011; Killu et al., 2014).

7.2. Device Availability and Technical Limitations

Limited device availability in busy emergency departments is another barrier. Some triage areas may lack dedicated ultrasound machines, and workflow interruptions can occur due to probe malfunction, battery failure, or competition for shared devices (Shokoohi et al., 2019).

Patient factors such as morbid obesity, subcutaneous emphysema, surgical dressings, or mechanical ventilation can degrade image quality and make acquisition more challenging (Brown et al., 2012). Restricted patient positioning—common among unstable or agitated patients—can also result in incomplete views and missed diagnoses.

7.3. Methodological Concerns and Quality of Evidence

Research evaluating ultrasound-supported triage demonstrates substantial heterogeneity regarding operator training, protocol adherence, patient populations, ED crowding, and device type. Because of this variability, many systematic reviews categorize the quality of evidence for POCUS-based triage as moderate rather than high (Maw, 2020; Alrajhi et al., 2012).

System-level constraints may confound study outcomes. For instance:

- delayed resuscitation,
- insufficient ICU bed availability,
- time lost in accessing or sharing ultrasound devices, and
- inaccurate interpretation of findings

can all influence results attributed to POCUS (Shokoohi et al., 2019).

Concerns have also been raised that indiscriminate ultrasound use may slow patient flow or increase workload, especially in low-acuity presentations (Moore & Copel, 2011). Conversely, when applied selectively to high-risk patients, ultrasound has been shown to expedite triage and reduce diagnostic uncertainty (Atkinson et al., 2018; Moss et al., 2023).

This ongoing debate highlights the need for well-defined indications and triage-specific ultrasound criteria.

7.4. Lack of Standardization

A major barrier to widespread adoption is the lack of universal standards defining:

- in which triage categories ultrasound should be mandatory or recommended,

- at what stage of triage ultrasound should be incorporated, and
- which clinical questions each protocol should address.

This variability leads to inconsistent integration of ultrasound into ED workflows. Some centers use POCUS as a standardized component of triage, while others apply it only at clinician discretion (Volpicelli et al., 2012; Maw, 2020).

Standardized frameworks for interpreting ultrasound findings within triage categories are also lacking. Without clearly defined decision thresholds, the reproducibility of ultrasound-supported triage diminishes.

During periods of ED overcrowding, clinicians may deviate from protocols, perform only abbreviated examinations, or fail to document images. Absence of systematic image archiving, quality assurance review, and competency monitoring weakens the reliability of ultrasound-supported triage (ACEP, 2023; Moore & Copel, 2011).

7.5. Legal and Ethical Considerations

In many healthcare systems, POCUS interpretation is considered the responsibility of the treating clinician, and radiology over-read is not required. While this expedites care, it also increases medicolegal exposure in cases of misdiagnosis (Blaivas, 2024).

Some institutions mandate image archiving for quality assurance and educational review. Failure to store images may impede medicolegal defense, limit audit processes, and hinder continued professional development.

AI integration adds further ethical considerations, including:

- accountability for AI-assisted decisions,
- transparency and explainability of algorithms,
- patient consent for data use, and
- protection of sensitive health information (Topol, 2019; Gichoya et al., 2022).

Current expert consensus emphasizes that AI should augment—not replace—clinician judgment. The clinician must remain the final decision-maker for image acquisition, interpretation, and patient management (ACEP, 2023; Blaivas, 2024).

8. CONCLUSION

Ultrasound-supported triage has become increasingly important in modern emergency departments, both clinically and operationally. Integrating ultrasound into the triage process significantly enhances early decision-making, particularly in time-sensitive conditions such as trauma, dyspnea, shock, and pediatric emergencies. Its rapid applicability, radiation-free nature, real-time bedside information, and high diagnostic value make it a powerful decision-support tool that complements the limitations of traditional triage systems.

Education and standardization remain the most essential components of this process. Competent practitioners—equipped with structured training and protocol adherence—can utilize the full potential of ultrasound to provide objective and accurate assessments. Nonetheless, it is crucial to recognize existing limitations, including operator dependence, equipment availability, and broader system-level barriers.

Looking ahead, artificial intelligence and automated image-recognition algorithms are expected to make ultrasound-supported triage faster, safer, more standardized, and more accessible. Innovations such as tele-ultrasound, automated protocol execution, and AI-driven risk scoring have the potential to transform the very nature of emergency triage. However, the literature still lacks large, multicenter, high-quality datasets and randomized controlled trials. Robust evidence on the impact of ultrasound-supported triage on mortality, morbidity, healthcare costs, and ED overcrowding will be essential to accelerate its integration into standard care pathways.

Despite its advantages, the method's inherent operator dependency underscores the need for ongoing education, standardization, quality assurance, and continuity. Variability in training levels can influence not only image quality but also the accuracy of triage decisions. Therefore, the reliable use of ultrasound in triage will only be possible when supported by structured training programs, regular competency assessments, and standardized protocols.

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CHAPTER 15

Diagnosis and Treatment of Hydatid Cyst

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INTRODUCTION

Hydatid cyst disease is a zoonotic parasitic infection caused by the larval stage of *Echinococcus granulosus sensu lato*, characterized by a slow clinical course and the potential to cause serious complications. Although the disease most commonly involves the liver and lungs, it has the capacity to affect almost any organ or tissue in the human body. Its prolonged asymptomatic course often leads to delayed diagnosis, with many cases being detected at advanced stages of the disease (1).

Cystic echinococcosis remains a significant public health problem worldwide. The World Health Organization (WHO) classifies hydatid disease among the “neglected tropical diseases” (NTDs), emphasizing not only its clinical consequences but also its substantial socioeconomic burden. The disease is endemic particularly in regions where animal husbandry is widespread and the dog–sheep transmission cycle is actively maintained. High-risk areas include the Mediterranean basin, the Middle East, Central Asia, South America, and certain regions of Africa (1,2).

Humans are accidental intermediate hosts for *Echinococcus granulosus*, and infection typically occurs through the oral ingestion of parasite eggs via food, water, or hands contaminated with infected dog feces. Although humans do not play a role in the natural biological cycle of the parasite, rendering the disease theoretically preventable, insufficient control programs have allowed the global burden of cystic echinococcosis to persist. The clinical significance of hydatid disease arises not only from the infection itself but also from the morbidity associated with cyst size, anatomical location, and the development of complications. Life-threatening conditions such as cyst rupture, secondary infection, communication with the biliary tract, and anaphylactic reactions may occur. Therefore, hydatid disease should be regarded as a complex clinical entity requiring a multidisciplinary approach rather than a simple parasitic infection.

In recent years, advances in imaging modalities—particularly the widespread adoption of ultrasonography-based classification systems such as those proposed by the WHO Informal Working Group on Echinococcosis (WHO-IWGE)—have led to a significant paradigm shift in the diagnosis and management of hydatid disease (1,3). Contemporary management strategies have moved away from a “one-size-fits-all” approach toward individualized treatment plans based on cyst stage, biological activity, and patient-specific characteristics.

This book chapter aims to comprehensively address hydatid disease in the context of epidemiological characteristics, parasite biology, pathogenesis, clinical manifestations, imaging-based classification systems, diagnostic

approaches, current treatment options, and preventive strategies, in light of contemporary literature and international guidelines.

1. EPIDEMIOLOGICAL CHARACTERISTICS

Cystic echinococcosis is a zoonotic disease with marked regional variation in prevalence worldwide and is closely associated with environmental, socioeconomic, and cultural factors. Its geographic distribution is primarily confined to areas where the life cycle of *Echinococcus granulosus* can be sustained, with animal husbandry practices, dog population control, and hygiene conditions serving as key determinants of transmission (4).

The disease is endemic in several regions, including the Mediterranean basin, the Middle East, the Balkans, Central Asia, western China, southern parts of South America, and selected areas of Africa. In these regions, annual incidence rates vary considerably, and prevalence may exceed 5% in certain rural communities. According to World Health Organization data, tens of thousands of new cases of hydatid disease are diagnosed globally each year, resulting in substantial economic losses related to healthcare costs and reduced productivity (3,5).

Turkey is among the countries where cystic echinococcosis is endemic, with a particularly high prevalence in rural areas and regions where livestock farming is common. Epidemiological studies conducted in Turkey have reported that the majority of surgically treated cases involve hepatic (60–70%) and pulmonary (20–30%) localization, while involvement of the spleen, kidneys, bones, and central nervous system is observed less frequently. Hydatid disease may occur in all age groups; however, clinical presentation varies according to age. In children, pulmonary involvement is relatively more common, which has been attributed to the elastic nature of lung tissue and the more rapid growth of cysts. In contrast, hepatic involvement is markedly more prevalent in adults. Although no consistent sex predominance has been established, some studies have reported a higher prevalence among women, possibly related to domestic animal husbandry, food preparation practices, and hygiene-related behaviors (7).

Socioeconomic factors play a decisive role in the epidemiology of cystic echinococcosis. Low income levels, limited education, uncontrolled home slaughtering practices, feeding dogs with raw offal, and restricted access to veterinary services are among the principal factors contributing to the persistence of endemicity. In this regard, hydatid disease should be considered not merely an individual infectious condition but also a major socioeconomic problem affecting public health and rural development. The global burden of cystic echinococcosis is not limited to morbidity and mortality alone. Surgical interventions, prolonged

medical treatment, loss of workforce productivity, and reductions in livestock production collectively impose a substantial economic burden. Budke et al. reported that the annual global economic cost of cystic echinococcosis reaches several billion US dollars. These data clearly underscore the necessity for integrated, sustainable, and multidisciplinary public health control programs aimed at disease prevention and eradication (6,8).

2. ETIOLOGY AND LIFE CYCLE

Hydatid disease is a parasitic infection primarily caused by *Echinococcus granulosus sensu lato*. The genus *Echinococcus* belongs to the family Taeniidae, and among the species infecting humans, *E. granulosus* and *E. multilocularis* are the most clinically relevant. *E. granulosus* is the etiological agent of cystic echinococcosis and is responsible for the vast majority of human cases worldwide. Although the adult form of *E. granulosus* measures only a few millimeters in length, its biological impact can lead to severe clinical consequences. The adult parasite resides in the small intestine of dogs and other canids, whereas the larval form responsible for human disease develops as hydatid cysts within tissues (4).

Molecular studies have demonstrated that the *E. granulosus sensu lato* complex comprises multiple genotypes (G1–G10). Among these, the G1 genotype (sheep strain) is the most common cause of human infection. Genotypic variation has been suggested to influence clinical manifestations, organ localization, and response to treatment (9).

The life cycle of *Echinococcus granulosus* involves definitive hosts, intermediate hosts, and accidental intermediate hosts and is fundamentally based on the dog–sheep transmission cycle. Dogs and other canids serve as definitive hosts, harboring the adult parasite in their small intestines. After maturation, parasite eggs are excreted into the environment via feces. These eggs are highly resistant to environmental conditions and can remain infective for weeks to months. Natural intermediate hosts typically include sheep, goats, cattle, and other herbivorous animals, which ingest parasite eggs while grazing on contaminated pastures. Once ingested, the eggs release oncospheres in the small intestine; these penetrate the intestinal wall, enter the circulation, and lodge primarily in the liver and other organs, where they develop into hydatid cysts.

Humans represent accidental intermediate hosts who are not biologically essential to the parasite's life cycle but may become infected through oral ingestion of eggs. Human infection most commonly occurs through direct contact with infected dogs, consumption of contaminated food or water, or inadequate hand hygiene (10).

2.1. Organ Localization and Filtration Mechanism

In humans, the liver is the first filter organ encountered by circulating oncospheres; consequently, approximately 60–70% of hydatid cysts are localized in the liver. The lungs act as the second filter, accounting for involvement in 20–30% of cases. Less frequently, the spleen, kidneys, bones, brain, and other organs may be affected. This distribution pattern is largely explained by portal circulation and capillary filtration mechanisms. However, alternative pathways, including lymphatic dissemination and right-to-left shunts, have been proposed to account for atypical organ localizations (1,7).

2.2. Structure and Biological Activity of the Hydatid Cyst

A hydatid cyst consists of three principal layers:

Pericyst: The outer fibrous layer formed by host tissue

Laminated membrane: A thick, acellular protective layer of parasitic origin

Germinal layer: The viable inner layer responsible for the formation of protoscoleces and daughter cysts

Cysts in which the germinal layer remains viable and metabolically active are referred to as fertile cysts and are primarily responsible for disease propagation and recurrence. Over time, the biological activity of the cyst may decline, leading to degeneration of cyst contents and transition to an inactive state. This biological evolution constitutes the foundation of current imaging-based classification systems and stage-specific treatment strategies for hydatid disease (1).

3. PATHOGENESIS AND HISTOPATHOLOGY

3.1. Pathogenesis

The pathogenesis of hydatid disease results from a complex host–parasite interaction that begins after the oral ingestion of *Echinococcus granulosus* eggs. Once the eggs hatch in the small intestine, the released oncospheres penetrate the intestinal mucosa and enter the portal circulation, subsequently reaching various organs, most notably the liver. At this stage, parasite localization is largely determined by the filtration mechanism of the portal venous system. Because the liver represents the first capillary filter, it is the most frequently involved organ. While a substantial proportion of oncospheres are trapped within the hepatic sinusoids, a smaller fraction passes into the pulmonary circulation and becomes lodged in the lungs. More rarely, oncospheres may enter the systemic circulation and give rise to hydatid cysts in organs such as the spleen, kidneys, bones, brain, and other tissues (11).

This distribution pattern accounts for the wide spectrum of clinical presentations observed in hydatid disease. Once established within tissues, oncospheres gradually develop into hydatid cysts. Cyst growth is typically slow and insidious, often remaining clinically silent for many years. However, as cyst volume increases, compression of adjacent structures, functional impairment, and the risk of complications become more pronounced. Due to the highly antigenic nature of cyst contents, rupture may precipitate severe allergic reactions, including anaphylaxis (1).

The ability of the parasite to evade the host immune system plays a pivotal role in the chronicity of the disease. The laminated membrane of the hydatid cyst largely prevents immune cell penetration, while certain parasite-derived molecules exert immunomodulatory and immunosuppressive effects on the host response. These mechanisms allow the cyst to remain viable for prolonged periods (11).

3.2. Histopathology

The histopathological architecture of the hydatid cyst is highly characteristic and provides important diagnostic clues. The cyst consists of two major components: host-derived tissue reactions and parasite-derived structures.

The pericyst is a fibrous layer formed as a result of the host's chronic inflammatory response to the parasite. Histologically, it contains collagen fibers, fibroblasts, lymphocytes, plasma cells, and occasionally eosinophils. The thickness of the pericyst and the intensity of the inflammatory infiltrate may vary depending on cyst localization and biological activity.

Lining the inner surface of the pericyst is the laminated membrane, a parasite-derived, thick, acellular, eosinophilic layer. This membrane exhibits a distinctive lamellar appearance on histological examination and is considered pathognomonic for hydatid disease. It serves as the primary mechanical and immunological barrier protecting the parasite from host defenses.

The innermost germinal layer represents the biologically active component of the cyst. Protoscoleces and daughter cysts develop from this layer and determine cyst fertility. Histological sections may reveal free protoscoleces and hooklets, commonly referred to as "hydatid sand." Loss of viability of the germinal layer leads to cyst degeneration and transition to an inactive state. Over time, degenerative changes such as cyst content breakdown, calcification, and fibrosis may occur. In inactive cysts, the laminated membrane becomes thickened, the germinal layer disappears, and the cyst contents assume an amorphous, necrotic appearance. These histopathological changes closely correlate with imaging

findings and form the basis of contemporary classification systems and stage-adapted treatment strategies (4).

4. CLINICAL MANIFESTATIONS

Hydatid disease exhibits a highly variable clinical course, with symptoms largely determined by cyst location, size, growth rate, biological activity, and the presence of complications. Given its typically slow progression, a substantial proportion of cases remain asymptomatic for prolonged periods, and cysts are frequently detected incidentally during imaging studies performed for unrelated reasons (11).

4.1. Hepatic Involvement

The liver is the most commonly affected organ in hydatid disease. Clinical manifestations are generally nonspecific and include right upper quadrant pain, a sensation of fullness, hepatomegaly, and dyspeptic symptoms. As cyst size increases, compression of adjacent structures may occur, particularly involving the biliary tree. This may result in serious complications such as obstructive jaundice, cholangitis, and pancreatitis. Intrabiliary rupture is of particular clinical importance, especially in large and centrally located hepatic cysts (4).

4.2. Pulmonary Involvement

The lungs represent the second most frequently involved organ and are particularly commonly affected in childhood. Typical symptoms include cough, chest pain, dyspnea, and hemoptysis. Due to the elastic nature of pulmonary tissue, lung cysts may reach large dimensions. Sudden rupture may lead to expectoration of cystic contents, acute respiratory distress, and allergic reactions (11).

4.3. Involvement of Other Organs

Although less common, hydatid disease may involve almost any organ or tissue. The spleen, kidneys, peritoneum, bones, and central nervous system constitute the most frequently reported atypical localizations. Cerebral involvement may present with headache, nausea, vomiting, epileptic seizures, and focal neurological deficits, whereas bone involvement is often associated with pathological fractures, chronic pain, and osteomyelitis-like presentations. In such locations, diagnosis is frequently delayed, and the disease may follow a more aggressive course (1).

4.4. Clinical Presentations Related to Complications

Cyst rupture is one of the most serious complications of hydatid disease and may occur spontaneously or secondary to trauma. Dissemination of cyst contents

into the circulation or serous cavities following rupture can provoke a broad spectrum of allergic reactions, ranging from urticaria to life-threatening anaphylactic shock. Secondary bacterial infection of the cyst may also occur, manifesting with fever, leukocytosis, and sepsis (4).

The heterogeneity of clinical manifestations in hydatid disease underscores the necessity for a multidisciplinary approach to diagnosis and management. Early diagnosis is critical for preventing complications and for selecting the most appropriate therapeutic strategy (11).

5. IMAGING AND WHO-IWGE CLASSIFICATION

Imaging modalities play a central role in the management of hydatid disease, not only for confirming the diagnosis but also for assessing biological activity, guiding therapeutic strategies, and monitoring disease progression. Currently, an imaging-based approach constitutes the cornerstone of clinical decision-making in the management of cystic echinococcosis (1).

5.1. Ultrasonography

Ultrasonography (US) is considered the first-line and most valuable diagnostic modality, particularly for hepatic hydatid disease. Owing to its noninvasive nature, ease of application, and repeatability, US is widely used for both diagnosis and follow-up. It allows detailed visualization of the internal architecture of the cyst, including septations, daughter cysts, membrane detachment, and calcifications, thereby enabling assessment of the biological stage of the cyst. One of the most important contributions of ultrasonography is its role as the foundation of the standardized classification system developed by the World Health Organization Informal Working Group on Echinococcosis (WHO-IWGE). This classification enables staging of cysts according to their morphological and biological characteristics and has established a “cyst stage-specific treatment” approach (1,12).

5.2. Computed Tomography

Computed tomography (CT) serves as an important complementary imaging modality, particularly in the evaluation of complicated cysts, detection of calcifications, and identification of pulmonary involvement. CT provides detailed information regarding anatomical relationships, mass effect on adjacent structures, and signs of rupture or secondary infection. However, CT is less discriminative than ultrasonography in assessing the biological activity of hydatid cysts (1,12).

5.3. Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is particularly advantageous in the evaluation of hepatic cysts with biliary involvement, central nervous system localizations, and soft tissue involvement. MRI is useful for characterizing cyst contents and assessing complications; however, it does not replace ultrasonography in routine clinical practice (1).

5.4. WHO-IWGE Ultrasonographic Classification

The WHO-IWGE classification is currently the most widely accepted system for hydatid disease and directly guides clinical management. This system categorizes cysts into three main groups based on biological activity: active, transitional, and inactive.

- **CE1 (Active cyst):** Appears as a unilocular, anechoic cyst. Hydatid sand may be present. The germinal layer is viable, and the cyst is considered fertile.
- **CE2 (Active cyst):** Characterized by a multivesicular, multiseptated structure with a typical “honeycomb” or “rosette” appearance. These cysts exhibit high biological activity.
- **CE3a (Transitional cyst):** Defined by detachment of the internal membrane, producing the characteristic “water lily sign.” Biological activity begins to decline.
- **CE3b (Transitional cyst):** Contains dense internal contents with solid components and daughter cysts and may continue to exhibit clinically active behavior.
- **CE4(Inactive cyst):** Displays heterogeneous, hyperechoic internal contents. The germinal layer has undergone degeneration, and the cyst is considered biologically inactive.
- **CE5(Inactive cyst):** Features a thick wall with calcifications, indicating loss of parasite viability.

This classification directly influences treatment strategies. Active cysts generally require medical, percutaneous, or surgical intervention, whereas inactive cysts may often be managed conservatively with follow-up alone, thereby preventing unnecessary invasive procedures (1,12).

6.DIFFERENTIAL DIAGNOSIS

Hydatid disease can mimic a wide range of pathologies from both clinical and radiological perspectives. Therefore, differential diagnosis should incorporate lesion localization, imaging characteristics, epidemiological background, and

serological findings. In endemic regions, every detected cystic lesion should be carefully evaluated for possible hydatid disease (1).

6.1. Cystic Lesions of the Liver

In the differential diagnosis of hepatic cystic lesions, simple liver cysts should be considered first. These lesions are typically thin-walled, anechoic, and lack internal septations. Unlike hydatid cysts, they do not demonstrate daughter cysts, membrane detachment, or hydatid sand. Pyogenic liver abscesses should also be considered, particularly in patients presenting with fever, leukocytosis, and prominent inflammatory findings. Abscess contents are usually heterogeneous, and the clinical course is more acute compared with hydatid disease.

Amebic liver abscesses are typically solitary and may be distinguished using serological testing. Biliary cystic lesions (e.g., choledochal cysts, biliary hamartomas) and cystic neoplasms (such as cystic hepatocellular carcinoma, biliary cystadenoma, and cystadenocarcinoma) are also important considerations. These lesions often demonstrate solid components, contrast enhancement, and atypical wall thickening (1,4,13).

6.2. Cystic Lesions of the Lung

In the differential diagnosis of pulmonary hydatid cysts, bronchogenic cysts, lung abscesses, congenital pulmonary airway malformations, and necrotizing pneumonias should be considered. Hydatid cysts typically appear as well-defined, round lesions; however, the development of air–fluid levels following rupture may complicate the diagnostic process (4).

6.3. Involvement of Other Organs

For central nervous system cysts, differential diagnoses include arachnoid cysts, cystic tumors, neurocysticercosis, and abscesses. In bone involvement, destructive lesions may resemble tuberculosis, metastatic disease, or primary bone tumors. In these atypical localizations, hydatid disease frequently leads to diagnostic delays (1,4).

6.4. Serological and Epidemiological Assessment

Although serological tests are useful adjuncts in the differential diagnosis, negative results do not exclude hydatid disease. Epidemiological factors such as residence in endemic regions, involvement in animal husbandry, and contact with dogs should always be incorporated into the diagnostic evaluation. Imaging findings consistent with the WHO-IWGE classification represent one of the most powerful determinants in differential diagnosis (1).

In conclusion, no single clinical or radiological criterion is sufficient for the differential diagnosis of hydatid disease. Accurate diagnosis requires a multidisciplinary and integrative approach.

7. TREATMENT AND PROGNOSIS

The therapeutic approach to hydatid disease should be individualized due to the heterogeneous nature of the condition. The currently accepted fundamental principle is that treatment should be planned by taking into account the biological activity and stage of the cyst (according to the WHO-IWGE classification), as well as its localization, size, presence of complications, and patient-specific clinical characteristics. This stage-adapted strategy has replaced the former “one-size-fits-all” surgical approach and has contributed to a significant reduction in unnecessary invasive interventions (1).

7.1. Observation (Watch and Wait)

Biologically inactive cysts (CE4 and CE5) may not require active treatment provided that they are asymptomatic and show no signs of complications. In such cases, a conservative “watch and wait” strategy with regular imaging follow-up is recommended. Several studies have demonstrated that the majority of inactive cysts remain stable over time and that the rate of reactivation is low (1,4).

7.2. Medical Therapy

Albendazole is the cornerstone of medical therapy and is used either as primary treatment in selected cases or as an adjunct to interventional procedures. Albendazole is particularly effective in small active cysts (CE1) and in the peri-procedural period before and after surgical or percutaneous interventions. Close monitoring of liver function tests and hematological parameters is mandatory during treatment. The therapeutic response to medical therapy varies depending on the cyst stage and biological activity (1).

7.3. Percutaneous Treatment Methods

The PAIR technique (Puncture–Aspiration–Injection–Reaspiration), performed under ultrasonographic or CT guidance, represents an effective and minimally invasive treatment option in appropriately selected cyst types. High success rates have been reported particularly in CE1 and selected CE3a cysts. Percutaneous treatment should always be combined with albendazole therapy. However, its use is limited in multiseptated cysts, cysts containing numerous daughter vesicles, or cysts with biliary communication (1,2).

7.4. Surgical Treatment

Surgical intervention is indicated in complicated, large, ruptured, secondarily infected cysts, or in cases that are unsuitable for medical or percutaneous approaches. The primary goals of surgery are complete eradication of the parasite, safe removal of cyst contents, and prevention of complications. Although conservative surgical techniques and minimally invasive approaches are increasingly favored, surgical treatment is still associated with a certain degree of morbidity and risk of recurrence (4).

8. PROGNOSIS

The prognosis of hydatid disease largely depends on early diagnosis, appropriate selection of treatment modality, and prevention of complications. Long-term outcomes are generally favorable in appropriately treated patients. While the life expectancy of patients with inactive cysts under surveillance is typically unaffected, the risk of morbidity increases in patients with active or complicated cysts (1).

Recurrence may occur particularly in fertile cysts, in cases of inadequate treatment, or following spillage of cyst contents. Therefore, long-term post-treatment follow-up is of paramount importance. A multidisciplinary approach and treatment strategies based on the WHO-IWGE classification have been shown to reduce recurrence rates and improve overall patient quality of life (12).

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CHAPTER 16

Royal Jelly as a Modulator of Gut Microbiota in Healthy Aging and Longevity

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1. Introduction

Aging is a biological process that is characterized by the accumulation of damage to molecules and cells, which leads to a decline in functionality and susceptibility to disease (Mathur & et al., 2024). Although there are many factors that affect aging, even with the elimination of certain factors such as somatic mutations, the human body still has the potential to last for a long time, that is, 430 years, which shows that other factors also affect this process (Efimov & et al., 2025).

According to the World Health Organization (WHO), the world population is ageing rapidly: by the year 2050, the number of people aged 60 years or above will increase to approximately 2.1 billion, nearly double the recorded 1 billion in 2020 (World Health Organization, 2015). This, therefore, calls for immediate understanding of the biological processes that drive aging. The prevalence of age-related diseases is closely connected with the gradual loss of tissue and organ function; therefore, physiological competence is a public health priority (Ling & et al., 2022).

The phenomenon of aging is thus described as a chronic, low-grade inflammatory response, enhancing a host susceptibility to infections and developing chronic diseases, although the exact biological pathway(s) accountable for this remains poorly documented (Caetano-Silva & et al., 2024; Chung & et al., 2019). Chronic low-grade inflammation influences the development and progression of several age-related disorders, including metabolic syndrome, type 2 diabetes and obesity, cardiovascular diseases (such as atherosclerosis), sarcopenia and osteoporosis, neurodegeneration and major depression, and altered mental health (Calder & et al., 2017; Castelo-Branco & et al., 2014).

Recent literature would suggest that inflammatory mechanisms impact on host physiology through the gut microbiome, one of the most active and influential human ecosystems, and as such, has been the focus of increasing scientific interest in recent years (Fava & et al. 2019; Franceschi & et al. 2018; Ragonnaud & Biragyn 2021). Reduced diversity within the gut microbiome has been associated with the development of chronic illness and accelerated physiological aging (Chatterjee & et al. 2025). The gut microbiota has the potential to have a significant impact on human health in general but in particular in older adults (O'Toole & Jeffery 2015).

The word 'microbiota' refers to the total community of organisms constituting a defined biological niche (Gyriki & et al., 2025). There exist over 100 trillion microorganisms in the human gut, which are crucial for sustaining general health

(Xu & et al., 2019). However, determining the exact impact of the microbiota is difficult to achieve because of marked interindividual differences (Clemente & et al., 2012).

In view of the fact that the intestinal microbiome seems to play an imperative role in the pathogenesis and progression of most disorders that are caused by the inflammation phenomenon, much interest has been directed to the study of this specific ecosystem (Biagi & et al., 2011). Indeed, recent studies suggest that the gut microbiome may play a key role in driving age-related inflammation, further justifying its importance in maintaining homeostasis across multiple physiological systems (Buford, 2017; Maffei & et al., 2017). Hence, these findings have provided evidence for compositional and functional changes of the gut microbiota throughout their lifetime. There is an increase in evidence suggesting that changes in gut microbiota composition with aging contribute to age-associated inflammatory conditions, which they do by participating in immune responses and systemic effects appearing later in life (Caetano-Silva et al., 2024). Environmental factors such as gut physiology (especially pertaining to transit time and luminal pH) largely affect microbial community composition. These combined effects of gut physiology and environmental factors create variation in microbial community composition between individuals (Procházková & et al., 2024).

Given that it is central to host physiology, understanding the gut microbiota is important when exploring possible interventions that could affect aging (Nagpal & et al., 2018). In this view, one of the most potent modulators of gut microbiota composition and function is diet (Rinninella & et al., 2023). Since bacterial communities are involved in most of the aspects of the aging process, dietary options can be considered one of the most useful and pragmatic ways to counteract or delay the aging phenotype. In addition, individuals who consume a healthy diet typically experience fewer incidences of age-related illnesses (e.g., cancers, neurodegenerative disorders, cardiovascular disease, and metabolic dysfunction). Furthermore, a healthy diet appears to slow the aging process, as well as enhance the resilience or strength of an individual's gut microbiome (Beaver & et al., 2025; Hu, 2024).

Due to this strong connection between diet, microbiota, and aging, there has been an increase in the focus on natural products as potential modulators for gut microbiota function (Beaver & et al., 2025; Demain & Vaishnav, 2011; Newman & Cragg, 2020). Since Serthürner, in 1805, became the first person to extract and isolate a naturally occurring chemical from opium (Morphine), the role of natural products in developing new pharmaceutical agents has been highly significant. This is followed, through continued technological advancement and scientific

exploration, by increasing importance of natural products in enhancing the scope of pharmaceutical research (Ahmad & et al., 2025). Natural products have been the backbone of traditional medicine; even more recently, they act as an important source for modern drug discovery (Atanasov & et al., 2021). In contrast to synthetic chemical libraries, natural products exhibit enormous structural diversity and biological relevance, serving as the direct source or inspiration for a great portion of modern pharmacotherapy (Newman & Cragg, 2020). Growing evidence has shown that the therapeutic action of such natural products is often bidirectional: the gut microbiota metabolizes bioactive natural products into more active and bioavailable derivatives, while these compounds simultaneously remodel the microbial community structure to revert age-related dysbiosis (Beaver & et al., 2025; Zhang & et al., 2023). The strategic use of naturally occurring interventions targeting the gut microbiome therefore offers a promising opportunity to foster healthy aging (Ghosh & et al., 2022).

Among the enormous sources of natural resources, functional foods and animal-derived bioactive protein- and lipid-rich products have emerged as potential candidates for such interventions (Cuchillo-Hilario & et al., 2024). Recently, the complex nature of the chemistry involved in natural products has made many scientists focus their work on certain sub-products, with a now-established volume of literature documenting the number of bioactive compounds and their modes of action (Ahmad & et al., 2025; Barba-Ostria & et al., 2022). Several recent studies have elucidated how natural compounds modulate a variety of signaling pathways that play a central role in what is normally referred to as the "aging process." The major types of compounds studied include polyphenols, terpenoids, flavonoids, and alkaloids (De Oliveira & et al., 2025).

In this growing field, Royal Jelly (RJ) is one of the most intensively investigated natural animal products because of its unique and complex profile of bioactive compounds. Here we review aging, gut microbiota, and diet, focusing on possible mechanisms that may be involved in how RJ affects age-related physiological changes. Although many studies have been performed on RJ's general biological effects, it will only be fully understood when the molecular mechanisms linking gut microbiota with aging are understood. Hence, prior to researching the molecular mechanism through which RJ can affect longevity, we should outline key processes of gut microbiota that cause age-related physiological decline. Results attained to this date evidence that there is an intimate connection between aging and diet-related changes in the gut microbial ecosystem. Dietary modulation of microbiota can propagate physiological decline or promote positive resilience to various stressors throughout life. With growing interest in the natural bioactive repertoire able to influence these

microbial pathways, RJ is one of the most exciting candidates because of its distinctive composition and broad spectrum of biological activities.

In this chapter, we aim to synthesize current evidence regarding the relationship between the gut microbiota and host aging, and to explore how RJ may influence the rate of aging and the organism's capacity to cope with stress. The following sections provide a conceptual framework (integrating microbiology, nutrition, and molecular geroscience) to better understand how RJ may function as a microbiota-dependent modulator of the aging process.

2. Mechanisms of Microbiota-Mediated Aging

The microbiome is essential during all stages of life for the development, performance and control of the immune system (Bosco & Noti, 2021). The human microbiome is made up of about 40 trillion microorganisms and thousands of microbial species, most of which are bacteria and includes fungal and viral members. Despite large interindividual variability, it plays a critical role within the maintenance of systemic homeostasis (Clark & Walker, 2018; Zhao & et al., 2023). The intestinal microbiota of humans is mostly dominated by the bacterial phyla *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria* (Clark & Walker, 2018). Due to its importance as a regulator of metabolism and immunity, even small changes within microbial populations could have significant physiological effects, especially with regard to aging (Cortés & et al., 2025).

Such shifts collectively accelerate immunosenescence and contribute to the low-grade, chronic inflammation commonly described as ‘inflammaging’ (Nguyen & Cho, 2025). In healthy adults, the composition of the gut microbiota is relatively unchanged, yet during the process of aging, there are many substantial changes to both the composition and function of the gut microbiota; one of the most significant is a substantial decrease in the diversity of the total microbial community (Bosco & Noti, 2021).

Historically, aging research and studies were conducted with focus on either factors associated with genetics, the environment, and life-styles (Ling, 2020). However, recent research indicates an ever more increasingly integral role for gut microorganisms. Current research unscrambles that some specific bacteria types, including *Bacteroides*, *Eubacterium*, and *Bifidobacterium*, were highly significantly associated with chronological aging and thus imply them as markers in aging status. It was suggested, as cited by Galkin & et al., (2020), as well as Ling (2020), that these were indicated. It can thus be seen that there would be implications and effects implied in aging research with regard to micro-aging as substantiated at Le Cosquer & et al. (2024).

Not only does microb-aging feature a decrease in these beneficial species *Clostridiales* and *Bifidobacterium*, but it is also distinguished by an enrichment of *Proteobacteria* and pathobionts such as *Enterobacteriaceae*. These shifts in composition, diversity, and function have been well documented in previous studies. There are known individual variations in environmental, dietary, and pharmacological factors, which influence microbiome profiles (O'Toole & Jeffery, 2015).

Diet is one of the most relevant external factors that can influence gut microbiota composition. With advancing age, impaired chewing and reduced salivation, changed digestion, and slowed intestinal passage may alter patterns of eating and the balance of macronutrient intake, potentially favoring the variations in the gut microbiota that accompany-and may contribute to-accelerated decline in age-related health (Ling & et al., 2022). These dietary changes might alter microbial metabolism, suppress the production of short-chain fatty acids, and promote systemic inflammation (Ney & et al., 2023).

Collectively, these microbiota shifts have functional consequences for host immunity and systemic inflammation (Wang & et al., 2025). Chronic low-grade inflammation is a key contributor to age-related diseases and is closely linked to gut dysbiosis in older adults. Gut microbiota alterations during aging can adversely affect intestinal barrier function, increase total body inflammation, and increase risk for developing an age-associated disease. The relative decline of beneficial microorganisms (for example, *Akkermansia muciniphila* and several species that produce short-chain fatty acids [SCFAs]) in conjunction with a relative increase in proinflammatory microorganisms promotes inflammation. SCFAs (butyrate, acetate, and propionate) play an important role in regulating energy metabolism, maintaining gut barrier integrity, and modifying immune responses. Several *Bifidobacterium* species also assist in maintaining immune homeostasis through their effects on such processes as promoting the production of anti-inflammatory cytokines; promoting maturation of immune cells; stimulating secretion of immunoglobulin A (IgA) and exerting antioxidant actions. Conversely, pro-inflammatory bacteria, such as *Escherichia coli*, *Eggerthella lenta*, and *Enterococcus* spp., produce endotoxins that activate inflammatory signaling pathways, contributing to age-related immunological dysfunction (Ren & et al., 2023).

3. Possible Impacts of Royal Jelly on Microbiota-Mediated Aging

Honey bees (*Apis mellifera* L.) are essential for sustaining ecological equilibrium and bolstering the global agricultural economy through their vital pollination services for several crops worldwide (Lin & et al., 2025). For thousands of years, they have benefited humanity by producing valuable

products, including honey, pollen, bee bread (perga), RJ, propolis, and bee venom, all of which have contributed to the preservation and/or improvement of human health (Papa & et al., 2022). Besides, these substances are significant Apitherapy products due to their varied composition of bioactive molecules exerting biological activities, such as antioxidant, antibacterial, antiviral, anti-inflammatory, and anti-tumoral effects (Martinello & Mutinelli, 2021). Currently, Apitherapy, which utilizes diverse honey bee products for treatment, is becoming recognized as a complementary and traditional medical approach (Kolayli & Keskin, 2020).

RJ, a major nutritional factor important for caste distinction and longevity in honey bees, is a yellowish-white and acidic secretion by the hypopharyngeal and mandibular glands of young adult worker honey bees (Shi & et al., 2018; Oršolić & Jazvinščak Jembrek, 2024). Although queen bees and worker bees share the same genome, they develop into either "queen" or "worker" honey bees depending on their diet. RJ is responsible for the transformation of the worker phenotype into the queen phenotype. Queen-worker differentiation is determined by nutrition during the larval stage. Queen honey bees consume RJ as a primary nutrient throughout their lives, including adulthood, but workers only consume worker milk when they are less than three days old. Namely, early honey bee diets affect the epigenome and phenotype. Two female castes are produced from identical genomes: a long-lived queen with fully developed ovaries and a short-lived functionally sterile worker, depending on the early larval diet. The maximum longevity of worker honey bees is a few months, but RJ supports queen honey bees to survive for years. Honey bees use nutritionally regulated epigenetic processes to create these distinct physiological and morphological variations between queens and workers. DNA methylation and histone acetylation are these processes. RJ may change DNA methylation and histone acetylation in honeybee larvae. This diet contains physiologically active substances (10-HDA (10-hydroxydecanoic acid)) that block the *de novo* methyltransferase (DNMT3A) or histone deacetylase 3 (HDAC3) to build and maintain the epigenetic state needed for larvae to become queens. (Haddad & et al., 2007; Wang & et al., 2014; Yang & et al., 2017; Alhosin, 2023). In addition, the gut microbiota of worker honey bees differs from that of queen honey bees, even though they share a habitat and are genetically quite similar. The queen honey bee microbiota is composed primarily of four bacterial species: *Lactobacillus* spp., *Bombella apis*, *Apilactobacillus kunkeei*, and *Commensalibacter* sp., with *Lactobacillus* being the only species consistently found in all worker honey bee stomachs. It has been suggested that the microbiota of worker bees and queens differ because the larvae that will become queens are fed RJ throughout their development (Zumkhawala-Cook & et al., 2024). A recent study has shown that queen bee larvae, an edible

byproduct of RJ, ameliorate symptoms related to D-galactose-induced aging in mice via modulating gut microbiota composition and amino acid metabolism (Zhao & et al., 2024).

RJ was first mentioned in ancient Greece as a contributor to the nectar that immortalized the gods of Olympus. The importance of RJ in bee society was initially discovered by Aristotle, whose research on queen bees showed that their ingestion increased physical strength and, most importantly, intelligence. RJ, an ancient Egyptian cosmetic, was Cleopatra's beauty secret. China has used RJ in traditional medicine for ages. This royal garden-only beekeeping product was related to ancient Chinese dynasties' longevity and sexual strength in old age. After identifying the royal cell's substance of nutrition, a Dutch scientist, microscopist, and entomologist discovered that the "beehive chief" was a queen until the seventeenth century. French scientist Queen Bee Nutrition identified "Royal Jelly" and connected it to the queen's exceptional growth. American beekeeping creator Reverend Langstroth chemically tested RJ for the first time in 1852, but his methods yielded no scientifically meaningful results. Langstroth sold RJ in the 1950s, especially in unsuccessful honey regions (Fratini & et al., 2016). From past to present, RJ is a highly sought-after product of beehives that beekeepers may benefit from because of its high market value and reputation for consistent production even in challenging environmental circumstances (Kanelis & et al., 2024). Commercial production of RJ has been going strong up until now, and beekeepers rely heavily on it as a source of revenue since its market value is so much greater than that of other bee products like pollen or honey. China's annual production of RJ is approximately 4,000 tons, with a market valuation exceeding \$2.5 billion. The nation constitutes 90% of global RJ production and primarily exports to Japan, Europe, and the United States (Ahmad & et al., 2020). RJ production methods, which generally involve transferring larvae taken from worker honey bee cells to numerous (artificial) queen bee cells using an artificial larval inoculation method, are being improved day by day. The reports indicate that methods have been devised in China to increase the yield of RJ. These methods involve maintaining a species of honey bees known for their high yield, employing specially designed equipment, prolonging the time it takes to produce RJ, honing manipulation skills, supplementing feeding, modifying the honeycomb structure, using the correct number of queen bee cups per colony, and raising larvae (Chen & et al., 2002; Hu & et al., 2019).

RJ, which is collected from queen bee cells, is whitish in color, which usually turns to yellow as it is stored. Its density is 1.1 g/mL, pH is in the range 3.4-4.5, and it has a sour taste with a strong odor (El-Guendouz & et al., 2020). On a chemical basis, fresh RJ was composed of water (50-70%), proteins (9-18%),

carbohydrates (7-18%), fatty acids and lipids (3-8%), mineral salts (ca. 1.5%), and minor levels of polyphenols and vitamins (Melliou & Chinou, 2014). RJ is rich in bioactive compounds of major RJ proteins (MRJPs) and lipids, mainly two fatty acids: 10-H2DA (10-hydroxy-trans-decenoic acid) and 10-HDA (10-hydroxydecanoic acid), peptides, amino acids, flavonoids, polyphenols, vitamins, and minerals (Civelek, 2022). The chemical composition of RJ varies by locality and is based on many external factors related to nectar sources, external conditions, methods applied to its harvesting, and honeybee species, leading to differences in quantity and quality (Khalfan Saeed Alwali Alkindi & et al., 2024).

Important components that form the backbone of RJ's structure are proteins. These complex components comprise over 50% of RJ's dry matter. Moreover, over 80% of the protein content is comprised of MRJPs. MRJPs are believed to play a crucial role in the development of the queen honey bee and larvae by supplying essential amino acids, which include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Meanly, MRJPs play an important role in the growth and longevity of queen bees. Nine MRJP members (MRJPs 1–9) have been found in the honey bee MRJPs 1–7 in RJ and MRJPs 8 and 9 in bee venom. MRJPs 1–9 are elevated in the spermathecal fluid of mated queen bees, whereas MRJPs 1–7 exhibit differential expression in the hypopharyngeal glands of nurse worker honey bees after bacterial consumption.

MRJP1, also known as apalbumin 1 or royalactin, is the main RJ glycoprotein and is important for determining both RJ freshness and quality (Bagameri & et al., 2022; Lee & et al., 2022; Xue & et al., 2025). According to studies, MRJP1 has immune-regulatory, antioxidant, and anti-tumor properties; MRJP2 and MRJP3 have immunomodulatory effects; and MRJP3 has potential as an anti-inflammatory and anti-allergic agent. MRJP4 contributes nutritional components to RJ; however, its expression in the hypopharyngeal gland is comparatively low relative to other MRJPs (Ghosh & et al., 2024). Besides, as another important RJ component, 10-hydroxy-2-decenoic acid, often referred to as Queen Bee acid, constitutes about 70% of the total fatty acids. 10-HDA is an alpha, beta-unsaturated carboxylic acid characterized by a hydroxylated 10-carbon chain at its terminus. The quantification of 10-HDA is a routine method for the quality assessment of RJ because of its unique occurrence and content in the lipid fraction. In addition, 10-HDA is used as a marker compound to detect fraud and authenticate the key quality of RJ. Due to its strong correlation with RJ's remarkable medical and pharmacological properties, such as estrogenic, neurotrophic, anti-tumoral, and glucose-regulating effects, 10-HDA may be considered a naturally occurring bioactive compound. Further research on its photoaging and skin-protective properties has also been conducted (Gasbarri & Angelini, 2025). In addition, jelleins, royalisin, royalactin, and apisimin are active

compounds in RJ which have fallen under the category of antimicrobial peptides (AMPs). Those peptides, considering their mode of action-which is interaction with the negatively charged bacterial membranes and leads to the death of the bacterial cell-have been considered a more natural alternative to conventional antibiotics (Spanidi & et al., 2022). A great number of structurally different flavonoids, a subclass of polyphenol, are present in RJ. In this category finds flavanones like hesperetin, naringenin, isosakuranetin, pinobanksin, and pinocembrin, as well as flavones like apigenin, luteolin, chrysin, and acacetin, flavonols like kaempferol, isorhamnetin, fisetin, galangin, quercetin, and rutin, isoflavonoids like coumestrol, genistein, and formononetin, and anthocyanins and chalcones. These compounds have been known for possessing potent antioxidant properties, which enable them to reduce free radicals via redox reactions (Sönmez, 2025).

RJ has been known as a commercial medical product for a long time owing to its multiple biological properties (Figure 1), such as antioxidant, antibacterial, anti-inflammatories, anti-aging, anticancer, estrogenic, fertility, neuroprotective, and immuno-stimulatory properties (Ali & Kunungi, 2020; Bălan & et al., 2020; Chi & et al., 2022; Yu & et al., 2023; Wu & et al., 2024). In preclinical and clinical researches, RJ has been proven to increase immunity, especially concerning wound healing, as well as alleviated effects on patients with chronic diseases, especially diabetes and heart conditions (Kumar & et al., 2024). Moreover, RJ is a highly prominent and in-demand active component used in the cosmetic industry owing to its skin moistening, decolorizing, anti-aging, and whitening properties (Toma & Băran, 2025).

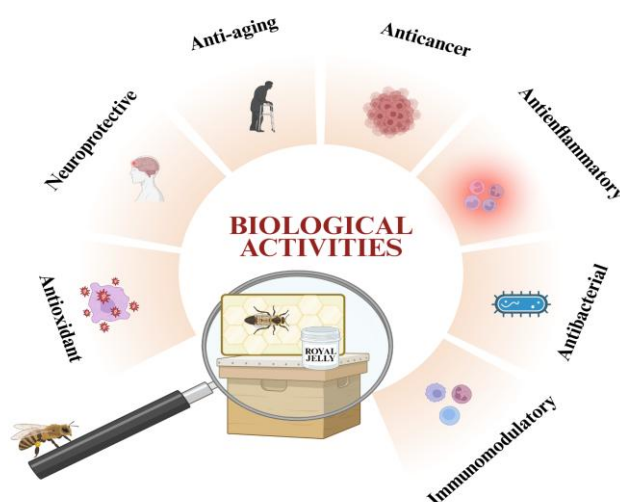


Figure 1. Biological activities of RJ

In recent years, individuals and researchers alike are becoming more interested in RJ as a healthy, functional food, particularly as a result of research that has revealed how RJ has been proven to affect pathogenic processes within disease models (Alu'datt & et al., 2025). Current research suggests that RJ supports healthy aging and longevity (Kunugi & Ali, 2019), reverses cognitive decline linked to oxidative stress and inflammation (Ali & Kunugi, 2020), reduces negative effects on immune function (Bouamama & et al., 2021), enhances antioxidant capacity and modulates intestinal microbiota (Chi & et al., 2021), and acts as an effective dietary approach for managing age-related frailty through gut microbiota modulation (Chen & et al., 2024). A comprehensive study from animal models to humans has demonstrated that RJ and its components extend lifespan by modulating age-related mechanisms, including downregulating insulin-like growth factors, targeting rapamycin, upregulating epidermal growth factor signaling, promoting dietary restriction, and enhancing antioxidant capacity. One of the ways that RJ acts is via the regulation of the Insulin/Insulin-like Growth Factor-1 (IIS) signaling pathway. A down-regulation of IIS leads to an increase in DAF-16 transcription factor, which is equivalent to the mammalian transcription factor FOXO. It then goes on to bind with critical co-regulators of DAF-16 like HCF-1, SIR-2.1, and 14-3-3 protein FTT-2. It then leads to an up-regulation of longevity-associated genes, causing an modulation in dietary restriction and mTOR pathways. It also enhances cellular processes associated with DNA repair, autophagy, antioxidant and anti-inflammatory response, stress resilience, and regulated cell division. The next way through which RJ acts as an anti-aging supplement is via the activation of epidermal growth factor receptor (EGFR) signaling pathway mediated by LET-23 receptor. It acts via phospholipase C- γ (plc-3) and inositol-3-phosphate receptor (itr-1) and leads to an enhancement of elongation factors and chaperonins. It leads to an increase in protein synthesis and proteasomal activity. The associated increase in protein turnover leads to an enhancement in ribosomal activity, muscle maintenance, and reduces lipofuscin levels, which are associated with aging. It acts as an anti-aging supplement and leads to an enhancement in cellular processes associated with detoxification, muscle maintenance, and reduction of lipofuscin levels. Skewing lipofuscin levels leads to aging (Kunugi & Ali, 2019).

There is an emerging perspective that microbiota-targeting therapies have therapeutic value for retarding aging and promoting better health and longevity in humans. However, there appear to be very limited reports exploring links among RJ, microbiota, and aging. Moreover, there is a need for more research on these links and the associated mechanisms. Findings from recent reports on the role of RJ for unmasking various associations among aging and microbiota have been summarized in Figure 2 and Table 1.

Table 1. Summary of *in vitro* and *in vivo* RJ studies

<i>RJ or bioactive components or based on RJ</i>	<i>Study type, length, and groups</i>	<i>Study design</i>	<i>Results</i>	<i>References</i>
RJ	<i>in vivo</i> study, Male Sprague Dawley (SD) rats	RJ (2.5 g/kg, dissolved in saline) via gavage	rebalancing gut–brain communication	Zhu & et al., (2025)
RJ	<i>in vivo</i> study, Male C57BL/6J mice (Middle-aged)	A daily dose of approximately 1500 mg per kg body weight (BW)	reducing TNF- α , IL-6, IL-1 β suppressed the p16, p21, p53	Chen & et al., (2024)
Major royal-jelly proteins (MRJPs)	<i>in vivo</i> study, Female C57BL/6 mice (6–8 weeks old)	250 mg/kg of MRJPs via gavage	preserved the stability of the natural gut microflora in mice	Wu & et al., (2024)
an Edible By-Product of RJ(Queen Bee Larva)	<i>in vivo</i> study, aging model Female C57BL/6J mice (18–22 g)	low-dose QBLP (1 g/kg/d), or high-dose QBLP (5 g/kg/d) 8-week treatment period	improved organ index and prevented pathological damage to brain tissue	Zhao & et al., (2024)
MRJPs	<i>in vivo</i> study, Kunming mice, (Eight-week-old, weighing 40–45 g)	0.25 g/(kg·bw) of RJ main protein, administered intragastrically for 30 days.	increased white blood cell count, IL-4 and IL-6 levels, and T/B-lymphocyte proliferation.	Wang & et al., (2023)
RJ	<i>in vivo</i> study, Female C57BL/6JNifdc mice (5-week-old, 14–17 g)	The RJ treatment groups: 1.0 g/kg, 1.5 g/kg, and 2.0 g/kg BW for 31 days	reduced IL-6 level, increased IL-10 and slgA, improved the colonic mucosal barrier and gut microbiota	Guo & et al., (2022)
SRJ (RJ with Se)	<i>in vivo</i> study, Male BALB/c mice (18–22 g)	3000 mg/kg day of RJ via gavage, for 30 days	increases T-AOC, total SOD and GSH-Px activities	Chi & et al., (2022)
10-hydroxydecanoic acid (10-HDAA)	<i>in vitro</i> study, Microglia BV-2 cells <i>in vivo</i> study, Male C57BL/6J mice (20–22 g)	orally administered 10-HDAA (80 mg kg ⁻¹ d ⁻¹), aspirin (40 mg kg ⁻¹ d ⁻¹)	reduced LPS-induced inflammation, downregulation of nitric oxide, COX-2, and IL-6	You & et al., (2022)

New evidence suggests that RJ may support restoring an impaired gut-brain connection. Research findings point out that RJ influences the expression of numerous genes, encompassing neuroactive ligand-receptor interactions, synaptic vesicle cycling, and various pathways associated with neuronal transmission. By correlation analysis, *Escherichia*, *Akkermansia*, and *Clostridium* were closely linked to gastrin-releasing peptide (Grp) gene modules and Rps6ka3 signaling pathways, suggesting an intrinsic gut-brain connection. Besides, *Escherichia* infection trials significantly reduced the social standing of RJ-treated rats, but several cerebral genes, such as Grpr and Grpel1, and prefrontal spine density were also altered, highlighting the importance of gut-brain connectivity in dyadic competition outcomes (Zhu & et al., 2025).

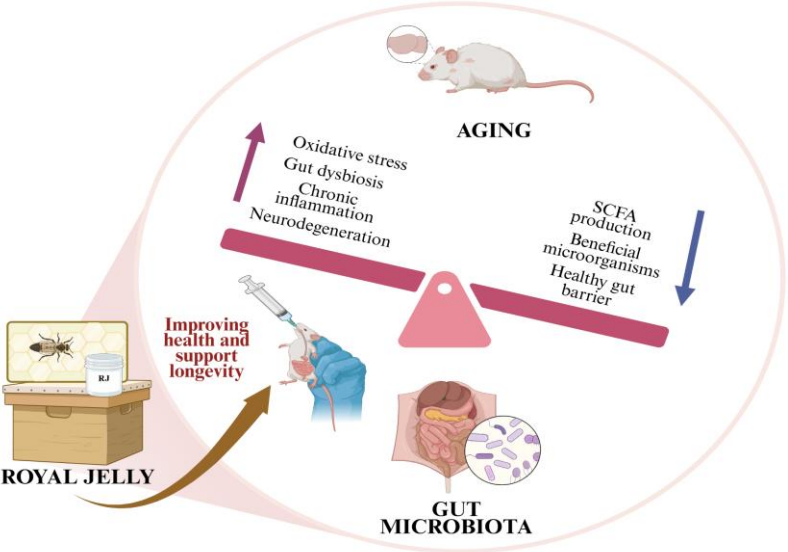


Figure 2. Schematic illustration of the potential protective effects of RJ on the aging process and accompanying physiological changes via modulation of intestinal microbiota.

Using a naturally aging mouse model, researchers looked at how RJ affected the intestinal barrier, the microbiota, the content of short-chain fatty acids (SCFAs), and potential mechanisms of action. It has been observed that RJ enhances intestinal function and helps manage age-related disorders (Chen & et al., 2024). The results showed that RJ helped regulate blood lipid profiles by lowering levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C). Additionally, it protects the liver by increasing antioxidant enzyme levels and decreasing inflammatory cytokines such as TNF- α , IL-6, and IL-1 β . Furthermore, cell cycle-dependent kinase inhibitors p16, p21, and p53 were downregulated by RJ. Two of the mechanisms by which RJ attenuated gut dysfunctions were reducing the amount of inflammatory cytokines in the colon and preventing the loss of tight junction protein. There was a shift in the gut microbiota, including beneficial bacteria like *Lachnospiraceae* NK4A136 and *Akkermansia* with a higher *Firmicutes-to-Bacteroides* (F/B) ratio. Several decades later, microbiota associated with aging changes includes increased subdominant species such as *Oscillospira* *Odoribacter*, and *Butyricimonas*, while dominant species such as *Ruminococcaceae*, *Lachnospiraceae*, and *Bacteroidaceae* are less abundant. With age, there is a reduction in beneficial bacteria, including *Christensenellaceae*, *Akkermansia*, and *Bifidobacterium*. The present study found RJ to reverse the effect of *Akkermansia*, which was less abundant in old control group mice. These kinds of results indicate that RJ can improve host physiology related to aging and intestinal health. The impact of MRPs on the mice's immune response and intestinal flora and estrogen levels was studied in one research.

The results indicated that the optimal dose through which the integrity of the gut's natural flora was maintained was 250 mg/kg MRJP. Also, at the same dose at which inflammatory cytokines IL-1 β and IL-6 production occurred, MRJPs suppressed the activation of splenocytes and mesenteric lymphocytes induced by LPS. Dietary MRJPs were found to stabilize the microbiota in the intestines of mice. These findings could potentially prevent and cure chronic inflammatory diseases (Wu & et al., 2024).

In a D-Galactose-induced aging model, supplementing queen bee larvae (QBL), an edible residue of RJ, increased the gut microbiota, enhanced amino acid metabolism, and improved cognitive impairment and postponed memory loss in aged mice (Zhao & et al., 2024). The findings from this research implied that the abundance of *Ruminococcaceae* and *Lachnospiraceae*, which are two critical bacterial families that are predominantly found in the gastrointestinal tract of animals, but are reduced with age, increased remarkably in the intestines of the mice that were approaching old age when administered with the supplement of

QBLP. *Rikenellaceae*, *Eubacterium*, *Ruminococcus*, *Roseburia*, and *Oscillibacter* were also increased by the treatment with QBLP. In particular, increased abundance of *Rikenellaceae* led to a decrease in visceral fat, which in turn increased the mass and life span of mice. SCFA production can further be enhanced by enriching mouse intestinal flora with more *Oscillibacter* spp., the major bacterial species in the gut that produces SCFA. These data imply that QBLP reduced the aging phenotype in mice by modulating the gut microbiota, specifically by enhancing favorable genera and families.

The results of the study in which MRJP was orally administered to mice that had been induced with cyclophosphamide showed that the medium-dosage group with 0.25 g/kg body weight of RJ parent protein significantly affected spleen and liver development, peripheral blood leukocyte count, immunoglobulin content, immune factor levels, and the proliferation of spleen lymphocytes. *Bacteroidetes*, the largest Gram-negative phylum in the human gut, plays a crucial role in sustaining healthy and complex colonic homeostasis. Gut ecological dysbiosis can modify immune function and metabolic processes. In this study, the MG + HM group exhibited a significantly higher percentage of *Bacteroidetes* compared to the MG, indicating that the elevated dosage of MRJP enhanced mouse immunity through the regulation of intestinal *Bacteroidetes*. The results of this study demonstrated that MRJPs have the potential to enhance the immune system in mice, increase the quantity and variety of gut flora, and imply that MRJPs contribute significantly by regulating the bacteria in the intestines that are linked with the immune system (Wang & et al., 2023).

In DSS-induced ulcerative colitis mice, treatment with RJ increased the expression of tight-junction protein, goblet cells, and their secretion of mucin, MUC2, which reduced intestinal permeability and improved symptoms and colonic cell death. RJ decreased the expression of the proinflammatory cytokine IL-6 while increasing the expression of the anti-inflammatory cytokine IL-10 and secretory immunoglobulin A (sIgA). DSS led to an increase in the relative abundance of *Parabacteroides*, *Erysipelotrichaceae*, *Proteobacteria* (including *Gammaproteobacteria*, *Enterobacteriales*, and *Enterobacteriaceae*), and *Escherichia* and *Shigella* in the colon, while decreasing the relative abundance of *Muribaculum*. The treatment with 2.0 g/kg RJ resulted in an enhancement of the relative abundance of the aforementioned intestinal flora in the RJ treatment group. The results showed RJ ameliorated the DSS-induced colitis by strengthening the colonic mucosal barrier (Guo & et al., 2022).

Scientists documented that when honey bees were fed with sodium selenite, the produced RJ was high in selenium, which, in turn was tested on mice to see its effects on gut microbiota. To understand how SRJ, RJ and SRJ were provided

for 30 days to mice, their antioxidant capacity and gut microbiota composition were evaluated. After the SRJ treatment, enhanced glutathione peroxidase, total antioxidant activity, superoxide dismutase in both the kidney and liver. SRJ-treated mice have a higher *Firmicutes/Bacteroidetes* ratio, a low level of *Helicobacteraceae*, an enhanced *Lachnospiraceae* and *Prevotellaceae*. SRJ treatment caused a reduction of *Alistipes* and enhanced *Oscillibacter*, the *Lachnospiraceae* NK4A136 group *Prevotellaceae* UCG 001, the *Rikenellaceae* RC9 intestine group and *Lachnospiraceae*. Based on the functional gut microbiota predictions, it has been suggested that SRJ may enhance amino acid metabolism. The correlation study indicated that SRJ can improve the function of gut microbiota and host microbiota interaction. Based on the findings, it has been suggested that SRJ may enhance health and reduce dysbiosis and oxidative stress (Chi & et al., 2022).

This study shows that 10-HDAA suppresses the activation of the NF- κ B pathway and, further targets PtgS-1/2, which is known to be the target of aspirin. So, a combinatory therapy of 10-HDAA and aspirin was performed. *In vitro* studies reported that the combinatory group reduced LPS-induced inflammation in BV-2 cells which was evident from the downregulation of nitric oxide, COX-2 and IL-6 when compared to the group treated with 10-HDAA or aspirin alone. The combinatory therapy lowered the pro-inflammatory mediator levels and inhibited glial cell overactivation, as revealed in *in vivo* studies. Gut injuries and microbiota dysbiosis caused due to aspirin were reduced by 10-HDAA (You & et al., 2022).

Taken together, these studies underscore the diverse role of RJ as a modulator of interactions between intestinal microbiota and the immune system, thereby providing a rationale for potential use in anti-aging treatment.

4. Conclusion

Evidence discussed in this chapter underlines the central role of gut microbiota as an executor or mediator of aging and its deep influence on immune balance, metabolic homeostasis, systemic inflammation, and organismal resilience. Age-related dysbiosis is characterized by reduced microbial diversity, loss of beneficial taxa, decreased SCFA production, and the expansion of pro-inflammatory pathobionts-all traits collectively driving immunosenescence and inflammaging. Therefore, strategies preserving or restoring microbiota integrity are promising ways to support healthy aging. In this context, RJ has attracted considerable interest as a natural product worthy of focused investigation. Its bioactive components target multiple molecular pathways implicated in longevity, including epigenetic regulation, antioxidant defense, anti-inflammatory signaling, and metabolic pathways such as SIRT1/FOXO, AMPK,

and mTOR. Emerging evidence further suggests that RJ and RJ-derived compounds may beneficially modulate gut microbial composition, enhance barrier integrity, and counteract processes underlying physiological decline associated with aging. Despite these promising findings, substantial knowledge gaps still exist-particularly with regard to microbiota-dependent mechanisms of RJ and how the effects of RJ are shaped by diet, genetics, sex, and lifestyle. Further research integrating metagenomics, metabolomics, and controlled clinical studies will be required to elucidate these processes and identify optimal strategies for RJ supplementation. The conjunction of microbiome science, nutritional gerontology, and apitherapy supports the suggestion that RJ holds considerable promise as a microbiota-modulating intervention to promote healthspan and support longevity. As this field continues to evolve, RJ may assume its place as a meaningful component of personalized strategies aimed at mitigating age-related decline and improving quality of life across the lifespan.

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CHAPTER 17

Burnout Among Nurses Working in Intensive Care and the Public Health Approach

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1. Introduction

Intensive care units are one of the most critical areas of the healthcare system, and nurses working in these units face a high level of responsibility, rapid decision-making, heavy workloads, and emotional pressure (Babkair et al., 2024; Bruyneel et al., 2022). Nurses working on the fine line between life and death constantly struggle with stressors such as high-risk patient care, communication with families, technological intensity, and time pressure (Özcanarslan and Önen, 2020).

This situation increases the risk of burnout syndrome developing in nurses, leading to physical, emotional, and mental exhaustion. This situation increases the risk of burnout syndrome developing in nurses, leading to physical, emotional, and mental exhaustion (Utlü et al., 2024). Burnout negatively affects not only nurses' individual health but also patient safety, quality of care, and the sustainability of healthcare services (Jun et al., 2021; Li et al., 2024). Therefore, understanding burnout in intensive care nurses and developing preventive strategies should not be limited to psychosocial support at the individual level; it should be addressed within a public health approach. The public health perspective has the potential to offer comprehensive solutions by considering not only the individual but also the institutional, social, and systemic dimensions of burnout.

2. Definition of Burnout

Burnout is a psychological syndrome characterized by emotional exhaustion, depersonalization (detachment from work), and reduced personal accomplishment, which develops as a result of prolonged work-related stress (Jun et al., 2021). Burnout syndrome is assessed in three main dimensions: emotional exhaustion, depersonalization, and reduced personal accomplishment. Emotional exhaustion is when an individual feels physically and mentally tired, worn out, and depleted due to the intensity of their work. In the dehumanization dimension, the person begins to display distant, negative, or sarcastic attitudes towards the individuals they serve. The decrease in the sense of personal accomplishment is related to the individual seeing themselves as inadequate, struggling to cope with problems, and negatively evaluating their professional competence (Pustulka-Piwnik et al., 2014). Burnout syndrome arises as a result of increased emotional exhaustion and depersonalization, coupled with a decrease in personal accomplishment (Kekeç and Tan, 2021).

Burnout is a long-term response to chronic emotional and interpersonal stress factors at work (Maslach and Leiter, 2016). Burnout, which has become one of the most significant psychosocial occupational hazards in today's society, generates significant costs for both individuals and organizations (Edú-Valsania et al., 2022; Epstein et al., 2020). Burnout, which results from chronic and persistent occupational stress, is associated with a range of adverse individual

health problems, including chronic pain, gastrointestinal disorders, depression, and even death. The prevalence rate of burnout syndrome among nurses working in hospitals varies from 5% to 50%, depending on their area of expertise and geographical region (Jun et al., 2021). Healthcare professionals working under intense stress are particularly vulnerable to this condition. Intensive care nurses are a group at risk for burnout due to their constant contact with critically ill patients, the high level of attention required for their tasks, and the time pressure they work under. However, this is not a personal problem, but a result of specific characteristics of the job activity (Bouza et al., 2020).

3. Burnout Among Nurses Working in Intensive Care

Burnout, a syndrome caused by chronic work stress in the workplace, is a common problem among clinicians working in healthcare institutions. ICU nurses are at risk for chronic work stress due to the complexity and pace of the intensive care environment (Rushton and Pappas, 2020). Studies show that the prevalence of burnout among ICU nurses has increased significantly. A study conducted in the Hail Region of Saudi Arabia found that the majority of intensive care nurses reported “high” or “very high” levels of burnout; a significant inverse correlation was found between burnout levels and all dimensions of quality of life (Villagrancia et al., 2025). A study by Ramírez-Elvira et al. (2021) found that burnout, particularly in the dimensions of emotional exhaustion and disengagement, was highly prevalent among intensive care nurses.

This increase is largely due to excessive workload, long working hours, a stressful work environment, pressure to make critical decisions, challenging patient communication processes, and a lack of organizational support. A study by Ramírez-Elvira et al. (2021) highlights that intensive care nurses experience a significant increase in emotional exhaustion levels due to high workload and long working hours. Studies conducted during the pandemic revealed that nurses' burnout levels increased due to the stress caused by this situation (Saravanan et al. 2023). Burnout rates increased among intensive care nurses, particularly during the pandemic, and this negatively affected nurses' quality of life (Villagrancia et al., 2025). Li et al. (2024) reported that communication difficulties with patients' relatives had a significant impact on nurses' job satisfaction and burnout levels. Al Sabei et al. (2025) stated that staff shortages increased nurses' emotional exhaustion and, when combined with technological stress, negatively affected job performance. A study by Quesada-Puga et al. (2024) found an inverse relationship between job satisfaction and burnout, revealing that low job satisfaction significantly increases the risk of burnout. A study conducted with 228 intensive care nurses in Turkey found a positive correlation between burnout levels and intention to leave the job; however, it was reported that psychological resilience did not fully demonstrate the expected mediating role in this relationship (Turunç et al., 2024). Nevertheless, organizational commitment was

found to be an important protective factor that reduced nurses' intention to leave their jobs. Furthermore, Al Sabei et al. (2025) noted that emotional exhaustion was more pronounced among young nurses and that this increased their intention to leave their jobs. Wang et al. (2023) emphasize that the relationship between emotional exhaustion and intention to leave is weaker among nurses with high levels of organizational commitment.

Burnout is reported to negatively affect not only nurses' individual mental health but also the quality of patient care. In a study by Quesada-Puga et al. (2024), it was reported that in intensive care units with high levels of burnout, the quality of patient care declined and patient satisfaction decreased. Similarly, a study by Kerlin et al. (2020) showed that as burnout levels increased among intensive care nurses, the quality of patient care decreased, which in turn further increased nurses' burnout levels. A meta-analysis conducted by Li et al. (2024) shows that increased burnout levels among nurses have direct negative effects on patient safety, satisfaction, and overall healthcare quality. Wudarczyk et al. (2025) emphasize that organizational support and effective stress management strategies are critical in reducing burnout. In this regard, strengthening organizational support mechanisms, balancing workloads, and implementing programs aimed at increasing psychological resilience emerge as key intervention areas in preventing burnout among intensive care nurses.

In conclusion, the professional factors triggering burnout are multidimensional and interrelated; therefore, developing strategies at both the individual and organizational levels is critical for intensive care nurses to cope with burnout.

4. Public Health and Intervention Approach

Intensive care nurses work under high levels of stress and emotional strain when caring for critically ill patients. This situation poses serious risks to both the physical and mental health of nurses and patient safety. Public health nurses play an important role in reducing these risks and improving the quality of healthcare services. In this context, strategies for education, psychosocial support, improvement of working conditions, and occupational health–community health integration are of great importance.

Public health nurses can raise awareness by organizing training sessions for intensive care nurses on stress management, recognizing signs of burnout, and emotional resilience. These training sessions help nurses perform their professional duties in a healthier manner while also increasing their individual awareness and self-care levels. In addition, group work, experience-sharing sessions, and discussion groups strengthen solidarity among nurses and collectively develop their stress coping skills (Pourneshad et al., 2025; Mathew et al., 2025). Continuing education and professional development opportunities increase nurses' knowledge and skills, thereby enhancing job satisfaction and

professional fulfillment. This can also reduce the risk of burnout (Yıldırım et al., 2023).

Psychosocial support and counseling services are effective tools in reducing the negative effects of burnout (Jaber et al., 2025). Individual counseling, group therapy, and stress management workshops boost nurses' morale and positively affect the quality of patient care (Kekeç & Tan, 2021). Such support mechanisms increase job satisfaction, reduce turnover rates, and offer an effective strategy for coping with emotional burnout (Kıssal & Dokgöz, 2024).

In addition, improving the work environment is a fundamental factor in reducing burnout levels. Balancing the workload, ensuring adequate rest periods, strengthening team support, and implementing morale-boosting practices help nurses maintain their physical and emotional health. Thus, nurses' professional satisfaction increases, and patient safety and quality of care improve in a sustainable manner (Erçevik & Bahçecik, 2024).

Finally, it should not be forgotten that burnout is not only an individual problem, but also has significant effects on public health. Public health nurses can develop preventive programs by regularly monitoring nurses' burnout levels. When nurses at high risk of burnout are identified through early warning systems and provided with the necessary support, they can perform their professional duties in a healthier, safer, and more sustainable manner (Sümen, 2021).

5. Conclusions and Recommendations

Burnout among intensive care nurses is a multidimensional problem that poses serious risks to both individual health and the quality of patient care. Current research indicates that factors such as heavy workloads, long shift hours, pressure to make critical decisions, challenging communication processes, and insufficient staffing trigger burnout. Burnout not only negatively affects nurses' psychological and physical health, but also leads to low job satisfaction, increased intention to leave the job, and decreased quality of patient care. Therefore, strategies to prevent burnout require a comprehensive approach at both the individual and organizational levels. The role of public health nurses is critical in reducing burnout through awareness-raising training, psychosocial support and counseling services, improvement of the work environment, and early warning systems. Furthermore, psycho-educational programs, meditation, support groups, leadership and organizational support, and the provision of continuous professional development opportunities will increase the emotional resilience of intensive care nurses and reduce the risk of burnout.

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CHAPTER 18

Evaluation of Patient Stay Lengths in Intensive Care Units

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Introduction and Conceptual Framework

Patient length of stay is a fundamental clinical metric in the healthcare system, referring to the time a patient spends in the hospital from admission to discharge or death. For critical care settings such as intensive care units (ICUs), this metric holds particular significance. ICUs are units where patients with respiratory failure, multiple organ failure, severe trauma, or major surgical operations have their vital functions monitored 24 hours a day using advanced medical equipment and are treated by a specialized team. Due to the nature of these units, patient length of stay not only reflects the patient's clinical course but is also a critical indicator of healthcare quality, resource utilization, and cost-effectiveness (OECD, 2025; Eskandari et al., 2022).

Patient length of stay data can be calculated hospital-wide or by a specific disease group. In this context, it is important to distinguish between length of stay (average number of days per patient admission) and "patient days" (total length of stay for all patients over a specific period). Patient length of stay provides direct information on the efficiency of care and the effectiveness of the hospital's operational policies, forming a cornerstone for managerial decisions (Baek et al., 2018).

Data on patient length of stay in the intensive care unit (ICU) are statistically complex. Traditionally, these data do not exhibit a normal distribution; rather, they show a highly skewed distribution. This leads to simple statistical measures, such as average length of stay, failing to accurately represent the true situation because they are influenced by the small number of patients who stay for very long periods. For example, the presence of patients who stay in ICU for more than 6 months or even a year can significantly inflate the overall average.

To overcome these statistical challenges, academic research suggests using more sophisticated approaches such as mixed distribution models (Peres, Hamacher, Oliveira, Bozza, & Salluh, 2021; Williford, Haley, McNutt, & Lazariu, 2020). These models enable more accurate predictions of patient length of stay by more accurately capturing the distributions of different subgroups in the data (Williford et al., 2020). Furthermore, data preprocessing techniques such as logarithmic transformation have been observed to improve model performance by reducing data skewness (Peres et al., 2021).

Patient length of stay data carries meanings far beyond a mere numerical value. While the majority of patients in ICUs have short stays, a small group of patients with prolonged stays consumes a significant portion of the hospital's resources (Stricker, Rothen, & Takala, 2003). This "long-term stay" phenomenon is associated with a number of negative clinical and economic consequences, including resource waste and increased healthcare costs, as well as an increased risk of hospital-acquired infections and higher mortality rates (Stricker et al., 2003; Vincent, 2003).

Therefore, efforts to reduce patient length of stay are not merely an economic goal to conserve hospital budgets, but a vital strategic step to improve patient safety and clinical outcomes. Analyzing data on patient length of stay is a critical management tool that offers hospital administration the opportunity to optimize resource allocation, identify characteristics of long-stay patients, and develop preventive interventions for these groups (Haddad et al, 2001). With this approach, patient length of stay should no longer be considered merely an "outcome" metric, but rather a reflection of optimal care processes.

International Standards: Guidelines and Research

Internationally, there is no directly defined and universally accepted 'standard' number of days for patient stay in intensive care. This is closely related to the fact that each patient's clinical condition and response to treatment are unique (Santos et al., 2025).

Clinical practice guidelines published by leading international organizations such as the Society of Critical Care Medicine (SCCM) focus on managing critical conditions that have been proven to directly impact patient length of stay, such as sepsis (SCCM, 2021), delirium, and post-intensive care syndrome (PCI, 2018; Sharma, 2019), rather than establishing patient length of stay as a primary goal. This focus represents a significant shift in modern critical care philosophy: the aim is not to reach a specific number of days, but to achieve the best clinical outcome by implementing evidence-based processes. Optimal processes often result in shorter and more efficient lengths of stay. This approach suggests that healthcare systems should treat patient length of stay as a reflection of a process metric.

International studies indicate that the average length of stay in the intensive care unit varies depending on the type of ICU and the patient population, but the general average is approximately 4 days (Garland, Olafson, Ramsey, Yogendran, & Fransoo, 2015). For example, a large-scale population analysis conducted in Canada found that the average length of stay for adult ICU patients was 3.96 days. However, it is emphasized that, in addition to these averages, some critically ill patients may remain in the ICU for longer than 6 months or even a year; prolonged stay may be associated with clinical characteristics such as critical illness severity, need for mechanical ventilation, and comorbidity burden (Miniksar & Keten, 2021). It has been reported that this duration may also be related to non-patient individual factors such as social support/caregiver availability, which can affect discharge planning, especially in patients requiring long-term mechanical ventilation (Ho, Lim, Pua, & Lew, 2020). Furthermore, the same Canadian study shows that the in-hospital mortality rate was 19.0% and that patients continued to use significant healthcare resources after discharge (41% were readmitted to the hospital, 10% were readmitted to the ICU) (Garland et al., 2015). These findings reveal that the effects of critical care extend beyond

the hospital and that patient length of stay is an indicator reflecting long-term patient outcomes, beyond in-hospital efficiency.

A randomized clinical trial conducted in 30 adult intensive care units in Brazil evaluated the impact of a “remote intensive care specialist support” model on clinical outcomes and length of stay in the intensive care unit. The study compared 6,012 patients in units receiving remote intensive care specialist support with those receiving conventional care; the median length of stay in the remote intensive care specialist support group was 2.9 days, compared to 3.0 days in the conventional care group. However, the length of hospital stay remained similar between the groups, and no significant superiority was shown in terms of hospital mortality. The findings suggest that remote intensive care specialist support should not be considered a standalone intervention that “shortens length of stay”; rather, strengthening implementation components such as integration with clinical protocols, team training, decision support processes, and harmonization of local workflows is necessary to enhance its impact (Pereira et al., 2024).

A national cohort study conducted in the Netherlands using the NICE (National Intensive Care Evaluation) registry compared the characteristics and outcomes of patients admitted to intensive care units (ICUs) in successive phases of the COVID-19 pandemic. The study showed a significant decrease in ICU stay as the disease progressed from early to later stages: the median ICU stay decreased from 20.6 days in the first phase to 7.6 days in the final phase; discharge rates also increased over time. This trend can be attributed to experience gained in clinical management, updates in treatment approaches, patient selection/referral profile, and variant/wave effects. The study emphasizes that health systems should use not only the number of beds but also the dynamic change in mean length of stay as a scenario parameter in “wave-phase” based ICU capacity planning (Termorshuizen et al., 2024).

This study, using Dutch NICE data, examined the differences in mortality and ICU stay between immunocompromised and immunocompromised COVID-19 patients admitted to the ICU during the Omikron period (February 2022–December 2023). Of the 1,491 patients, 29.5% were classified as immunocompromised; this group had higher in-hospital and ICU mortality. Immunocompromised patients also reported longer ICU stays (e.g., 12.2 days vs. 9.1 days). The findings suggest that even during the pandemic’s “lower mortality” variants, immunocompromised groups were able to utilize ICU resources for longer periods; therefore, surveillance, targeted containment strategies, and risk-based capacity planning (especially with regard to bed turnover rate/patient stay parameters) should be continued (Pander et al., 2025).

A single-center retrospective study in South Korea evaluated determinants associated with prolonged ICU stay (≥ 2 weeks) in 96 patients admitted to the ICU

for severe COVID-19 between March 2021 and February 2022 and receiving high-flow nasal cannula or higher oxygen therapy. Twenty-three patients had an ICU stay of ≥ 2 weeks; in multivariate analyses, mechanical ventilation requirement and day 5 P/F ratio were found to be independent predictors of prolonged stay. These results suggest that monitoring respiratory support level and oxygenation response from the early days of admission can identify patients at high risk of prolonged stay, thus allowing for more proactive design of bed management, staffing planning, and rehabilitation/separation strategies (Han et al., 2023).

A secondary analysis of a multicenter observational dataset of 537 sepsis patients in 27 intensive care units in South Korea compared closed and open intensive care units. Mortality was found to be lower in closed intensive care units, and the length of stay was also reported to be significantly shorter: the median was 13 days in closed units and 18 days in open units. Although sepsis bundle compliance rates were similar, the reduction in patient length of stay and mortality suggests that the organizational model (high-intensity/expert-led care) can reduce length of stay by influencing clinical workflow and decision-making speed. The study suggests that not only clinical protocols but also organizational design can be considered as a leverage for reducing patient length of stay in intensive care service delivery (Jeon et al., 2025).

Situation in Türkiye: National Standards and Research

The current legislation and guidelines published by the Ministry of Health of the Republic of Turkey do not contain a specific standard or legal regulation regarding patient length of stay in intensive care units. For example, the "Private Hospitals Regulation" focuses more on general operational standards of hospitals such as architecture, personnel, and licensing (Republic of Turkey Ministry of Health, 2025). Similarly, the Turkish Intensive Care Society, in parallel with international standards, publishes guidelines for clinical processes such as sepsis management instead of directly defining patient length of stay (Turkish Intensive Care Society, 2022; Turkish Intensive Care Society, 2019).

The only relevant regulation is the Emergency Department Circular, which states that the observation period in the emergency department should not exceed 2 hours (Republic of Turkey Ministry of Health, 2023). This can be interpreted as an effort to optimize patient flow between the ICU and the emergency department, but it is not a direct regulation regarding the length of stay in the ICU.

Research in Turkey focuses on a more specific patient population compared to international averages. A retrospective study conducted in a tertiary ICU in Ankara examined patients who stayed for 30 days or more. The median length of stay for this patient group was found to be 45 days, with the longest stay recorded at 328 days. In the study, the most frequent reason for admission was respiratory

failure (38.7%), and the mortality rate in this patient group was found to be as high as 51.20% (Cankar Dal et al., 2022).

Another national study found that the mortality rate from Ventilator-Associated Pneumonia (VAP) increased to 58% in patients who stayed in intensive care for more than 7 days, and to 75% in those who stayed for more than 10 days. This finding emphasizes that prolonged stay is not only a cost issue but also a serious mortality risk factor (Arısoy, Demirkıran, Günbatır, Ekin, & Sertoğullarından, 2013).

A retrospective cohort study examining the impact of delayed ICU admission under overcrowded conditions in intensive care units (ICUs) analyzed 273 adult patients admitted to the ICU of a university hospital in 2019. The rate of delayed admission, where the time from ICU consultation to transfer to the ICU exceeded 6 hours, was 72.8%. While the study did not demonstrate that delayed admission independently increased mortality in multivariate models, an association was reported between delay and prolonged ICU stay and adverse long-term outcomes; furthermore, it was emphasized that transfer delay was associated with a heavier disease burden (APACHE-II, Charlson). The authors suggest that, in terms of reducing the length of stay in the intensive care unit, not only the rapid admission indicator but also the combined assessment of the initial severity of the disease and resource limitations in triage decisions, and process improvements (flow/transfer protocols, bed management) are critical (Aydemir et al., 2025).

In contrast to international averages, a significant portion of research conducted in Turkey highlights the high mortality and resource concentration in national ICUs by specifically examining this "prolonged stay" subgroup in detail (Çevik & Geyik, 2019; Sabaz & Aşar, 2021; Cankar Dal et al., 2022). For example, in a large data analysis conducted at a tertiary center in Istanbul, patients who stayed in the ICU for ≥ 14 days constituted 18.7% of all admissions, but consumed 59.7% of total ICU stay days and 66.3% of mechanical ventilator days; furthermore, ICU mortality was found to be higher in this group (Sabaz & Aşar, 2021). Similarly, in a study evaluating patients hospitalized for ≥ 30 days in a tertiary ICU in Ankara, the median length of stay was 45 days (30–328) and mortality was reported as 51.2% (Cankar Dal et al., 2022). These findings highlight the strategic importance of strengthening the transition of care to appropriate levels (high dependency/intermediate care units, palliative care, home care, and rehabilitation services) in a healthcare system operating with limited resources (Çevik & Geyik, 2019). Indeed, structured planning of post-critical illness rehabilitation and referral to appropriate services after discharge have the potential to improve patient outcomes, reduce readmissions, and decrease hospital/ICU stay durations (National Institute for Health and Clinical Excellence, 2009).

Factors Affecting Length of Stay

Clinical and Patient-Related Factors

The main factors determining the length of stay are the patient's initial disease severity (e.g., APACHE-II), the underlying clinical diagnosis/complication profile, and the advanced life support treatments applied (Siddiqui, Ahmed, & Manasia, 2005; Çevik & Geyik, 2019). In large-scale analyses, especially in trauma patients, it has been shown that the strongest clinical determinants that prolong the length of stay in the intensive care unit are renal failure (+8.1 days), sepsis (+7.8 days), and respiratory failure (+4.9 days); in addition, treatment requirements such as massive transfusion (+3.3 days) and invasive mechanical ventilation (+3.1 days) have been shown to significantly increase the length of stay (Böhmer et al., 2014).

Similarly, it has been reported that sepsis upon presentation, need for mechanical ventilation, renal replacement therapy, and high APACHE-II scores are risk indicators for prolonged stay in surgical intensive care patients; and that the length of stay increases as the clinical condition worsens (Arslan, Altunbay, & Şahin, 2024).

Furthermore, a study based on data from tertiary-level mixed ICUs in Turkey reported that high APACHE-II scores, mechanical ventilation duration, need for renal replacement therapy, and the need for invasive procedures/treatments such as tracheotomy were associated with prolonged stay (Çevik & Geyik, 2019).

While age can be a significant predictor of prolonged stay among demographic variables, some national data have shown that gender does not make a statistically significant difference in prolonged stay in the intensive care unit (Çevik & Geyik, 2019).

In the study conducted by Haddad et al. (2001) in the adult medical-surgical intensive care unit of King Fahad National Guard Hospital in Riyadh, Saudi Arabia, the factors that contributed to the prolonged length of stay in the intensive care unit were non-emergency/non-elective admission, readmission, admission due to respiratory or trauma reasons, and signs of infection in the first 24 hours, oliguria, coagulopathy, and the need for mechanical ventilation or vasopressors; furthermore, APACHE II and SAPS II scores were found to be somewhat higher in the prolonged stay group.

Institutional and Managerial Factors

The organizational structure and managerial strategies of intensive care units directly affect patient length of stay. Critical care nursing workforce standards published in many countries emphasize that nurse/patient ratios of 1:1 for level 3 patients and 1:2 for level 2 patients are a fundamental threshold for minimum safe care (UK Critical Care Nursing Alliance [UKCCNA], 2024). Furthermore,

systematic reviews and meta-analyses support the finding that increasing the number of nurses in ICUs can reduce mortality and be associated with shorter length of stay (Kane, Shamliyan, Mueller, Duval, & Wilt, 2007).

Closed ICU models (where care is provided by a single team led by a specialist in intensive care) have been shown to have the potential to improve patient outcomes and, in particular, shorten ICU stay; systematic reviews and meta-analyses report that ICU mortality and ICU stay can be better in closed ICU formats compared to open models (Vahedian-Azimi et al., 2021). Similarly, studies in surgical intensive care samples have reported that switching to a closed model may be associated with improvements in clinical outcomes (such as mortality and/or length of stay) (Chittawatanarat & Pamorsinlapathum, 2009).

Preventing hospital-acquired infections plays a vital role in shortening patient length of stay (CDC, 2002). In particular, the use of invasive devices (catheters, mechanical ventilation, etc.) in intensive care units can increase the risk of healthcare-associated infections, and these infections can create a significant morbidity and mortality burden (Vincent, 2003). Therefore, measures such as hand hygiene, the use of disposable medical supplies, environmental cleaning, and standardized infection control practices are among the fundamental strategies; the WHO indicates in its guidelines that hand hygiene is a critical practice in reducing healthcare-associated infections (WHO, 2009). For example, ventilator-associated pneumonia (VAP) is a significant complication in intensive care patients and is associated with adverse outcomes (Mumtaz et al., 2023); similarly, central catheter-related bloodstream infections are also associated with poor clinical outcomes and additional burden (Elangovan et al., 2024).

The relationship between factors affecting length of stay often functions more as a “vicious cycle” than a one-way cause-and-effect chain: a severe initial clinical picture increases the need for invasive procedures; invasive procedures and intensive care conditions increase the risk of infection/complications; developing complications prolong the length of stay; and a prolonged stay further increases the likelihood of new complications (Vincent, 2003). Treating the underlying disease alone is not sufficient to break this cycle. Multi-component approaches such as routine screening and prevention of delirium, optimization of sedation, and early mobilization are recommended for adult ICU patients (Devlin et al., 2018). The ICU Liberation (A–F) package, one of the frameworks for implementing this approach, aims to improve patient outcomes by standardizing care processes in accordance with PADIS principles (Society of Critical Care Medicine [SCCM], n.d.). Furthermore, standardizing appropriate enteral nutrition and nutritional therapy in the early stages is among the important components of quality of care in critically ill patients and is addressed in detail in guidelines (McClave et al., 2016; Singer et al., 2019).

Clinical and Economic Consequences of Length of Stay

Effects on Survival and Mortality

Prolonged length of stay in the intensive care unit has been associated with higher short/long-term mortality in many cohort studies; in particular, it has been shown that the risk of 1-year mortality increases with the number of days spent in the ICU in elderly ICU patients who are discharged (Moitra, Guerra, Linde-Zwirble, & Wunsch, 2016).

Similarly, a population-based Canadian analysis reported that in-hospital mortality was 19.0%, while 6-month mortality rose to 21.7%; the mean ICU stay was 3.96 days, and readmission to the hospital (41%) and readmission to the ICU (10%) remained significantly high in the post-discharge period (Garland, Olafson, Ramsey, Yogendran, & Fransoo, 2015).

These findings indicate that the effects of critical care are significant not only in the hospital but also post-discharge, influencing patient outcomes and healthcare utilization.

Healthcare Costs and Resource Utilization

In terms of healthcare costs, ICUs are among the most expensive hospital units due to their high technology, intensive workforce, and invasive support requirements (Dasta, McLaughlin, Mody, & Piech, 2005).

Total costs increase with longer ICU stays; resource-intensive interventions such as mechanical ventilation significantly contribute to the increase in daily costs (Dasta et al., 2005).

Direct cost analysis conducted using standardized methods across different European countries has reported the direct cost of a day in the ICU to range from €1168 to €2025 (Tan et al., 2012). Furthermore, the distribution of resource utilization is uneven: a small group of patients staying in the ICU for >7 days has been shown to consume more than 50% of the bed-day and care workload (e.g., nursing/treatment intensity) (Stricker, Rothen, & Takala, 2003). Therefore, initiatives aimed at optimizing patient length of stay are not only a clinical necessity but also an economic one (Dasta et al., 2005; Stricker et al., 2003).

Institutional and managerial arrangements can also affect patient length of stay and outcomes. Systematic reviews and meta-analyses comparing open and closed ICU models report that closed-format (intensivist-led) ICUs may be associated with better performance in some outcomes (e.g., ICU mortality and ICU length of stay) (Vahedian-Azimi et al., 2021). In this context, ICU length of stay should be evaluated as a multidimensional performance indicator intertwined with mortality, cost, and resource utilization (Moitra et al., 2016; Tan et al., 2012; Vahedian-Azimi et al., 2021).

Conclusion

Patient length of stay in intensive care units is not simply an answer to the question "how many days were spent in the hospital?"; it is a critical performance indicator shaped by the patient's initial clinical severity, diagnostic and complication burden, invasive supportive treatments applied, and the organizational capacity of the institution. Therefore, length of stay should be considered a fundamental measure in monitoring resource utilization and service quality as well as clinical outcomes.

As highlighted in this section, ICU length of stay data often exhibit skewed distributions; mean values can be significantly affected by a small number of "extra-long-stayed" patients. This methodological problem necessitates the use of sophisticated approaches, such as mixed distribution models, and appropriate data preprocessing techniques to more accurately model length of stay.

Furthermore, the lack of a universally accepted "standard day" threshold for ICU length of stay suggests that length of stay should not be considered merely a target number, but rather an outcome variable reflecting the success of evidence-based clinical processes (sepsis management, delirium management, etc.).

The most critical finding from a clinical and economic perspective is that while a significant proportion of ICU patients have short stays, a small group of patients with prolonged stays places a disproportionate burden on bed-days and care workload. This situation must be considered in conjunction with increased costs, increased exposure to hospital-acquired infections, and adverse clinical outcomes.

Indeed, the relationship between infections, complications, and length of stay often follows a "vicious cycle": severe clinical conditions increase the need for invasive procedures, invasive procedures increase the risk of infection/complications, developing complications prolong the length of stay, and a prolonged stay makes new complications more likely.

Therefore, strategies to improve length of stay should focus not only on reducing the number of days in hospital, but also on reducing the underlying causes that prolong the stay (such as infection prevention, early diagnosis and management of delirium, early mobilization, and nutritional support).

The findings in Turkey highlight the association between the clinical burden and high mortality of the "prolonged stay" patient group, exceeding international averages. This situation points to a systemic problem that needs to be addressed not only through increasing the number of beds at the national level, but also through patient flow management, strengthening intermediate care/rehabilitation/palliative care options, and planning for continuity of post-discharge care.

Finally, the finding that ICUs are among the most expensive units within the healthcare system, that total costs increase with length of stay, and that direct daily costs are reported across a wide range in Europe, reinforces the "economic necessity" dimension of length of stay.

In this context, improvements at both clinical and managerial levels (e.g., adequate nursing workforce planning, transition to a closed ICU model, standard infection control practices, net turnover/transfer criteria) have the potential not only to reduce mortality but also to optimize resource utilization and strengthen sustainability.

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CHAPTER 19

Cost Awareness Among Doctors and Nurses

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1. Introduction

Healthcare services, as in the rest of the world, constitute a significant pressure on economic systems in Türkiye. Technological advancements, an aging population, the increasing burden of chronic diseases, and rising patient expectations make it difficult to control healthcare expenditures (Zhou, Ampon-Wireko, Antwi, Xu, Salman, & Antwi, 2020).

In Turkey specifically, the share of healthcare expenditures in GDP and the out-of-pocket payments individuals have to make constitute a significant financial burden (Sülkü & Caner, 2012; Çınaroğlu, 2024). This economic picture reveals the reality of scarce resources and the necessity of using these resources in the most efficient way.

At the heart of this complex equation are clinicians—physicians and nurses—who make countless decisions in their daily practice. Decisions such as which tests to order, which treatments to administer, which materials to use, and how long the patient will stay in the hospital have a direct and decisive impact on the total cost (Nethathe, 2015). However, clinical education and practice processes often disregard these economic dimensions, prioritizing only medical accuracy and patient safety.

Studies have shown that healthcare professionals often have inadequate knowledge of the financial consequences of their decisions. For example, Abuhanoğlu et al. (2013), when examining physicians' attitudes towards cost-effectiveness, reported deficiencies related to shortcomings. Similarly, another study conducted on surgical teams revealed a significant lack of knowledge regarding common expenditures used in the operating room (Sorber et al., 2020). In this case, it is seen that cost loss is not only an individual knowledge gap but also a systemic problem.

A lack of cost awareness can lead to two main negative consequences: waste of resources and defensive medicine practices. Unnecessary expensive tests or ineffective treatments result in wasted resources (Hussey, Wertheimer, & Mehrotra, 2013), while defensive medicine practices implemented by physicians due to legal concerns are also a significant factor increasing costs (Kılıçarslan and Narıcı, 2022; Özer and Aslan, 2022).

This study aims to fill a critical gap at this very point. Cost awareness among physicians and nurses aims to build a bridge between the abstract world of health economics and the concrete realities of the clinic. By synthesizing current literature in Türkiye and worldwide, this study aims to show physicians and nurses what cost awareness is, why it is important, and how they can make cost-effective decisions while providing value-based care. Therefore, this study is a guide that aims not to cut costs, but to produce the highest health value with limited resources.

2. Cost Dynamics in the Turkish Healthcare System

Healthcare expenditures are a source of concern at both national and global levels, and every country develops various cost control policies to ensure the sustainability of its healthcare system. Turkey is no exception to this process. The Healthcare Transformation Program, implemented particularly since the 2000s, has fundamentally changed access to healthcare services, but it has also reshaped cost dynamics. This section will examine the key cost dynamics in the Turkish healthcare system, current policies, and the specific factors influencing these dynamics.

A systematic review of effective cost control policies shows that countries generally use a combination of strategies such as budget caps, price controls, regulation of reimbursement mechanisms, and promotion of market competition (Stadhouders, Kruse, Tanke, Koolman, & Jeurissen, 2019). Türkiye's healthcare system also uses a mixed model incorporating such mechanisms. However, for these policies to be implemented on the most rational basis, it is necessary to know which healthcare technology (drug, device, procedure) is cost-effective.

At this point, "Health Technology Assessment" (HTA) plays a critical role. HTA is a process that systematically analyzes the clinical and economic value of new and existing health technologies. An analysis by Atikeler, Fasseeh, and Mantel-Teeuwisse (2023) examines the current state of HTA in Türkiye and its future application perspectives in detail. This study reveals that Türkiye is developing its HTA capacity, but that stronger integration of these assessments into reimbursement decisions is essential for the rational use of resources. Clinicians' cost awareness is precisely the practical reflection of these HTA decisions; because even if a national decision is made on which technology is valuable, the decision to use that technology where needed rests with the physician and nurse.

The macroeconomic cost dynamics of the system directly affect individuals' access to healthcare services. Increased healthcare expenditures are reflected in out-of-pocket payments (Çınaroğlu, 2024) and healthcare needs that cannot be met due to cost. A national study conducted in Türkiye by Davas and Etiler (2024) revealed gender differences in healthcare needs that cannot be met due to cost. This study shows that women may be more likely to avoid receiving healthcare services due to financial barriers, and highlights that cost is not just a budget item, but also a social equality issue.

To understand the cost structure in Türkiye, it is necessary to examine both chronic and predictable expenditure items as well as sudden systemic shocks. For example, Tatar, Ergin, and Ecdar (2013) analyzed the costs of private hemodialysis centers in Türkiye, demonstrating the continuous burden on the budget of managing high-cost chronic diseases such as chronic kidney failure. On the other hand, Öksüz et al. (2021) analyzed healthcare costs and hospital stays

during the initial peak of the COVID-19 pandemic. This study revealed the sudden and enormous financial burden created by the pandemic, showing how vulnerable the system can be to unexpected shocks. Finally, Park et al. (2022), examining the cost-effectiveness of a stress management intervention developed for Syrian refugees hosted by Türkiye, discussed the economic impacts of humanitarian crises and migration on the healthcare system and cost-effective solutions that could be developed in this area.

Therefore, the Turkish healthcare system faces multifaceted cost dynamics, including the ongoing funding needs for chronic diseases (such as dialysis), the shocks created by acute crises like COVID-19, the financial pressure of new technologies (managed with STDs), and socio-economic inequalities in access to healthcare (such as gender and refugee status). This complex picture necessitates a simultaneous consideration of cost and quality for the sustainability of the system.

3. Balance between Cost, Quality, and Value

In healthcare, a common but often misleading belief exists among both patients and professionals: "More expensive is better." It's assumed that a new drug, a cutting-edge imaging technique, or an expensive surgical instrument will always deliver superior clinical outcomes compared to cheaper alternatives. However, health economics and quality research have repeatedly shown that this relationship is not as linear and simple as commonly believed. This section will examine the complex relationship between cost and quality and discuss the concept of "value-based care," which has become a focal point of modern healthcare.

Systematic reviews examining the relationship between cost and quality clearly highlight the confusion in this area. A comprehensive systematic review by Hussey, Wertheimer, and Mehrotra (2013) analyzed studies examining the relationship between healthcare quality and cost. The findings of this review clearly demonstrated that "high cost does not guarantee high quality." While in some cases high-cost hospitals were seen to deliver better outcomes, many studies revealed that this relationship is weak, inconsistent, and even sometimes negative (i.e., higher cost may be associated with worse outcomes). Similarly, a more recent systematic review by Jamalabadi, Goudarzi, and Vogt (2020) focused on the relationship between hospital cost/price and quality of care and confirmed that this relationship remains unclear. These findings underscore the importance of distinguishing between "low-value care," which leads to resource waste, and "high-value care," which utilizes resources efficiently.

At this point, the understanding has emerged that the focus in healthcare should be on increasing value rather than reducing cost. Value-Based Healthcare (VBHC) is defined as the patient health outcome obtained per unit of cost incurred ($\text{Value} = \text{Outcome} / \text{Cost}$). In this paradigm, the goal is not to blindly

cut costs, but to deliver clinically meaningful outcomes for the patient (e.g., faster recovery, fewer complications, better quality of life) at the most reasonable cost (Stammen et al., 2015).

However, to measure value, it is necessary to accurately measure both sides of the equation, namely both outputs (quality) and costs. A systematic review by Leusder, den Exter, and Evers (2022) examined the methodologies and challenges of cost measurement in the context of value-based healthcare. This study demonstrates that it is impossible to talk about value without accurate cost data, and that cost awareness is not merely a matter of consciousness but also a technical necessity requiring accurate measurement techniques.

Furthermore, the concepts of value and cost are not limited to factors determined solely within hospital walls. Mohan and Gaskin (2024) examined the relationship between healthcare expenditures and social determinants of health (education, housing, food security, etc.) in the US. This study revealed that investments in non-health social factors can significantly reduce healthcare expenditures in the long run and increase the overall "value" of health. This demonstrates that clinicians evaluating the patient not only as a biological entity but also within their social environment is an indirect but important part of cost-effective care.

In conclusion, the balance between cost and quality is not simply a comparison of price tags. High cost does not guarantee quality (Hussey et al., 2013; Jamalabadi et al., 2020). The real goal is to achieve the best clinical outcome for the patient through the most efficient use of resources, i.e., to provide "high-value care." To achieve this goal, physicians and nurses need to understand not only the clinical effectiveness but also the economic dimension of their decisions, and to center the concept of "value" when making these decisions. The first step in doing this is to know how costs are generated and measured within the hospital.

4. Cost Management and Analysis in Healthcare Institutions

The effective management of the value equation ($\text{Value} = \text{Output} / \text{Cost}$) depends on the accurate and transparent measurement of the cost, which is the denominator of the equation. This section will examine traditional and departmental approaches to how healthcare organizations analyze and manage costs, which form the organizational basis of cost awareness.

Healthcare organizations inherently have highly complex cost structures. Numerous items, such as human resources, medical supplies, medications, depreciation for expensive equipment, laboratory services, and administrative expenses, are used in common for different patients and procedures. Fairly allocating the cost of these shared resources (overhead expenses) to a specific service or patient is the most challenging part of cost analysis.

Academic literature in Türkiye shows a growing interest in cost management in healthcare institutions to overcome this challenge. A review of Turkish literature from 2004-2021 by Çalık and Ülkü Akpınar (2022) reveals that cost analysis, cost determination systems, and budgeting in healthcare institutions are being studied more and more. This is important because it shows that cost awareness is developing at the institutional level and that efforts are being made to create an academic infrastructure for it.

A concrete example of how cost analysis is applied in practice is the study conducted by Soylular and Büyükkayıkçı (2016) in a secondary state hospital. In this study, the researchers identified the hospital's cost centers (e.g., outpatient clinics, inpatient wards, operating rooms, laboratories, etc.). Then, they allocated general and administrative expenses such as staff salaries, rent, electricity, and cleaning to these cost centers using specific allocation keys (e.g., square footage, number of personnel, amount of materials used). As a result of this analysis, the unit cost to the hospital of an outpatient examination, a surgery, or a day of inpatient treatment could be calculated. It is the first step in raising awareness for physicians and nurses to know that each service they request or each decision they make directly impacts the institutional cost determined by these detailed calculations.

Cost management involves not only identifying costs but also using this information to monitor and improve organizational performance. Yüksel (2021) conducted a study examining the relationship between departmental cost management and performance in healthcare institutions. According to this approach, each clinical department (e.g., cardiology, orthopedics, or intensive care) is treated as a unit responsible for its own budget and resource utilization. The department's performance is measured not only by the number of patients treated or clinical success rate but also by the efficiency of the resources used to achieve that success. This model combines clinical responsibility (typically carried out by a physician or head nurse) with financial responsibility, bringing cost awareness directly to the agenda of decision-makers.

In conclusion, increasing academic interest, as seen in the review by Çalık and Ülkü Akpınar (2022), traditional hospital cost analyses as seen in the practice of Soylular and Büyükkayıkçı (2016), and departmental performance management as in the study by Yüksel (2021) are vital tools for the financial sustainability of healthcare institutions.

However, these traditional methods often aggregate costs based on "departments" or "types of services." Yet patient care is a complex process moving between these departments. Traditional methods may struggle to capture the true cost of the entire process, especially including staffing and resource activities; this includes the patient's journey from outpatient clinic A to laboratory B, then to unit C (possibly radiology), and finally to ward D.

5. Activity-Based Costing (ABC) and its Application in the Clinic

For physicians and nurses to understand the financial consequences of their decisions, costs need to be tracked at the activity level. A modern approach that addresses this need is Activity-Based Costing (ABC).

Unlike traditional systems, ABC is based on the assumption that costs are consumed by the activities that make up healthcare, not by patients or procedures. Resources (staff salaries, medical supplies, depreciation) are primarily allocated to activities (patient registration, blood sampling, surgical preparation, ward rounds); then patients or procedures bear the cost of the activities they consume (Şenyurt & Torun, 2022). This methodology ensures a much fairer and more accurate distribution of overheads to each step in the patient care process.

The complex and time-intensive nature of healthcare has given rise to Time-Based Activity-Based Costing (TAB-ABC), a further developed version of ABC. TAB-ABC recognizes that the most expensive resource in healthcare is the time of physicians, nurses, and other healthcare personnel. This model calculates the cost of one minute of work for each personnel category (e.g., specialist physician, operating room nurse, technician). It then determines the cost by identifying how many minutes each staff member spends on a patient throughout the patient's care process (Martin et al., 2018).

Numerous case analyses demonstrate the clinical power of TAB-ABC. Martin et al. (2018) piloted this method as a value platform component for three different procedures: colonoscopy, aortic valve replacement, and carpal tunnel release. The researchers created detailed process maps for each procedure, recording how many minutes each staff member (physician, nurse, technician) spent at each step, from patient admission to the room, anesthesia preparation, procedure execution, and patient recovery. This allowed them to clearly identify which steps in the process were the most costly and where inefficiencies (unnecessary waiting times, incorrect staffing, etc.) were present.

Similarly, Au and Rudmik (2013) used the TAB-ABC method to examine the cost of outpatient endoscopic sinus surgery from a Canadian government perspective. This study revealed the true cost of the procedure by accounting for the time spent not only by the surgeon but also by nurses, administrative staff, and cleaning personnel. This detailed data showed clinicians which activities increased costs the most and how much savings could be achieved with a small change in the process flow (e.g., shortening room preparation time).

Another example is the TAB-ABC analysis for cataract surgery conducted by Sadri et al. (2021). High-volume and standardized procedures like cataract surgery are ideal for TAB-ABC application. By measuring staff time at different steps of the process, the study revealed which points should be focused on to increase efficiency and reduce costs.

These applications demonstrate why TAB-ABC is a critical tool for physicians and nurses. While traditional costing tells the clinician, "Your department spent a lot this month," TAB-ABC can say, "The cost increased by X lira because preparing the patient for this procedure took 25 minutes instead of 10." This level of transparency empowers clinicians not only to be aware of costs but also to directly influence process improvements that impact costs. The question is no longer just how much is spent, but how time and resources are spent.

6. Cost Awareness and Attitudes Among Physicians

At the heart of all systems is an actor who decides on the use of the vast majority of resources: the physician. Decisions such as prescribing medication, ordering laboratory tests, choosing an imaging method, planning a surgical procedure, or determining a patient's length of hospital stay are all made by the physician. Therefore, the cost awareness that physicians possess when making these decisions has a direct impact on the overall cost and efficiency of the system. This section will examine physicians' levels of cost awareness, their attitudes towards cost-effectiveness, and the factors influencing these attitudes, in light of studies in both Turkish and international literature.

Research consistently shows that physicians often lack sufficient knowledge about the economic consequences of their decisions. A cross-sectional study among physicians in Jordan revealed varying levels of cost awareness and significant knowledge gaps (Yasin et al., 2022). Similarly, a multicenter observational study among intensive care specialists in France found that physicians had low awareness of the costs of common treatments and procedures used in daily clinical practice (Lehut et al., 2024).

This situation can be even more pronounced in surgical specialties. A study by Sorber et al. (2020) showed that the entire surgical team, including not only surgeons but also operating room nurses and technicians, has a severely inadequate awareness of the costs of commonly used surgical equipment. Interestingly, the same study also revealed that despite this lack of information, the teams have a strong desire to learn more about costs and increase their awareness. This finding points to the problem being more of a training and access to information issue rather than a matter of indifference or carelessness.

A study conducted specifically in Turkey offers an opportunity to analyze attitudes and behaviors regarding this issue in greater depth. Abuhanoğlu et al. (2013) evaluated physicians' attitudes and behaviors regarding cost-effectiveness during medical procedures. This study showed that physicians theoretically consider the concept of cost-effectiveness important, but have difficulty reflecting this knowledge in their practical decisions. Physicians may believe that their primary responsibility is to meet the patient's medical needs and that cost should be secondary in this decision. This situation also brings with it ethical

dilemmas: Is the physician responsible only to the patient in front of them or to the entire society (in terms of managing scarce resources)?

Factors influencing physicians' awareness levels and attitudes are also being increasingly researched. Nethathe (2015), while examining cost awareness among healthcare professionals in South Africa, highlighted deficiencies in economics education and the system's failure to encourage physicians to act cost-consciously. A more recent European study added a different dimension to the issue. Lüdemann, Schreyögg, and Wübker (2024) examined human capital (education, experience) and stress levels as factors influencing cost awareness in medical personnel. This study suggests that high stress levels can negatively impact physicians' ability to make rational and cost-effective decisions. A physician under high stress may opt for unnecessary tests to be sure or lose the capacity to consider secondary factors such as cost.

In conclusion, physicians are key decision-makers in healthcare spending. However, studies both in Türkiye and internationally show that physicians in this critical role generally have low cost awareness. This deficiency stems from inadequacies in medical education (Nethathe, 2015), lack of systemic incentives, high stress levels (Lüdemann et al., 2024), and lack of transparent access to cost information (Sorber et al., 2020). However, the success of cost management in healthcare does not depend solely on physicians. The role of nurses, who are an integral part of the care process and are involved in many critical points from material management to discharge planning, is at least as important as that of physicians.

7. The Critical Role of Nurses in Health Economics

Discussions on cost and resource management in healthcare often focus on physicians, who are at the center of decision-making (Abuhanoğlu et al., 2013; Yasin et al., 2022). However, healthcare is a team effort, and at the heart of this team are nurses, who are the 24/7 implementers and coordinators of the patient care process. As healthcare professionals who have the longest contact with patients, nurses have a tremendous impact on the efficient use of resources. This section will examine the critical, yet often overlooked, role of nurses in health economics.

In her review examining the relationship between nursing and health economics, Çınar (2019) clearly demonstrates that the role of nurses is not only to provide care but also to manage resources. Nurses directly influence costs in multiple areas:

- a. **Direct Material Use:** Nurses are the ones who directly decide on and implement the use of medical supplies (syringes, gloves, dressings, catheters). The decision of which material to use, in what quantity, and how often, while seemingly small in terms of unit costs,

represents a huge share of the overall hospital budget. Unnecessary material use or waste directly leads to increased costs (Çınar, 2019).

- b. ***Efficiency of Care Processes:*** Nurses' care planning and implementation directly affect the patient's length of hospital stay. For example, effective wound care shortens the patient's stay by preventing infections (nosocomial infections) and avoids additional antibiotic costs. Similarly, effective discharge planning and patient education reduce the burden on the system by preventing the patient from returning to the hospital after going home (Çınar, 2019).
- c. ***Human Resource (Time) Management:*** Time-Based Activity-Based Costing (TAB-APC), which we discussed in previous sections (Martin et al., 2018), showed that staff time is the most valuable resource. The efficiency of a nurse's workflow affects not only their own time but also the time of the physician and other staff. For example, a nurse who prepares materials in advance and completely for a procedure prevents the entire team (and an expensive operating room) from wasting time.

Studies showing low cost awareness among physicians are unfortunately also true for nurses and other team members. A study conducted by Sorber et al. (2020), which included the entire surgical team (surgeons, nurses, technicians), strikingly illustrates this. The study found that nurses lacked as much, or sometimes even more, knowledge than surgeons regarding the cost of commonly used materials in the operating room (e.g., a specific suture material, a stapler, an energy device).

This lack of information is a critical problem because nurses are often the intermediaries who request supplies and manage inventories. Using a material without knowing its cost, simply out of habit or preference, can lead to waste when a cheaper and clinically equivalent alternative exists. A key finding in Sorber et al.'s (2020) study is that, like physicians, nurses and technicians also have a strong desire to learn about costs. This indicates that there is room for behavioral change if information is provided.

In conclusion, nurses are like invisible catalysts in the health economy. The micro-level daily decisions they make (using a glove, changing a dressing, educating a patient) directly affect the financial health of the hospital and the sustainability of the system at the macro level (Çınar, 2019). Cost awareness training should be directed not only at physicians but at the entire multidisciplinary team, especially nurses. However, one of the biggest obstacles to clinicians (physicians and nurses) making rational and cost-effective decisions is not only a lack of information but also the legal concerns created by the system.

8. Defensive Medicine: Fear, Cost, and Ethics

In previous sections, we saw that a lack of cost awareness can increase costs through unnecessary material use or inefficient processes. However, ignorance is not the only factor that increases costs. Sometimes, even when physicians are aware of the cost, they deliberately overuse resources. The underlying motivation for this behavior is not medical necessity, but fear. This phenomenon is called "defensive medicine."

Defensive medicine is the set of decisions and practices where physicians prioritize their own legal safety over the patient's medical benefit in order to protect themselves from a potential malpractice lawsuit or complaint (Kılıçarslan and Narcı, 2022).

These practices place a significant burden on healthcare systems, both clinically and economically. Defensive medicine manifests itself in two main ways (Kılıçarslan and Narcı, 2022):

a. Positive Defensive Medicine (Assurance Behavior): This is when a physician requests extra tests or consultations, prescribes unnecessary medications, or hospitalizes a patient even when the medical indication is weak or unnecessary, simply to avoid missing anything or to prove they have done everything. This is the most common and costly type of defensive medicine.

b. Negative Defensive Medicine (Avoidance Behavior): This is when a physician avoids treating high-risk patients or procedures that they believe carry a high risk of complications or are likely to be subject to litigation. This hinders a patient's access to appropriate treatment, leading to indirect costs and serious ethical problems.

A multicenter observational study by Kılıçarslan and Narcı (2022) examined physicians' awareness levels and attitudes towards defensive medicine. The study revealed that a significant portion of physicians were aware of such practices and that malpractice concerns directly influenced their decisions. Physicians feel the need to protect themselves due to increasing patient expectations, erosion of trust in the patient-physician relationship, and the pressure created by legal processes.

The economic cost of these behaviors is enormous. A retrospective study by Özer and Aslan (2022) focused precisely on this issue, aiming to determine the cost of defensive medicine practices. This study retrospectively calculated only the cost of positive defensive medicine practices (unnecessary laboratory tests, radiological imaging, consultations, etc.). The findings showed that these extra procedures performed out of defensive concerns constitute a significant share of total healthcare expenditures.

Defensive medicine directly contradicts the concepts of cost awareness and value-based care, which are the main themes of this study. When a physician

makes a positive defensive decision, they are actually opting for low-value care (Hussey et al., 2013). That is, they are making a decision that provides little (or no) clinical benefit to the patient, but imposes a cost on the system. Moreover, these unnecessary interventions are not only costly but also harmless. An unnecessary invasive procedure has the potential to directly harm the patient by leading to complications, an unnecessary radiological imaging by causing radiation exposure, or further investigation of an incidentally discovered, insignificant finding.

In conclusion, while we strive to increase cost awareness among physicians and nurses, we cannot ignore the systemic issues (legal pressures, stress, lack of trust) that push them towards defensive medicine (Lüdemann et al., 2024). Simply saying "think only of cost" can put the physician in a dilemma between "the patient's benefit" and "their own legal security." Therefore, cost management must be addressed in conjunction with a fair legal framework, a strong patient-physician relationship, and the robust application of evidence-based medicine guidelines.

9. Cost Comparisons in Surgical Branches

Surgery is one of the areas where hospital costs are highest, combining expensive technologies, disposable materials, and a large human resource base (surgeons, anesthesiologists, nursing staff). Decisions made in this area have a huge impact on cost-effectiveness.

The study by Sorber et al. (2020), cited in previous sections, clearly demonstrated how critical clinician awareness is: the entire operating room team (surgeons, nurses, technicians) was largely unaware of the costs of the common materials they used. This section will show what this lack of awareness means, through specific surgical comparisons.

a. Traditional Method and Minimally Invasive: A Laparoscopy Example

One of the classic comparisons in health economics discussions is between open surgery and laparoscopic (minimally invasive) surgery. Keskin (2005) directly poses this question under the title "Is laparoscopic cholecystectomy cheaper?". At first glance, laparoscopic surgery appears more expensive: it requires more complex equipment, specially trained personnel, and sometimes a longer operating time. However, cost analysis is not limited to the money spent in the operating room. Laparoscopic surgery generally results in less post-operative pain, shorter hospital stays, and a faster return to work for the patient. Keskin (2005) emphasizes that these factors change the equation when calculating the total cost (direct costs + indirect/social costs).

A recent example from Türkiye is the study by Aydoğdu et al. (2019). This study compared laparoscopic and open appendectomy in terms of both clinical outcomes and cost. While the researchers found no significant difference in clinical success between the two methods, they observed differences in cost analysis. Such studies convey the message to the physician: If two methods yield similar clinical results, choosing the more cost-effective one is a requirement for providing high-value care.

b. High-Volume Procedures and Package Payment Models: Orthopedics

One of the areas most focused on by cost-effectiveness analysis is joint replacement surgeries (hip, knee) in orthopedics. These surgeries are high-volume, standardized, and high-cost procedures (due to the cost of the prosthesis). Navathe et al. (2017) examined joint replacement costs within the scope of package payment models.

A bundled payment, unlike the traditional pay-per-service model, involves a single, predetermined fee paid to the institution for the patient's entire care process (preoperative assessment, surgery, hospital stay, post-discharge physical therapy). This model shifts the financial risk from the insurance company to the healthcare institution and, consequently, to the clinicians. Navathe et al. (2017) demonstrated that this model forces institutions to increase efficiency to reduce costs (e.g., standardization of materials, optimization of the discharge process, prevention of complications). In this system, cost awareness becomes a financial necessity, not a preference. The surgeon's choice of prosthesis and how well the nurse prepares the patient for discharge directly impact the department's budget.

c. The Peak of Cost: Intensive Care Unit (ICU)

Surgical departments frequently transfer patients to intensive care units (ICUs), especially after major and complex surgeries. ICUs are the most expensive departments in a hospital. A multicenter observational study by Lehut et al. (2024) examined the cost awareness of intensive care physicians in their daily clinical practice. This study confirmed that the general lack of physician awareness discussed in our study is also valid in this most costly area. Every decision made by an intensive care physician (which ventilator setting to choose, which antibiotic to start, which blood tests to order daily) places a significant cost burden on the system.

In conclusion, whether it's a common procedure like appendectomy (Aydoğdu et al., 2019) or cholecystectomy (Keskin, 2005), a high-cost surgery like joint replacement (Navathe et al., 2017), or the post-operative intensive care process (Lehut et al., 2024), every decision in surgical specialties has an economic

consequence. It is essential for the sustainability of the system that these decisions are based not only on clinical rationality but also on economic rationality. This situation is not unique to surgery.

10. Cost-Effectiveness in Internal Medicine and Interventional Procedures

Cost discussions, as in the surgical specialties examined in the previous section, generally revolve around high-tech operating rooms and expensive implants (Navathe et al., 2017). However, a very large portion of healthcare expenditure is driven by chronic diseases (such as dialysis) managed by internal medicine (Tatar et al., 2013), expensive drug treatments, and increasingly frequent interventional procedures. Decisions made outside the operating room play a crucial role in the economic sustainability of the system, at least as much as surgical decisions.

This section will examine two critical cost-effectiveness dynamics in the field of internal medicine and interventional procedures: a comparison of high-tech interventions and the economic value of preventive medicine/screening programs.

a. Interventional Procedures: The Dilemma of High Technology, High Cost, and Value

Perhaps the most studied area in cost-effectiveness analysis within internal medicine is interventional cardiology. In particular, the cost-effectiveness comparison between Percutaneous Coronary Intervention (PCI, i.e., stent placement) and Coronary Artery Bypass Grafting (CABG, i.e., open-heart surgery), two fundamental approaches to treating coronary artery disease, is a classic decision point for clinicians.

A significant study by Magnuson et al. (2022) compared the cost-effectiveness of PCI and CABG in patients with multivessel disease. Such analyses demonstrate the complexity of the decision-making process. PCI is less invasive, has a shorter hospital stay, and generally has a lower initial cost. However, the long-term durability of CABG (less need for repeat interventions) may be better in some patient groups. The findings of Magnuson et al. (2022) provide evidence showing which strategy is valuable for which patient subgroup. Here, the cost awareness of the cardiologist and cardiovascular surgeon is not just about knowing the price tag of the two procedures, but about being able to choose the treatment of highest value based on the results of such long-term cost-effectiveness studies.

Another dimension of cost is not the procedure itself, but the subsequent complications and readmissions. Kwok et al. (2021) examined the costs of coronary syndrome patients treated with PCI and the economic burden of unplanned readmissions over 30 days. This study revealed that unplanned readmissions create a huge financial burden on the system. This finding shows how much cost awareness depends not only on the physician but also on the nurse. The quality of the nurse's post-discharge education (medication adherence, lifestyle changes) directly affects these readmissions and therefore the total cost, as much as the technical success of the physician's procedure. Quality care is cost-effective care.

b. Preventive Medicine

The impact of internal medicine on costs is not limited to high-tech interventions. Preventive medicine and screening programs lie on the other side of the value equation. These programs impose a cost on the system today (e.g., the cost of screening tests), but in the future they provide enormous savings by preventing the treatment of advanced-stage diseases, which would otherwise be far more expensive.

Fisher et al. (2022) examined the healthcare costs of colorectal cancer screening and events detected during this screening (e.g., polyp removal or early-stage cancer treatment). This study shows that catching the disease in the early stages through screening or treating precancerous lesions (polyps) is far more cost-effective than the complicated and extremely expensive treatment (chemotherapy, surgery, immunotherapy) of advanced-stage colorectal cancer.

Here, the physician's cost awareness manifests itself differently. Their role is not to cut costs, but to ensure spending is done in the right place, in accordance with evidence-based guidelines. Just as ordering unnecessary screenings (e.g., in a low-risk young patient) out of defensive medicine concerns constitutes low-value care, so too does failing to recommend screening for a high-risk patient due to a lack of time or awareness (because it opens the door to higher costs in the future).

In conclusion, physicians and nurses in internal medicine departments face cost decisions that are different from, but at least as critical as, those of surgeons. These decisions require choosing between high-tech interventions (Magnuson et al., 2022), balancing short-term costs against long-term complication costs (Kwok et al., 2021), and weighing today's preventive costs against future treatment costs (Fisher et al., 2022).

11. Cost-Conscious Maintenance Training

Although physicians and nurses are key decision-makers in healthcare spending, they often lack significant awareness and knowledge about the financial consequences of their decisions (Abuhanoğlu et al., 2013; Yasin et al., 2022; Lehut et al., 2024). This deficiency is even widespread, encompassing the entire operating room team (Sorber et al., 2020). One of the primary reasons for this is not clinician indifference, but a fundamental deficiency in medical and nursing education curricula (Nethathe, 2015). Healthcare professionals are trained to do what is clinically correct, but they do not receive systematic training to choose what is valuable, i.e., what is cost-effective.

This section will examine education as the most fundamental and sustainable way to close the cost awareness gap. But can cost-conscious care be taught? A comprehensive systematic review published in JAMA by Stammen, Stalmeijer, Paternotte et al. (2015) provides a clear answer to this question. This review analyzed interventions aimed at educating physicians to provide high-value, cost-conscious care. The study's findings reveal that this education is possible and that certain strategies are more effective than others.

According to Stammen et al. (2015), successful educational interventions are not limited to abstract health economics courses taught in medical school lecture halls. The most effective methods are practical and actionable strategies integrated into clinicians' daily workflows. Some of these strategies include:

a. *Cost Transparency and Feedback:* A primary reason for physicians' low cost awareness (Sorber et al., 2020) is their lack of access to information. Successful interventions include systems that show clinicians the true costs of the tests they order, the medications they prescribe, or the materials they use, preferably at the time of decision (e.g., via an electronic health record system). In addition, providing physicians with regular, confidential, and non-punitive audit and feedback reports that compare their resource usage habits (e.g., frequency of ordering an MRI for a particular diagnosis) to the averages of their colleagues is one of the most powerful tools for creating behavioral change (Stammen et al., 2015).

b. *Integration into Clinical Case Discussions:* Cost awareness should move beyond being a separate course and become an integral part of existing education. Case discussions, morning meetings, or morbidity-mortality boards, where questions such as "What was the contribution (value) of the third CT scan requested for this patient's treatment?" or "Would a lower-cost antibiotic option have achieved the same clinical outcome?" are asked, make cost-effective thinking a standard part of clinical reasoning.

c. *Clinical Guidelines and Health Technology Assessment (HTA):*

Education should include not only individual awareness but also the use of systemic tools. The results of developing HTA reports in Türkiye (Atikeler et al., 2023) need to be integrated into national and institutional treatment guidelines. Clinicians can use these guidelines to make evidence-based decisions about which technology is cost-effective.

These educational interventions should go beyond the review by Stammen et al. (2015), which focused solely on physicians, and encompass the entire multidisciplinary team. Given the critical role of nurses in health economics (Çınar, 2019), nursing curricula must also include topics such as material management, resource planning, and the impact of discharge education (by preventing readmissions) on costs. The overall lack of knowledge among surgical teams (Sorber et al., 2020) indicates that the solution lies in interprofessional training.

In conclusion, cost awareness is not an innate trait, but a learned skill. Teaching physicians and nurses that resources are scarce and that they are custodians of those resources should be presented not as a form of restriction or rationing, but as part of their professional responsibility. Education is the primary tool for achieving this change in mindset. However, even an educated clinician cannot be expected to succeed alone in a system that pushes them towards defensive medicine or operates inefficiently. Education is the first step in individual responsibility; but the final step is the transformation of the system itself.

12. Conclusion

In light of the information presented, the solution to the lack of cost awareness lies not in blaming a single actor, but in creating a sense of "shared responsibility."

a. *The Responsibility of Physicians and Nurses:* The clinician's responsibility is not to prioritize financial gain over the patient's well-being. Responsibility means adopting the role of resource custodian. This means choosing not the lowest cost option, but the one that has proven cost-effectiveness, i.e., the one with high value (Stammen et al., 2015). This means protecting the patient from potential harm by not ordering unnecessary tests (avoiding defensive medicine). This means the nurse preventing costly readmissions through effective discharge education (Kwok et al., 2021). This means being open to learning and developing cost awareness as a skill. The clinicians' desire to know the costs (Sorber et al., 2020) shows their readiness to assume this responsibility.

b. Responsibility of the System: However, leaving physicians and nurses to bear this responsibility alone is unfair. Clinicians may make inefficient decisions not because they fail to act cost-effectively, but because they work within a system that prevents or hinders them from doing so. The system's (health authority, hospital management, educational institutions) responsibility is threefold:

Educating: Making cost-conscious care (Stammen et al., 2015) and the fundamentals of health economics an integral part of the curriculum, starting from medical and nursing schools.

Ensuring Transparency: Providing clinicians with the cost data they need to know (Sorber et al., 2020) at the time of decision-making. Making it clear how much each procedure costs through methods like FTM (First Aid and Testing).

Providing Assurance: Establishing a fair legal framework to protect clinicians from legal pressures that push them towards defensive medicine. Supporting rational decisions made with evidence-based tools such as Health Technology Assessment (Atikeler et al., 2023).

In conclusion, cost awareness among physicians and nurses is not a call for budget cuts or profit maximization. It is an ethical call for the fair and efficient use of scarce resources. Cost awareness is not a cold managerial imperative, but rather, in a modern interpretation of the Hippocratic Oath, a part of professional responsibility not only to the patient in front of us, but to the entire society that awaits resources. The goal is not to save money, but to maximize health with the resources available. This goal can only be achieved through the awareness of clinicians and the cooperation of a system that supports them.

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CHAPTER 20

A Neurodegenerative Disorder: Diagnosis, Pathophysiology and Current Therapeutic Approaches in Parkinson's Disease

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INTRODUCTION

Parkinson's disease (PD) is a slowly advancing neurodegenerative condition associated with aging, whose clinical features were originally documented by James Parkinson in the early 19th century. Today, Parkinson's disease is regarded as one of the most prevalent neurodegenerative conditions worldwide, second only to Alzheimer's disease (Selkoe, 2004; York, 2017). Idiopathic PD is characterized by the progressive loss of dopamine-producing nerve cells located within the substantia nigra pars compacta (SNpc) of the midbrain, accompanied by the accumulation of Lewy bodies composed mainly of misfolded α -synuclein. These neuropathological features play a key role in shaping both the movement-related symptoms and the diverse non-motor clinical manifestations observed in Parkinson's disease.

The etiology of PD remains incompletely understood. Current evidence supports a multifactorial pathogenesis, where genetic susceptibility interacts with environmental exposures such as pesticides, heavy metals, rural living, and synthetic opioid toxins (e.g., MPTP). Risk increases markedly with age, and a positive family history accounts for a subset of cases in which mutations in genes such as Synuclein Alpha (SNCA), Leucine-Rich Repeat Kinase 2 (LRRK2), PTEN-Induced Kinase 1 (PINK), and Parkin contribute to disease vulnerability (Poewe et al., 2017).

Clinically, PD is characterized by a constellation of hallmark motor manifestations, including rest tremor, muscular rigidity, slowness of movement, and impaired postural control, all of which reflect dopaminergic denervation within the nigrostriatal pathway. However, PD is now understood as a multisystem disorder, affecting far more than motor control. Non-motor symptoms, including autonomic dysfunction, depression, anxiety, sleep disturbances, sensory impairments, and these features frequently appear long before the onset of motor signs, imposing a substantial burden on daily functioning and negatively impacting overall well-being (Jankovic & Tan, 2020).

Over the past several decades, notable progress has been achieved in how PD is diagnosed, monitored, and treated in clinical practice. Medication-based approaches, particularly those aimed at restoring dopaminergic function, continue to serve as the primary means of managing symptoms. Additionally, advanced surgical interventions—most notably deep brain stimulation (DBS)—have markedly improved clinical outcomes in appropriately selected individuals with later-stage disease. Despite these therapeutic gains, no available intervention has yet demonstrated the ability to halt or reverse the underlying neurodegenerative process. Thus, PD continues to impose substantial functional, emotional, and socioeconomic burdens on patients, caregivers, and healthcare systems worldwide.

These realities underscore the critical need for comprehensive, multidisciplinary care approaches and for the development of innovative disease-

modifying strategies. Efforts to integrate personalized medicine, digital biomarkers, artificial intelligence–assisted monitoring, and next-generation molecular diagnostics represent promising avenues for improving early detection and tailoring treatment. Increasing awareness of PD and enhancing dissemination of updated clinical guidelines among healthcare providers are essential for optimizing patient outcomes and advancing public health.

1. PATHOPHYSIOLOGY

Parkinson's disease (PD) was first recognized in 1960 as a disorder fundamentally rooted in a neurotransmitter deficiency, marking it as the earliest neurological condition attributed to a specific chemical deficit in the brain (Balestrino & Schapira, 2020). The pathophysiology of PD is multifactorial, involving the interplay of dopaminergic neuronal loss, α -synuclein aggregation, mitochondrial dysfunction, impaired autophagy–lysosomal pathways, oxidative stress, neuroinflammation, and synaptic failure (Frake et al., 2015; Finkbeiner, 2020; Poewe et al., 2017).

The hallmark of the disorder is the progressive degeneration of dopaminergic neurons within the SNpc, which leads to a profound reduction in dopamine levels in the striatum. This dopaminergic deficit disrupts basal ganglia function, resulting in the characteristic motor manifestations of PD (Nixon, 2013; Poewe et al., 2017). The pathogenesis of PD cannot be explained by a single pathological mechanism. Instead, it emerges from the convergence of multiple biological dysfunctions, including:

- Loss of protein homeostasis
- Mitochondrial energy impairment
- Insufficient autophagic clearance
- Oxidative damage
- Activation of inflammatory signaling cascades

This multifaceted profile has led to the conceptualization of PD in modern neurobiology as a **“multi-stage disorder”**, wherein progressive cellular failure across several pathways ultimately culminates in neurodegeneration.

1.1. Deterioration of Dopaminergic Neural Circuits Within the Nigrostriatal System

A hallmark of PD pathology is the marked depletion of dopamine-producing cells within the SNpc—often exceeding half of the neuronal population—alongside the progressive deterioration of their projections that innervate the striatum (Poewe et al., 2017). As neuronal death progresses, striatal dopamine concentrations significantly decline, reducing dopaminergic receptor signaling and impairing the functional dynamics of the basal ganglia circuitry.

This dopaminergic deficit disrupts both D1 receptor–mediated direct pathways and D2 receptor–mediated indirect pathways, giving rise to abnormalities in signal transmission, impaired inhibitory–excitatory balance within glutamatergic pathways, and maladaptive changes in motor control loops (Menzies et al., 2015).

These alterations form the biological foundation of the core motor symptoms of PD, which ultimately give rise to the characteristic movement abnormalities of the disorder—such as tremor at rest, slowed voluntary motion, muscle stiffness, and impaired postural control. The progressive dysfunction of the nigrostriatal system is therefore considered central to understanding the motor phenotype of PD.

1.2. Lewy Bodies and α -Synuclein Aggregation

A prominent histopathological feature of PD involves the formation of Lewy bodies, intracellular deposits that predominantly consist of aberrantly folded α -synuclein. Abnormal accumulation, misfolding, and oligomerization of α -synuclein result in neuronal toxicity by disrupting essential cellular processes (Singleton et al., 2003; Winslow et al., 2010).

Misfolded α -synuclein exerts several detrimental effects, including:

- Disruption of mitochondrial functions
- Impairment of synaptic vesicle trafficking and neurotransmitter release
- Inhibition of autophagic and proteasomal degradation pathways
- Disturbance of intracellular calcium homeostasis.

These pathogenic events position α -synuclein not merely as a by-product but as a primary driver of neurodegeneration, contributing both to intracellular toxicity and the propagation of pathology between neurons (Finkbeiner, 2020; Poewe et al., 2017).

1.3. Mitochondrial Dysfunction and Impaired Energy Metabolism

Mitochondrial impairment represents one of the most widely supported mechanisms in the pathogenesis of PD. Deficits in mitochondrial complex I activity, impairments in the cellular energy-producing machinery, accompanied by elevated generation of reactive oxygen species (ROS), have been closely linked to the degeneration of dopaminergic neurons (Valente et al., 2004; Geisler et al., 2010).

A critical element of mitochondrial homeostasis is PINK1–Parkin-mediated mitophagy, a selective autophagic process responsible for identifying and removing damaged mitochondria. When this pathway is disrupted, several pathological events ensue:

- Accumulation of mitochondrial DNA damage
- Excessive oxidative stress
- Energetic failure and metabolic crisis within neurons (Pickrell & Youle, 2015)

Given that SNpc dopaminergic neurons have exceptionally high metabolic demands and relatively low antioxidant capacity, deficits in mitochondrial quality control accelerate their vulnerability to degeneration.

1.4. Autophagy–Lysosomal System Dysfunction

Autophagy is a fundamental catabolic process required for a key cellular mechanism responsible for removing aberrantly folded proteins and dysfunctional organelles. In PD, both macroautophagy and mitophagy pathways are impaired, contributing directly to α -synuclein accumulation and neuronal death (Frake et al., 2015; Deretic & Kroemer, 2022).

Several molecular defects contribute to autophagy–lysosomal failure in PD:

- LRRK2 mutations, which disrupt autophagic flux (Kwan et al., 2017)
- Mislocalization of ATG9, a critical membrane-trafficking protein necessary for initiating and building autophagic vesicles (Winslow et al., 2010)
- Defective lysosomal acidification, impairing enzymatic degradation (Bolos et al., 2016)
- Accumulation of p62/SQSTM1, indicating impaired cargo processing (Fecto et al., 2011)

Importantly, excessive α -synuclein itself further overloads and inhibits autophagic pathways, promoting a self-amplifying cycle of protein aggregation and cellular toxicity.

1.5. Oxidative Stress and Neuronal Damage

Dopaminergic neurons in the SNpc are intrinsically vulnerable to oxidative stress due to dopamine metabolism. Dopamine undergoes auto-oxidation, producing reactive quinones that bind to cellular proteins and disrupt their function, thereby increasing neuronal susceptibility to injury (Nixon, 2013).

Excessive ROS production contributes to several pathological events (Dias et al., 2013):

- Mitochondrial dysfunction and respiratory chain impairment
- DNA oxidation and lipid peroxidation
- Acceleration of α -synuclein aggregation and oligomerization
- Further disruption of autophagic pathways

Thus, oxidative stress functions as both a cause and consequence of neurodegeneration, forming a self-perpetuating vicious cycle that progressively amplifies cellular damage in PD.

1.6. Neuroinflammation

Chronic neuroinflammation and microglial activation are well-established pathological components of PD. Activated microglia release a wide array of pro-inflammatory mediators, perpetuating neuronal toxicity.

Activated microglia:

- Secrete pro-inflammatory cytokines, including TNF- α and IL-1 β
- Produce nitric oxide (NO) and additional ROS

- Enhance the propagation and extracellular spread of α -synuclein aggregates (Hickman et al., 2018).

This creates a bidirectional interaction between inflammation and neurodegeneration, in which neuronal injury triggers microglial activation, and inflammatory signaling further accelerates neuronal loss (Subramaniam & Federoff, 2017).

1.7. Synaptic Dysfunction and Impaired Cellular Signaling

Synaptic abnormalities contribute significantly to the clinical manifestations of PD. Because α -synuclein plays a central role in regulating synaptic vesicle dynamics, its aggregation disrupts synaptic transmission and neuronal connectivity (Kim & Guan, 2019).

Loss of synaptic integrity precedes overt neuronal death and is considered a key driver of early-stage motor and non-motor symptoms. Impaired neurotransmission within basal ganglia circuits further exacerbates motor dysfunction, reflecting a complex impairment of both pre- and postsynaptic signaling mechanisms (Schirinzi et al., 2016).

1.8. Gene–Environment Interactions

Emerging evidence indicates that PD results from a complex interplay between genetic predisposition and environmental exposures, rather than being attributable solely to either factor (Poewe et al., 2017).

Examples of key genetic–environmental interactions include (Kalia & Lang, 2015):

- LRRK2 mutations, which disrupt autophagy and mitochondrial dynamics
- PINK1 and Parkin mutations, leading to impaired mitophagy and accumulation of dysfunctional mitochondria
- SNCA gene duplication or triplication, resulting in excessive α -synuclein expression and aggregation
- Environmental toxins such as MPTP and paraquat, which inhibit mitochondrial complex I and induce oxidative stress

These interacting pathways converge on dopaminergic neurons, which have high metabolic demands and low intrinsic antioxidant capacity, rendering them disproportionately vulnerable to combined genetic and environmental insults.

2. DIAGNOSTIC APPROACHES IN PARKINSON'S DISEASE

Parkinson's disease (PD) is primarily a clinical diagnosis, heavily reliant on characteristic findings observed during neurological examination. Although no single biomarker currently exists to definitively confirm the disease, a combination of clinical assessment, ancillary neuroimaging, biochemical markers, genetic testing, and sensorimotor functional evaluations contributes significantly to diagnostic accuracy (Postuma et al., 2015; Poewe et al., 2017). The diagnostic foundation is grounded in the clinical manifestations resulting from dopaminergic neuronal loss within the SNpc.

A comprehensive overview of contemporary diagnostic modalities used in PD is presented below.

2.1. Clinical Evaluation

2.1.1. Motor Symptoms

The diagnosis of PD is principally supported by the presence of at least three of the four cardinal motor features, with bradykinesia being mandatory (Postuma et al., 2015):

- Bradykinesia (mandatory criterion)
- Resting tremor
- Rigidity
- Postural instability (typically emerging in later stages)

Bradykinesia represents the most distinctive hallmark of PD and must be present for diagnosis. Clinically, it manifests as a delay in movement initiation, generalized slowness, and reduced amplitude of repetitive movements (Berardelli et al., 2001).

2.1.2. Non-Motor Symptoms

Non-motor manifestations may precede motor symptoms by several years, providing valuable insight into the prodromal phase of PD. Key prodromal indicators include:

- REM sleep behavior disorder (RBD)
- Olfactory loss (hyposmia)
- Constipation
- Autonomic dysfunction
- Depression and anxiety
- Mild cognitive impairment

These features significantly enhance the early recognition of PD before the onset of classical motor signs (Berg et al., 2015).

2.2. International Diagnostic Criteria

2.2.1. MDS Clinical Diagnostic Criteria for Parkinson's Disease

The most widely accepted international diagnostic framework is the 2015 Movement Disorder Society (MDS) Clinical Diagnostic Criteria (Postuma et al., 2015).

These criteria consist of **three major components**:

- **Mandatory criterion:** Presence of bradykinesia
- **Supportive criteria:** Dramatic response to levodopa, presence of resting tremor, abnormal dopaminergic imaging findings
- **Exclusion criteria:** Cerebellar signs, supranuclear gaze palsy or other oculomotor abnormalities, Early and severe autonomic failure (e.g., multiple system atrophy).

The MDS criteria integrate both clinical manifestations and imaging findings, providing high diagnostic accuracy and reducing misclassification, especially in early disease stages.

2.3. Neuroimaging Techniques

Although PD is primarily a clinical diagnosis, modern neuroimaging methods offer substantial value in differential diagnosis, reducing diagnostic uncertainty, and distinguishing idiopathic PD from atypical parkinsonian syndromes (Postuma et al., 2015). While no imaging modality alone can confirm PD with absolute certainty, several techniques provide critical insights into dopaminergic degeneration and associated structural or functional changes.

➤ **Dopamine Transporter Imaging (DaTScan – SPECT):** DaTScan, based on single-photon emission computed tomography (SPECT), visualizes presynaptic dopamine transporter (DaT) density, enabling the assessment of nigrostriatal dopaminergic integrity. In PD, the hallmark finding is a marked reduction of radiotracer uptake in the striatum, reflecting dopaminergic terminal loss (Hutchison et al., 2021).

DaTScan is particularly useful in:

- ❖ Differentiating PD from essential tremor
- ❖ Distinguishing idiopathic PD from drug-induced parkinsonism
- ❖ Supporting diagnostic accuracy in early or equivocal cases

➤ **Positron Emission Tomography (PET):** Positron Emission Tomography is considered one of the most sensitive techniques for evaluating neurochemical and metabolic alterations and is widely used in research settings (Nixon, 2013).

Major PET applications include:

❖ **18F-DOPA PET:** Measures dopaminergic terminal function with high sensitivity, effectively demonstrating presynaptic dopaminergic loss.

❖ **FDG-PET:** Assesses cortical glucose metabolism, identifying characteristic hypometabolic patterns in atypical parkinsonian syndromes such as multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD).

❖ **Amyloid/Tau PET:** Useful in detecting coexisting Alzheimer-type pathology and cognitive decline in advanced PD.

PET imaging continues to gain importance in differential diagnosis, disease phenotyping, and multimodal biomarker research (Poewe et al., 2017).

➤ **Magnetic Resonance Imaging (MRI):** Conventional MRI is generally normal in idiopathic PD and therefore is not a primary diagnostic tool. However, MRI plays a crucial role in excluding structural, vascular, or alternative neurodegenerative conditions (Postuma et al., 2015). MRI is particularly valuable in differentiating:

- ❖ Vascular parkinsonism
- ❖ Multiple system atrophy (MSA)
- ❖ Corticobasal degeneration (CBD)

➤ **Advanced MRI Techniques:** Recent advances in high-resolution MRI have enabled noninvasive visualization of substantia nigra pathology:

❖ **Susceptibility-Weighted Imaging (SWI):** SWI provides enhanced sensitivity to iron deposition and microstructural tissue alterations. In PD, elevated paramagnetic signals within the SNpc correlate with dopaminergic neuronal loss and disease progression (Poewe et al., 2017).

❖ **Nigrosome-1 Imaging (“Swallow Tail Sign”):** Nigrosome-1 appears as a hyperintense, swallow-tail-shaped structure within the dorsolateral substantia nigra on T2* or SWI sequences. Loss of this signal is considered a highly specific imaging marker of early PD (Blazejewska et al., 2013). The disappearance of the “swallow tail sign” helps distinguish PD from essential tremor and other parkinsonian syndromes with high diagnostic accuracy.

Together, SWI and Nigrosome-1 imaging represent promising future biomarkers, with strong potential for incorporation into standardized diagnostic criteria.

➤ **Transcranial Sonography (TCS):** Transcranial sonography is a noninvasive, rapid, and cost-effective imaging modality that assesses the echogenicity of the substantia nigra. The presence of hyperechogenic areas (≥ 4 mm²) in the substantia nigra is strongly associated with PD (Berg et al., 2015).

Advantages of TCS include:

- ❖ High applicability in patients with adequate acoustic bone windows
- ❖ Practical use as a radiation-free screening tool
- ❖ Detection of substantia nigra hyperechogenicity in prodromal and early-stage

TCS is increasingly used for risk stratification, early detection, and monitoring high-risk populations, making it a valuable adjunct in modern PD diagnostic workflows (Poewe et al., 2017).

2.4. Biological Biomarkers and Genetic Testing

Although no single definitive biomarker is currently available for routine clinical diagnosis of PD, increasing research over the past decade has highlighted several molecular indicators with potential diagnostic and prognostic value. Among these, ***α -synuclein***—a central component of PD pathology—remains the most extensively investigated. Measurements of oligomeric and phosphorylated α -synuclein levels in cerebrospinal fluid (CSF), as well as synucleinopathy-associated aggregates detected in serum or plasma, have shown promise as early-stage biomarkers of PD (Magalhães & Lashuel, 2022). However, standardized cut-off values and universal clinical thresholds have yet to be established, limiting their current applicability in routine practice.

Another increasingly important biomarker is ***neurofilament light chain (NfL)***. Elevated NfL concentrations are particularly informative in distinguishing PD from atypical parkinsonian disorders such as MSA and progressive supranuclear palsy (PSP), in which NfL levels are markedly higher. In contrast, individuals with idiopathic PD typically exhibit normal or only mildly elevated NfL concentrations, making this marker useful for differential diagnosis (Poewe et al., 2017).

Given the central role of mitochondrial dysfunction in PD pathology, biomarkers related to ***PINK1–Parkin-mediated mitophagy***, mitochondrial DNA damage, and altered mitochondrial protein expression are being actively explored. These molecular signatures are under investigation as potential indicators of disease onset, subtype differentiation, and progression in ongoing clinical and translational research (Pickrell & Youle, 2015).

In addition to molecular biomarkers, genetic testing can provide significant diagnostic support, particularly in early-onset PD. Mutations in genes such as ***LRRK2***, ***PARKIN (PARK2)***, ***PINK1***, ***DJ-1***, and ***SNCA*** are known to increase susceptibility to PD and may influence phenotypic severity (Klein & Westenberger, 2012). Although genetic findings do not currently alter standard therapeutic strategies, genetic analysis plays a critical role in counseling families, risk stratification, and advancing the implementation of personalized medicine in PD.

2.4. Olfactory Testing

In addition to molecular biomarkers, olfactory testing serves as an important clinical tool in the prodromal detection of PD. Hyposmia occurs in approximately 80–90% of patients, often emerging years before the onset of motor symptoms, making it one of the earliest and most reliable clinical indicators of neurodegeneration (Doty, 2012).

Standardized olfactory assessments such as the University of Pennsylvania Smell Identification Test (UPSIT) and the Sniffin' Sticks test battery have demonstrated significant utility in both early detection and in identifying individuals at elevated risk for developing PD (Doty, 2012; Berg et al., 2015). These tests allow for quantitative evaluation of odor identification, discrimination, and threshold sensitivity, providing valuable insight into olfactory pathway dysfunction.

Although olfactory tests alone are not diagnostic, they represent an increasingly important component of multimodal diagnostic approaches. When combined with biochemical, imaging, and genetic markers, olfactory dysfunction contributes to early disease detection, phenotypic subtyping, and monitoring longitudinal progression in PD.

2.5. Electrophysiological and Sensorimotor Testing

Electrophysiological and sensor-based assessment methods contribute valuable information to the diagnostic evaluation of PD, particularly for the differentiation of tremor phenotypes. Using electromyography (EMG), accelerometers, and various movement sensors, tremor frequency and amplitude can be quantified objectively. These measures enable more accurate distinction between Parkinsonian tremor and essential tremor, as well as between rest tremor and action tremor, improving diagnostic precision (Poewe et al., 2017).

In addition to tremor analysis, oculomotor assessment provides further diagnostic insight. Patients with PD frequently exhibit reduced saccadic velocity

and slowed smooth pursuit eye movements, findings that are even more pronounced in atypical parkinsonian syndromes such as PSP (Postuma et al., 2015). These oculomotor abnormalities aid in distinguishing idiopathic PD from parkinsonian conditions characterized by early and severe gaze palsy.

2.6. Artificial Intelligence and Digital Biomarkers

In recent years, artificial intelligence (AI)–based digital biomarkers have gained increasing prominence in the early detection and monitoring of PD. Novel digital health technologies enable continuous, real-world assessment of motor and non-motor function, offering objective, scalable, and highly sensitive diagnostic opportunities.

Innovative approaches include gait pattern analysis via smartwatches and wrist-worn sensors, monitoring of typing speed and touchscreen interactions, digital voice analytics for detecting bradyphonia, AI-based models for facial and motor assessment, and passive in-home sensors for continuous tracking of activity and gait stability (Lipsmeier et al., 2018).

These AI-driven tools show high potential not only for early and prodromal detection of PD but also for objective longitudinal tracking of disease progression (Polvorinos-Fernández et al., 2024). As such, digital biomarkers are increasingly viewed as valuable complementary tools to traditional clinical assessments, helping to refine diagnosis, personalize monitoring strategies, and support remote care models in modern neurodegenerative disease management.

3. THERAPEUTIC APPROACHES IN PARKINSON’S DISEASE

The management of Parkinson’s disease focuses on alleviating both motor and non-motor manifestations, enhancing overall functional well-being, and slowing the progression of clinical decline. Despite advances in therapy, currently available treatments are primarily symptomatic, as none have yet been shown to completely stop or reverse the neurodegenerative mechanisms driving the disorder. (Poewe et al., 2017). Modern therapeutic strategies are broadly categorized into *pharmacological, surgical, rehabilitation-based, and experimental approaches*.

3.1. Pharmacological Therapies

Pharmacologic management of PD is designed to compensate for dopaminergic deficiency, alleviate motor symptoms, and enhance daily functioning. The core of treatment includes agents that increase dopamine availability, stimulate dopamine receptors, or modify dopamine metabolism. The principal drug classes and contemporary clinical considerations are outlined below.

➤ Levodopa and Combination Therapies (The Gold Standard):

Levodopa remains the most effective pharmacological agent for addressing dopamine deficiency and continues to serve as the cornerstone of PD management (Olanow & Schapira, 2013). To minimize peripheral conversion to dopamine and reduce adverse effects, levodopa is co-administered with carbidopa

or benserazide. Long-term levodopa therapy is associated with motor fluctuations (“on–off” phenomena) and dyskinesias, necessitating dose adjustments as disease progresses (Ahlskog & Muentner, 2001).

➤ **Dopamine Agonists:** Agents such as pramipexole, ropinirole, and rotigotine directly stimulate dopamine receptors. Although associated with a lower risk of motor complications compared to levodopa, dopamine agonists carry increased risk of hallucinations, sudden sleep episodes, and impulse control disorders (Weintraub et al., 2010).

➤ **Monoamine Oxidase-B MAO-B Inhibitors:** Selegiline, rasagiline, and safinamide inhibit MAO-B, thereby reducing the breakdown of endogenous and exogenous dopamine. These agents provide modest symptomatic benefit in early-stage PD and may be added to levodopa regimens to reduce motor fluctuations (Oertel & Schulz, 2016).

➤ **Catechol-O-Methyl Transferase (COMT) Inhibitors:** Entacapone and opicapone block the enzyme COMT, prolonging the plasma half-life of levodopa. These medications are particularly useful for patients experiencing levodopa-related motor fluctuations (Takeda et al., 2021).

➤ **Anticholinergic Agents:** Trihexyphenidyl and related anticholinergic medications are most effective in reducing tremor. However, adverse effects such as cognitive impairment, dry mouth, and constipation limit their use, especially in older adults (Katzenschlager et al., 1996).

➤ **Amantadine:** Amantadine, an NMDA receptor antagonist, reduces glutamatergic overactivity and is particularly effective in managing levodopa-induced dyskinesias. It may also provide moderate improvement in motor symptoms (Fox et al., 2018).

3.2. Surgical Therapies

Surgical interventions play a critical role in the management of advanced PD, particularly in patients who continue to experience motor fluctuations, levodopa-induced dyskinesias, or medication-refractory tremor despite optimized pharmacological treatment. Among the available approaches, **Deep Brain Stimulation (DBS)** remains the most widely used and efficacious option. In addition, continuous **infusion–based advanced therapies** have gained increasing clinical relevance.

➤ **Deep Brain Stimulation (DBS):** DBS, targets pathological motor circuit activity by delivering high-frequency electrical stimulation to specific subcortical nuclei, most commonly the subthalamic nucleus and the globus pallidus internus. Deep brain stimulation (DBS) represents a beneficial therapeutic option for patients in the advanced stages of Parkinson’s disease who develop prominent motor complications as a consequence of long-term levodopa treatment (Benabid et al., 2009).

Clinical evidence demonstrates that DBS:

- Produces significant improvement in motor symptoms
- Enhances activities of daily living
- Reduces levodopa requirements

- Provides sustained therapeutic benefit in appropriately selected patients

However, patient selection is crucial. Individuals with substantial cognitive impairment, advanced age, or significant psychiatric comorbidities—such as severe depression or psychosis—require careful preoperative evaluation to ensure safety and optimal outcomes (Fasano & Lozano, 2015).

Beyond DBS, several continuous dopaminergic delivery systems offer important therapeutic alternatives for managing advanced motor complications.

➤ **Levodopa–Carbidopa Intestinal Gel (LCIG):** LCIG allows continuous jejunal infusion of levodopa–carbidopa, reducing plasma fluctuations and providing more stable dopaminergic stimulation. Clinical studies indicate that LCIG:

- Decreases the duration of “off” episodes
- Reduces motor fluctuations
- Improves quality of life in late-stage PD (Olanow et al., 2014).

➤ **Continuous Subcutaneous Apomorphine Infusion:** Apomorphine infusion delivers continuous dopaminergic stimulation through subcutaneous administration. As a potent dopamine agonist, apomorphine has been shown to:

- Reduce “off” periods
- Improve global motor function
- Provide an alternative for patients not suitable for DBS (Trenkwalder et al., 2015).

While these surgical and advanced therapeutic strategies provide valuable benefits in the management of motor complications, treatment selection must be highly individualized. Patient-specific factors—including clinical phenotype, comorbidities, cognitive status, and therapeutic goals—should guide decision-making to optimize outcomes.

3.3. Rehabilitation and Multidisciplinary Care Approaches

Parkinson’s disease (PD) is not solely a movement disorder confined to the motor system; rather, it is a complex neurodegenerative condition that affects cognitive, autonomic, emotional, and sensory domains. For this reason, treatment cannot rely exclusively on pharmacological or surgical interventions. Given the complex and heterogeneous nature of Parkinson’s disease, optimal management depends on coordinated, multidisciplinary care rather than isolated therapeutic strategies. Current evidence demonstrates that integrated care models lead to meaningful improvements in both motor and non-motor domains and contribute to better overall patient well-being (Poewe et al., 2017; Fasano & Lozano, 2015; Krause et al., 2022).

A comprehensive multidisciplinary team for PD typically includes physiotherapists, occupational, speech and language therapists, neuropsychologists, nutrition specialists, and social workers, each contributing to improved functional independence and patient-centered outcomes.

Physiotherapy plays a central role in addressing key motor impairments such as postural imbalance, bradykinesia, gait instability and rigidity. Evidence-based physiotherapy programs incorporate:

- Aerobic exercise
- Balance training
- Resistance and strength exercises
- Gait training supported by external sensory cues

Such therapeutic approaches contribute to decreased motor slowness, better postural control, and improved overall mobility (Fox et al., 2018).

Occupational therapy focuses on optimizing activities of daily living (ADLs) through environmental modification, energy conservation strategies, assistive devices, and training in functional task performance. Such interventions help maintain independence and reduce disability burden (Poewe et al., 2017). Speech and language disturbances, including hypophonia and reduced speech intelligibility, are common in PD. The Lee **Silverman Voice Treatment (LSVT-LOUD)** program has demonstrated robust improvements in vocal loudness, articulation, and overall communication effectiveness (Fox et al., 2018).

Neuropsychological interventions address motor and non-motor psychiatric symptoms such as:

- Apathy
- Depression
- Cognitive decline
- Anxiety

These approaches are essential for **improving emotional well-being and supporting cognitive functioning** (Weintraub et al., 2010).

Nutritional counseling plays a critical role in:

- Managing dysphagia
- Reducing constipation
- Mitigating medication–food interactions (particularly with levodopa)

These strategies help optimize treatment efficacy and overall health status (Oertel & Schulz, 2016).

Education and support for caregivers are crucial, as they contribute directly to the sustainability of home-based care and reduce caregiver burden. Social services provide resources, counseling, and support programs to enhance both patient and caregiver well-being (Fasano & Lozano, 2015).

The integration of these professional interventions forms the foundation of comprehensive PD management. Coordinated multidisciplinary care improves patient adaptation to disease progression, enhances functional outcomes, and has come to be regarded as a fundamental element of contemporary clinical management.

3.4. Emerging and Experimental Therapies: Gene Therapy, Stem Cell Applications, and Neuroprotective Agents

While current therapies for PD primarily focus on symptomatic control, no available treatment has yet been shown to stop or counteract the progressive neuronal deterioration that characterizes the disease. Consequently, recent translational research has increasingly focused on gene therapy, stem cell–based interventions, and neuroprotective agents, all of which hold significant promise for disease modification. These approaches aim to target the cellular and molecular mechanisms driving PD pathology and have the potential to revolutionize future therapeutic paradigms (Bartus et al., 2014; Kalia & Lang, 2015).

3.4.1. Gene Therapy Approaches

Gene therapy aims to enhance neuronal function or correct biochemical pathways that contribute to neurodegeneration by delivering specific genetic material directly into the brain. In PD, three major gene therapy strategies have emerged:

➤ **Gene Therapy to Enhance Dopaminergic Synthesis:** This approach targets the restoration of dopamine production by delivering genes involved in dopamine biosynthesis—aromatic L-amino acid decarboxylase (AADC), tyrosine hydroxylase (TH), or GTP cyclohydrolase-1 (GTPCH-1)—into the striatum using viral vectors. Clinical trials of AAV2-AADC have demonstrated favorable safety profiles in Phase I/II studies and have shown significant improvements in motor function, supporting the feasibility of this strategy (Christine et al., 2022).

➤ **Glutamatergic Modulation in the Subthalamic Nucleus (STN):** A second strategy involves modulating overactive basal ganglia circuitry by increasing inhibitory neurotransmission in the STN. Delivery of the glutamic acid decarboxylase (GAD) gene using an AAV2 vector enhances GABA production, thereby reducing abnormal hyperactivity within motor pathways. Phase II clinical trials of AAV2-GAD have reported meaningful improvements in motor symptoms, indicating its potential as a disease-modifying intervention (LeWitt et al., 2011).

➤ **Neurotrophic Factor–Based Gene Therapy:** Neurotrophic molecules such as glial cell line–derived neurotrophic factor (GDNF) and neurturin (NRTN) exert strong trophic and protective effects on dopaminergic neurons. Early clinical evaluation of AAV2-NRTN (CERE-120) demonstrated initial promise; however, subsequent Phase II/III trials did not achieve the anticipated clinical efficacy (Marks et al., 2010). These outcomes highlight critical considerations—such as limited distribution of the therapeutic protein, insufficient dosing, and variability related to disease stage—that continue to challenge this therapeutic approach. Gene therapy in PD therefore remains experimental, and long-term safety and efficacy require further validation.

3.4.2. Stem Cell and Cell Replacement Therapies

The progressive loss of dopaminergic neurons is a defining pathological hallmark of PD, making stem cell-based replacement therapies a compelling avenue for potential disease modification. The fundamental aim of these approaches is to transplant functional dopaminergic neurons capable of restoring dopamine synthesis and re-establishing physiological activity within basal ganglia motor circuits. Current research focuses on several promising cell sources, including *embryonic stem cells (ESCs)*, *induced pluripotent stem cells (iPSCs)*, and *mesenchymal stem cells (MSCs)* (Kikuchi et al., 2017; Takahashi & Yamanaka, 2013).

➤ **Embryonic Stem Cell-Derived Dopaminergic Neurons:** ESC-derived dopaminergic neurons have demonstrated robust graft survival and functional motor improvement in preclinical animal models. Early clinical investigations indicate that transplantation of ESC-derived cells into the human striatum can be performed safely, with encouraging evidence of graft viability and dopaminergic function (Kikuchi et al., 2017). These findings mark an important step toward clinical translation.

➤ **Induced Pluripotent Stem Cells (iPSCs):** The use of induced pluripotent stem cell (iPSC) technology allows researchers to derive dopaminergic neurons directly from an individual's own cells, thereby minimizing immunological incompatibility and circumventing ethical concerns associated with embryonic cell sources. Initial clinical applications and primate studies have shown that iPSC-derived dopaminergic grafts can survive long-term, integrate into host neural circuits, and exhibit favorable safety profiles (Takahashi & Yamanaka, 2013; Hallett et al., 2015). This personalized approach offers high potential for future clinical use.

➤ **Mesenchymal Stem Cells (MSCs):** MSCs possess neuroprotective properties, anti-inflammatory, and immunomodulatory. Preclinical PD models have reported significant motor improvements following MSC administration. However, human studies remain limited, and long-term efficacy has yet to be conclusively demonstrated (Ramo et al., 2015). Thus, MSC therapy is still considered investigational.

Despite the promise of stem cell therapeutics, several major challenges hinder large-scale clinical translation:

- Risk of tumorigenesis following transplantation, particularly with pluripotent stem cell-derived grafts
- Limited long-term survival and insufficient synaptic integration of transplanted cells
- Ethical controversies, especially concerning embryonic stem cell use
- Lack of standardized, clinically compliant, and homogeneous cell lines suitable for transplantation
- Insufficient long-term safety and efficacy data from large-scale randomized clinical trials

These limitations indicate that although stem cell-based treatments are scientifically compelling, further refinement, long-term monitoring, and

methodological standardization are necessary before they can become routine therapeutic options for PD (Takahashi & Yamanaka, 2013; Ramo et al., 2015; Kikuchi et al., 2017).

3.4.3. Neuroprotective Agents

Neuroprotective treatment strategies in PD aim to counteract the major pathogenic mechanisms underlying dopaminergic neuronal loss, including oxidative stress, mitochondrial dysfunction, neuroinflammation, and protein aggregation. The overarching goal is to slow the progression of motor and non-motor symptoms, preserve dopaminergic cell populations, and ultimately contribute to disease modification. Numerous pharmacological candidates have been explored across preclinical models and clinical trials, with several demonstrating promising findings.

Given the central role of mitochondrial impairment in PD pathogenesis, preservation of mitochondrial function has been a major therapeutic target. Agents such as Coenzyme Q10 (CoQ10), creatine, and other mitochondrial antioxidants have been investigated due to their potential to support energy metabolism and reduce oxidative damage. Although preclinical data were encouraging, large-scale clinical trials failed to show significant clinical benefit (Beal, 2011). Nonetheless, research into more targeted, mitochondria-specific antioxidants continues to advance.

Oxidative stress and excess production of reactive nitrogen species sensitize dopaminergic neurons to degeneration. Antioxidants, nitric oxide modulators, and free radical scavengers have shown neuroprotective effects in experimental models, though their translation to clinical practice remains preliminary. Ongoing investigations aim to develop agents with greater CNS penetration and mechanistic specificity (Percário et al., 2020; Stykel, & Ryan, 2022).

Glucagon-like peptide-1 (GLP-1) receptor agonists have attracted substantial interest due to their anti-inflammatory, mitochondrial stabilizing, and neurotrophic properties. Exenatide demonstrated a slowing of motor symptom progression and signs of disease-modifying potential in a Phase II randomized controlled trial (Athauda et al., 2017). These findings position GLP-1 agonists as some of the most promising pharmacological candidates for neuroprotection in PD.

Immunotherapeutic approaches aim to reduce pathological α -synuclein aggregation by targeting misfolded species with specific monoclonal antibodies. Prasinezumab, an anti- α -syn monoclonal antibody, showed encouraging signals of slowed progression in certain subgroups within a Phase II trial, with a favorable safety profile (Pagano et al., 2022). However, larger and longer-term trials are required to clarify its therapeutic impact.

Small-molecule anti-aggregants designed to inhibit the misfolding, oligomerization, or accumulation of α -synuclein and tau proteins represent another promising research area. These compounds aim to restore proteostasis by directly interfering with early steps of toxic protein aggregation. Although

preclinical results are encouraging, no such agents have yet reached clinical approval.

Collectively, research into neuroprotective agents holds substantial promise for the development of disease-modifying therapies in PD. Progress in this field relies on integrative, multidisciplinary efforts and the accumulation of robust long-term clinical evidence. As mechanistic understanding deepens, these emerging therapies may reshape the future landscape of PD treatment.

4. CONCLUSION AND FUTURE PERSPECTIVES

Parkinson's disease (PD) is a slow-progressing, multifaceted neurodegenerative disorder characterized by a complex interplay of pathophysiological mechanisms. Contemporary evidence highlights that disease progression is not solely driven by dopaminergic neuronal loss but also by a network of interconnected biological processes, including α -synuclein aggregation, mitochondrial dysfunction, impaired proteostasis, neuroinflammation, and genetic susceptibility (Poewe et al., 2017; Jankovic & Tan, 2020). Consequently, the management of PD has evolved well beyond traditional dopaminergic replacement therapies, expanding toward multidisciplinary care models, advanced surgical approaches, innovative pharmacological strategies, and experimental disease-modifying interventions.

Pharmacological interventions remain the primary means of managing clinical symptoms in Parkinson's disease; however, prolonged use frequently leads to complications such as motor fluctuations and medication-related adverse effects, highlighting the growing need for individualized treatment approaches. Although DBS can produce substantial improvement in well-selected individuals with advanced disease, its use is constrained by factors such as advanced age, cognitive impairment, and coexisting psychiatric disorders (Fasano & Lozano, 2015). These therapeutic challenges have intensified ongoing efforts to develop disease-modifying strategies that directly address the fundamental biological mechanisms driving neurodegeneration in PD.

Among emerging therapeutic modalities, gene therapy, stem cell-based replacement strategies, and neuroprotective agents represent some of the most promising avenues. Gene transfer techniques aimed at restoring dopaminergic function, immunotherapies designed to neutralize pathological α -synuclein species, and mitochondria-targeted compounds intended to preserve cellular bioenergetics have shown encouraging early results, suggesting that future treatments may slow disease progression more effectively (Athauda et al., 2017; Pagano et al., 2022). Nonetheless, questions regarding long-term safety, durability of clinical benefit, neuronal integration, and ethical considerations remain to be fully resolved.

Looking ahead, the integration of personalized medicine into PD care is expected to become increasingly pivotal. Advances in digital biomarkers, AI-based motor assessments, in-home passive sensor technologies, and next-generation molecular biomarkers derived from biofluids are transforming early

diagnosis, risk stratification, and longitudinal disease monitoring (Lipsmeier et al., 2018). The incorporation of these digital and biological tools into clinical practice promises to enhance the precision of disease characterization and treatment evaluation.

In conclusion, although a definitive cure for PD has yet to be realized, rapid progress in understanding disease biology, the emergence of powerful biomarker platforms, and the development of innovative therapeutic strategies offer substantial optimism for the future. Achieving effective disease modification will require multidisciplinary collaboration, long-term clinical trials, and rigorous translational research efforts. In the coming years, advances in genetics, molecular biology, artificial intelligence, and neurotechnology are poised to fundamentally reshape the landscape of PD management.

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CHAPTER 21

Common Oral and Dental Health Problems in Elderly Individuals

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INTRODUCTION

Global life expectancy has increased significantly, leading to an aging population. It is projected that by 2030, approximately one in every six individuals worldwide will be 60 years of age or older. While high-income nations, such as Japan—where nearly 30% of the population is already over 60—have experienced population aging earlier, the most substantial demographic shifts are now occurring in low- and middle-income countries. By 2050, around two-thirds of the global population aged 60 and above is expected to reside in these regions (WHO, 2021).

Aging is a natural and inevitable stage of life that follows development and maturation, characterized by complex physiological and psychological changes arising from the highest level of interaction between genetic factors and the environment, and observed in all living organisms alongside other life stages such as childhood, adolescence, and adulthood (Yerli, 2017).

Elderly individuals are increasingly recognized as a population at risk of receiving insufficient or inadequate dental care, highlighting the urgent global need for oral health services targeted at this age group. As people age, their demand for dental care rises due to factors such as anatomical alterations, cumulative wear and tear of teeth, changes in surrounding oral tissues, existing restorations, and the presence of dentures (Pietrokovski, 2006).

Oral health is closely interconnected with the overall health of older adults. The commonly used expression that "the mouth reflects overall health" emphasizes how oral conditions can serve as indicators of systemic well-being. Changes in the oral mucosa, for example, may signal underlying conditions such as diabetes, dermatological disorders, immune deficiencies, hematological abnormalities, allergic or toxic reactions, gastrointestinal issues, and deficiencies in vitamins or minerals. Conversely, systemic diseases—including cardiovascular disorders, diabetes, respiratory conditions, rheumatic diseases, and periodontal disease—can also negatively affect oral health (Radwan-Oczko et al., 2022).

1. CHANGES IN ORAL TISSUES DUE TO AGING

1.1. Age-Related Changes in Teeth

With advancing age, physiological enamel wear (attrition) can lead to morphological changes in the teeth. This process may range from minor surface abrasions to events that result in significant structural loss (Nazliel et al., 2007). Perikymata and imbrication lines disappear, giving the enamel surface a smoother appearance with fewer details compared with newly erupted teeth. As dentin changes over time in both thickness and structural quality, it gradually loses its natural translucency. Tooth color can also be affected by pigmentation of anatomical irregularities, the presence of corrosion products, and poor oral

hygiene. Age-related alterations in enamel primarily result from ion exchange processes. Additionally, the nitrogen content of enamel tends to increase with age, and enamel may become darker or develop stains due to the accumulation of organic material (Razak et al., 2014).

Dentin undergoes two primary age-related changes: a decrease in overall size due to the ongoing deposition of secondary dentin—which can, in some instances, lead to the complete obliteration of the pulp chamber—and the development of dentin sclerosis resulting from continued formation of peritubular dentin. Both processes are associated with dental caries and mechanical wear. Sclerotic changes in dentin may affect the effectiveness of adhesive dental materials, while sclerosis in the radicular dentin can render tooth roots more brittle. With aging, the dental pulp becomes less vascularized, less cellular, and increasingly fibrotic, leading to diminished reparative capacity and reduced responsiveness to injury (Carvalho et al., 2017).

1.2. Periodontium

The periodontium, which includes the supporting structures of the teeth such as the gingiva, cementum, alveolar bone, and periodontal ligament, functions as a cohesive unit (Nazliel et al., 2007; Bozdemir & Amasya, 2019). Aging can lead to thinning of the gingival epithelium and a reduction in its keratinization. These changes may increase the permeability of the epithelium to bacterial antigens, decrease resistance to functional stress, or both, potentially influencing long-term periodontal health outcomes (Bhadbhade, 2015). Nevertheless, some studies have reported no significant histological alterations in epithelial cell size, shape, or organization. Additionally, aging is associated with gingival connective tissues becoming denser and coarser. Concurrently, the number of fibroblasts within the gingival connective tissue may decline with age (Andreescu et al., 2013; Huttinet et al., 2009; Krieger et al., 2013).

Cementum commonly thickens with age, with increases potentially ranging from five- to tenfold due to continued deposition after tooth eruption (Sinecan & Sarıbaş, 2021). This thickening is particularly notable in the apical and lingual regions. As individuals age, the fluoride concentration in cementum rises, while its permeability decreases. Although cementum has a limited capacity for remodeling, the presence of accumulated resorption lacunae contributes to greater surface irregularity (Bhadbhade, 2015). Furthermore, the fiber and cellular content of the periodontal ligament declines with age, and its structural arrangement becomes increasingly irregular (Huttinet et al., 2009).

Gingival recession can occur as a consequence of age-related loss of periodontal attachment. When the gingiva recedes, the root surfaces are exposed to the oral environment, increasing the risk of plaque accumulation. Aggressive or improper toothbrushing can damage the exposed cementum, revealing the

underlying dentin. The presence of plaque on this exposed dentin may ultimately contribute to the formation of root caries lesions (Carvalho & Lussi, 2017).

1.3. Changes in the Oral Mucosa

The oral mucosa, comprising both the epithelial layer and the underlying connective tissue, serves essential protective functions. The stratified epithelial layer consists of closely packed cells that create a physical barrier against the infiltration of toxins and microorganisms. In addition, mucosal epithelial cells produce key molecules, including keratin and laminin, which are vital for the maintenance and integrity of the mucosal surface (Razak et al., 2014). These protective functions play a significant role in overall health. However, as individuals age, the effectiveness of these defenses declines, potentially increasing exposure to harmful substances and facilitating the entry of pathogens into the oral cavity.

In elderly individuals, the oral epithelium tends to become thinner, while collagen production in the underlying connective tissue may decline. These alterations can reduce the capacity for tissue regeneration and lower resistance to pathogenic microorganisms (Khanagar et al., 2021). The tongue may also undergo visible clinical changes, appearing smoother due to a loss of filiform papillae (Razak et al., 2014).

Advancing age is also associated with a higher likelihood of sublingual varicosities, an increased vulnerability to conditions such as candidal infections, and slower wound healing (Razak et al., 2014). Furthermore, research by Mozafari et al. (2012) indicated that almost all elderly individuals living in nursing homes exhibited poor oral mucosal health.

1.4. Masticatory and Perioral Muscles

Aging influences multiple aspects of oral physiology that are important for chewing, though the degree of impact varies. Reduced muscle activity with age is associated with a longer mastication process, indicating that older adults may adjust their chewing patterns to accommodate these changes (Mioche et al., 2004). Masticatory function is a critical oral function in the elderly, and its preservation is essential for maintaining nutrition and overall quality of life (Gaszynska et al., 2014).

Radiographic studies show that the X-ray density of masticatory muscles decreases with age, reflecting an increase in intramuscular fat and fibrous tissue. Atrophy in these muscles can impair chewing efficiency and make mastication more challenging. Similar to other skeletal muscles, aging reduces muscle tone and performance in the masticatory muscles, accompanied by decreases in the number and activity of muscle cells, as well as in the number and size of muscle fibers (Bozdemir & Amasya, 2019).

In an electromyographic study, Galo et al. (2006) evaluated the masticatory system in two age groups, 23–30 years and 60–75 years, under functional conditions including testing, positioning, and chewing. The temporalis and masseter muscles were monitored, and during masticatory activities such as chewing peanuts, older adults achieved up to 79% of their maximum voluntary contraction, whereas younger adults reached 108%. During swallowing, the left masseter muscle demonstrated approximately 20% activity in older adults compared to around 7% in younger adults.

1.5. Salivary Glands

The salivary glands primarily function to produce saliva, which is essential for lubrication, digestion, immune defense, oral health, and maintaining overall bodily homeostasis. Aging causes irreversible degeneration in nearly all organs, including the salivary glands, characterized by a gradual loss of physiological integrity, reduced functional capacity, and increased vulnerability to external stress or injury (Choi et al., 2013; Li et al., 2018). Common age-related changes in the salivary glands include a decrease in acinar volume, an increase in ductal volume, slower rates of protein and mRNA synthesis, and diminished salivary flow (Affoo et al., 2015; Li et al., 2018). Factors such as reduced blood supply, impaired neural signaling, age-related health conditions, and medication use can further impair salivary gland function in older adults (Toan & Ahn, 2021). These irreversible changes in the glands can significantly restrict oral functions (Li et al., 2018).

Sialin, a multifunctional anion transporter abundant in salivary glands, may play a key role in maintaining their function. Research by Li et al. (2018) on both mice and humans showed that protein and mRNA expression of sialin declines with age. Expression in the striated and excretory ducts decreased in both species, and whereas sialin was present in the basal cytoplasmic membranes of acini and in some myoepithelial-like cells in young glands, it was almost entirely absent in older glands. These changes are thought to contribute to age-related alterations in salivary gland physiology (Li et al., 2018).

Saliva itself is a complex biological fluid that is naturally secreted in the oral cavity, playing a crucial role in food processing by forming a cohesive, smooth, and swallowable bolus. It also facilitates sensory perception by diluting taste and aroma components so they can interact with taste buds (Xu, Laguna, & Sarkar, 2019). Saliva contributes to the elasticity, lubrication, and cohesiveness of the bolus, which are essential for initiating the swallowing reflex (Doyennette et al., 2011). Beyond aiding mastication, salivary secretion maintains oral moisture and has antibacterial properties (Xu, Laguna, & Sarkar, 2019).

A decrease in salivary flow, known as hyposalivation, can result in dry oral mucosa, difficulty forming and transporting a bolus, tooth demineralization,

mucosal ulceration, alterations in the oral microbiome, dysphonia, impaired taste and smell during eating, and general oral discomfort (Affoo et al., 2015).

2. COMMON ORAL AND DENTAL PROBLEMS IN ELDERLY INDIVIDUALS

2.1. Tooth Loss

Although the global proportion of edentulous individuals has decreased in recent decades, tooth loss remains more likely with advancing age. Improvements in dental care techniques and the widespread adoption of preventive measures have delayed the onset of tooth loss, resulting in a declining overall prevalence. Age is consistently identified as the primary factor associated with tooth loss worldwide (Tramini et al., 2007). Other risk factors include male sex, advanced age, and tobacco use (Hirotsu et al., 2012). In older adults, tooth loss may result from dental caries, periodontal disease, prosthetic or orthodontic treatments, trauma, endodontic or periapical pain, as well as behavioral and attitudinal factors (Müller et al., 2007). Adequate intake of calcium and vitamin D has also been linked to lower risk of tooth loss (Krall et al., 2019), while lower socioeconomic status and limited education are associated with higher prevalence of edentulism (Gaio et al., 2012). Although dental caries is the leading cause of tooth loss among adolescents and younger adults, periodontal disease becomes a more significant contributor later in life. Additionally, both the frequency and type of dental care received throughout life influence the number of teeth retained in old age (Laguzzi et al., 2016).

Research by Musacchi et al. (2007) indicated that edentulism was highly prevalent among elderly Italians, with women exhibiting higher rates than men. In women, tooth loss was associated with age, reproductive and hormonal factors (such as pregnancies and menopause), and living alone. In men, aging and smoking were significant determinants, with edentulism also linked to an increased risk of hypoalbuminemia. Chewing difficulties were related to the type of missing teeth. Similarly, Negreiros et al. (2025) reported that complete tooth loss was more common among elderly individuals with lower socioeconomic status, smokers, those who consumed sugar-sweetened beverages, and those who did not engage in regular physical activity.

2.2. Tooth Decay

Dental caries is one of the most prevalent oral health issues among older adults (Janto et al., 2022). In the United States, most elderly individuals have at least one decayed tooth. The prevalence of caries in older populations differs significantly between developed and developing countries. For instance, in India, approximately 82% of older adults are affected by dental caries, whereas in Germany, the prevalence among the elderly is around 30% (Chan et al., 2021).

Caries remains the leading cause of tooth loss in older adults (Nelson et al., 2020). Its high prevalence is associated with age-related changes in saliva, medication-induced dry mouth, poor dietary habits, gingival recession exposing root surfaces, and the extended retention of natural teeth due to dental interventions (Janto et al., 2022). Moreover, older adults with psychiatric disorders may experience higher rates of dental decay due to frequent medication use and inadequate oral hygiene (Yang et al., 2021).

Since the introduction of fluoride toothpaste in the early 1970s, many of today's elderly did not benefit from its use until after their permanent teeth had erupted. Limited exposure to fluoride and a tendency toward more invasive dental treatments may explain the higher incidence of cavities, restorations, and tooth fractures observed in current older populations compared to younger generations (Diep et al., 2023). Therefore, regular dental check-ups, professional cleanings, oral hygiene education, and dietary counseling are crucial for maintaining oral health in older adults (Chan et al., 2021).

2.3. Periodontal Diseases

Periodontal diseases, including gingivitis and periodontitis, are chronic inflammatory conditions that compromise the structural integrity of tissues supporting the teeth. Due to their high prevalence, these conditions represent a significant public health concern (Lasica et al., 2024). In older adults, periodontitis is closely linked to malnutrition, functional disability, and a reduced quality of life, although the precise mechanisms behind these associations are not fully understood (Li et al., 2020).

Periodontal disease affects over 60% of elderly individuals and is considered a serious non-communicable condition (Chu et al., 2025). In addition to biological changes related to aging, social determinants such as low income and limited education contribute to poorer oral care, increasing the risk of periodontal disease. Loss of manual dexterity, cognitive decline, and dementia further exacerbate oral health challenges, leading to higher rates of tooth loss, dental caries, and periodontal problems. Nutritional deficiencies can influence the inflammatory immune response and tissue repair, potentially triggering periodontal disease. Obesity has also been linked to systemic inflammation, which may contribute to periodontal pathology. Xerostomia, common in older adults, promotes disease progression by reducing salivary protection. Tobacco use further aggravates periodontitis by stimulating immune-mediated inflammation and disrupting the subgingival microbiome, promoting dysbiosis (Batwa et al., 2025).

Psychological stress in elderly individuals increases susceptibility to periodontitis through mechanisms such as poor oral hygiene, elevated glucocorticoid levels, immune suppression, and insulin resistance. Periodontitis is frequently associated with other chronic systemic conditions, including

cardiovascular disease, rheumatoid arthritis, and atherosclerosis, partly due to inflammatory mediators entering the circulation (Preethanath et al., 2025). More than half of older adults with diabetes develop periodontitis as an oral complication (Li et al., 2020). Chronic hyperglycemia and systemic inflammation can enhance oxidative stress in the periodontium, elevating inflammatory mediator levels and promoting alveolar bone loss, which contributes to periodontitis (Mirzaei et al., 2021). The bidirectional relationship between periodontitis and type 2 diabetes can disrupt glucose homeostasis and increase mortality risk, highlighting the systemic impact of oral inflammation in this population (Chu et al., 2025).

2.4. Xerostomia

Xerostomia refers to the subjective sensation of a dry mouth, typically resulting from reduced salivary flow or impaired salivary gland function (Korn et al., 2002). It is not classified as a disease itself but is a symptom associated with various pathological conditions that can considerably affect quality of life. Xerostomia may interfere with essential oral functions such as chewing, swallowing, denture use, and speech (Fornari et al., 2021; Villa et al., 2014). Additionally, it can increase the risk of oral complications, including fungal infections such as candidiasis, dental caries, halitosis, taste disturbances, and oral burning sensations (Fornari et al., 2021).

This condition is particularly common among older adults, largely due to the high prevalence of polypharmacy in this population (Scully, 2003; Ship et al., 2002). Medications frequently associated with xerostomia include anticholinergics, sympathomimetics, sedative-hypnotics, opiates, antihistamines, and muscle relaxants. Beyond medication-related causes, xerostomia may also arise from radiation therapy in patients with head and neck cancers or from autoimmune conditions such as Sjögren's syndrome (Gil-Montoya et al., 2016; Ship et al., 2002).

2.5. Denture Stomatitis

Denture stomatitis refers to diffuse or localized erythematous inflammation occurring in areas of the oral mucosa that are in contact with a denture. This condition is highly prevalent, affecting between 20% and 80% of denture wearers worldwide (McReynolds et al., 2023). Risk factors include advancing age and the associated decline in immune function, systemic diseases, prolonged denture use, aging dentures, and inadequate denture hygiene, which facilitates plaque accumulation on the prosthesis (Bilhan et al., 2009).

Clinically, denture stomatitis presents as inflammation and redness of the mucosal tissues beneath the denture. It is estimated that over two-thirds of removable complete denture users may experience this condition. Despite its high prevalence, denture stomatitis is frequently asymptomatic; only a minority of

patients report discomfort, itching, or a burning sensation. The condition is often first identified during clinical examination as erythema or swelling of the mucosa beneath the denture (Gendreau & Loewy, 2011).

Management strategies focus on addressing the underlying causes, which may include disinfecting the denture, ensuring proper denture fit, and treating fungal colonization if present (McAlpine & Culton, 2024).

2.6. Oral Mucosal Lesions

Aging is associated with physiological changes, an increased prevalence of systemic diseases, polypharmacy, reduced salivary flow, inadequate oral hygiene, poor nutrition, and lifestyle factors such as tobacco and alcohol use, all of which make the oral mucosa more vulnerable to lesions (Chan et al., 2021). Given their higher incidence in older adults, it is essential to identify oral mucosal lesions, manage contributing risk factors, and establish appropriate treatment strategies.

Oral mucosal lesions are commonly observed in the elderly and are clinically significant because they may reduce quality of life, represent precancerous conditions, or serve as indicators of systemic disease. Denture use and smoking are two major risk factors for oral lesions, both of which are prevalent in this population. Additionally, diminished awareness of oral changes or the inability to report symptoms due to cognitive decline, delirium, social isolation, or limited access to dental care can exacerbate the condition (Ewan & Staines, 2008).

Bozdemir et al. (2019) reported that most elderly individuals had at least one oral mucosal lesion, with prevalence rates of 46.3% in men and 41.3% in women. The most frequently observed lesion in both genders was sublingual varicosities. Similarly, Lynge Pedersen et al. (2015) found that the most common oral lesions in older adults included lingual varicosities (28.3%), denture stomatitis (12.7%), candidiasis (11.8%), fissured tongue (9.1%), and frictional keratosis (8.4%). These lesions were generally associated with smoking and reduced salivary flow.

2.7. Angular Cheilitis

Angular cheilitis is an inflammatory condition that affects the corners of the mouth, beginning at the mucocutaneous junction and sometimes extending to the surrounding skin. Clinically, it presents with erythema, moist maceration, ulceration, and crusting in the affected areas (Pandarithodiyil et al., 2021).

This condition is commonly observed in older adults, particularly those who wear complete dentures, and is often associated with denture stomatitis or glossitis. The prevalence of angular cheilitis among denture-wearing individuals over the age of 65 ranges from 10% to 25% (Talmaç, 2018). Contributing factors include a reduced vertical facial dimension, excessive moisture at the mouth corners, lip-licking, thumb-sucking, repetitive biting of the oral commissures, and

tissue sagging at the lip corners (Pandarthodiyil et al., 2021). Nutritional deficiencies, particularly iron and B vitamins, diabetes, and long-term tetracycline therapy are also recognized as common underlying causes (Febrine & Azzaim, 2023).

Management of angular cheilitis focuses on addressing predisposing factors and behavioural habits, as well as applying barrier protection using petroleum jelly, emollients, or lip balms. Antifungal and antibacterial therapies may be employed when indicated (McAlpine & Culton, 2024).

2.8. Temporomandibular Joint Problems

Temporomandibular joint (TMJ) disorders are characterized by craniofacial pain involving the head and neck, masticatory muscles, or their neural innervations. Common contributing factors include parafunctional habits, occlusal discrepancies, psychological stress and anxiety, trauma or microtrauma, mandibular instability, postural imbalances, and abnormal physiological conditions (Bitiniene et al., 2018). In older adults, posterior tooth loss, edentulism, and denture use may further contribute to the development of TMJ disorders (Jouhadi et al., 2025).

The prevalence of TMJ problems increases with age, driven by reduced masticatory muscle strength, degenerative changes in joint surfaces, and increased dental issues. Osteoarthritis, dysplasia, joint noises, and difficulties in mastication are among the most commonly observed TMJ disorders in elderly individuals. Age-related alterations in collagen structure and reduced muscle-tendon elasticity contribute to impaired joint function, while tooth loss and poorly fitting prostheses negatively impact joint biomechanics, thereby heightening the risk of TMJ dysfunction (Dimitroulis, 2018).

TMJ disorders can have broad consequences, including anxiety, stress, depression, sleep disturbances, social isolation, reduced work capacity, increased social and healthcare costs, physical disability, and diminished economic productivity due to frequent medical needs (Bitiniene et al., 2018). Consequently, early detection and management of TMJ disorders are crucial for maintaining health and quality of life in older adults.

3. RECOMMENDATIONS FOR PROTECTING THE ORAL AND DENTAL HEALTH OF ELDERLY INDIVIDUALS

- Family physicians should integrate routine oral examinations into the general health checkups of elderly patients.
- When prescribing medications, physicians should consider the potential for xerostomia and, if possible, choose alternatives with minimal effects on salivary flow.

- Older adults should be encouraged to maintain oral moisture by drinking water regularly or using saliva substitutes.
- Maintaining proper oral and denture hygiene should be emphasized among elderly individuals.
- Caregivers should be educated and encouraged to assist older adults with oral and denture care (Khanagar et al., 2021).
- Oral healthcare professionals should provide patient education on oral diseases and strategies for their prevention.
- Attention should be given to ensuring that elderly individuals follow a nutritionally adequate and balanced diet.
- Consumption of sugary or sticky foods between meals should be minimized to reduce the risk of dental problems.
- Regular toothbrushing should be performed after meals or at least once daily in the evening.
- Selection of toothbrushes should prioritize ergonomic design, comfort, and ease of use for elderly individuals.
- Routine dental visits should be encouraged to maintain oral health and promptly address any emerging dental issues (Tangade & Yusuf, 2018).

CONCLUSION

As with aging, physiological and pathological changes occur in the oral mucosa and teeth, as in all other structures of the body. Factors such as age-related systemic diseases, polypharmacy, denture use, and decreased salivation can increase the incidence of oral mucosal lesions in elderly individuals. However, oral and dental health problems in elderly individuals are often overlooked. Oral and dental health problems in elderly individuals can not only physically affect them but also impact their quality of life. Therefore, oral and dental health assessments should be included in the examination and evaluation of elderly individuals.

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CHAPTER 22

Limitations of the DMFT Index and a New Approach: The Weighted DMFT (W-DMFT) Model

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1. INTRODUCTION

According to the World Health Organization (WHO), between 60% and 90% of adults experience at least one episode of dental caries during their lifetime (1). This high prevalence necessitates the development of reliable and comparable indicators for assessing oral health.

Oral health is an integral part of an individual's overall health and quality of life. Many oral diseases—particularly dental caries and periodontal diseases—affect large segments of the population and represent significant public health problems. Objectively measuring the prevalence, severity, and treatment needs of these diseases is critically important for establishing national oral health policies and conducting epidemiological comparisons.

The most widely used measure for assessing dental caries prevalence is the DMFT (Decayed, Missing, Filled Teeth) index (2). However, although DMFT was suitable for the epidemiological needs of the period in which it was developed, its ability to reflect modern clinical understanding has been increasingly questioned (3). Despite the significant biological and functional differences between enamel-, dentin-, and pulp-level caries, DMFT reduces all of these stages to a single numerical value; moreover, it records even successfully restored teeth as indicators of disease.

For these reasons, this study identifies the conceptual and methodological limitations of the classical DMFT index and subsequently proposes the Weighted DMFT (W-DMFT) model as a modern alternative in which teeth are weighted according to their biological condition.

2. HISTORICAL DEVELOPMENT AND CONCEPTUAL FRAMEWORK OF THE DMFT INDEX

The DMFT index (Decayed, Missing, Filled Teeth) was developed in the late 1930s to standardize oral health epidemiology and quickly gained international acceptance. Considering the conditions of that period, DMFT provided an important innovation by expressing caries prevalence with a single numerical value and facilitating comparisons between different populations (4).

The basic structure of the DMFT index consists of three components:

- **D (Decayed):** Number of decayed teeth
- **M (Missing):** Number of teeth lost due to caries
- **F (Filled):** Number of restored teeth

The sum of these components forms the DMFT score. In the classical approach, each tooth is assigned an equal weight of **1 point**.

2.1. Reasons for the Widespread Use of DMFT

Historically, DMFT has been widely accepted for several main reasons:

- It is easy to apply,
- It can be assessed with minimal clinical equipment,
- It is suitable for international comparisons,
- It has decades of accumulated data.

These advantages have made DMFT the “common language” of oral health research worldwide.

2.2. Limitations of the Conceptual Structure

However, the period in which DMFT emerged was one in which both treatment approaches and the biological understanding of caries differed significantly from today. Therefore, the index has fallen behind modern clinical needs.

The main limitations are summarized below:

2.2.1. Inability to Reflect Caries Severity

In DMFT, an initial enamel lesion and a pulp-involved deep lesion carry the same value. This significantly distorts biological reality and underestimates the true caries burden of a population.

2.2.2. Inability to Measure Restorative Success

A successfully restored tooth is included in the “F” category and thus counted as “disease present.”

However, the disease has been treated, and the tooth is functionally healthy.

2.2.3. Inability to Distinguish Causes of Tooth Loss

The “Missing” category cannot distinguish whether tooth loss is due to:

- caries,
- periodontal disease,
- trauma,
- orthodontic extraction,
- or congenital absence (agenesis).

This reduces the epidemiological validity of the measure, especially in populations with high periodontal disease prevalence.

2.2.4. Incompatibility with Modern Treatment Approaches

Minimal intervention dentistry promotes non-invasive management of early lesions.

However, DMFT records even clinically manageable enamel lesions as “disease,” which:

- does not encourage preventive treatment,
- may lead to unnecessary restorations,
- and fails to reflect contemporary clinical philosophy.

2.2.5. Neglect of Functional Value

Assigning each tooth a value of 1 regardless of its biological or functional condition prevents accurate assessment of:

- functional loss,
- treatment need,
- occlusal integrity.

2.3. Current Epidemiological Needs

Today, when assessing population oral health, it is necessary to consider not only the presence of caries but also:

- biological depth of the lesion,
- treatment need,
- functional loss,
- restorative treatment success,
- future caries risk profile.

Since DMFT cannot meet these needs, the demand for more advanced, biologically based, treatment-oriented models has become increasingly evident. The W-DMFT model offers a solution that addresses this gap.

3. CLINICAL AND EPIDEMIOLOGICAL LIMITATIONS OF THE DMFT INDEX

Although the DMFT index has been widely used for many years, it has several limitations that fail to adequately reflect modern clinical realities (5,6,7). These limitations weaken the quality of epidemiological data and may lead to

misprioritization in community oral health policies. The major issues of DMFT are systematically presented below.

3.1. Inability to Distinguish Caries Severity

The fundamental structural problem of DMFT is that it does not account for the biological depth of caries (8,9). Coding both a superficial enamel lesion and a pulp-involved painful deep caries as “D = 1”:

- underestimates the actual caries burden of the population,
- fails to reflect treatment needs accurately,
- makes severity-based risk assessment impossible.

This is particularly disadvantageous in identifying high-risk populations.

3.2. Successful Restorations Counted as Disease

In DMFT, every restored tooth is recorded in the “F” category and thus considered as “disease present.”

However, modern restorative treatments:

- eliminate disease,
- restore function and aesthetics,
- create a biologically healthy condition.

Thus, treating restored teeth as indicators of disease weakens the clinical validity of DMFT.

3.3. Inability to Distinguish the Causes of Tooth Loss

The “Missing” category is one of the most problematic components of DMFT because tooth loss may result from:

- periodontal disease,
- trauma,
- orthodontic extraction,
- prosthetic planning,
- congenital absence (agenesis),

yet all are coded identically.

This can lead to misinterpretation of caries prevalence, especially in populations with high periodontal disease rates (10).

3.4. Inability to Provide Information About Treatment Needs

DMFT measures only the outcome, not the treatment need.

For example:

- An early enamel lesion

and

- A severe lesion requiring endodontic treatment

both fall under the same category.

Thus, DMFT does not adequately guide:

- health policy planning,
- resource allocation,
- the design of preventive programs.

3.5. Inability to Assess Functional Status

The functional condition of the tooth—such as chewing capacity, occlusal integrity, or aesthetic requirements—is completely ignored by DMFT (11,12,13). This limitation is especially relevant in:

- elderly populations,
- individuals with multiple missing teeth,
- denture users.

3.6. Incompatibility with Minimal Intervention Dentistry

Modern dentistry promotes non-invasive or micro-invasive management of early caries lesions.

However, DMFT codes even stabilized early enamel lesions as “D,” which:

- does not encourage preventive care,
- may lead to unnecessary restorative procedures,
- falls behind contemporary minimally invasive philosophy.

3.7. Problems with Interpretation Over Time

Although DMFT allows basic comparisons between populations, it becomes difficult to interpret over time due to:

- changes in treatment policies,
- advances in restorative materials,
- increased life expectancy,

- varying reasons for tooth extraction.

Two populations with the same DMFT score may have entirely different clinical profiles.

3.8. Misrepresentation of Clinical Burden

For two individuals with the same DMFT score:

- One may have only a few minor enamel lesions,
- while the other may have several pulp-involved caries and extraction indications.

The fact that these two individuals receive the same score clearly demonstrates DMFT's inability to reflect true clinical burden.

These limitations show that despite its historical importance, DMFT is insufficient for meeting modern epidemiological needs. Therefore, a more detailed, biologically based, and treatment-oriented model is necessary. The W-DMFT model was developed in response to this need.

4. THEORETICAL STRUCTURE AND WEIGHTING LOGIC OF THE W-DMFT MODEL

The limitations of the DMFT index highlight the need for a more advanced model that reflects the biological depth and functional impact of caries. The Weighted DMFT (W-DMFT) model was developed to address this need. While preserving the basic framework of the classical DMFT index, W-DMFT is a modern caries measurement system that assigns a weight to each tooth according to its biological severity and functional condition.

4.1. Theoretical Basis of W-DMFT

The fundamental philosophy of W-DMFT is:

“Not all teeth share the same biological condition; therefore, they should not carry equal value in epidemiological assessment.”

This approach takes into account the biological progression of caries—enamel → dentin → pulp—and acknowledges that each stage has different implications for treatment needs and functional impact.

By assigning different weights to teeth that would all be given a value of 1 in the classical DMFT system, W-DMFT provides a measure that aligns more closely with biological and clinical reality.

4.2. Weighting System of W-DMFT

In the W-DMFT model, each tooth is assigned a value between **0 and 3**, based on biological depth, treatment history, and functional condition:

- **0:** Sound tooth (caries-free and without restoration)
- **1:** Enamel-level lesion or small restoration
- **2:** Dentin-level advanced caries or dentin restoration
- **3:** Pulp-involved caries, endodontically treated tooth, or tooth loss due to caries

This classification:

- differentiates stages of caries severity,
- reflects treatment needs,
- categorizes restorations based on biological significance,
- models tooth loss as the final stage of disease severity.

4.3. Why a 0–3 Weighting Scale?

Three fundamental factors guided the choice of this weighting range:

1. Biological Depth

The hierarchical progression of caries from enamel → dentin → pulp is the primary biological basis.

This progression parallels the increasing need for treatment.

2. Increase in Functional Loss

As lesion depth increases, impairments occur in:

- chewing function,
- esthetics,
- occlusal integrity,
- pain levels.

3. Epidemiological Interpretability

A 0–3 scale is:

- simple,
- scalable,

- suitable for population comparisons,
- clinically meaningful.

4.4. Key Differences Between W-DMFT and Classical DMFT

Feature	Classical DMFT	W-DMFT
Weight per tooth	1	0–3
Caries severity differentiation	None	Present
Treatment need representation	Weak	Strong
Functional status consideration	Weak	Included
Evaluation of restorations	Counted as disease	Based on functional/biological state
Clinical realism	Low	High

These differences show that W-DMFT is a modern indicator that addresses current epidemiological needs more effectively.

4.5. Theoretical Advantages of W-DMFT

1. Compatibility with Biological Realit

Quantifies the biological stages of caries progression.

2. Suitability for Clinical Prioritization

Weighted severity helps determine treatment priorities more accurately.

3. Removal of Restorations from “Penalty” Category

Modern restorative outcomes are evaluated based on functional health, not as disease indicators.

4. More Accurate Reflection of Community Oral Health

Prevents two populations with the same DMFT score from being misinterpreted despite different clinical burdens.

5. Ease of Long-Term Monitoring

The weighting system provides greater sensitivity for assessing the effectiveness of preventive programs.

5.APPLICATION METHOD AND CALCULATION SYSTEM OF W-DMFT

The W-DMFT model offers a modern, radiographically based approach to evaluating the biological depth and functional impact of dental caries. The model is based on assigning a weight between 0 and 3 to each tooth according to its radiographic condition, and the sum of these weights reflects the individual’s caries burden.

Since this version relies on radiographic analysis rather than intraoral examination for weighting, it is particularly suitable for epidemiological studies, community oral health screenings, and retrospective record reviews. This section explains, step by step, how W-DMFT is applied entirely based on radiographic evaluation.

5.1. Radiographic Assessment Steps

In W-DMFT, evaluation is performed using panoramic, bite-wing, or periapical radiographs.

The goal is to reliably identify the biological damage level of each tooth based on its radiographic appearance.

The evaluation is carried out in three main steps:

1. Radiographic Examination of Each Tooth

Each tooth is examined individually on the radiograph. During this examination, the following are assessed:

- initial enamel lesions,
- caries extending into dentin,
- pulp proximity or pulp involvement,
- signs of periapical pathology,
- presence of endodontic treatment,
- tooth loss due to caries.

This step is critical for correctly identifying caries progression based on radiographic findings.

2. Assigning an Appropriate Weight (0–3) to Each Tooth Based on Radiographic Findings

Weighting in W-DMFT is determined entirely by radiographic data:

- **0:** Sound tooth (no caries or restoration visible radiographically)
- **1:** Enamel-level superficial lesion or small restoration
- **2:** Dentin-level advanced caries or dentin restoration
- **3:** Pulp-involved caries, endodontically treated tooth, or tooth loss due to caries

This classification converts the biological stages of caries into a numerical structure consistent with radiographic evidence.

3. Summing the Weights to Obtain the W-DMFT Score

The values (0–3) assigned to each tooth are summed, and the total constitutes the individual's W-DMFT score.

5.2. W-DMFT Calculation Formula

The mathematical expression used in W-DMFT is:

$$W\text{-DMFT} = \sum_{i=1}^{28/32} w_i \quad \text{where } \{W\text{-DMFT}\} = \sum_{i=1}^{28/32} w_i$$

Where:

- w_i = the weight assigned to each tooth based on radiographic findings
- $28/32$ = number of evaluated teeth

This formula produces a more biologically and functionally meaningful result than the classical DMFT.

5.3. Radiographic Evaluation of Missing Teeth

Evaluation of missing teeth depends on the availability of radiographic or historical data:

- If previous radiographs or treatment records indicate tooth loss due to caries → **3 points**
- If tooth loss is due to non-carious reasons → may be:
 - excluded from evaluation,
 - assigned **0 points**,
 - or reported as a separate category.

This flexibility allows adaptation to various epidemiological study designs.

5.4. Radiographic Classification of Restorations

Restorations are categorized radiographically according to depth and biological relevance:

- Small restorations limited to enamel → **1 point**
- Dentin-level restorations → **2 points**
- Endodontically treated teeth → **3 points**

This approach provides a more accurate assessment of restorative treatment impact compared to DMFT.

5.5. Ease of Application and Compatibility

Radiographic-based W-DMFT offers several practical advantages:

- No additional measurement equipment required
- Allows retrospective analysis using digital archives
- Field workers familiar with DMFT can easily adapt
- Generates standardized data in epidemiological screenings
- Offers high accuracy in estimating treatment needs

5.6. Interpretation at the Individual and Population Levels

Individual Level: W-DMFT integrates:

- caries severity,
- biological depth,
- treatment need

into a single measurement.

Population Level: W-DMFT:

- reflects caries burden more accurately,
- shows the impact of preventive programs more sensitively,
- is more reliable for inter-population comparisons than DMFT.

6. EPIDEMIOLOGICAL ADVANTAGES OF W-DMFT AND COMPARATIVE ANALYSIS WITH DMFT

The W-DMFT model was developed to address the long-debated biological and epidemiological limitations of the classical DMFT index. While DMFT measures caries merely as a presence/absence condition, W-DMFT provides a weighted approach that incorporates the biological depth of the lesion, functional impacts, and restorative processes. This section examines the superiority of W-DMFT in population-based studies through a comparative analysis with DMFT.

6.1. Ability to Reflect Biological Depth

In DMFT, all caries—whether enamel-, dentin-, or pulp-level—receive the same value (1).

This approach cannot reflect the biological progression of the lesion.

W-DMFT, however:

- assigns low weight to enamel lesions (1),

- moderate weight to dentin involvement (2),
- high weight to pulp involvement (3),

thereby measuring biological severity in a graded and realistic manner.

This is especially beneficial in populations with high caries burden, where biological damage must be evaluated accurately.

6.2. Ability to Express Treatment Need Numerically

DMFT only provides the number of carious teeth; it offers no information regarding severity or treatment need.

W-DMFT, on the other hand:

- assigns low weight to early lesions,
- moderate weight to lesions requiring restoration,
- high weight to teeth requiring endodontic or advanced treatment,

thus numerically expressing treatment needs at both the individual and population levels.

This feature is highly valuable for planning community oral health services.

6.3. Increased Epidemiological Sensitivity

Because DMFT assigns equal value to different biological stages, variations within populations may remain hidden.

W-DMFT:

- creates a broader score distribution,
- highlights severity differences,
- enables more sensitive tracking of changes over time.

Therefore, its epidemiological sensitivity is considerably higher than that of DMFT.

6.4. Incorporation of Restorative Success and Biological Condition

DMFT evaluates all restorations under a single category.

W-DMFT classifies restorations according to biological relevance:

- enamel restorations → 1
- dentin restorations → 2
- endodontic treatments → 3

This approach reflects restorative procedures not as mere numerical counts but as indicators of severity and functional impact.

6.5. Improved Comparability Between Populations

Two populations with identical DMFT scores may have very different caries severity profiles.

W-DMFT:

- quantifies biological severity,
- distinguishes between shallow and deep lesions,
- considers restoration depth,

resulting in more reliable, sensitive, and meaningful inter-population comparisons.

This makes W-DMFT superior to DMFT in identifying oral health inequalities and high-risk groups.

6.6. Advantages of a Radiography-Based System

Reliance on radiographic data provides significant methodological benefits:

- objective and reproducible results,
- ability to conduct retrospective studies using digital archives,
- high inter-observer agreement,
- reduced clinical examination errors,
- reliable detection of lesion depth.

These features make W-DMFT highly suitable for large-scale epidemiological research.

7. RESEARCH APPLICATIONS OF W-DMFT AND DISCUSSION ON MEASUREMENT VALIDITY AND RELIABILITY

The W-DMFT model is designed as a modern measurement tool that provides both quantitative and qualitative data in caries epidemiology. Its weighted structure allows it to be used in different research designs, and it offers significant advantages over classical DMFT in terms of validity and reliability. This section discusses the methodological characteristics and research potential of W-DMFT.

7.1. Research Areas of Application

W-DMFT offers strong compatibility and advantages in the following types of research:

- **Epidemiological population surveys**

- Graded measurement of severity allows more accurate identification of risk distribution.

- **Clinical research**

- Since it includes clinically significant indicators such as treatment need, pulp involvement, and restoration depth, it can be used in studies examining treatment effectiveness.

- **Health service planning and policy development**

- As a system that grades treatment burden, it provides more sensitive information than DMFT for resource planning.

- **Digital archive and retrospective studies**

- Radiographic-based scoring makes it possible to extract data from past digital records.

7.2. Evaluation of Measurement Validity

The W-DMFT model has stronger validity than the DMFT index. The main reasons include:

- **Content validity**

- All clinical stages of caries progression (enamel–dentin–pulp) are represented in a weighted manner.

- The biological scope missing in DMFT is fully captured in W-DMFT.

- **Construct validity**

- The weighting system reflects the biological hierarchy of caries progression, making the model conceptually coherent.

- **Criterion validity**

- Strong correlation with clinical criteria such as caries severity, pulp involvement, and depth of restorations increases the model's alignment with biological reality.

7.3. Evaluation of Measurement Reliability

The radiographic foundation of W-DMFT provides substantial advantages for reliability:

- **High inter-observer reliability**

- Different evaluators can assess the same radiograph with high consistency.

- **High test–retest reliability**

- Since digital radiographs do not change over time, repeated measurements yield consistent results.

- **Reduction of clinical examination variability**

- Subjective inconsistencies in clinical DMFT examinations are minimized.

These properties are especially important in large-scale population studies.

7.4. Interpretability of Research Findings

The wider scoring range of W-DMFT offers several advantages:

- clearer visibility of differences in caries severity between individuals,
- more meaningful comparisons between groups,
- more sensitive analysis of changes over time,
- increased statistical power in modeling (regression, risk analysis, etc.).

Thus, W-DMFT provides greater interpretability in epidemiological modeling.

7.5. Scientific Advantages Compared with DMFT

From a measurement theory perspective, W-DMFT is:

- more compatible with biological reality,
- more sensitive,
- more reliable,
- more suitable for epidemiological analysis,
- reusable in digital environments.

These characteristics indicate that W-DMFT has the potential to replace DMFT in the long term.

8. LIMITATIONS OF W-DMFT APPLICATION AND RECOMMENDATIONS FOR FUTURE RESEARCH

Although the W-DMFT model offers significant advantages over DMFT, it has certain limitations, as is the case with any epidemiological or clinical measurement tool. Identifying these limitations is important for strengthening the methodological structure of the model and guiding future research.

8.1. Limitations of a Radiography-Based System

While radiography provides important advantages, it also introduces certain limitations:

- Radiographic resolution and device quality can affect diagnostic accuracy.
- In large population studies where bite-wing or periapical imaging cannot be used, panoramic radiographs may not always detect early enamel lesions clearly.
- Early superficial lesions may remain undetected with some imaging techniques.

Therefore, standardization of imaging protocols is particularly important for evaluating enamel lesions.

8.2. Evaluation Based on a Single Image

When W-DMFT scoring is based on a single radiographic image:

- the three-dimensional structure of the lesion may not be fully assessed,
- perceived lesion depth may vary depending on the angle and exposure of the radiograph,
- minimal secondary caries beneath restorations may not always be visible.

Thus, multi-angle imaging or validation studies using volumetric digital imaging (e.g., CBCT) are valuable.

8.3. Radiographic Limitations in Distinguishing Restoration Depth

Radiographs may not always clearly reveal whether restorations are:

- restricted to enamel,
- extending into dentin,
- or associated with minimal secondary caries.

This can occasionally limit the precision of restorative categorization.

8.4. Dependence on Standardization Across Studies

For W-DMFT to be comparable across research studies, the following need standardization:

- imaging type,
- weighting protocol,
- definitions of evaluation criteria,
- number of teeth assessed.

Without standardization, cross-study comparability may be reduced.

8.5. Need for Training and Calibration

Evaluator experience may influence results, including:

- interpretation of radiographic features,
- assessment of pulp proximity,
- identification of restoration depth.

Calibration between observers is recommended to ensure reliability.

8.6. Recommendations for Future Research

To further develop W-DMFT, future research may focus on:

1. Validity studies across different age groups

Creating normative datasets for children, young adults, adults, and the elderly.

2. Comparisons of radiographic techniques

Evaluating the influence of panoramic, bite-wing, and periapical imaging on W-DMFT scoring.

Investigating digital technologies and AI-assisted analysis.

3. Longitudinal (follow-up) studies

Assessing caries progression over time using W-DMFT to test model sensitivity.

4. Studies in diverse populations

Evaluating W-DMFT performance across socioeconomic groups, rural–urban settings, and high-risk populations.

5. Relationship between restoration success and W-DMFT

Examining long-term restoration outcomes in relation to W-DMFT categories.

6. Additional measurement theory studies

Including construct validation, factor analyses, and comparisons with other indices.

Identifying these limitations does not weaken the scientific value of W-DMFT; rather, it guides future improvements and strengthens its methodological foundation.

9. CONCLUSION AND EVALUATION

The W-DMFT model offers a modern approach that incorporates important biological and epidemiological elements overlooked by the classical DMFT index. By quantifying not only the presence of caries but also its biological depth, functional impact, and restorative status, the model becomes a more comprehensive and sensitive measurement tool compared with DMFT.

The radiography-based weighting system enables objective differentiation of the enamel–dentin–pulp continuum, allowing the biological severity of caries to be accurately reflected. In this respect, W-DMFT more effectively defines treatment needs at the individual level and more precisely evaluates caries burden and distribution at the population level.

Key epidemiological advantages of the W-DMFT model include:

- strong alignment with biological reality,
- increased measurement sensitivity,
- incorporation of restorative processes into the index,
- objectivity provided by radiographic assessment,
- greater reliability in inter-population comparisons.

However, reliance solely on radiographic data may limit the visibility of early enamel lesions, which underscores the importance of image quality and standardized imaging protocols. These limitations provide an important framework for future research aiming to refine the model.

Overall, W-DMFT is more than an alternative to the classical DMFT; it represents a conceptual update to the philosophy of measuring caries epidemiology. Because it successfully reflects biological and functional reality, the model is well positioned for broader use in community oral health surveys, clinical studies, and health planning efforts in the future.

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CHAPTER 23

The Institutionalization of Hygiene and Public Health Education in Turkey: Professor Julius Hirsch's Contributions

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1. Introduction

The modernization of medical education in Turkey evolved in parallel with the broader structural reforms of the early Republican era. Within this framework, the University Reform of 1933 not only reshaped higher education as an institution but also transformed the scientific foundations of medical instruction. Among the foreign scholars recruited to Istanbul University Faculty of Medicine after the reform, Ordinarius Professor Julius Hirsch played a significant role in redefining hygiene—positioning it not as a narrowly microbiological field, but as a comprehensive scientific discipline shaped by biological, environmental, and socio-economic determinants relevant to public health policies.

Hirsch's perspective conceptualized hygiene as a discipline that transcended individual preventive measures, emphasizing instead population-based health indicators, the interpretation of epidemiological data, and community-oriented research. This approach reshaped public health education in Turkey, uniting laboratory-based microbiology instruction with field-oriented social medicine. His emphasis on laboratory infrastructure aimed not merely to teach experimental microbiology, but to establish a sustainable environment in which research and education could be systematically integrated.

In this context, Hirsch's contributions to hygiene education can be articulated across three fundamental dimensions: (i) the institutionalization of laboratory-based and practical training, (ii) the incorporation of epidemiological observation and data-driven social hygiene into the curriculum, and (iii) the integration of microbiological research, chemotherapy, and antibacterial studies into teaching. Together, these elements not only enhanced the quality of medical education but also supported the development of public health policies grounded in scientific evidence.

2. Hirsch's Arrival in Istanbul and the Reorganization of the Hygiene Chair

Following the 1933 University Reform, the dissolution of the Ottoman-era Darülfünun and the establishment of Istanbul University required not only the reconstitution of academic personnel but also the structural reorganization of existing chairs with respect to scientific productivity and instructional methodology. Within this process, Ordinarius Professor Julius Hirsch—previously affiliated with the Institute of Hygiene at the University of Berlin—was appointed to lead the hygiene chair at the Istanbul University Faculty of Medicine. His recruitment was not merely the replacement of a vacant position; it was a strategic decision aimed at aligning hygiene education in Turkey with contemporary scientific standards.

At the time of Hirsch's arrival, hygiene, microbiology, and parasitology were institutionally distinct chairs functioning independently within the faculty. Hirsch

challenged the perception of hygiene as a secondary component of preventive medicine, asserting instead that hygiene encompassed a broad spectrum of inquiry, including environmental health sciences, epidemiological analysis, and laboratory-based research. This holistic view mandated a redefinition of the discipline as a central preclinical science grounded in laboratory investigation and public health-oriented field studies.

One of Hirsch's earliest administrative initiatives was to prioritize the training and recruitment of local Turkish assistants rather than requesting foreign specialists. His stance was based on the notion that the long-term sustainability of hygiene as a scientific discipline depended on the development of native academic personnel. This decision not only reduced structural dependence on foreign expertise but also laid the foundation for a permanent institutional culture centered on local scientific capacity.

Additionally, the acquisition of Professor Martin Hahn's extensive library collection for the chair—facilitated through Hirsch's initiative—provided access to a comprehensive body of contemporary German-language literature in hygiene and microbiology. This transfer was not merely an act of donation; it represented the systematic integration of Turkish medical education into the international scientific network. The availability of this literature played a crucial role in shaping the intellectual environment of the chair, supporting both education and research on a modern and globally informed basis.

Thus, Hirsch's appointment must not be interpreted solely as a personnel replacement. Instead, it signified the structural reconfiguration of hygiene as a scientific discipline in Turkey, integrating laboratory infrastructure, field-based observation, and interdisciplinary methodology into a coherent institutional framework.

3. Laboratory-Based Instruction and the Institutionalization of Hygiene as an Experimental Science

Professor Julius Hirsch's educational strategy in Istanbul was founded on the conviction that hygiene could not be taught merely through theoretical instruction. Instead, it required a laboratory-centered pedagogical model in which epidemiological inquiry, microbiological observation, and chemical analysis functioned as inseparable components of scientific training. Hirsch's approach rejected the traditional separation of theoretical lectures from practical applications, establishing instead an integrated structure where experimentation constituted the basis of both learning and research.

To achieve this, Hirsch reorganized the hygiene laboratories according to standards derived from leading European institutes, particularly those in Germany. Within this restructured system, the curriculum incorporated microbiological culture techniques, antibacterial susceptibility testing, chemical

evaluation of water and food quality, environmental sanitation analyses, sterilization procedures, and epidemiological sampling methods. Through these practices, hygiene education in Turkey was transformed from an ancillary preventive field into a research-based scientific discipline capable of producing empirical data and informing public policy.

Hirsch's insistence on incorporating microbiological and chemical experimentation into preclinical instruction marked a methodological shift for medical education in Turkey. Students were required not only to identify pathogenic microorganisms but also to observe their behavior under varying environmental and chemical conditions. This included examining microbial proliferation, transmission mechanisms, and responses to disinfectants or chemotherapeutic substances. Such instruction enabled students to understand hygiene not simply as a technique of disease prevention, but as a scientific investigation into the biological and environmental determinants of disease control.

The scientific rigor of this laboratory-centered instruction had broader implications beyond medical pedagogy. As research developed within the hygiene chair, laboratories began to generate technical data relevant to national and municipal public health administration. Water sanitation standards, food inspection parameters, sterilization protocols, and environmental health regulations began to rely on laboratory-tested guidelines rather than empirical observation or imported recommendations. In this respect, the hygiene chair evolved into a reference institution capable of advising local authorities and contributing to policymaking through empirical evidence.

Through these reforms, Hirsch transformed the hygiene chair into an academic and technical institution where education, research, and state-level public health administration converged. Hygiene education in Turkey thus became not merely a preclinical requirement, but a cornerstone of scientific public health practice grounded in experimental methodology.

4. The Social Hygiene Approach and the Integration of Epidemiology into the Curriculum

One of the most distinctive aspects of Julius Hirsch's contribution to hygiene education in Turkey was his introduction of a social hygiene perspective grounded in systematic epidemiological analysis. This approach redefined hygiene not merely as the study of pathogenic agents but as a discipline examining the interplay between disease and the broader social, economic, and environmental conditions that shape public health outcomes. In contrast to traditional preventive medicine—often limited to individual measures—Hirsch emphasized population-level health determinants, assigning a central place to epidemiology within the curriculum.

Hirsch argued that the emergence, distribution, and persistence of diseases could not be explained solely by biological causes. Instead, they were influenced by demographic dynamics, patterns of urbanization, occupational conditions, migration, nutritional status, housing quality, and sanitary infrastructure. Consequently, students were required to study data collection methods, statistical analysis, and field-based observational techniques as integral components of hygiene education. Through this integration, epidemiology ceased to be a supplementary subject and became a core methodological instrument in the training of medical students.

Importantly, Hirsch rejected the reduction of epidemiology to abstract statistical procedures. In his pedagogical framework, epidemiology operated as a bridge between field observations and laboratory investigations. Students were expected to correlate quantitative data obtained from communities with microbiological laboratory results, thereby producing scientifically grounded evaluations of public health conditions. This interdependence of field and laboratory methodologies constituted a defining characteristic of Hirsch's model.

Furthermore, Hirsch promoted the notion that public health challenges must be examined through nationally representative data rather than localized or anecdotal accounts. His advocacy for field studies conducted in rural Anatolia exemplified this principle. These studies were not conceived as singular research expeditions but as essential extensions of public health training, enabling students to engage directly with diverse regional health profiles and socio-economic contexts. Thus, fieldwork became a pedagogical tool that anchored public health education within the real-life conditions of the Turkish population.

Through these innovations, Hirsch institutionalized a discipline in which epidemiology and social hygiene operated not as peripheral themes but as foundational principles. Under his influence, public health education in Turkey gained a scientific identity rooted in population science, empirical investigation, and the methodological convergence of laboratory research with community-based inquiry.

5. The Integration of Microbiology, Chemotherapy, and Antimicrobial Research into Hygiene Education

A defining dimension of Julius Hirsch's scientific influence in Turkey was his emphasis on the integration of microbiology, chemotherapy, and antimicrobial research into the pedagogical structure of hygiene. By doing so, Hirsch challenged the conventional separation between basic microbiological knowledge and therapeutic practice, arguing that the scientific investigation of infectious diseases required a comprehensive understanding of pathogenic mechanisms, antimicrobial action, and chemotherapeutic interventions. This approach positioned hygiene as a discipline contributing not only to prevention but also to research-driven public health treatment strategies.

Hirsch's laboratory courses introduced students to experimental assays involving disinfectants, antiseptics, and early chemotherapeutic agents. He incorporated analyses of microbial growth inhibition, bacteriostatic mechanisms, and the chemical properties of antimicrobial compounds. These experiments were not supplementary demonstrations but functioned as core components of laboratory education, requiring students to observe the effects of chemical interventions on various bacterial species under controlled conditions. Such instruction aimed to cultivate a new generation of physicians capable of drawing scientific connections between microbiological processes and therapeutic applications.

During the years preceding and following World War II, Hirsch expanded the laboratory's capacity for experimental work on antibacterial substances, fostering studies on sulfonamides, antiseptic solutions, and later penicillin. The hygiene laboratory became an institutional site where developments in chemotherapy were monitored, tested, and adapted to local scientific needs. Hirsch's publications on bacterial culture, microbial proliferation, and chemotherapeutic effects contributed to disseminating modern knowledge within the Turkish medical community and strengthened the scientific identity of the hygiene chair.

Through this integration of microbiological science and chemotherapeutic research, Hirsch advanced an educational model in which hygiene no longer functioned as an exclusively preventive or descriptive discipline. Instead, it became an applied experimental science contributing to therapeutic decision-making, laboratory diagnostics, and the scientific evaluation of antimicrobial agents. This methodological expansion ensured that hygiene instruction in Turkey evolved alongside global developments in infectious disease science, chemotherapy, and antibiotic research.

6. Field Studies in Anatolia and the Development of National Public Health Training

A central feature of Julius Hirsch's concept of hygiene education was the notion that the scientific study of public health required direct engagement with the lived conditions of the population. For Hirsch, empirical public health training could not remain confined to the laboratory or the urban environment. Instead, it necessitated systematic fieldwork in diverse rural regions of Anatolia, where epidemiological profiles, socio-economic structures, and sanitary conditions differed significantly from metropolitan centers. Hirsch viewed these investigations as essential pedagogical tools, enabling students to confront a more accurate representation of the nation's health realities.

Field studies organized under Hirsch's leadership did not function as temporary excursions or isolated research missions. Rather, they were structured educational practices designed to train students in population-based observation, survey techniques, sanitary inspection, and epidemiological documentation.

During these studies, students collected data on infectious disease prevalence, nutritional deficiencies, living standards, waste management, water supply systems, and the availability of medical services. These observations were then correlated with laboratory analyses performed upon return to Istanbul, reinforcing Hirsch's principle of methodological integration between fieldwork and laboratory science.

Importantly, these field investigations contributed to the formation of a national public health perspective that was grounded in empirical evidence rather than theoretical assumption or imported models. By exposing students to public health disparities across different regions, Hirsch sought to cultivate a generation of physicians capable of developing policies reflective of Turkey's demographic and socio-economic diversity. In doing so, he advanced the idea that effective public health planning required localized data collection and context-specific epidemiological evaluation.

The impact of these field studies extended beyond medical pedagogy. The data gathered during such investigations informed municipal and provincial health authorities, supporting the development of programs in sanitation, disease prevention, water regulation, and rural health administration. Thus, the hygiene chair did not merely educate students—it became an advisory institution shaping public health governance on a national level.

Through these efforts, Hirsch established a model of public health training in Turkey that relied on a dynamic relationship between laboratory science, epidemiological analysis, and field-based inquiry. This model served as a precursor to later public health initiatives institutionalized in the country, demonstrating the enduring influence of Hirsch's pedagogical framework.

7. Institutional Legacy: Continuity of Public Health Education after Hirsch

The institutional reforms initiated by Julius Hirsch did not conclude with his tenure or personal presence at Istanbul University. Instead, they cultivated a lasting academic framework that shaped the development of public health education in Turkey for subsequent generations. Hirsch's emphasis on laboratory-based methodology, epidemiological training, and field-oriented inquiry became embedded within the structural organization of the hygiene chair, ensuring its continuity through the work of his students and academic successors.

Among the figures who carried Hirsch's legacy forward, Professor Kemal Erel stands out as a significant contributor to the post-war consolidation of public health education in Turkey. Erel, who had been trained under Hirsch's mentorship, maintained the integration of laboratory science and community-based investigation as central components of educational practice. Under his direction, the hygiene chair continued to expand its laboratory capabilities while

strengthening its advisory function to municipal and national health authorities. This sustained the reciprocal relationship between scientific research and public health administration that Hirsch had originally established.

The continuation of this institutional model ensured that hygiene and public health education remained aligned with contemporary scientific developments, particularly in bacteriology, chemotherapy, epidemiology, and environmental health regulation. The integration of these fields into the curriculum created a stable academic identity for hygiene as a discipline deeply rooted in experimental methods and evidence-based policy formation.

Beyond educational continuity, Hirsch's legacy contributed to the broader professionalization of public health in Turkey. By training physicians capable of simultaneously functioning as researchers and administrators, he helped shape a generation of public health professionals who could translate scientific knowledge into practical measures. This alignment of science and administration established a foundation for later institutions, including state-level health services and university-based public health departments, whose operations relied on empirical data and laboratory expertise.

In this regard, Hirsch's legacy was not limited to the modernization of medical education. It extended to the institutionalization of a scientific public health culture in Turkey—one that valued empirical inquiry, laboratory-grounded decision-making, and community-based research as the core instruments of health policy and medical training.

Conclusion

The contributions of Ordinarius Professor Julius Hirsch to the institutionalization of hygiene and public health education in Turkey represent a turning point in the development of modern medical instruction and scientific public health practice. By redefining hygiene as an interdisciplinary, laboratory-based, and population-oriented discipline, Hirsch transformed both the pedagogical and structural foundations of public health training at Istanbul University. His integration of microbiology, epidemiology, chemistry, and field investigation produced an educational model that linked theoretical knowledge with empirical research and community-based observation.

Hirsch's insistence on cultivating local academic expertise, constructing modern laboratory facilities, and expanding field studies across Anatolia established a sustainable institutional structure capable of producing scientific evidence relevant to national health planning. The hygiene chair, under his direction, became not only a center of pedagogical innovation but also a technical advisory institution that informed municipal and state health authorities. By aligning laboratory science with public health policy, Hirsch contributed to the

emergence of a professional culture in which scientific research functioned as the basis for administrative decision-making.

The continued development of this model through the work of Hirsch's students and successors ensured the long-term stability of public health education in Turkey. This legacy resulted in a durable institutional framework where hygiene and public health were shaped by experimental methods, epidemiological research, and scientifically grounded intervention strategies. In this context, Hirsch's contributions transcended individual instruction and played a formative role in shaping the intellectual, institutional, and professional foundations of public health in the early Republican period.

Hirsch may thus be regarded as one of the central figures who transformed hygiene from a preventive medical practice into a scientifically autonomous discipline capable of informing public health policies. His work stands not only as a milestone in the modernization of medical education but also as a foundational step in the creation of a national scientific public health tradition in Turkey.

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CHAPTER 24

Powered Enstruments in Septorhinoplasty

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Rhinoplasty is one of the most commonly performed surgical procedures in the field of facial aesthetics worldwide. Due to its central position on the face and its critical role in aesthetic harmony, the nose lies at the intersection of both functional and aesthetic expectations. Rhinoplasty procedures are primarily categorised under two main categories: aesthetic rhinoplasty and functional rhinoplasty (Whitaker, Karoo, Spyrou, & Fenton, 2007).

The goal of aesthetic rhinoplasty is to reshape the nasal dorsum, tip, alar structure, and nasal surface contours, which determine the patient's appearance, to align with the patient's facial proportions. In functional rhinoplasty, the goal is to optimize airflow by correcting anatomical pathologies that impair breathing, such as septal deviation, nasal valve stenosis, collapse, nasal cavity stenosis, and turbinate hypertrophy.

In contemporary rhinoplasty practice, integrating both aesthetic and functional objectives represents a central guiding principle. Consequently, the outcome of rhinoplasty surgery is closely linked to the surgeon's depth of anatomical knowledge, technical skill, and the surgical planning. Furthermore, the variety and precision of surgical instruments, along with their capacity to minimize tissue trauma, play a critical role in achieving successful results.

As the frequency of rhinoplasty procedures continues to rise globally, a growing body of research has emerged, aiming to optimize patient comfort, maximize surgical effectiveness, and minimize complications. Extensive literature exists regarding both pharmacological approaches including corticosteroids, decongestants, tranexamic acid, fibrin sealants, local anesthetics with epinephrine, and herbal or enzymatic agents such as arnica montana, papain, melilotus, bromelain, and α -chymotrypsin and surgical strategies, such as intraoperative cooling, controlled hypotension, postoperative head elevation, and drainage. These interventions are primarily focused on reducing common postoperative complications, including edema, pain, and ecchymosis (Mehdizadeh et al., 2018; Ong, Farhood, Kyle, & Patel, 2016).

Moreover, many modifications have been proposed to improve the components of the surgical technique. These innovations encompass adjustments in the order of lateral osteotomy steps, selection between external and internal methods, and perforated or continuous osteotomies. Further enhancements involve the development of subperiosteal tunnels, preservation of periosteal attachments, and the adoption of advanced instruments such as powered micro-osteotomes, specialized sawing systems known as "magic saws," piezosurgery devices, and diamond burrs.

Success in rhinoplasty surgery is not limited solely to the surgeon's skill; the impact of the instruments used on the tissue, the level of control, and safety are also crucial. Because nasal bone and cartilage structures are thin, fragile, and exhibit significant anatomical variation among individuals, accurate planning of

the osteotomy line, dorsal hump resection, controlled application of lateral and medial osteotomes, and selection of appropriate instruments for smoothing bone surfaces directly impact postoperative edema, ecchymosis, pain, and the healing process.

Osteotomes, rasps, and manual saws have been the primary tools in traditional rhinoplasty techniques for many years. However, with the integration of technology into surgical instruments over the past two decades, the use of energy-based systems such as piezoelectric devices, microsaws, and high-speed burrs has increased. These modern devices provide more controlled and minimally traumatic intervention on the bone, improving intraoperative control and reducing damage to surrounding soft tissues (Bayrakci, Aricigil, Eryilmaz, Eravci, & Arbağ, 2025; Ozucer & Özturan, 2016).

Microsaw systems offer high accuracy, especially for lateral osteotomies, controlled incisions in the keystone area, and dorsal hump resection. The surgeon can precisely control incision direction and depth, which lowers the risk of uneven bone fractures and healing. High-speed burr systems also offer key advantages. They help gradually open the osteotomy line, correct nasal bone irregularities, and precisely shape the nasal contour. Burrs of various diameters and shapes help correct radial asymmetries through controlled abrasion of the bone surface.

These modern energy-based devices offer clear advantages over traditional instruments, including a more stable surgical field, less edema and ecchymosis, reduced tissue trauma, more precise osteotomies, and faster recovery. However, given device and use costs, as well as certain clinical limitations, many surgeons now take a hybrid approach, combining conventional and energy-based instruments.

In conclusion, advances in rhinoplasty instruments have enhanced precision, minimized complications, and improved outcomes. This section details the technical specifications of basic conventional instruments, modern energy-based devices, and especially the microsaw and burr systems.

Rinoplasty History and Technological Development

The earliest record of nasal surgery is found in the Edwin Smith Papyrus, dating back to 3000 BC. These texts contain fundamental statements on the treatment of nasal trauma. In the 5th century BC, Hippocrates, in his work *Mochlicon*, first systematically classified nasal fractures. Galen, by describing nasal anatomy and nasal functions, laid the foundation for surgical practice in the centuries that followed (Bayrakci, 2023; Whitaker et al., 2007).

One of the most striking developments in nasal reconstruction stems from methods described in India in the 6th century BC. Sushruta, in his *Samhita*, detailed tissue transfer from the forehead or cheek to the nose, which is

considered the first known reconstructive nasal surgery. Known as the Indian Method, this technique later spread to the Middle East and eventually to Europe (Goldwyn, 1982). Although surgery remained in the background in Europe throughout the Middle Ages, nasal surgery reemerged with the Renaissance. The modern form of rhinoplasty dates to the 19th century, when the re-creation of nasal contours and reconstruction with living tissue became fundamental surgical principles (Adamson & Gantous, 2019; Whitaker et al., 2007). In 1887, John O. Roe, a pioneer of rhinoplasty for aesthetic purposes, performed the first modern hump resection and introduced the endonasal approach to the literature.

The first descriptions of open rhinoplasty were made by Jacques Joseph, and the concept of the nasal valve was introduced by Mink in 1903. The columellar incision, described by Rethi in 1921, was a significant step that laid the foundation for today's open techniques. Throughout the 20th century, Gorla's perforated osteotomy method and Sheen's understanding of structural rhinoplasty significantly advanced both the functional and aesthetic dimensions of modern nasal surgery

(Adamson & Gantous, 2019; Bayrakçı, 2023). Today, rhinoplasty has become one of the most refined facial surgeries, both aesthetically and functionally, as a result of the combined knowledge accumulated over history, modern instruments, and advanced surgical techniques.

For many years, scalpels, osteotomes, and rasps were the primary surgical tools in rhinoplasty. These manual techniques made intraoperative control challenging and increased trauma, leading to more edema, ecchymosis, and uncontrolled fractures.

The use of electrical and mechanical power marked a major advance in rhinoplasty. Motorized cutting systems, first used in orthopedics and neurosurgery in the mid-20th century, were adopted in facial surgery from the 1980s onward (Bayrakçı, 2023; Becker, Park, & Toriumi, 1999).

One of the most striking innovations in rhinoplasty over the last 20 years has been the increasing use of piezoelectric surgical devices. The selective cutting feature of piezoelectric systems, which targets only mineralized tissues while protecting soft tissues, has facilitated safe work in intranasal structures and has been reported in the literature to significantly reduce edema, bleeding, and surrounding tissue damage during nasal osteotomies (Becker et al., 1999; Gerbault, Daniel, & Kosins, 2016).

With the widespread use of micromotor-assisted systems, microsaws and high-speed burr tips have also been incorporated into rhinoplasty practice. These devices were initially tested in maxillofacial surgery. Since the late 1990s, they have become increasingly preferred in aesthetic rhinoplasty. They provide controlled bone resection, smoother cutting surfaces, and minimal soft tissue

trauma. Burrs, in particular, allow for high precision in bone contouring. This gives significant advantages in dorsum adjustment and asymmetry correction. Today, energy-based instruments, with their more compact designs, lower heat generation, and reduced vibration, are widely used in both primary and revision rhinoplasty. This technological advancement in microsaw, piezo, and high-speed burr systems allows the surgeon to achieve superior control over the bone while also improving patient comfort, complication rates, and recovery (Bayrakci et al., 2025).

Modern powered instruments with options that adapt to varying bone densities, ergonomically designed handpieces, and safety-focused motor designs enable controlled bone shaping. They demonstrate significant superiority over traditional manual techniques, particularly in terms of nasal dorsum reconstruction, prevention of greenstick fractures, ensuring smooth osteotomy lines, and minimally traumatic surgical procedures.

Piezelektric Surgery History and Technological Development

The foundation of piezoelectric surgery was laid in 1880 by Jean and Marie Curie, who discovered that certain crystals and ceramics generate ultrasonic vibrations by changing shape under an electric charge (Pavlíková et al., 2011). The identification of the piezoelectric effect later enabled the development of modern surgical devices capable of selectively cutting mineralized tissues. This physical principle became a technology applicable to clinical surgery nearly a century later, and the concept that ultrasonic energy could cut bone without damaging soft tissue was first applied in the 1970s.

Pioneering clinical studies on the use of piezoelectric energy in surgery were conducted by Horton and colleagues (Horton, Tarpley Jr, & Wood, 1975) in 1975. Researchers introduced piezoelectric ultrasonic vibration for gentler, more controlled cutting of alveolar bone, demonstrating that this technology provided a smoother cutting surface and safer operation compared to mechanical osteotomies. This application marked the beginning of the widespread adoption of piezoelectric devices in oral and maxillofacial surgery. Many operations, such as sinus lift, bone graft preparation, crest splitting, endodontic surgery, and impacted tooth extractions, have become safer with this technology. The primary reasons for the rapid acceptance of piezoelectric surgery include selective bone cutting, preservation of neurovascular structures, minimal heat generation, and a low risk of osteonecrosis.

While the clinical use of piezoelectric devices was initially limited to oral and maxillofacial surgery, their surgical application has expanded significantly over time. Procedures such as third molar extraction, cyst excision, sinus lift, bone graft preparation, ridge splitting, implant site preparation, and periosteal elevation have become safer and more controlled with this technology. Piezoelectric systems have subsequently expanded into a broader spectrum of maxillofacial

surgery and have been effectively applied in numerous complex procedures, including mandibular sagittal split osteotomies, temporomandibular joint surgery, cranial bone harvesting, and maxillary Le Fort I osteotomies. Piezoelectricity is primarily effective in craniofacial surgery because it allows the surgeon to perform large osteotomies without damaging adjacent neurovascular structures or the underlying dura mater. Accordingly, piezoelectric applications have been adopted in many surgical fields, including otologic surgery (chain replacement, stapedectomy, and facial nerve decompression), orthopedic surgery (osteotomy and hardware removal), and rhinoplasty. Various piezoelectric study methods have been developed for current indications (Pribitkin, Lavasani, Shindle, & Greywoode, 2010).

Piezoelectric technology was introduced to rhinoplasty in the early 2000s. In 2007, Robiony and colleagues published the first study of piezoelectric energy in nasal osteotomy. The study showed that the device made a more controlled fracture line, allowed cutting without harming nearby soft tissue, and left a smoother bone surface (Robiony et al., 2007). Subsequently, Pribitkin and colleagues reported in 2010 that piezosurgery offers a safe, adjustable, and highly precise cutting method for rhinoplasty. These two studies marked a critical turning point in the acceptance of piezoelectric surgery in rhinoplasty, paving the way for greater interest in the technology's underlying mechanisms. The operating principle of piezoelectric devices is based on the transmission of ultrasonic vibrations generated by a ceramic transducer to specially designed tips, which abrade mineralized tissue at micrometric levels. Piezoelectric systems operate in the 25–29 kHz range, a frequency sufficient only for cutting bone. Because the frequency required for cutting soft tissues exceeds 50 kHz, mucosa, skin, nerves, and vascular structures are preserved during the procedure. This selectivity minimizes trauma and supports favorable surgical outcomes, making piezoelectric surgery particularly attractive for rhinoplasty. (Pribitkin et al., 2010).

Piezoelectric devices in rhinoplasty give surgeons greater control over the osteotomy line. They reduce irregular fracture lines, bone fragmentation, and soft tissue trauma common with traditional osteotomies. This leads to less postoperative edema and ecchymosis, faster recovery, and more predictable aesthetic outcomes. The vibration-free motion of the piezo-powered tips also enables precise adjustments in the dorsum and radix regions, benefiting both primary and revision rhinoplasty cases.

The Role of Piezoelectric Surgery in Rhinoplasty and Clinical Results

In recent years, piezoelectric surgery has brought significant innovations to bone-processing techniques in rhinoplasty. Cadaver studies by Gerbault et al. have demonstrated that osteotomies performed with a piezo device yield more predictable, stable results, particularly in achieving dorsal narrowing and nasal

symmetry. Radial fractures extending from the osteotomy line to adjacent bones, right-left asymmetries, and uncontrolled fracture lines are common in conventional osteotomies, whereas these undesirable fractures were not observed with piezo surgery. Furthermore, it has been emphasized that piezo incisions are more precise, limited, and controlled. The high cutting selectivity of the piezo device, specific to mineralized tissues, allows for precise osteotomies in both thin bones and mobilized segments. Preserving the periosteum to a large extent during osteotomy allows for stable, complete osteotomies. Because hammering, as used in conventional techniques, is not required in piezo surgery, assistant-induced variability is eliminated (Gerbault et al., 2016). One of the first clinical reports on the use of piezoelectric in rhinoplasty was made by Robiony, who demonstrated that the method was associated with less bleeding, edema, and ecchymosis. Cochran reported using a piezo device in lateral osteotomy (Cochran & Roostaeian, 2013), Pribitkin (Pribitkin et al., 2010) in hump resection. Piezoelectric systems cut only mineralized tissue and spare soft tissue, thereby causing minimal trauma to structures such as vessels, nerves, and mucosa. This selectivity minimizes vascular damage and lymphatic trauma, significantly reducing postoperative edema and ecchymosis.

Histological studies show that piezoelectric osteotomies do not cause bone necrosis and preserve osteocytes. These findings support the notion that piezoelectric surgery is a tissue-friendly and safe method. However, piezosurgery also has its drawbacks.

The high cost of the device and the extensive dissection required for complete visualization of the osteotomy line, which adds an average of 30 minutes to operative time, are significant limitations. However, this additional time decreases significantly with increasing surgeon experience.

Effects of Piezo Surgery on Edema and Ecchymosis

In clinical studies comparing ultrasonic osteotomy with conventional techniques, it is generally accepted that piezo surgery causes less soft tissue trauma in the early postoperative period.

İlhan et al. compared ultrasonic and conventional internal osteotomy and found that both edema and ecchymosis scores were significantly lower in the ultrasonic osteotomy group on day three. On the seventh day, piezo surgery was again found to be superior for ecchymosis, while only one rater found a statistically significant difference in edema (İlhan, Cengiz, & Caypinar Eser, 2016).

Kocak et al. edema was significantly lower in the piezo group on day two, but no difference in edema was observed on days four and seven. Ecchymosis scores were substantially lower in favor of ultrasonic osteotomy on all days (I Koçak, Doğan, & Gökler, 2017). Koç et al. found edema and ecchymosis to be lower in

the ultrasonic osteotomy group on both the first and seventh days (Koc, Koc, & Erbek, 2017).

Troedhan et al. reported that edema, ecchymosis, and pain scores were better with ultrasonic surgery on the seventh postoperative day, and ROE scores at six months were also significantly higher in the piezo group (Troedhan, 2016).

Robiony et al. compared percutaneous conventional osteotomy with percutaneous piezo osteotomy in an extensive series of 183 patients; studies have reported that edema and ecchymosis were significantly reduced, and scar formation was less frequent with piezo (Robiony, Franz, Costa, Bianchi, & Marchetti, 2016).

When all these studies are evaluated, it appears that ultrasonic osteotomy is associated with less edema, ecchymosis, and soft-tissue trauma, especially in the early postoperative period, due to the device's specific cutting properties for mineralized tissue.

Effects of Piezo Surgery on Pain

Studies examining the effects of piezo surgery on postoperative pain similarly indicate a comfortable recovery process. Tirelli and colleagues reported that 80% of patients undergoing piezotherapy had low VAS scores, and most did not require analgesics. In the control group, 75% of patients needed analgesics (Tirelli, Tofanelli, Bullo, Bianchi, & Robiony, 2015).

Koç et al. VAS scores were lower in favor of the piezo group on the first day, but no difference was found between the two groups on the seventh day (Koc et al., 2017).

Troedhan measured analgesic tablet consumption during pain assessment and found that the piezo group used significantly less analgesics by the seventh day (Troedhan, 2016).

Micro-Saw and Powered Micro-Saw Systems

The sculpting of bone plays a crucial role in achieving both aesthetically pleasing and functionally successful outcomes in rhinoplasty. Although traditional osteotomy techniques have long served as the mainstay in this field, they present notable challenges, including limited control over bone cuts, unpredictable fracture lines, and inadvertent injury to surrounding soft tissues. These persistent limitations have driven the pursuit of innovative surgical methods that prioritize precision, control, and minimal trauma. In response, advancements in technology have led to the introduction of the micro-saw and, subsequently, the powered micro-saw system, which are now recognized as significant instruments in contemporary rhinoplasty practice.

History and Technological Development

Micro-saw technology was initially developed for precise bone resection in maxillofacial surgery and has demonstrated success, particularly for highly precise incisions in the mandible and craniofacial bones. Thanks to its thin, sharp blade design, high vibration speed, and controlled movement, this device could be adapted for rhinoplasty in the late 1990s.

The adoption of microsaws in rhinoplasty practice has accelerated due to their ability to create smoother cutting surfaces, particularly on the nasal dorsum, and to better guide the surgeon in defining the osteotomy line. With the evolution of technology, powered microsaws, motorized versions of micro-saw systems, have been developed, providing a more stable, faster, more ergonomic, and less traumatic cutting process for surrounding tissues. Motorized systems reduce the manual force required by the surgeon, significantly reducing unwanted vibrations on bone and soft tissues.

The micro-saw's most significant contribution was its ability to produce a thinner, more linear, and more predictable cutting line in bone. The characteristics of micro-saw blades developed during this period were highlighted by their thinner blade structure of 0.1–0.2 mm, reduced vibration, narrower cutting line, and minimal bone loss. Since the 2000s, micro-saw technology has evolved into a new generation instrument, the powered micro-saw, supported by electric motor systems. These devices feature a high-frequency oscillating blade structure and offer significant advantages to surgeons: more controlled, rapid cutting; stable blade movement; reduced heat generation; minimal soft-tissue trauma; shorter operative times; and increased surgical accuracy (Avşar, 2012; Bayrakçı, 2023).

The electric micro-saw has been noted for its ability to prevent irregular fracture lines that can occur with manual osteotomies, particularly in small and delicate areas. This has increased the surgeon's technical foresight in both primary and revision rhinoplasty.

Clinical Application and Advantages

In an extensive series of 259 cases of hump reduction using a powered micro-saw, patient satisfaction was very high, with only 10 cases requiring revision (Avşar, 2009). The high precision of the micro-saw enables bone hump shaping to be performed in a controlled and predictable manner, allowing the surgeon to more accurately determine the amount of bone to be removed before and during the procedure—this level of control results in a significant reduction in edema and ecchymosis, both intraoperatively and postoperatively. Another significant advantage of the powered micro-saw is its rapid healing process. The literature reports that approximately 90% of patients experience considerable resolution of swelling within 1–2 weeks. The self-protective design of the micro-saw blades ensures that only one-third of the bone circle is cut, particularly

in the radix region; the outer two-thirds of the circle are safely protected against the skin. In addition, the dorsal retractor protects the skin and subcutaneous tissues against blade contact and increases the surgical field of view.

With a thickness of just 0.1 mm, the micro-osteotomy blade generates a minimal bone gap—around 0.15 mm—that promotes quick healing and rapid callus development. Additionally, the use of simultaneous cold irrigation in microsaw systems plays a crucial role in minimizing intraoperative swelling. Osteotomies utilizing a powered saw have been shown to offer superior protection for the nasal mucosa. Typically, the osteotomy line encompasses 80–90% of the bone, deliberately preserving a thin bone bridge adjacent to the mucosa, thereby reducing the risk of mucosal injury (Avşar, 2009).

In a comprehensive series involving 1,374 patients, Avşar employed low-to-low lateral osteotomy alongside endoscopy-guided transverse osteotomy. The video-endoscopy-guided approach demonstrated an exceptionally low incidence of uncontrolled fracture lines, with rocker and step deformities being nearly eliminated. Moreover, occurrences of mucosal tears were minimal, and both postoperative edema and ecchymosis resolved more quickly. Nonetheless, it has been noted that the endoscopic technique incurs higher costs due to its requirement for a single-site approach (Avşar, 2012).

Recent research examining the impact of microsaw technology on rhinoplasty outcomes suggests that these devices confer considerable clinical benefits compared to conventional osteotomy techniques.

Pamuk et al. reported that powered microsawing produces more predictable incisions than conventional osteotomy, significantly reduces the rate of uncontrolled fracture lines, and provides a much clearer field of view during surgery. The statistically significant reduction in postoperative edema and ecchymosis, in particular, demonstrates the value of powered micro-saw technology in rhinoplasty (Pamuk, Pamuk, & Kandemir, 2023).

Bayrakcı et al. compared conventional osteotomy with the electrically assisted micro-sawing method in a prospective study. According to the results of the study, patients using powered micro-sawing experienced significantly lower edema, ecchymosis, and pain scores in the first postoperative days. Minimal soft-tissue trauma was cited as the primary reason for this clinical difference. These findings demonstrate that the powered micro-sawing system offers a significant advantage, especially in terms of patient comfort in the early period (Bayrakci et al., 2025).

Bilal et al. evaluated the effects of microsaw and conventional osteotomy techniques on vestibular and auditory functions. While previous studies have found no significant impact of the osteotomy technique on the balance and auditory systems, this study demonstrates that technical differences can have

substantial effects on the vestibular system. Greater changes were observed in balance tests after conventional osteotomy, and it has been reported that there may be an increased susceptibility to benign paroxysmal positional vertigo (BPPV). Therefore, the microsaw technique is considered a safer option for the vestibular system (Bilal, Orhan, Turna, Doğaner, & Oruk, 2023).

Burr (Rotating Bur) in Septorhinoplasty

While conventional techniques utilize osteotomes and hammers, high-speed rotating burrs have come to the fore in recent years, offering the potential for more controlled bone removal and minimal trauma. These devices provide precision in lateral, paramedian, and various bone modifications, allowing surgeons to achieve more predictable results.

History and Technological Development

The rotary burr has long been recognized by otolaryngologists, particularly for its application in temporal bone surgery. There are numerous techniques and instruments available for nasal dorsum surgery, each offering distinct advantages. Goodman's study from the 1980s highlighted that the use of a rotary bur resulted in reduced swelling and bruising compared to other methods. Additionally, rotary burs produced smoother bone contours, thereby enhancing cosmetic results (Goodman, 1981). Since the early 2000s, technological advancements in micromotors have paved the way for microburr systems that operate at higher speeds, generate less heat, and offer improved safety. Over the past twenty years, the introduction of diamond burr tips has provided surgeons with greater control when working with bone. Both clinical and cadaver studies have shown that employing the principle of "abrasion" instead of "fracture" during lateral osteotomies allows for more precise and controlled bone modifications.

Ishida et al. introduced a burr-assisted abrasion method designed for the precise sculpting of nasal cartilages, recognizing their crucial influence on both nasal aesthetics and function. In their approach, a powered burr drill enabled selective weakening of targeted cartilage regions. When abrasion was limited to a single surface, the cartilage naturally bent away from the treated side. This technique has proven especially effective in addressing septal cartilage deviations, as applying gentle abrasion to the concave side encourages realignment of the septum toward the midline. By carefully thinning the alar cartilages with the burr, surgeons can not only highlight the natural curvature but also, when needed, entirely reshape the alar cartilages. The authors highlighted that this approach typically necessitates only minimal removal of septal cartilage, and, in most cases, avoids the need for alar cartilage resection altogether (Ishida, Ishida, Passos, & Ferreira, 2006).

Ghassemi et al. investigated the intraoral technique for lateral osteotomy—a particularly challenging and traumatic aspect of rhinoplasty—by employing a

1.5-mm diamond burr. Their findings demonstrated that this method is straightforward to implement, ensures mucosal protection, enhances safety, and yields consistent, predictable outcomes (Ghassemi, Riediger, Hölzle, & Gerressen, 2013).

Koçak et al. introduced an alternative method designed to minimize reliance on traditional double osteotomies when correcting I-shaped nasal deviation. In this technique, the lateral portion of the long nasal bone is carefully thinned with a rasp or burr, allowing the bone to be repositioned toward the maxilla without actually performing an osteotomy. Among the 26 patients studied, postoperative axis deviation was substantially reduced, and no recurrences were observed at the one-year follow-up. Compared to standard double osteotomy, this approach offers improved control over fracture lines, reduces trauma to mucosal and periosteal tissues, and is associated with fewer complications. Collectively, these results indicate that burr-assisted osteoplasty represents a more conservative, safer, and reliable option for the surgical correction of nasal deviation (Ilker Koçak & Senturk, 2017).

Aysel et al. evaluated early postoperative complications by comparing the use of a conventional osteotome with a power-assisted surgical burr in patients undergoing primary open septorhinoplasty. Seventy patients were included in the study; 36 underwent a conventional guided osteotome, and 34 underwent a lateral osteotomy with a round surgical burr. Evaluations performed on postoperative days 1, 3, and 7 revealed significantly less periorbital ecchymosis in the burr group. A significant advantage was observed in the burr group on the first day only, with no difference between groups on days 3 and 7. Pain scores were lower in the burr group at all time points. These results indicate that surgical burr osteotomy is associated with less ecchymosis, edema, and pain in the early period, suggesting that the use of a burr offers a significant advantage in terms of patient comfort (Aysel, Karatan, & Müderris, 2022).

In a separate prospective, randomized, and controlled trial, Aysel et al. examined the potential impact of burr-assisted techniques used during hump resection and osteoplasty on cochlear function. The study involved two groups: 20 patients who underwent burr-assisted septorhinoplasty and 20 patients who had septoplasty. All participants were evaluated using pure-tone audiometry, distortion product otoacoustic emissions (DPOAE), and tympanometry. The analysis revealed no significant differences in bone conduction thresholds between the two groups. Additionally, no notable changes in air-conduction thresholds were detected between 500 and 8000 Hz, either before or after surgery, and signal-to-noise ratio measurements were comparable across groups. While the study group showed minor changes at only two frequencies (2000 Hz in the right ear and 500 Hz in the left ear), otoacoustic emissions persisted. These results indicate that the use of burrs during rhinoplasty exerts only mild and clinically

insignificant effects on hearing, with no evidence of significant hearing loss. Therefore, burr-assisted surgical techniques can be considered safe with respect to auditory function (Zorlu, Aysel, Aydin, & Catli, 2023).

Conclusion

Currently, the adoption of powered instruments—such as piezoelectric devices, microsaw systems, and surgical burrs—is transforming the field of rhinoplasty. These advanced tools grant surgeons enhanced precision, greater control, and improved preservation of bone and cartilage tissue during surgical procedures.

Piezoelectric surgery, renowned for its selective bone-cutting ability, stands out as one of the least traumatic surgical options. By preserving soft tissues, blood vessels, and mucosa, it minimizes collateral damage. The precise osteotomy lines achieved through microvibration technology offer notable benefits, especially in maintaining the dorsal aesthetic line, reducing contour irregularities, and lowering the risk of surgical revisions. Clinical research consistently demonstrates that piezoelectric surgery leads to marked reductions in postoperative edema, ecchymosis, and pain.

The powered microsaw enables highly controlled osteotomies due to its thin blade and self-limiting cutting depth. This precision allows for predictable bone cuts with minimal trauma to soft tissues, which in turn leads to fewer uncontrolled fractures, more rapid healing, and reduced early complications compared to traditional osteotomy techniques. Literature reports consistently show that microsaw procedures result in less postoperative swelling, bruising, and discomfort. Surgical burr systems also deliver exceptional accuracy when working with both bone and cartilage. Procedures such as lateral osteotomies and dorsal hump reductions performed with diamond burrs yield smoother bone contours and decrease the likelihood of mucosal injury. Burrs have become essential, especially for tasks like osteoplasty, bone thinning, contour refinement, and correction of curvatures. Importantly, studies on auditory outcomes confirm that the use of burrs does not significantly affect hearing, affirming their safety profile.

- Collectively, powered systems offer several key advantages:
- More controlled and linear osteotomy lines
Preservation of mucosal and soft tissue integrity
- Reduced risk of uncontrolled fractures and undesirable deformations
- Better surgical field of vision
- Significantly reduced edema, ecchymosis, and pain
- Accelerated postoperative recovery

➤ Reduced revision rates

Piezoelectric devices, microsaws, and burr systems are increasingly regarded as complementary innovations that facilitate safer, more predictable, and more refined bone procedures in both aesthetic and functional rhinoplasty. As surgical device technologies continue to evolve, it is anticipated that these tools will become integral to the standard of care in rhinoplasty.

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CHAPTER 25

The Patient Rights Unit: Reflections from a Dental Teaching Hospital in Türkiye

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Introduction

Patient Rights

Formalized in 1948, the Universal Declaration of Human Rights recognizes “the inherent dignity” and the “equal and unalienable rights of all members of the human family”. The notion of patient rights was developed based on the fundamental dignity and equality of all human beings. In other words, what is owed to the patient as a human being, by physicians and by the state, took shape largely due to this understanding of the basic rights of the person. Patient rights, as defined, pertain to the rights attributed to individuals seeking healthcare. They can be seen as reflections of the medical profession's standards, which establish the code of practice governing patient-physician relationships (Universal Declaration of Human Rights, 1948; WHO Human Rights and Health, 2017; Cohan&Ezer, 2013).

Patients occupy a distinctive position within healthcare systems due to their illness-related vulnerabilities, which often result in heightened physical, mental, and social needs compared to the general population. These vulnerabilities may limit patients’ capacity to meet their own needs independently and render them more dependent on healthcare professionals and institutional structures. For this reason, the recognition and protection of patient rights constitute a fundamental ethical and legal obligation in healthcare delivery (Aultman, 2014; Clark&Preto, 2018). At the core of patient rights lies the principle of respect for people, which requires that patients be adequately informed about their health status. This includes clear and comprehensible information regarding the nature of their illness, the anticipated diagnostic and therapeutic interventions, potential risks and benefits, and available alternatives. Equally important is the right to access accurate and complete documentation of the care received, which supports transparency, accountability, and continuity of care (Annas,1973; Aydın, 2004).

Beyond information and documentation, patient rights encompass a broad range of protections, including the right to privacy and confidentiality, the right to life and to quality healthcare services, and the right to be free from discrimination on any grounds. Patients must also be safeguarded against torture and cruel, inhumane, or degrading treatment, regardless of their medical condition, social status, or personal characteristics. Healthcare delivery should therefore be guided not only by clinical effectiveness but also by ethical sensitivity (Boldt, 2019). Furthermore, patients have the right to considerate and respectful care, provided in a timely manner and responsive to their expressed needs and requests for services. Such care should be organized in a way that ensures continuity, minimizes fragmentation, and acknowledges the patient as a whole person rather than merely a clinical case. In this sense, patient rights function as both ethical standards and practical guides for humane, equitable, and patient-centered healthcare practice (Aydın, 2004; Cohan&Ezer, 2013).

Since the introduction of the Human Rights Act by the United Nations in 1948, legislation on patient rights has been enacted globally. Notable examples include the 1964 Helsinki Declaration on research subjects and the 1978 Alma-Ata Declaration on Primary Health Care. The term “patient rights” was officially introduced in 1981 with the Lisbon Declaration of the World Medical Association (WMA Declaration of Helsinki, 1964; WHO Declaration of Alma Ata, 1978; WMA Declaration of Lisbon 1981).

Patient Rights in Türkiye

In most countries, healthcare organizations have established regulations or charters for patient rights, announcing and implementing them to achieve greater patient satisfaction. In Türkiye, the Patient Rights Regulation was prepared on the basis of international documents and issued in 1998 (and updated in 2014) by the Ministry of Health (Turkish Patient Rights Regulation, 1998). This Regulation was influenced by the 1994 Declaration of Amsterdam on the Promotion of Patient Rights in Europe (WHO, 1994) and by the Declaration of Lisbon on the Rights of the Patient, as revised in 1995 in Bali (World Medical Association, 1995).

The Turkish Patient Rights Regulation articulates a comprehensive framework of patient rights designed to safeguard human dignity and ensure ethically sound healthcare delivery, particularly in light of patients’ heightened vulnerability. These rights encompass equitable and non-discriminatory access to healthcare services, ensuring that all individuals may benefit from available services under equal conditions. Central to this framework is the patient’s right to be informed, which includes receiving clear, accurate, and sufficient information about one’s health status, proposed diagnostic and therapeutic interventions, possible alternatives, and associated risks. Closely linked to this is the right to privacy and confidentiality, as well as the requirement for informed consent, granting patients autonomy to accept, refuse, or withdraw from treatment at any stage. The Regulation further recognizes patients’ rights to select and change healthcare institutions and personnel, thereby reinforcing freedom of choice and trust in the therapeutic relationship. Respect for personal dignity is emphasized through rights related to being treated with consideration, maintaining comfort, receiving visitors, and having a caregiver present when appropriate. In addition, patients are entitled to security within healthcare settings and to the uninterrupted continuity of care, which is essential for both clinical effectiveness and ethical integrity. The Regulation also acknowledges patients’ cultural and spiritual dimensions by protecting the right to perform religious obligations. Importantly, procedural rights, such as the right to apply to relevant authorities, to file complaints, and to pursue legal action, serve as mechanisms of accountability and legal protection. Taken together, these rights move beyond a narrow clinical focus and establish a patient-centered model of care grounded in

equity, autonomy, respect, and continuity, reflecting both ethical principles and legal obligations within modern healthcare systems (Turkish Patient Rights Regulation, 1998).

Patient Rights Units in Türkiye

In Türkiye, the issue of patient rights began to attract sustained academic and public attention following the publication of the Patient Rights Regulation on August 1, 1998. Although this Regulation provided a clear definition of patients' fundamental rights, uncertainties remained regarding the concrete responsibilities of healthcare institutions and professionals, as well as the mechanisms for implementing and safeguarding these rights in daily clinical practice. In response to these gaps, the Ministry of Health issued the "Patient Rights Implementation Directive in Healthcare Facilities" in 2003, which was subsequently revised in 2005 (Health's Directive on Practice of Patient Rights in Healthcare Facilities, 2003). This directive aimed to enhance both awareness and operationalization of patient rights within healthcare services and led to the establishment of Patient Rights Commissions (PRCs) in hospitals. With further legal reforms in 2014, these bodies were restructured and renamed as Patient Rights Units (PRUs), reflecting a more institutionalized and continuous approach to patient rights protection (Turkish Patient Rights Regulation, 1998).

Currently, PRUs operate within the framework of the Patient Rights Regulation and related legal provisions. Their core responsibilities include informing patients and their relatives about patient rights, receiving and evaluating verbal or written complaints, investigating alleged violations, and facilitating communication between patients and healthcare professionals (Turkish Patient Rights Regulation, 1998).

Complaints submitted to PRUs are reviewed by unit staff and, when necessary, relevant healthcare professionals are consulted. Resolution is sought through reconciliation mechanisms or formal responses, thereby providing an accessible channel for accountability. In addition to complaint management, PRUs play an educational role by organizing training activities on professional ethics and patient rights, often using anonymized complaint data as learning material (Kadioglu, 2018).

Awareness of patient rights has been shown to yield significant benefits, including improved quality of healthcare services, reduced healthcare costs, shorter hospital stays, faster recovery, and a decreased risk of irreversible physical or psychological harm (Mastaneh&Mouseli, 2013; Erer, et al., 2018). Most importantly, it enhances patient dignity by empowering individuals to participate actively in decisions regarding their care. Over time, Turkey has followed global trends by integrating patient rights into medical education, supporting the establishment of patient rights organizations, and increasing public

visibility of patient rights violations through the media (Erer, et al, 2008; Güven&Sert, 2010, Eşiyok et al, 2014).

Nevertheless, PRUs have faced notable criticism. The performance-based healthcare system has intensified workloads and strained patient-provider communication, while PRUs are often perceived as focusing on individual healthcare workers rather than addressing systemic deficiencies. This has raised concerns that prioritizing patient satisfaction may inadvertently overlook healthcare professionals' burnout and structural constraints (Kadioglu, 2016). Therefore, the long-term effectiveness of PRUs depends on stronger institutional support, adequate resources, and policies that balance patient rights protection with sustainable working conditions for healthcare professionals.

Determinants of Rising Patient Complaints in Türkiye

In Turkish legal and medical literature, a substantial body of research indicates that applications and complaints submitted to Patient Rights Units have steadily increased over the years (Erer et al, 2008; Mastaneh&Mouseli, 2013; Eşiyok et al, 2014; Friele et al, 2015; Önal&Civaner, 2015; Erer et al, 2018). Notably, one of the most frequently reported complaints concerns “not being treated in a respectful manner”. The prominence of this complaint suggests that problems related to respect, communication, and human dignity are not isolated incidents but rather reflect systemic shortcomings within healthcare services. In this respect, the expectations of patients and their relatives appear to be persistently unmet, particularly regarding interpersonal aspects of care (Eşiyok et al, 2014; Friele et al, 2015; Önal&Civaner, 2015; Erer et al, 2018).

Interestingly, patients rarely submit complaints concerning violations of classical patient rights, such as confidentiality, informed consent, or access to necessary healthcare services. Instead, both in Türkiye and internationally, the most common complaints are closely associated with dignity-related expectations, including respectful treatment and adequate, comprehensible information. This pattern highlights that patients tend to react more strongly to perceived moral and relational failures than to technical or procedural deficiencies (Kadioglu, 2018).

The increasing number of complaints may partially be explained by heightened awareness of patient rights and the strengthening of legal regulations. However, these factors alone do not fully account for the trend. Since 2003, the Turkish healthcare system has undergone profound restructuring under the Health Transformation Program, which aimed to reorganize, finance, and deliver healthcare services in a more efficient, productive, and equitable manner. Central to this reform was the gradual introduction of privatization and a performance-based payment system, grounded in the assumption that market-oriented mechanisms would enhance efficiency and patient satisfaction (Önal&Civaner, 2015; Kadioglu, 2016; Kavas et al, 2025).

While these reforms have increased service volume and accessibility, they have also significantly reduced the time allocated per patient. As a result, establishing trust-based relationships and effective communication has become increasingly difficult, while the risk of medical errors has grown. Revenue-driven pressures may lead healthcare professionals to rush examinations, pay insufficient attention to patients' concerns, and engage in rapid diagnosis and overprescription. Moreover, framing healthcare within a business model has, in practice, transformed patient rights into consumer rights, while complaint mechanisms tend to individualize responsibility by holding physicians and dentists accountable for systemic failures. Consequently, dissatisfaction, reduced patient satisfaction, and rising complaint rates appear as structural outcomes of this transformation rather than merely individual misconduct (Kadioglu, 2016; Kavas et al, 2025).

The Dental Teaching Hospital Patient Rights Unit in the Case of Türkiye

Although it was not mandatory for university hospitals, a Patient Rights Unit was established in December 2012 within the Teaching Hospital of Çukurova University Faculty of Dentistry in Adana, Türkiye, with the aim of preventing violations of patients' rights. In this Unit, both written and verbal complaints of patients are initially received and recorded. Subsequently, in accordance with established legal procedures, dentists, nurses, students, and clinic secretaries involved in the complaint are systematically invited to present their verbal and written defenses. At the end of each month, the complaints are regularly re-evaluated and classified, as the systematic examination of applications submitted to PRUs provides valuable insight into patients' priorities, expectations, and needs (Kadioglu, 2018).

By examining the written and verbal applications submitted to the PRU between December 2013 and December 2024, it is possible to make the following observations. Based on data obtained from 1,320 files, 45% of the complainants were female, and the age range of applicants varied between 18 and 67 years. Using the Reader's Patient Complaints Taxonomy (Reader et al, 2014), the primary reasons for complaints submitted to the Patient Rights Unit were identified as "communication conflicts" (36%), "rude behavior toward patients" (32%), "failure to inform patients adequately" (20%), and "overcrowded appointment schedules" (12%). No data were identified under the categories of "clinical quality" or "clinical safety." Furthermore, it was determined that 90% of the applications were resolved "on-site" within the PRU. Most patients who filed complaints stated that they primarily expected a sincere apology from their dentists, a clear explanation of what had occurred, and information regarding measures proposed to prevent similar incidents from recurring. A smaller proportion of complainants expressed a desire for their physicians to be formally punished (Kadioglu, 2022).

Within the framework of a “culture of learning from medical errors,” error reporting manifests in practice as two distinct but interconnected processes. At the institutional level, this involves the formal reporting of errors, while at the level of the patient–physician relationship, it includes the disclosure of the error and the offering of an apology. When an error occurs during a medical intervention, healthcare providers have an ethical obligation to inform patients about the error and its potential consequences. Nevertheless, physicians often hesitate to disclose such errors due to fears of litigation, concerns about professional reputation, or feelings of shame and guilt. However, empirical evidence indicates that timely and transparent disclosure, accompanied by expressions of regret, empathy, and apology, may reduce the likelihood of litigation and significantly improve patient satisfaction (Kadioğlu, 2024).

From a legal perspective, the primary priority of the Patient Rights Unit is to prevent violations of patient rights, while simultaneously providing professional ethics education to healthcare professionals, including students and clinic secretaries who are involved in complaint processes. Cases derived from patient complaints, following anonymization, are actively utilized as educational materials in dental ethics training.

Conclusion

The protection of patient rights facilitates access to healthcare services in a manner grounded in respect and human dignity. Patient rights are an indispensable component of proper medical and dental practice and should be actively defended by both patients and healthcare professionals in order to sustain a professional and ethical healthcare environment. It is essential to integrate human rights education into the training of healthcare providers at all levels. Together with other normative standards governing patient care, human rights principles contribute to healthcare systems functioning as spaces of treatment, care, and support for all individuals. In this sense, human rights complement public health ethics -a field closely related to medical ethics- that seeks to ensure public health interventions maximize benefit, minimize harm, respect individual dignity and rights to the greatest extent possible, and are implemented in a fair and just manner. All patients should be informed of both their rights and responsibilities; therefore, improving the effectiveness and practical functioning of Patient Rights Units (PRUs) within dental teaching hospitals is strongly recommended to ensure supervision, monitoring, effective communication, and the observance of patient rights.

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CHAPTER 26

Histology of Epithelial Tissue

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The constituent cells of epithelial tissues are referred to as epithelial cells. Epithelial cells can derive from all three embryonic germ layers: ectoderm, endoderm, and mesoderm. These cells are physically and functionally interrelated and interdependent; they interact via ions and metabolic byproducts. Epithelial cells are designated various functions and adopt distinct forms based on their roles. The principal tasks of epithelial cells include: covering the exterior surface of the organism and the inside surfaces of hollow organs, secretion, contraction, and sensation reception and transmission.

Epithelial tissues are categorized into four types based on their functions. 1. Protective epithelium, 2. Glandular epithelium, 3. Contractile epithelium, and 4. Neuro-epithelium.

Epithelial tissues lack blood vessels. Their sustenance is obtained through the passage of blood contents via the arteries situated beneath them (in the covering epithelium) or around them (in the epithelial tissues).

PROTECTIVE (COVERING) EPITHELIUM

The covering epithelium envelops the external or interior surfaces, or both, of organs comprised of different tissue types, safeguarding these organs from mechanical, physical, and chemical stimuli. Due to its protective function, it is referred to as the protective epithelium. Covering epithelia, which envelop the surfaces or cavities of the body and organs, have surfaces oriented towards these places designated as apical surfaces, while the opposing surfaces are referred to as basal surfaces. The surfaces of adjacent cells that confront one another are referred to as the lateral surfaces.

The epithelium that constitutes the outer surface of the body is referred to as the epidermis. The epithelium that lines the inner surfaces of systems exposed to the external environment, such as the digestive, respiratory, and urogenital systems, is referred to as mucosal epithelium. Mesothelial cells are the type of cells that line the outside borders of body cavities, including the abdominal and thoracic cavities, as well as the cavity housing the heart, and the organs situated within these cavities. The tissue that lines the inner surface of the circulatory system, including the heart and blood and lymph vessels, is referred to as the endothelium.

The apical surfaces of epithelial cells display diverse cell surface differentiations, including microvilli, kinocilia, stereocilia, and flagella, contingent upon their specific functions.

Classification of epithelial tissue

Epithelial tissue is categorized according to the stratification and morphology of the constituent epithelial cells. Epithelia are categorized into three types based on their layers: 1) Simple epithelium, 2) Pseudostratified epithelium, and 3)

Stratified epithelium. 4) Transitional epithelium. Epithelia are classified into four categories based on their morphology: a) squamous epithelium, b) cuboidal epithelium, c) columnar epithelium, d) pyramidal epithelium. These two properties are evaluated concurrently; there are 8 types of epithelial tissue that can be seen in the organisms.

1- Simple squamous epithelium	5- Pseudostratified columnar epithelium
2- Simple cuboidal epithelium	6- Stratified squamous epithelium
3- Simple columnar epithelium	7- Stratified columnar epithelium
4- Simple pyramidal epithelium	8- Stratified transitional columnar epithelium

Table 1: Types of the Epithelial Tissue

1. Simple squamous epithelium: In this epithelial tissue type, all cells are aligned adjacent in a single layer, each supported by a basement membrane on one side. The cells' width significantly exceeds their height. They are polygonal and, hence, polyhedral. This is observable in sections examined parallel to the surface. In certain squamous epithelia, the cell borders exhibit indentations and ridges, whereas in others, they remain smooth. In sections observed perpendicularly to the surface, the cells exhibit a rectangular and angular morphology, whereas in parallel sections, they provide a polyhedral form. Their nuclei are elliptical and extend into the core area of the cytoplasm. The internal surfaces of bodily cavities, the circulatory system, and the lung alveoli are lined with simple squamous epithelial cells. Likewise, certain renal tubules are also coated with this type of epithelium.

Owing to their slender cytoplasm, single-layered squamous epithelial cells are optimally adapted for material exchange. The membranes that line bodily cavities (peritoneum, pleura, and pericardium), together with the inner surfaces of blood arteries and certain renal tubules, are composed of squamous epithelial cells. Pinocytotic vesicles, representing material exchange, are prevalent in the cytoplasm of these epithelial cells. Additionally, the surfaces of squamous cells that line bodily cavities (mesothelial cells) possess microvilli, which augment absorption.

2-Simple cuboidal epithelium: These cells, organized in a singular row, are polygonal in shape; however, due to their equal width and height, they seem square in vertically viewed sections. Their spherical nuclei are situated at the center of the cells. Single-layered cuboidal epithelium is located in the connective ducts of the kidneys, anatomical salivary glands, beginning ducts of the pancreas, bronchioles of the lungs, choroid plexus, and the front surface of the lens of the eye.

3- Single-layered columnar epithelium consists of cells arranged in a singular row atop the basement membrane. The cell dimensions may range from flat columnar to tall columnar, contingent upon the specific organ in which they reside. In cross-sections, they manifest as polygonal and so polyhedral. Their nuclei are elongated and oval, corresponding to the cell morphology. They are typically situated toward the center of the cytoplasm, albeit slightly displaced from the basal half. Simple columnar epithelium is prevalent throughout the body. The remainder of the digestive system, from the stomach onward, is lined by columnar epithelium.

4- Simple columnar epithelial cells exhibit functional polarization. The apical half displays distinct characteristics based on its function and the specific organ involved. This section of the stomach contains a mucus-like discharge. This material infiltrates the lumen and forms a thin coating across the stomach surface, safeguarding the gastric mucosa from physical and chemical irritants. The apical surfaces of the columnar cells in the small intestine are covered with many microvilli. Consequently, these cells efficiently execute resorption. Ciliated columnar epithelium is also located on their apical surfaces. The columnar epithelial cells, which are single-layered and line the bronchi (except their terminal segments), possess kinocilia. Additionally, certain epithelial cells in the uterine tubes, uterus, and efferent ducts possess kinocilia. The apical portions also comprise undifferentiated columnar epithelial cells. The epithelial cells that comprise the inner lining of the kidney's collecting ducts and the gallbladder are of this kind. In certain single-layered, columnar epithelia, like the intestinal epithelium, goblet cells are present amid the epithelial cells (refer to pseudostratified epithelium for these cells).

4- Simple cuboidal epithelium: The quintessential illustration of this epithelium type is the epithelial cells of the proximal and distal tubules of the kidney. The surfaces of these cells in contact with the basement membrane are broader than those oriented towards the lumen. This imparts a pyramidal configuration to the cells; a cross-section perpendicular to the base resembles a triangle.

Their nuclei are round and positioned near the middle of the cytoplasm. The apical surfaces of the cells of the proximal tubules possess many microvilli.

Pyramidal-shaped lining epithelial cells are hardly referenced in traditional histology texts. However, upon examination of the renal tubules in a meticulously prepared kidney specimen, it is observed that the majority of the cells, particularly those constituting the proximal tubules, exhibit a pyramidal morphology rather than a pyramidal shape. Indeed, due to the broader bases of the cells lining the tiny channels compared to their luminal surfaces, it is more precise to classify them as truncated pyramids rather than true pyramids.

5-Pseudostratified columnar epithelium: This epithelial tissue is a single layer, with all cells resting on the basement membrane on one side; however, due to the varied lengths of the cells, only some can extend into the lumen. As a result, the cell nuclei are arranged in multiple layers, stacked vertically, instead of in a single row. This configuration of the nuclei designates this type of epithelium as stratified epithelium. The nuclei of the elongated cells are oval, whereas those of the shorter cells are spherical.

The cell termini that extend into the lumen are adorned with *kinosylvos*. All cells within the pseudostratified columnar epithelium possess a columnar morphology. While the cells adjacent to the lumen are columnar, the cells of disparate sizes occupying the interstitial areas are predominantly pyramidal. Moreover, a cell cannot consistently maintain a uniform shape; it must alter its form to some degree based on its purpose.

The pseudostratified epithelium, a type of covering epithelium, also comprises *secretory* cells. The cells, situated sporadically within the tissue, exhibit distended midsections, although their basal portions taper as they near the basement membrane. Due to their resemblance to a goblet, these cells are termed goblet cells. The apical portions of goblet cells are filled with a mucous secretion; their nuclei, often triangular or crescent-shaped, are situated in the basal portions.

The respiratory system, from the nose to the terminal bronchi, is lined by pseudostratified epithelium. The vibrissae of this epithelium endeavor to obstruct foreign substances from infiltrating the deeper airways. Goblet cells generate mucus that coats cell surfaces, safeguarding them from the desiccating effects of fluctuating air humidity during inhalation and exhalation. They also facilitate the adhesion of dust particles. Alongside the pseudostratified epithelium of the respiratory tract, goblet cells are prevalent in the simple columnar epithelium of the intestines. The role of these cells in this region is to lubricate the intestinal surface and safeguard the epithelial cells from the damaging effects of enzymes.

A different variety of pseudostratified epithelium is located in the epididymal duct (ductus epididymidis). The cells inside this tissue that project into the lumen are elongated (highly columnar), and their apical surfaces are equipped with nonmotile cilia (stereocilia). Goblet cells are not present in this tissue.

6-Stratified squamous epithelium: This epithelial tissue, subjected to mechanical and chemical factors, demonstrates a genuine multilayered structure. Only the cells in the basal row of the tissue are in contact with the basement membrane. The individuals in the superior rows lack any association with the basement membrane.

In all varieties of stratified epithelium, the epithelial layer directly above the basement membrane is stratified. Stratified epithelia are categorized into squamous, columnar, and transitional types according to the morphology of the surface cells within the epithelial tissue that encases the lumen. If the cells in the upper layers are flat, the tissue is termed stratified squamous epithelium; if they are columnar, it is designated as stratified columnar epithelium; and if they are deformable, it is referred to as stratified variable epithelium.

Mucus, also referred to as mucin, is a viscous material composed of glycoproteins and proteoglycans.

The epithelial cells in the central layers of the stratified epithelium are polygonal in shape. These cells are absent in stratified columnar epithelium; they constitute 3-5 layers in multilayered epithelium; in stratified squamous epithelium, they include a greater number of layers. The columnar cells on the basement membrane undergo mitosis to generate the cells in the superior layers.

Besides the epidermis, the outermost layer of the skin, stratified squamous epithelium also constitutes the inner surfaces (laminae epithelialis) of the cutaneous mucosae that line the initial segments of hollow organs interfacing with the external environment (oral cavity, pharynx, larynx, esophagus, anus, vagina, etc.). The surface cells of the squamous epithelium in these regions are hardened due to the presence of many tonofibrils; this type of epithelium is referred to as keratinized epithelium.

The stratified squamous epithelium is more robust than any other epithelial tissues, such as the epidermis and the lamina epithelialis of cutaneous mucosae. The thickness impedes the passage of nutrients to cells located near the surface. To enhance feeding, the underlying connective tissue sometimes forms digitiform indentations inside the tissue. These structures are referred to as tiny papillae. The connective tissue within the papillae is abundant in capillaries. Substances from these capillaries, which infiltrate the epithelial tissue without amalgamating due to the intervening basement membrane, can access the higher surfaces.

Microscopic papillae also guarantee the secure adhesion of the epithelial tissue to the underlying connective tissue. The anterior surface of the cornea is also enveloped by a stratified covering epithelium. Nonetheless, the superficial epithelial cells present are nonkeratinized. The epithelial tissue's minimal thickness precludes the presence of papillae.

The polygonal cells of stratified squamous epithelial tissue are organized loosely to enhance nutrient transport inside the tissue. This epithelial coating, because to its position, is often subjected to mechanical forces. Consequently, the polygonal cells are interconnected via cytoplasmic bridges. The connections are desmosomes. The cells are additionally fortified by tonofibrils.

7- Stratified columnar epithelium: This tissue is distinguished by the columnar morphology of the cells oriented towards the lumen. This variety of stratified epithelial tissue is relatively thin. It typically consists of epithelial cells organized in two layers. The primary illustration of stratified columnar epithelium is the epithelium of the main duct in anatomical glands.

8-Stratified transitional lining epithelium is located on the surface of the excretory ducts, extending from the renal pelvis to the terminus of the urethra. This tissue is characterized by cells that alter their form in response to the organ's fullness. The surface cells are significantly larger than those in other layers. Excretory organs, such as the urine bladder, exhibit a columnar shape when devoid of contents. (As the organ accumulates urine, it begins to contract and expand; when entirely full, it becomes flattened.) As the organ evacuates and constricts, the cells revert to a columnar morphology.

The columnar cells have several folds on their luminal surfaces; the flattening and expansion of these surfaces are facilitated by the unfolding and flattening of these folds, observable at the electron microscopic level.

In the literature, transitional epithelium is designated as transitional epithelium or mixed epithelium. The term "transitional" originates from the capacity of surface cells to alter their shape. During preparations, these surface cells may be observed in different states of transformation (including prismatic, cuboidal, and squamous).

In variable epithelium, superficial cells alter their morphology based on functional status, resulting in fluctuations in the thickness of the epithelial tissue. This transpires due to the polygonal cells constituting the intermediate layers of the tissue, which are flexibly interconnected, allowing lateral movement over one another.

In transitional epithelium, the superficial cells are large, possess enlarged nuclei, and contain double the typical amount of DNA, indicating polyploidy. Certain cells possess two or more nuclei with intact chromosomes. The cytoplasm of these cells is abundant in mitochondria and glycogen. It is believed that to safeguard against the detrimental effects of hypertonic urine, the cells synthesize and exude a form of mucus derived from glycogen. Moreover, their cytoplasm has greater density than that of cells in the inferior layers, resulting in a darker staining. Microfilaments are also located in the surface regions of the cells.

2. SECRETORY EPITHELIUM

All cells inside an organism engage in the exchange of chemicals to maintain life. Secretory cells, besides this function, also manufacture and secrete chemicals that influence processes affecting the entire organism. This synthesis is termed secretion, the produced compounds are referred to as secretions, and the process of releasing these substances from the cells is known as extrusion. Secretion is not an exclusive process of secretory epithelia. Certain epithelial cells, including the columnar epithelial cells that line the stomach, exhibit a secretory function alongside their protective roles. 1) Consequently, a definitive separation between epithelia and glandular epithelia is not always feasible. Secretory epithelia fundamentally arise from glandular epithelium during embryonic development. Aggregates of secretory epithelial cells are referred to as glands. Glands either release their secretions via a singular excretory duct to a relatively distant site (the body surface, the lumens of hollow organs) or directly into adjacent blood vessels (capillaries). The initial category of glands is termed exocrine glands, whilst the subsequent category is referred to as endocrine glands. Exocrine glands interact with the overlying epithelium through their excretory channels. Endocrine glands are devoid of excretory ducts and sever their link to the covering epithelium during embryonic development.

Exocrine and endocrine glands differ in the arrangement of their secretory cells. In exocrine glands, a certain quantity of glandular epithelial cells—typically pyramidal—organize around a tiny cavity (lumen) to create several units. Each of these is referred to as a corpus glandulae. If the quantity of corpus glands in an exocrine gland is minimal, it remains microscopic (for instance, the labial, buccal, palatal, and lingual glands). If the gland comprises multiple corpus glands, it is discernible to the naked eye; it is anatomical (for instance, the sublingual, parotid, and mandibular salivary glands, the pancreas, and the liver).

The arrangement of endocrine glands is entirely distinct. Cells constituting an endocrine gland, excluding the thyroid, do not congregate around a lumen to create a glandular structure. The epithelial cells of the gland aggregate in a three-dimensional configuration to create cords, which subsequently intertwine.

Dilated capillaries (sinusoids) occupy the interstitial spaces, and the cells discharge their secretions—hormones—into these capillaries. The epithelial cells constituting the endocrine glands are diverse, typically cuboidal in the thyroid.

In endocrine glands aside from the thyroid, the epithelial cells of the glands lack polarity. These predominantly polygonal cells facilitate nutrition exchange and expel secretions through the surface next to the arteries. The epithelial cells constituting exocrine glands display polarization. They get chemicals from their basal surface and discharge their secretions into the gland lumen via their apical surface.

The morphology of glandular epithelial cells fluctuates according on various degrees of activity. They are elongated during secretion and excretion phases and compressed during rest.

Glandular epithelial cells secrete their products through three mechanisms. In certain glandular epithelial cells, and hence in glands, the expulsion of secretion does not lead to any cytoplasmic loss. This secretion is referred to as merocrine or eccrine secretion. Eccrine secretion transpires through two mechanisms: either the secretory granules dissolve within the cytoplasm, allowing their contents to be expelled from the cell via diffusion; or the secretory granules translocate to the cell surface, where the membranes of the cell and granule amalgamate and rupture, discharging the granule contents into the lumen. This second form of secretion is referred to as exocytosis. All endocrine glands and most exocrine glands are classified as eccrine glands.

The second category of secretion is referred to as apocrine secretion. In this form of secretion, a portion of the cytoplasm is expelled. The produced secretory substance accumulates at the apex of the cytoplasm; this segment pinches off, detaches with the secretory substance, and descends into the lumen. The membrane encasing the cytoplasm disintegrates, allowing the secretory material to be released.

The cytoplasmic decrease in apocrine secretion is replenished by the cell prior to the initiation of a new secretory cycle.

The mammary glands, sweat glands in non-primate mammals' hairy skin regions, axillary glands in humans, glands in the labium majora, and perianal glands are responsible for secreting aphrodisiacs.

The third category of secretion is termed holocrine secretion. In holocrine secretion, the complete cell is expelled. The cell accumulates secretory material, undergoes apoptosis, disintegrates, and is ejected from the gland. Immature cells in the glands proliferate by mitosis to replace the deceased ones. The quintessential example of holocrine glands is the sebaceous glands located in the epidermis. The gonads, comprising the ovaries and testes, function as holocrine glands. However, they produce gametes (oocytes and spermatozoa) instead of secretory substances. Consequently, gonads are referred to as cytogenic glands.

Exocrine glands: These glands are situated at varying depths within the body. The most basic type is the goblet cells (*cellula caliciformis*) located in the lamina epithelialis of the glandular mucosa. These are unicellular glands situated intermittently inside the epithelial covering. Their form resembles a cup. They produce a mucous secretion. Goblet cells are located in the respiratory tract and the intestines. All exocrine glands, except from these, consist of many cells (multicellular glands) and are referred to as exoepithelial glands.

Multicellular exocrine glands are categorized into two classes according to the branching of their excretory ducts: a) simple glands and b) compound glands. Simple glands consist of one or more glandular corpuscles, all of which discharge into a singular excretory duct. This duct may occasionally be lacking. The corpus glands are linked to the covering epithelium by a singular neck (collum glandule). Compound glands possess a singular primary excretory duct, which undergoes multiple bifurcations until it reaches the corpus glandules, with a reduction in diameter at each division. The majority of compound glands are macroscopic anatomical structures, possessing multiple glandular corpuscles.

In certain anatomical glands, the primary excretory duct may be bifurcated (as shown in the pancreas of most mammals) or possess several branches (as seen in the mandibular and auxiliary genital glands).

The principal excretory duct of a complex gland at the anatomical level is referred to as the ductus excretorius or ductus glandularis. The duct, extending from the organ lumen to the glands, bifurcates into many branches within the gland, referred to as the pars excretory. These two duct types are coated with a bilayer of columnar epithelial cells. Each of the pars excretory divides into multiple finer branches known as the pars secretory or pars citriata. The parotid secretory cells consist of a singular layer of elongated columnar epithelial cells. These cells possess microvilli on their apical surfaces and invaginations on their basal surfaces. Threadlike mitochondria travel parallel to one another between these crevices. This organelle imparts a striated look to the basal half of the cell, therefore the designation citriata. Each pars secretory bifurcates into multiple slender branches, referred to as the pars initialis or pars intercalata. The first segments of the pars initialis consist of single-layered cuboidal epithelium, but the terminal segments, which link to the corpus glandules, are lined with single-layered squamous epithelium.

The excretory ducts of certain compound glands not only release the secretion generated in the corpus glandules but also affect its quantity and quality. For instance, reabsorption in the pars secretoria can concentrate the secretion, or alternatively, the primary secretion may be diluted by the release of a serous secretion—thus the designation secretoria. These ducts facilitate secretion by releasing specific active chemicals or integrating ions from the bloodstream into the secretion.

Exocrine glands are categorized into three types according to the morphology of their glandular corpuscles: a) tubular glands; b) alveolar (acinar) glands; c) tubulo-alveolar glands

These three categories of corpus glands are present in both simple and compound glands. In tubular glands, the corpus glands are cylindrical, whereas in alveolar glands, they are spherical (acinus). In tubulo-alveolar glands, certain corpus glands exhibit a tubular morphology, whereas others possess a grape-like

configuration. The most basic tubular glands are the fundic glands, characterized by their unbranched, straight tubular structure. The glands are situated in the lamina propria of the mucosa; they are devoid of excretory ducts and discharge directly into the lamina epithelialis via the collum glandular segments (see to the particular histology section for mucosal layers). Another category of uncomplicated tubular glands is devoid of excretory ducts; yet, the secretory segment of the gland diverges into several corpus areolules. All corpus glands discharge onto the surface, creating a singular column gland (b). These glands are situated in the lamina propria. The gastric and uterine glands belong to this category. Another group possesses an excretory duct analogous to the initialis; the glandular corpus is unbranched yet coiled or convoluted. The skin pores are of this glandular kind.

Vertebrates do not possess unbranched simple alveolar glands. These glands are located in the integument of invertebrates, including amphibians. The sebaceous glands in the skin of vertebrates are simple alveolar glands, which are branching.

Simple tubulo-alveolar glands are prevalent throughout the body. These glands discharge into the excretory duct at multiple locations. Certain corpus glands are tubular in structure, whilst others are alveolar (acinus). Simple tubulo-alveolar glands are situated in the submucosa. Examples of such glands include microscopic glands in the oral mucosa and those located in the submucosa of the esophagus, respiratory tract, and duodenum.

The excretory duct system in compound glands is highly developed and elongated. Consequently, these glands are situated at varying distances from the locations where they release their secretions. A compound gland is partitioned by connective tissue into lobes, which are further subdivided into lobules. Each lobule comprises many glandular corpuscles and excretory channels.

In anatomical glands, the connective tissue that occupies the interstitial spaces between the corpus glandules and lobules, as well as encasing the glands, is referred to as the stroma, as it constitutes the structural framework of the gland. The functional components, namely the corpus glandules and ducts, are termed the parenchyma.

The longest excretory ducts are located in the testes and kidneys, which are regarded as compound tubular glands in certain aspects. The corpus glands are elongated tubes. The mammary glands exemplify compound alveolar glands most prominently. The corpus glandules of these glands consist of expanded alveoli. The excretory ducts of these two types of compound glands exhibit structural differences from the aforementioned duct segments and are designated distinct names. The predominant complex glands in the body are the tubuloalveolar glands. In these, certain corpus glands are tubular, whilst others are alveolar. The

anatomical salivary glands and the pancreatic exemplify this type most prominently.

Exocrine glands are categorized into groups according to the type of secretion generated by their corpus glands. Glands that release a watery (serous) secretion, regardless of being simple or compound, are termed serous glands. If the corpus glands produce a viscous (mucus) secretion, they are classified as mucous glands. In certain glands, one section of the corpus secretes serous fluid, while another section secretes mucous; alternatively, inside a corpus glandule, some glandular epithelial cells produce serous, and others produce mucous. These glands are referred to as mixed (sero-mucous) glands.

Epithelial cells of serous and mucous glands exhibit distinct structural and morphological characteristics. In both gland types, the cells have a flattened or highly pyramidal morphology; however, in serous gland epithelial cells, the nucleus is spherical and positioned centrally, while the cytoplasm exhibits pronounced staining (black tone). This is attributable to the cytoplasm's abundance of ergastoplasm and the presence of proteinaceous secretory granules known as zymogen granules, which contain enzymes. The lumens of corpus glands, consisting of such cells, are thin because of the fluidity of the secretion.

In mucous gland epithelial cells, the nuclei are typically flattened (oval) and positioned against the basement membrane (shown by arrows); the cytoplasm exhibits poor staining (light hue); the lumens of the glands are dilated due to the opacity of the secretory substance.

The lighter appearance of mucous cells, which do not absorb the stain, in contrast to serous gland epithelial cells that stain effectively, is attributed to the inadequate preservation of carbohydrate-rich secretory granules (mucous granules) by fixative solutions. Zymogen granules, conversely, are firmly fixed and hence retain the dyes. Moreover, ergastoplasm, which predominantly facilitates the effective staining of serous cells, is not significantly developed in mucous cells.

All exocrine glands are encased by a basement membrane. The cells constituting both the glandular corpuscles and the excretory tubes of the glands adhere to this basement membrane on one side. The basement membrane is an extension of the basement membrane that provides support to the overlying epithelium to which the glands are affixed.

Exocrine glands receive innervation from the autonomic nervous system, comprising both parasympathetic and sympathetic divisions. Parasympathetic nerves function as activators, whilst sympathetic nerves serve as inhibitors. This transpires as follows: in a quiescent cell, the osmotic pressure within and outside the cell (in the interstitial matrix) is equilibrated. Intracellular pressure is modulated by colloidal substances (proteins) prevalent within the cell, while

intercellular pressure, which is minimal, is governed by crystalloid substances (sodium and chloride ions). Cells release secretory substances when this equilibrium is disturbed, adversely affecting intercellular pressure, specifically when intracellular pressure rises. Acetylcholine is the factor that elevates intracellular pressure. This material is released by parasympathetic nerve terminals that encircle the glands in a network external to the basement membrane. Acetylcholine enhances the permeability of gland cell membranes, hence promoting the influx of crystalloids from the external environment into the cell. Crystalloids entering the cell elevate intracellular density, prompting water from the external environment to influx into the cytoplasm, resulting in cellular swelling. Elevated intracellular pressure induces the movement of secretory chemicals towards the apical region of the cell, subsequently leading to their excretion. In certain glands, secretion is modulated by both cerebral inputs (acetylcholine) and locally synthesized hormones.

The suppression of secretion transpires as follows: sympathetic nerve endings, positioned within the interstitial spaces of the corpus glands, terminate in the walls of the many blood vessels there. The sympathetic nerves release epinephrine or norepinephrine as neurotransmitters. These chemicals restrict the vascular walls. As a result, the corpus glands are unable to obtain adequate fluid and crystalloids, compelling the cells to diminish or cease their activity.

Endocrine glands are not associated with the lining epithelium due to their lack of ducts. They are situated deep within the body. In these glands, the glandular epithelial cells organize into bundled cords of cells instead of forming glandular corpuscles. Because glands other than the thyroid gland lack lumens, they leak directly into blood arteries (sinusoids). In the thyroid gland, distinct from other endocrine glands, a specific number of epithelial cells align in a single row, creating spherical, hollow structures known as thyroid follicles. The secretion is initially collected and matured within the lumens of these follicles; subsequently, it is reabsorbed and discharged into the adjacent blood vessels.

The regulation of endocrine glands is more intricate than that of exocrine glands. These glands function partially under the regulation of autonomic nerves and partially under the influence of other endocrine glands, particularly the pituitary gland. As this latter element indicates, endocrine glands possess the ability to regulate one another.

These glands also substantially affect several tissues and organs throughout the body. Similarly to the nervous system, the endocrine glands facilitate the dissemination of stimulating substances—termed hormones in the endocrine system and neurotransmitters in the nervous system—throughout the body, so enabling organs to regulate (activate or inhibit) one another. This function is rapidly and efficiently accomplished via the neurological system, albeit for a brief

duration, whereas it can be attained more gradually but sustained over extended durations by the hormonal pathway.

Endocrine glands possess less ergoplasm due to their slower functioning compared to exocrine glands; consequently, the secretory chemicals generated are concentrated in the cytoplasm, resulting in a higher apparent quantity of secretory granules than in exocrine glands.

3. MUSCULAR EPITHELIUM = MYOEPITHELIUM

A specialized form of epithelial cell is located between the basal surfaces of glandular epithelial cells and the outer basement membrane in the corpus glandules of some serous and mucous glands, including salivary glands, mammary glands, and sweat glands. These are referred to as myoepithelial cells (muscular epithelial cells). In cross-sections, these cells have a densely pigmented, flattened nucleus encircled by a small quantity of cytoplasm. The cells possess many cytoplasmic extensions emanating from their body.

The muscular epithelia encasing a corpus glandule wrap the gland with their extensions, resembling an octopus.

These cells possess contractile filaments (actin and myosin) within their cytoplasm. This organelle provides myoepithelial cells with their contractile capability. By contracting, they compress the glandular corpus and assist the transfer of the sap accumulated in the lumen into the excretory ducts.

4. SENSORY EPITHELIUM = NEUROEPITHELIUM

These epithelial cells are specialized for sensory reception and are situated within the covering epithelium. They are accountable for perceiving some tactile experiences, in addition to the senses of taste and hearing. The cells that detect tactile sensations are typically spherical, but those that perceive aural stimuli are either bottle-shaped or prismatic. The apical ends of the sensory epithelial cells of the latter two types project into the organ cavities, where they generate stereocilia. The basal portions of the sensory epithelia are encircled by the terminals.

CHAPTER 27

Molecular Basis of Gallic Acid-Mediated Anticancer Activity

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INTRODUCTION

Cancer is a heterogeneous group of diseases characterized by the uncontrolled proliferation of cells, invasion of surrounding tissues, and metastasis to distant organs (Brown et al., 2023). This group of diseases is characterized by multiple biological processes, including genetic mutations, epigenetic changes, dysregulation of cell-cycle control, immune evasion, angiogenesis, and interactions with the tumor microenvironment (Vlierberghe, 2024). The accumulation of genetic and/or epigenetic alterations in normal cells is highlighted as the first step in cancer development. In this context, the conversion of proto-oncogenes into oncogenes or the inactivation of tumor suppressor genes are of vital importance (Bayramova et al., 2024; Vlierberghe, 2024). Mutations or aberrant expression of genes, in particular TP53, PTEN, APC, and KRAS have been directly linked to cellular proliferation, genomic instability, and tumor development (Yan et al., 2024). Additionally, epigenetic modifications-such as DNA methylation, histone modifications, and non-coding RNAs-contribute to tumor formation by altering chromatin structure and gene expression. These epigenetic changes play an important role not only in tumor initiation but also in tumor progression, metastasis, and resistance to treatment (Capp et al., 2025). Another prominent feature of cancer cells is their development of immune evasion mechanisms. While the immune system can normally detect and destroy abnormal cells, many tumor cells evade this defense by manipulating immune checkpoints (e.g., by increasing PD-L1 expression), disrupting antigen presentation, or secreting cytokines that create an inflammatory microenvironment (Ghorani et al., 2023). This allows the tumor to survive for an extended period and is associated with treatment resistance and tumor recurrence. Another critical factor in tumor growth and spread is angiogenesis. Cancer foci must establish their own blood vessel networks to continue growing and metastasizing. In this process, growth factors secreted by cells in the tumor microenvironment or by cancer cells (e.g., VEGF) play a key role (Vlierberghe, 2024). Additionally, the tumor microenvironment can increase metastatic potential through cell-cell and cell-matrix interactions, enzymatic remodeling of the extracellular matrix, and reorganization of signaling pathways (Biray Avci et al., 2024). Furthermore, expression of various proteins, such as the oncogenic transcription factor Forkhead box M1 (FOXM1) (Yildiz et al., 2024) and SIRT family proteins, affects disease course and cancer prognosis (Özden, 2017; Ozden and Park, 2021; Park et al., 2016; Song et al., 2018).

In 2022, approximately 19.96 million new cancer cases and 9.74 million cancer-related deaths were reported worldwide, according to cancer epidemiology. These data indicate that cancer is the second most common cause

of death after cardiovascular diseases (Zhang et al., 2024). Demographic changes, specifically population aging and increasing population density indicate that the cancer burden will increase further in the coming years. Some projections indicate that the annual number of cases could reach 35 million by 2050. According to 2022 data, the most common cancers were lung, breast, colorectal, prostate, and stomach, with approximately 2.48 million, 2.29 million, 1.93 million, 1.46 million, and 0.97 million new cases recorded, respectively (Bizuyehu et al., 2024). These types also have the highest mortality rates (Zhang et al., 2024). The burden of cancer varies significantly between countries. While mortality rates are lower in high-income countries due to more widespread early diagnosis and treatment options, they remain higher in low- and middle-income countries because of screening program deficiencies, limited access to healthcare, and the high prevalence of infection-related cancers (Zhang et al., 2024).

In the field of cancer treatment and prevention, research into natural compounds is gaining increasing attention alongside traditional chemotherapy and radiotherapy approaches. Plant-derived polyphenols, flavonoids, and phenolic acids have attracted attention due to their inhibitory effects on cell proliferation, as well as their antioxidant and anti-inflammatory properties. It has been suggested that, by affecting genetic and epigenetic regulation in cancer cells, these compounds can activate mechanisms such as triggering apoptosis, arresting the cell cycle, inhibiting angiogenesis, and modulating the immune response. Recent studies have shown that gallic acid, a phenolic compound, may have anticancer effects across different cancer types, highlighting the need for further research in this area. In this context, a detailed understanding of the molecular mechanisms of natural compounds is considered an important step towards developing new strategies for cancer prevention and treatment.

1. Gallic Acid Sources

Gallic acid (GA) is a fundamental phenolic compound that occurs naturally in many plants. Tea leaves (Xiang et al., 2024), oak bark (Valencia-Avilés et al., 2018), and various tree and shrub species (Lamien et al., 2005; Rodríguez et al., 2008) have frequently been reported as GA-rich. It is also present in significant quantities in fruits and other foods, such as walnuts, apples, strawberries, pineapples, bananas, blackberries, lemons, and grapes (Dadwal et al., 2022; Junior et al., 2021; Saifullah et al., 2019; Suleria et al., 2020), indicating that GA has considerable potential for both biological research and therapeutic applications.

GA can be found in plants both in its free form and in various conjugated structures. Among these, esterified derivatives are common, and gallates-either derived from catechins or formed directly as esters-are also known to occur.

Alkyl esters such as methyl gallate, propyl gallate, octyl gallate, and dodecyl gallate are among the best-known derivatives of GA (Kosuru et al., 2018).

2. Applications of Gallic Acid

GA is a compound widely used in the pharmaceutical industry, notable for its pronounced anti-inflammatory and antioxidant properties. These biological properties have been demonstrated in numerous *in vivo* and *in vitro* studies conducted in humans, animal models, and cell cultures (Bak et al., 2013; Cai et al., 2022, 2020; Hsu and Yen, 2007; Yang et al., 2016). In traditional medicine, GA has long been used as a component in the treatment of various gastrointestinal disorders, such as diarrhea, dysentery, and internal bleeding. It is also considered a supportive agent for reducing inflammation and managing diseases of the digestive system (Cai et al., 2022, 2020).

GA's capacity to neutralize free radicals effectively represents a potential therapeutic approach for limiting cellular damage associated with aging (Luo et al., 2009). Furthermore, owing to its antiseptic properties, it plays an important role in cleaning and disinfecting wounds and in supporting the healing process. These advantages enable its use as an effective component to reduce the risk of infection (Yang et al., 2016). In addition, GA has recently emerged as a molecule of interest in cancer treatment and oncology research because of its anticancer potential against various cancer types.

3. Anticancer Mechanisms of Gallic Acid

Modulation of PI3K/Akt/mTOR and MAPK/ERK Signaling Pathways:

GA exhibits anticancer properties by targeting critical to cancer cell survival. GA directly affects cell growth, proliferation, and survival by modulating the PI3K/Akt/mTOR and MAPK/ERK pathways (Peng et al., 2022; Xiang et al., 2024). PI3K activity promotes cell growth and metabolic processes, while Akt proteins play a central role in cellular survival and proliferation mechanisms. Furthermore, the two subcomplexes of mTOR, mTORC1 and mTORC2, play fundamental roles in regulating protein synthesis and cell growth. GA inhibits the overactivity of these pathways, thereby inducing apoptosis in cancer cells and reducing tumor cell resistance.

Mitochondrial Apoptosis and Cell Cycle Regulation: GA triggers mitochondria-mediated apoptosis by increasing oxidative stress, decreasing anti-apoptotic Bcl-2 levels, and increasing the expression of pro-apoptotic proteins such as Bax, Endo G, and AIF. These biochemical alterations result in the activation of caspases 3 and 9, thereby initiating cell death (Lo et al., 2011). Furthermore, GA acts on cell-cycle control points, causing cells to arrest at the G2/M phase. In this process, activation of Chk1 and Chk2 results in inactivation

of Cdc25C and Cdc2, thereby blocking the cell cycle (Chen et al., 2009). These mechanisms suppress cancer cell proliferation and effectively induce apoptosis.

Anticancer Mechanisms of Gallic Acid According to Cancer Types: GA and its derivatives exhibit significant anticancer effects in many tumor types, including prostate cancer, melanoma, leukemia, oral cancer, colon cancer, lymphoma, and breast cancer (Keyvani-Ghamsari et al., 2023; Subramanian et al., 2015, 2016). For instance, in DU145 prostate cancer cells, GA triggers mitochondria-mediated apoptosis and induces DNA damage by elevating the production of reactive oxygen species (ROS) (Liu et al., 2015). In PC3 prostate cancer cells, inhibition of the JNK, PKC, p38, and PI3K/Akt signaling pathways decreases MMP-2 and MMP-9 enzyme activities (Subramanian et al., 2015). In A375S2 melanoma cells, GA inhibits cell proliferation in a dose- and time-dependent manner, resulting in decreased mitochondrial membrane potential, release of cytochrome c, and activation of caspases (Lo et al., 2011; Subramanian et al., 2015). In HCT-15 colon cancer cells, GA has been observed to induce apoptosis, characterized by morphological changes such as cell shrinkage and loss of adhesion (Keyvani-Ghamsari et al., 2023). In leukemia cells (HL-60, HL-60RG, and K562), GA promotes apoptosis by inhibiting ribonucleotide reductase, causing DNA damage, releasing cytochrome c, arresting the cell cycle in the G0/G1 phase, and activating caspase (Chandramohan Reddy et al., 2012; Htay et al., 2002; Madlener et al., 2007). In HeLa cervical cancer cells, GA causes a decrease in glutathione (GSH) levels, a reduction in mitochondrial membrane potential, and suppression of the EGFR, ERK/p-ERK, and Akt/p-Akt signaling pathways. As a result of these effects, caspases are activated and the cell cycle is arrested in the G1 phase (You et al., 2010).

The Synergistic Effect of Gallic Acid with Chemotherapy and Radiotherapy: GA exhibits anticancer effects alone, and its anticancer activity is further enhanced when combined with chemotherapeutic drugs. For example, the combination of GA with cisplatin produces a stronger therapeutic response in non-small-cell lung cancer. Similarly, in breast cancer and melanoma models, cells treated with administered immediately after low-level laser therapy (LLLT) exhibited higher apoptotic responses than cells treated with GA alone (Ashrafizadeh et al., 2021; Khorsandi et al., 2020). Furthermore, GA reduces the proliferation of hepatocellular carcinoma cells by suppressing IL-8 levels, indicating an important biological role in controlling tumor progression (Aborehab et al., 2021; Lima et al., 2016).

4. Antitumor Activities of Gallic Acid According to Cancer Types

Anticancer Effects on Prostate Cancer: GA is an anticancer agent that suppresses proliferation of DU145 prostate cancer cells. GA triggers

mitochondrial apoptotic processes by increasing production of reactive oxygen species (ROS), thereby reducing cell survival. Additionally, GA activates Chk1 and Chk2 kinases, arresting the cell cycle in the G2/M phase and inactivating Cdc25C and Cdc2 proteins (Chen et al., 2009). The autooxidative effect of GA is particularly pronounced in prostate cancer cells. Elevated ROS levels lead to a loss of mitochondrial membrane potential and the release of cytochrome c into the cytoplasm. These events initiate a chain reaction in which caspase-3, -8, and -9 are activated and dose-dependent, progressive apoptosis occurs (Russell et al., 2012). The antitumor effects of GA on PC3 prostate cancer cells have also been carefully studied. These studies reveal that GA can cause DNA damage and activate certain DNA repair genes. Furthermore, GA application reduces metastatic potential by limiting migration and invasion of PC3 cells (Liu et al., 2011). During this process, significant inhibition of the JNK, PKC, p38, and PI3K/AKT signaling pathways is observed, and the enzymatic activities of MMP-2 and MMP-9 are also suppressed (Subramanian et al., 2015).

Anticancer Effects on Melanoma Cells: GA significantly suppresses proliferation and triggers apoptosis in A375S2 human melanoma cells (Kondo et al., 2009). The rate of cell proliferation decreases with GA application, depending on the dose and duration of treatment. The apoptotic process is triggered by a decrease in anti-apoptotic Bcl-2 protein levels and an increase in pro-apoptotic Bax levels. GA also a time-dependent decrease in mitochondrial membrane potential and promotes activation of caspase-9 and caspase-3 by increasing cytochrome c release. This accelerates apoptosis. Furthermore, GA promotes increased expression of endonuclease G (Endo G) and apoptosis-inducing factor (AIF) (Lo et al., 2011). Another study in melanoma cells revealed that GA exerts a significant regulatory effect on matrix metalloproteinases (MMPs). Decreases in MMP gene expression and protein levels following GA application to A375S2 cells indicate the antimetastatic potential of GA's. Furthermore, GA suppresses MMP-2 activity by regulating the Ras and p-ERK signaling pathways, thereby significantly limiting the metastatic capacity of melanoma cells (Subramanian et al., 2015).

Anticancer Effects in Colon Cancer: Various experimental models have detailed the antitumor activities of GA against colon cancer cells. Research on COLO 205 cells demonstrated that GA induces DNA fragmentation and morphological alterations associated with apoptosis. These findings suggest that GA has the potential to effectively trigger apoptosis (Yoshioka et al., 2000). Similarly, research conducted on the HCT-15 cell line reported that GA reduces cell viability in a dose-dependent manner and that treated cells exhibited features indicative of apoptosis, such as marked cell shrinkage, rounding, and detachment

from the surface (Devi et al., 2014). Notably, the biological effects of GA and its derivatives on colon cancer cells are mediated by common mechanisms. Due to its antioxidant capacity and polyphenolic structure, GA suppresses proliferation and induces apoptosis in many colorectal cancer cell lines, including HCT-15, HCT116, HT-29, SW620, COLO 205 and Caco-2 (Khaledi et al., 2011; Lin et al., 2021; Subramanian et al., 2016). These apoptotic processes generally proceed via the mitochondrial pathway and are characterized by events such as cytochrome c release, caspase-3 activation, and DNA fragmentation (Subramanian et al., 2016).

GA has also been shown to influence various cellular behaviors involved in colon cancer progression. It has been proposed that GA suppresses cell adhesion, migration, and invasion, and that this effect may be related to the inhibition of the integrin/FAK signaling axis and to microRNA regulation. Specifically, a recent study reported that GA can reduce metastatic potential through microRNA-mediated integrin/FAK modulation (Huang et al., 2024). Furthermore, GA has been reported to regulate transcription by directly interacting with DNA and by binding to telomeric regions or oncogene promoter sequences (Sanchez-Martin et al., 2022). Studies aimed at increasing its therapeutic efficacy are also ongoing. The results of studies combining GA with various drug delivery systems are promising. For example, chitosan oligosaccharide-gallic acid (COS-GA) conjugates have been found to increase antiproliferative activity against colon cancer cells (Saetang et al., 2023). Moreover, GA has demonstrated a regulatory influence on immune cells in MC38 adenocarcinoma models in C57BL/6 mice. This effect has been shown to enhance the antitumor immune response, particularly by modulating the Treg cell phenotype. This finding suggests that GA may have significant potential for combination with immunotherapy strategies (Deng et al., 2022).

Anticancer Effects on Leukemia: Studies conducted in leukemia cells have demonstrated that GA exhibits anticancer activity through various mechanisms. Research has shown that this compound specifically arrests the cell cycle at the G0/G1 phase and exerts a significant antiproliferative effect in HL-60 and HL-60RG promyelocytic leukemia cells, and in K562 chronic myeloid leukemia cells. These effects of GA have been linked to molecular processes, such as inhibition of ribonucleotide reductase, increased DNA damage, and DNA fragmentation (Htay et al., 2002; Madlener et al., 2007). One of the main effects of GA on leukemia cells is the induction of apoptosis via the mitochondrial pathway. This process begins with the release of cytochrome c into the cytosol, a decrease in levels of the anti-apoptotic protein Bcl-2, and the upregulation of the mitochondrially derived pro-apoptotic factors EndoG and AIF. As a result,

sequential activation of caspase-4, caspase-9, and caspase-3 was achieved, and programmed cell death was effectively triggered (Locatelli et al., 2008; Madlener et al., 2007). The anticancer effect of GA on leukemia cells is not limited to apoptotic mechanisms; it also targets some critical oncogenic signaling pathways. Specifically, in K562 cells, GA was found to reduce cell proliferation and increase apoptosis by inhibiting BCR/ABL tyrosine kinase activity. Additionally, it has been reported that GA inhibits the activation of the NF- κ B signaling pathway, increases Bax, Fas ligand, and p53 levels, and ultimately promotes cell death (Chandramohan Reddy et al., 2012; Yeh et al., 2011). These findings demonstrate that GA has potent anticancer activity by targeting multiple molecular pathways in leukemia cells.

Anticancer Activity in Cervical Cancer: Studies conducted on HeLa cervical cancer cells have revealed that GA exerts significant anticancer effects by disrupting cellular oxidative balance and suppressing critical signaling pathways. Following GA administration, a decrease in glutathione (GSH) levels was observed in the cells, leading to a significant drop in mitochondrial membrane potential. This mitochondrial dysfunction has been considered an important step in initiating apoptotic processes (You et al., 2010). Furthermore, GA has been shown to inhibit key signaling pathways that enable cancer cell proliferation and survival, such as EGFR, ERK/p-ERK, and AKT/p-AKT. This inhibition contributed to the cessation of cellular growth and facilitated apoptosis. At the molecular level, processes such as caspase activation and poly(ADP-ribose) polymerase (PARP) cleavage indicate that GA effectively triggers apoptotic mechanisms. As a result of these processes, HeLa cells were found to arrest in the G1 phase of the cell cycle (Zhao and Hu, 2013). Overall, these findings demonstrate that GA exerts a multifaceted anticancer effect on cervical cancer cells by targeting oxidative stress mechanisms and proliferative signaling pathways (You et al., 2010; Zhao and Hu, 2013).

Anticancer Activity in Mammary Tumor: Studies examining the effects of GA on breast cancer cells have demonstrated that this compound exerts potent anticancer effects both alone and in combination with other therapeutic agents. Research using the combination of GA and metformin has demonstrated substantial inhibition of cell proliferation in MCF-7 cells (Haghshenas et al., 2023). During this treatment, increases in reactive oxygen species (ROS), cell-cycle arrest in the G1 and S phases, and marked increases in autophagic activity have been reported. Additionally, this combination enhanced apoptotic cell death. Another study evaluating the synergistic effects of GA with chemotherapeutic agents found that combining GA with cisplatin significantly increased apoptosis in MCF-7 cells. GA at low toxicity levels enhanced the efficacy of cisplatin and markedly increased the apoptotic response (Mamat et al., 2025). On the other

hand, antiproliferative effects were also observed in studies where only GA was administered. For example, GA alone has been reported to inhibit growth in MCF-7 cells, increase ROS production, and cause cell cycle arrest in the G1 phase. Furthermore, GA has been reported to activate autophagy and, through this process, promote apoptotic cell death (Shruthi et al., 2023).

Anticancer Activity in Lung Cancer: Researchers have extensively investigated the antineoplastic potential of GA in lung cancer cells. Kang et al. (2025) discovered that GA inhibited the proliferation of lung cancer cells, elevated intracellular reactive oxygen species (ROS), and halted the cell cycle at the G1 phase. Furthermore, GA enhances apoptotic processes by activating autophagy (Kang et al., 2025). Tian et al. (2023) demonstrated that the GA form found in the abrownin inhibits autophagy in non-small cell lung cancer (NSCLC) cells, thereby reducing proliferation and migration. This finding suggests that GA may be effective against tumor progression and metastatic potential (Tian et al., 2023). Chen et al. (2025) reported that the complex of GA and soy lecithin triggers ferroptosis in NSCLC cells, thereby increasing sensitivity to radiotherapy. This effect has been attributed to regulation of the Nrf2/SLC7A11/GPX4 signaling pathway (Chen et al., 2025). The use of GA in combination with other chemotherapeutic agents has also yielded significant results. Wang et al. (2016) demonstrated that the simultaneous administration of GA and cisplatin significantly increased apoptosis and enhanced treatment response in small cell lung cancer (SCLC) H446 cells. Yamaguchi et al. (2025) found that GA and 3,5-dihydroxy-4-methoxybenzyl alcohol (DHMBA) together inhibited proliferation of the A549 cell line and increased cell death.

Anticancer Activity in Neuroblastoma: Research examining the therapeutic efficacy of GA in neuroblastoma cells has demonstrated that this compound reduces tumor cell viability and induces apoptosis. A study by He (2022) reported that GA significantly reduced the viability of tumor cells by inducing programmed cell death in human neuroblastoma (NB) cells. This apoptotic effect has been reported to be associated with GA's ability to regulate various intracellular signaling pathways (He, 2022).

In conclusion, the findings in this section demonstrate that GA has significant anticancer potential across different cancer types because of its multifaceted biological effects. The cellular effects of GA include mechanisms, such as suppression of cell proliferation, disruption of cell cycle control, regulation of oxidative balance, activation of mitochondrial apoptotic pathways, regulation of autophagy, and inhibition of metastasis-related signaling pathways. Research on diverse cancer cell lines unequivocally illustrates that GA can augment anticancer

efficacy, both as a monotherapy and in conjunction with chemotherapeutic agents.

Data obtained from patients with diseases such as melanoma, colon cancer, leukemia, cervical cancer, breast cancer, lung cancer, and neuroblastoma show that GA can suppress tumor progression by targeting similar biological processes in different tumor types. This suggests that GA may have a role in cancer treatment across a broad therapeutic spectrum. Furthermore, some studies indicate that GA may exhibit immunomodulatory effects in the tumor microenvironment, and these findings open new avenues for future research into the use of the compound in combination with immunotherapies. However, since the current literature is largely based on *in vitro* data, critical parameters for clinical application, such as GA's bioavailability, metabolic stability, tissue distribution, and toxicological profile, have not yet been fully elucidated. Therefore, to evaluate the clinical applicability of GA, well-designed clinical trials are required in addition to comprehensive *in vivo* studies.

Overall, GA, which is of natural origin and has a multi-targeted activity profile, is an important compound that could be considered a complementary or supportive agent in cancer treatment. Advanced research conducted in this direction will provide a deeper understanding of GA's therapeutic potential and contribute to the development of innovative strategies for cancer treatment.

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CHAPTER 28

Visual Illusions

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Visual illusions, which are a condition that manifests with symptoms such as distortion of visible objects that disappear/persist on covering one eye, double vision that does not change on cover-ring either eye, multiple images that do not change on covering either eye, seeing a persistent afterimage of an object (or a previously viewed object reappears minutes later), moving objects leave behind multiple images or a “comet trail,” and moving objects look stationary and have many possible underlying causes, are like a gap between perception and reality and even cast a shadow over the reliability of our senses (Ullman T., 2024, Westheimer G., 2008, Pane et al., 2025).

Considering the simple physical equations of visual physiology and their processes in the brain, it becomes clear that the primary deceptive mechanisms involve the retrieval and matching of visual information stored in memory. The ability of two people looking at the same image to focus on different objects, depending on their viewing angle, is related to the visual system's simultaneous operation of at least two information channels at different spatial scales (Greg et al., 2023, Ronald et al., 2023). Visual illusions, a fascinating phenomenon that goes far beyond the physiological equation of vision, can be considered a pattern or perceptual phenomenon of the brain's complex internal mechanisms that we have not yet fully understood (Gregory, 1997, Eagleman D. M., 2001, Ramachandran and Hubbard, 2001, Lotto and Purves, 2002, Costa et al., 2023, Zhang and Yoshida, 2024). These complex mechanisms include contrast and motion illusions, contrast perception, the Hermann grid, and Ebbinghaus illusions (Figure 1) (Westheimer G., 2008, Notredame et al., 2014, King et al., 2017, Costa et al., 2023, Ronald et al., 2023). Although not fully deciphered, even from a distance, you might recognize the painting in Figure 1 as Vinci's Mona Lisa. However, in this painting, high-spatial-frequency components are presented with a reduced pixel count. Despite this dual component, the visual input of the larger image is distorted in the visual system, but the image can still be recognized. From this, we understand that these dual input channels operate simultaneously and are transmitted simultaneously, leading us to marvel at the magnificently designed anatomy of the retina (Ronald et al., 2023).



Figure 1. 13×20 pixel filtered image of the Mona Lisa painting.

(Ronald et al., 2023)

Although more pronounced with the use of colors, some illusions can create a sense of movement despite the absence of movement (Figure 2). This perception of movement is achieved through shimmering phenomena, retinal shifts, or automatically moving phenomena. (Barton and Leff, 2021).

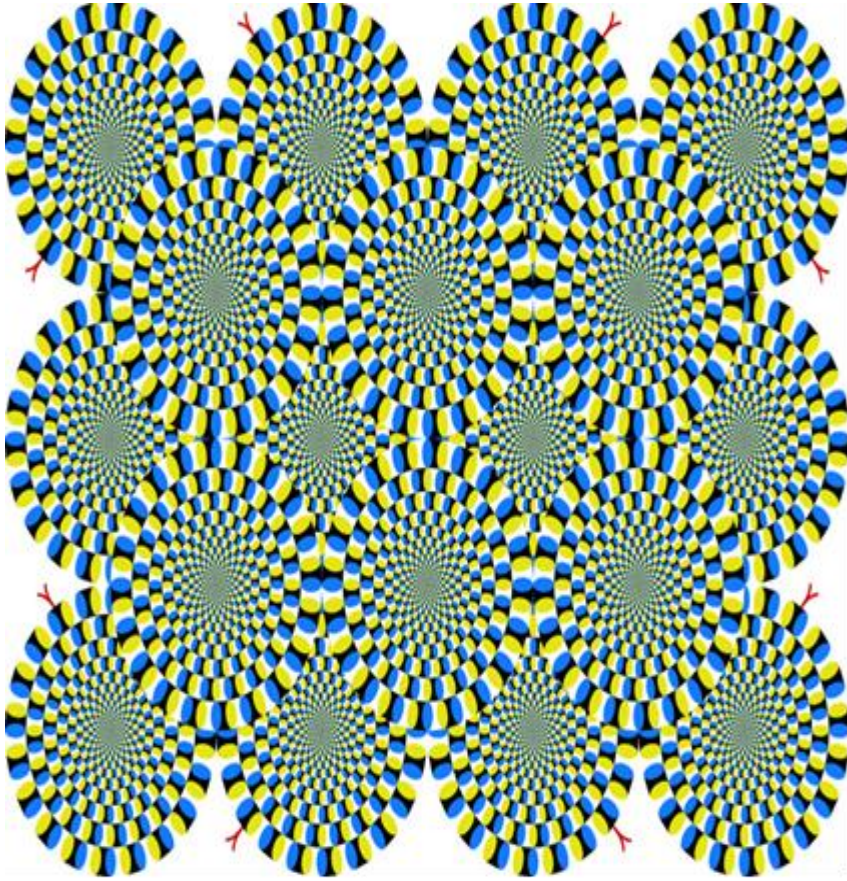


Figure 2. The illusion of movement (rotation) of a static image

(Barton and Leff, 2021).

Are illusions optical illusions, perceptual mismatches, or misleading contours? Geometric illusions, which are implied and masqueraded as real, also elicit neuronal activity in the visual cortex (Von der Heydt et al., 1984, Westheimer G., 2008, Petry, S., & Meyer, 2012). This collection of errors in perception actually provides us with information about the cognitive processes of vision (Westheimer G., 2008). Furthermore, visual illusions play an important role in the developmental processes of memory and attention. They enable increased concentration skills by focusing on a visual object. The recognition/recall and interpretation of illusions also feed into memory processes. While the perception process consists of stages such as color, shape, depth, and movement, including the eyes' optical processes and their organization and interpretation, visual illusions, in addition to these stages, also involve the individual's psychoemotional experiences. The curiosity and sense of discovery triggered by the enriched visual effects in illusions also lead to new experiences

(Adresyuk B., 2024). In the pattern in Figure 3, which contains three columns of black and white triangles on a single-plane background, multiple perceptual modes were created by changing the brightness phenomenon in regions A and B, thus transforming it into a single perceptual term. The image in Figure 3, composed of small black and white triangles designed to appear as if it has multiple views, was obtained with three columns. Area (a) is between the left column and the middle column, while area (b) is between the middle column and the right column. Importantly, the area labeled (a) appears as a dark surface in bright light, while the area labeled (b) appears as a light surface in shade (Shapiro and Hedjar, 2019).

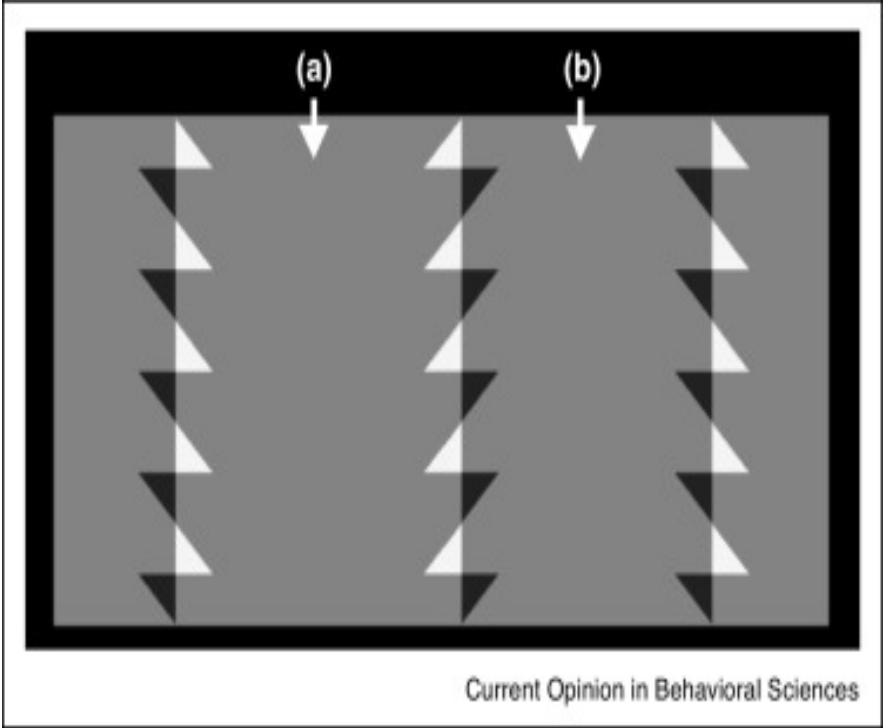


Figure 3. Obtaining multiple views with brightness differences on a uniform area
(Shapiro and Hedjar, 2019).

It is suggested that visual illusions are influenced by many physiological factors. The results of studies to date generally suggest that, just as in general vision, visual illusions decline with age, and that individual differences are also important in this regard. Interestingly, ecological factors are also stated to influence visual illusions. It has been suggested that growing up in an urban environment may result in a more linear perspective compared to a rural environment (Brislin R.W., 1974, Segall et al., 1963, Stewart V.M., 1973; Cretienoud et al., 2020). The results of a recent meta-analysis revealed that the magnitude of the Müller-Lyer, Pog-gendorff, and Vertical-Horizontal illusions

tends to decrease with age, while the magnitude of the Ebbinghaus and Ponzo illusions generally tends to increase with age (Wincza et al., 2024). Therefore, although sensitivity to visual illusions varies according to age, ecology, and other factors, the inconsistent results found among studies need to be investigated in more detail and with the same methodological analyses.

Findings from studies in the literature suggest that children with cognitive-motor disorders, such as developmental coordination disorder, are not only more important than auditory and tactile input but also more dependent on visual information (Bair et al., 2011, Miller and McIntosh, 2013, Nobusako et al., 2021; Shahbaz et al., 2024). Data such as these suggest that visual illusions are not limited to the visual cortex but also activate metacognitive and motor regions (Schwarzkopf and Rees, 2013; Pane et al., 2025).

The necessity of inferring visual information to interpret sensory signals was proposed by Von Helmholtz (von Helmholtz 1866, Gregory R. L., 1997, Cristalli C., 2020). It is known that optical imaging is an analytical collection of information derived from various information such as the physical properties of objects, their geometric identity, the ambient illumination, brightness, the angle of incidence of light, the position of the observer's eyes, the geometric properties of the contours, and contrast polarity (Westheimer G., 2008, Todorović, D., 2020). However, the definition of visual illusions is still a matter of debate (Von Gal et al., 2023). Elaborate cognitive illusions of objects in visual symbols, such as an object made of plastic, are cognitively made to appear as if it were wood by misleading information (Gregory T. L., 1997). Visual illusions, such as the perception that a taller or thinner glass will hold more, establish cognitive shortcuts using contextual stimuli in portion size estimations and the perception of volume. Horizontal-vertical illusions are also reported to influence this portion size perception (Van Ittersum and Wansink, 2007). An interesting study has shown that reptiles perceive only the portion related to photoreceptor activation when decoding visual information (Santacà et al., 2019).

In many images created using repetitive asymmetric patterns, motion is hypothetically observed despite the absence of motion. This is due to the illusion of peripheral drift. This phenomenon can be explained by the similar effect of activity in the lateral geniculate nucleus, as if the same situation were occurring in the real visual system or in the central nervous system, where directional information of motion is decoded. This is defined as high-contrast phase advance of contrast-driven motion (Backus and Oruç, 2005). The physiological basis of this mechanism lies in photoreceptor activation. Following this, there are steps such as luminance and contrast imaging mechanisms, speed inference and direction selection, and global motion perception

The visual thalamus, where the dorsal lateral geniculate nucleus (LGN) is located, is in contact with direction-selective (DS) retinal ganglion cells.

However, the potential effects of thalamic inputs, and thus selective thalamocortical inputs, on the direction selectivity of these direction-selective retinal ganglia in the visual cortex remain unclear. A very recent study suggested that LGN-DS cells may contribute to simple cell responses in layer 4 of the visual cortex in the preferred direction (Su et al., 2025).

Consequently, there is a need for detailed investigation of optical illusion formation methodologies and large cohort studies investigating the impact of individual differences on these illusions. It is also suggested that investigating whether differences exist between individuals in the data obtained is crucial. A more detailed understanding of the mechanisms of visual illusions will enable the identification of deep but unexplored areas of cognitive processes, thereby providing a wealth of knowledge that can guide the elucidation of cognitive disorders.

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CHAPTER 29

Febril Seizures

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Febrile seizures, a common childhood seizure disorder, occur with fever (mostly above 38°C). The highest incidence occurs between 12 and 18 months of age (Muthaffar et al., 2024, Whitney et al., 2025, Woo et al., 2025, Wu et al., 2025). These seizures develop without an underlying metabolic disorder or central nervous system infection (Leung et al., 2018, Baso et al., 2025, Woo et al., 2025). They are classified as simple febrile seizures and complex febrile seizures. Characteristics of simple febrile seizures include generalized tonic-clonic seizures without focal features, seizures lasting less than 15 minutes, not recurring within 24 hours, and spontaneously resolving. Complex febrile seizures, on the other hand, are characterized by focal neurological symptoms, last 15 minutes or longer, tend to recur within the same febrile illness, and require anticonvulsant treatment to control the seizures. Fever status epilepticus, the most severe type of complex febrile seizure, is also observed (Capovilla et al., 2009, Harini et al., 2015, Leung et al., 2018, Baso et al., 2025, Germain et al., 2025, Wu et al., 2025). Complications frequently observed in febrile status epilepticus include white matter maturation disorders and mesial temporal sclerosis (Pujar et al., 2017, Yu et al., 2017, Gordon E.E., 2025).

Approximately 20-35% of febrile seizures are complex seizures (Ferretti et al., 2024). The presence of electrographic and behavioral parameters of febrile seizures, along with clonic movements, allows families to recognize that their child is experiencing a seizure (Capovilla et al., 2009; Canpolat et al., 2018; Gordon E.E., 2025). Although seizures are brief, their recurrence rate is high. Despite having a benign prognosis, the wide range of recurrence rates can lead to prolonged febrile seizures that can lead to neurodevelopmental disorders in later life. More importantly, an examination of sudden unexplained death (SUDC) rates in childhood has revealed a significant prevalence of febrile seizures (Krous et al., 2005, Hesdorffer et al., 2015, Gould et al., 2023). A family history of febrile seizures is reported in approximately half of SUDC cases (Hefti et al., 2016, Smith et al., 2019, Sharma et al., 2025).

A new study suggests that prenatal and postnatal complications may play a significant role in the etiology of febrile seizures and may also influence the development of complex febrile seizures, suggesting that family history should be considered in terms of genetic predisposition. In addition to family history, it is known that an accompanying neuropsychiatric comorbidity can worsen the clinical course (Baso et al., 2025). Furthermore, studies demonstrate neurophysiological changes and anomalies in individuals with a family history of complex-form febrile seizures (Ito et al., 2005, Feghiu L., 2020, Contrucci et al., 2024).

Results from numerous studies examining the profiles of children with febrile seizures cover a wide range of factors, including prenatal maternal smoking and/or alcohol intake, developmental delay, genetic predisposition,

gastroenteritis, fever, daycare attendance, delayed neonatal discharge, family history, family education level, and geographic and socioeconomic characteristics (Bet-hune et al., 1993, Camfield and Camfield, 2015, Ferretti et al., 2024). However, there is no consistency across studies regarding all these factors. Some studies also report that no associations exist between factors such as daycare attendance, family history, vaccination status, and febrile seizures (Rahman and Fatema, 2020, Hossain and Saha, 2021, Eilbert and Chan, 2022, Muthaffar et al., 2024). It has been found that children with febrile seizures have more problems with fine motor skills than children without seizures (Ha et al., 2025). It is also possible that neuronal excitability in children with febrile seizures may be induced by fever, infections, underlying genetic disorders, genetic variations, or perhaps a combination of all these conditions (Figure 1) (Si-sodia S., 2014).

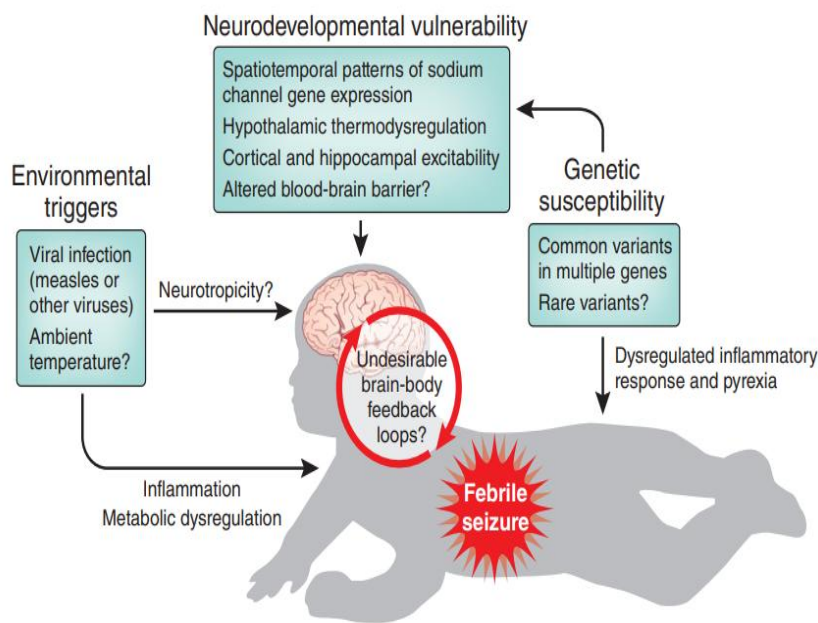


Figure 1. Possible causes of febrile seizures
(Sisodia S., 2014)

While the pathogenetic mechanisms underlying febrile seizures are still not fully understood, the potential association between genetic and environmental factors is being emphasized (Figure 2). However, a close relationship with viral infections has been noted. These infections primarily include influenza, herpesvirus, metapneumovirus, adenovirus, parainfluenza, rhinovirus, rotavirus, enterovirus, and measles. Additionally, seizures with high fever can frequently occur following diphtheria, tetanus, pertussis, and measles vaccinations (Francis et al., 2016, Canpolat et al., 2018, Carman et al., 2019, Hossain and Saha, 2021,

Sawires et al., 2022, Casabona et al., 2023). The presence of a positive family history and the high prevalence of febrile seizures in siblings of a child with febrile seizures indicate genetic heterogeneity, with the mechanism of inheritance varying between polygenetic and autosomal dominant. For example, an autosomal dominant locus for familial febrile seizures has been identified on chromosome 8q13-q21 (OMIM #121210) (Hauser et al., 1985, Fetveit A., 2008, Camfield and Camfield, 2015, FEB1 2021).

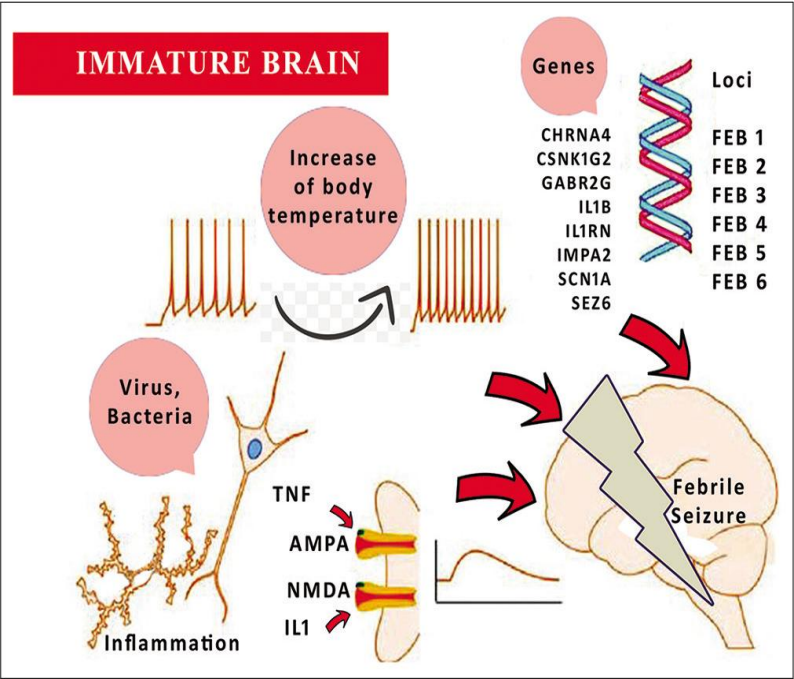


Figure 2. Genetic and environmental factors involved in the etiology of febrile seizures (Hossain and Saha, 2021)

These seizures, which occur at an early age, may occur as a result of the lowering of the excitability thresholds of developing neurons due to infection, which may explain why febrile seizures are so common in infants (Vezzani et al., 2015, Sharawat et al., 2016, Gordon E.E., 2025). Additionally, infection-induced hyperthermia is among the leading mechanisms in further excitation of neurons and the occurrence of febrile seizures (Fetveit A., 2008; Sawires et al., 2022; Gordon E.E., 2025). Although a relationship between neuroinflammatory mechanisms and seizures has been demonstrated in the recurrence of febrile seizures, the pathophysiological mechanisms are still not fully understood (Kong et al., 2025). Proinflammatory cytokines, which are present at high levels during inflammation, have been found to be elevated in the serum of patients with febrile seizures (Figure 3). It has been suggested that this elevation may increase

neuronal excitability (Vezzani and Viviani, 2015, Mosili et al., 2020, Maniu et al., 2021, Sawires et al., 2022, Hautala et al., 2023, Dong et al., 2024, Wu et al., 2025).

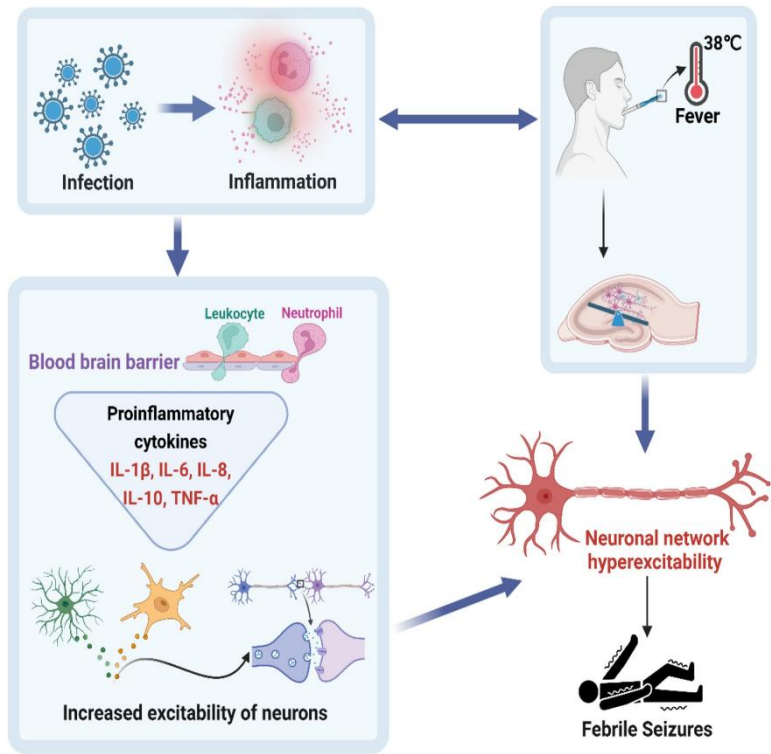


Figure 2. Relationship between neuroinflammation and febrile seizure pathophysiology (Wu et al.,2025)

The duration of exposure to a febrile seizure can lead to long-term consequences such as seizure-related neuronal damage and neuronal death, leading to developmental delays, learning disabilities, and developmental coordination disorders (Gillberg et al., 2017, Germain et al., 2025, Gordon E.E., 2025). Studies in children with prolonged febrile seizures indicate that hippocampal learning and functional deficits that occur affect normal neuronal functions (Baram and Shinnar, 2001, Chang et al., 2021, Dubé et al., 2009, Holmes G.L., 2025). The CA1 region of the hippocampus, known to play an active role in memory and learning processes, has been shown to exhibit adaptive changes with therapeutic potential by reducing epileptogenesis following febrile seizures. Furthermore, the same study suggests that this reduction in excitatory transmission may lead to cognitive impairments (Griflyuk et al., 2022, Postnikova and Zaitsev, 2025). The prevalence of neurodevelopmental and neurocognitive disorders in these individuals increase in the future due to the prolonged duration of complex-form febrile seizures. Among these disorders,

schizophrenia, attention deficit hyperactivity disorder (ADHD), and autism spectrum disorders are notable (Vestergaard et al., 2005, Bertelsen et al., 2016, Salehi et al., 2016, Gillberg et al., 2017, Griflyuk et al., 2025). Considered from this perspective, the strategies pediatricians implement in the management of febrile seizures are crucial. Recent studies recommend management priorities for this condition, such as terminating the seizure, excluding critical differential diagnoses, investigating the etiology of fever, and providing adequate counseling to families (Ferretti et al., 2024, Leung J. S. C., 2024).

Considering the age and frequency of febrile seizures, and even the possibility of them evolving into complex seizures, we recommend holding informational meetings to raise awareness of febrile seizures among babysitters, preschool teachers, and those in close contact with infants. We also recommend displaying public service announcements on television and billboards to reduce families' fear of febrile seizures and to demonstrate what can be done when faced with these seizures. Furthermore, equipping families with the skills to manage this traumatic process will have a positive impact on their quality of life.

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CHAPTER 30

Common Errors and Artifacts in Intraoral Indirect Digital Imaging

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1. PHOTOSTIMULABLE PHOSPHOR PLATE SYSTEMS

The first intraoral PSP system (Digora, Orion Co./Soredex, Helsinki, Finland) was introduced in 1994. PSP systems consist of a PSP plate that stores the exposure, a scanner that reads the plate, a computer that records the data, and a dedicated software for image processing. PSP plates consist of a polyester base coated with a delicate lattice of europium-doped barium fluorohalide, which acts as the luminescent material. During exposure, x-ray photons are absorbed and stored as latent energy in the phosphor layer. In the subsequent scanning process, the PSP plate is excited with red or green laser light, and the phosphor releases the stored light and converts it into an electrical signal, which is then digitized. Before reusing, the plate must be exposed to bright visible light to erase any remaining latent energy and to prevent ghost images and overlapping with subsequent exposures (Hildebolt, Couture, & Whiting, 2000).

Each receptor type has its own unique advantages and disadvantages (Ludlow & Mol 2014). Unlike CCD and CMOS sensors, which are relatively difficult to place in the oral cavity due to their thickness, PSP plates are similar in size and thickness to conventional films, do not contain electrical wires, and are more flexible. Therefore, they are more acceptable to patients (Hildebolt *et al.*, 2000; Macdonald, 2001; Parks & Williamson, 2002; Petrikowski 2005; Wakoh & Kuroyanagi, 2001; Wenzel, Frandsen, & Hintze, 1999). In addition, PSP plates are compatible with intraoral film holders used for conventional films. These similarities make the transition from film-based systems to digital PSP imaging systems easy, especially in institutions that provide dental education and clinics with heavy workloads (Kalathingall *et al.*, 2010).

Despite these advantages, PSP systems also have some limitations. A dedicated scanner is required for image processing. Depending on the type and resolution of the scanner, the scanning process takes from a few seconds to several minutes (Mol, 2019; Wenzel & Møystad, 2010). Exposure of the plate to ambient light or scanner-related variations may lead to emergence of artifacts that reduce diagnostic accuracy (Tashiro, Nakatani, Sugiura, & Nakayama, 2023). Even with careful handling, a progressive loss in image quality has been reported (Peker & Yapıcı, 2016; Tashiro *et al.*, 2023).

PSP plates are subject to mechanical and chemical damage during repeated use. Mechanical forces such as excessive bending, the pressure from film holding devices, or biting forces may damage the phosphor layer (Bedard, Davis, & Angelopoulos, 2004; Kalathingall *et al.*, 2010; Ludlow & Mol, 2014; Macdonald, 2001; Mol, 2019; Roberts & Mol, 2004; Wenzel & Møystad, 2010). Moreover, PSP plates cannot be heat sterilized or completely immersed in high-level disinfectants; contact with disinfecting chemicals or body fluids such as saliva, blood, or skin oil can also damage the phosphor layer (Bedard *et al.*, 2004; Gumru, Tarcin, & Idman, 2021; Kalathingall *et al.*, 2010; MacDonald &

Waterfield, 2011; Negron, Mauriello, Peterson, & Arnold, 2005; Roberts & Mol, 2004; Wenzel, Kornum, Knudsen, & Lau, 2013; Wenzel & Møystad, 2010).

2. CLASSIFICATION OF PHOTOSTIMULABLE PHOSPHOR SYSTEM ERRORS AND ARTIFACTS

Technical errors and improper manipulations during both image acquisition and processing may lead to radiographic errors and artifacts. Like any newly emerging technology, the integration of digital radiography into dental radiographic practices has presented new and diverse challenges, including new types of image artifacts that remain an issue for dental practitioners (Chiu *et al.*, 2008; Çalışkan & Sumer, 2017).

Intraoral digital imaging artifacts are more common in PSP plates compared to direct digital systems. This difference can be attributed to several factors. Direct digital sensors are structurally thicker and more rigid than PSP plates which are thin and flexible like conventional films; therefore, PSP plates are more vulnerable to external forces such as bending, scratching, and biting. In addition, while in direct digital systems the image is displayed immediately on the computer screen after exposure, PSP systems require an additional scanning step in which the latent image is converted into a digital image using a laser. This two-stage process increases the likelihood of artifacts due to both the time elapsed between exposure and scanning, and scanner-related artifacts (Peker & Yapıcı, 2016).

Artifacts, defined as undesirable image features on radiographic images that are unrelated to the anatomical structures being examined, and errors are well-documented in film-based radiography, however fewer studies have investigated PSP-related artifacts, particularly in dentistry (Chiu *et al.*, 2008; Çalışkan & Sumer, 2017; Deniz & Kaya, 2019; Elkhateeb, Aloyouny, Omer, & Mansour, 2022; Gulsahi & Secgin, 2016; İncebeyaz, Eren, & Öztaş, 2022; Tashiro *et al.*, 2023; Yurttaş, 2024). Furthermore, there is currently no consensus on a standardized classification system for PSP system artifacts, with considerable variation observed among the proposed systems.

Chiu *et al.* (2008) proposed a three-group classification consisting of operator errors, scanning machine artifacts, and PSP plate artifacts. Errors and artifacts were classified into four categories by Çalışkan and Sumer (2017), including operator- and patient-induced errors, ambient light-induced artifacts, PSP plate-induced artifacts, and scanner-induced artifacts. The classification was expanded by Deniz and Kaya (2019) into five categories based on the underlying causes, as operator errors, ambient light artifacts, plate artifacts, scanner artifacts, and software artifacts. Elkhateeb *et al.* (2022) categorized artifacts into three groups as operator errors, plate artifacts, and scanning artifacts. In their review study, İncebeyaz *et al.* (2022) proposed a four-stage classification based on the artifact formation time as before irradiation, during irradiation, after irradiation, and

caused by the scanner. Tashiro *et al.* (2023) classified PSP-specific image defects into six categories, including white borders, rotation/tilt, overlap of two images, superimposition, horizontally flip, and high-intensity/finger-like regions. Most recently, Yurttaş (2024), building on the work of Çalışkan and Sumer (2017), used a four-category system consisting of technical errors, plate-related artifacts, scanner-induced artifacts, and ambient light artifacts.

Taken together, these studies demonstrate that although classification systems vary considerably, there appears to be a broad agreement that errors and artifacts can broadly be grouped into four main categories, including operator/technical errors, plate-related artifacts, scanner-induced artifacts, and ambient light-related artifacts.

3. PHOTOSTIMULABLE PHOSPHOR SYSTEM ERRORS AND ARTIFACTS

3.1. Operator/Technical Errors

Operator/technical errors - such as plate bending, improper positioning of the plate in the oral cavity, incorrect vertical or horizontal angulation of the x-ray beam, underexposure or overexposure, motion, reversed image, and inadequate coverage of the detector by the x-ray field - can occur in both conventional and digital methods (Tashiro *et al.*, 2023; Yurttaş, 2024).

Many studies have identified operator-related errors, particularly cone-cut and projection geometry problems, as the most common errors, and underscored the substantial influence of operator technique on image quality and the need for continuous training and standardized radiographic procedures (Chiu *et al.*, 2008; Deniz & Kaya, 2019; Elkhateeb *et al.*, 2022; Yurttaş, 2024).

Since operator-related errors generally necessitate re-exposure, comprehensive training of practitioners in radiographic techniques is crucial in reducing operator errors when using PSP systems (Chiu *et al.*, 2008; Elkhateeb *et al.*, 2022).

3.2. Plate-related Artifacts

In the routine repeated use of PSP plates, mechanical stress, including tough handling, excessive bending, film holder pressure, patient bite forces, forceful insertion into the scanner, and stresses exerted by the scanner roller, may lead to cracks, scratches, or bite marks (Çalışkan & Sumer, 2017; Deniz & Kaya, 2019; Kalathingal *et al.*, 2010; Pamukcu, Tetik, Peker, Karadag Atas, & Zafersoy Akarslan, 2022; Yurttaş, 2024). Such excessive forces can cause damage to the photostimulating luminescence layer and cracking in the supporting polyester layer of the PSP plate, creating areas that no longer respond to x-rays and ultimately producing signal voids that appear as white or radiopaque artifacts in the resulting image (Thang *et al.*, 2020).

Prolonged use of the plates and wiping with chemical disinfectants may cause peeling of the protective and light-stimulating phosphor layer, particularly at the edges (Wenzel *et al.*, 2013; Yurttaş, 2024). Contamination (dust particles, glove powder, fingerprints, saliva, blood, adhesive material for the protective sheaths) damaging the active phosphor layer also causes artifacts. Dirt on the active surface of the plate blocks the laser light, causing radiopaque artifacts (Çalışkan & Sumer, 2017; Gulsahi & Secgin, 2016; Pamukcu *et al.*, 2022; Yurttaş, 2024).

Damage to the phosphor layer, which causes signal voids appearing as radiographic artifacts, leads to permanent deterioration of all subsequent images recorded with the plate, compromised diagnostic quality, and repeated imaging inconsistent with the ALADA (as low as diagnostically acceptable) principle, while also increasing chair time and costs (Bedard *et al.*, 2004; Çalışkan & Sumer, 2017; İncebeyaz *et al.*, 2022; Roberts & Mol, 2004; Wenzel & Møystad, 2010; Yurttaş, 2024). Damage accumulated from continued use progressively degrades the quality of the radiologic images, so the plates need to be discarded and replaced (Thang *et al.*, 2020).

The most common plate artifact was identified as peeling (53.4%) by Çalışkan & Sumer (2017), and bite marks (40.3%) by Elkhateeb *et al.* (2022). In another study, Yurttaş (2024) reported cracks and scratches as the predominant plate-related artifacts (77.1%).

Manufacturers recommend the routine use of protective covers to minimize mechanical damage to the plates. While disposable barrier sheaths aid infection control and reduce ambient light exposure, they do not prevent mechanical damage (e.g., bending, film holding device pressure, or bite marks) that can lead to artifacts (Chiu *et al.*, 2008; Roberts & Mol, 2004). Reducing such artifacts requires strict compliance with proper handling protocols by all users. These protocols include gentle handling PSP plates gently during and after exposure and scanning and limiting cleaning to gently wiping only when visible surface contamination is present to extend plate longevity (Elkhateeb *et al.*, 2022; Kalathingall *et al.*, 2010). Regularly checking the integrity of PSP plates is also extremely important (Elkhateeb *et al.*, 2022). PSP plates should be checked daily for scratches, and if surface damage is detected, a test image should be taken and the plate replaced if necessary (Deniz & Kaya, 2019).

3.3. Scanner-induced Artifacts

Scanners are a frequent source of image artifacts in PSP systems. Short-term variations in laser scanning intensity may cause horizontal radiopaque lines to appear in different regions of the scanned plate. The dust or dirt particles along the path of the laser light in the scanner blocks the laser light, causing areas devoid of signals, and results in parallel radiopaque lines in the scanning direction that appear consistently at the same location in every scanned image (Çalışkan & Sumer, 2017; Yurttaş, 2024). Zigzag radiopaque lines may appear if dust or dirt

particles move slightly during scanning (Çalışkan & Sumer, 2017; Peker & Yapıcı, 2016; Yurttaş, 2024). Radiolucent lines can appear due to electromagnetic interference caused by disruptions in scanner performance. Furthermore, Çalışkan and Sumer (2017) denoted this artifact as a ridging artifact, resulting from the fast variations in the intensity of the laser light, as well as the loss of harmonization between rapid scan cycles and image plate movement.

The scanner artifact rate was found to be 52% by Tashiro *et al.* (2023), while Çalışkan and Sumer (2017) reported 53% of PSP artifacts as scanner-related, the most frequent of which was straight lines (42.2%). Elkhateeb *et al.* (2022) stated that the most common scanning errors were non-uniform density or bright images (39.9%). Chiu *et al.* (2008) documented scanner-related artifacts in only 4.5% of PSP images, mostly in the form of horizontal radiopaque lines. Yurttaş (2024) reported a much higher frequency, with parallel or zigzag radiopaque lines observed in 98.7% of the images. Differences in reported artifact rates may be explained by the differences in the artifact classification criteria, institutional practices, equipment characteristics, number of plates examined, and experience levels of operators (Yurttaş, 2024).

The authors emphasized that, unlike many other types of errors and artifacts, some scanner-induced artifacts may be resolved by rescanning, thereby preventing additional radiation exposure from image repetitions (Chiu *et al.*, 2008; Yurttaş, 2024). To prevent the formation of radiolucent lines, measures such as appropriate electromagnetic shielding, a stable voltage source, and uninterrupted power supply have been recommended (Çalışkan & Sumer, 2017; Deniz & Kaya, 2019; Yurttaş, 2024). In addition, periodic maintenance, cleaning, and calibration of scanners will significantly reduce the number of scanning errors (Elkhateeb *et al.*, 2022).

3.4. Ambient Light-related Artifacts

PSP artifacts may also originate from ambient light (Yurttaş, 2024). If scanning of an exposed PSP plate is delayed without protection from ambient light, the information stored on the plate gradually fades due to increased signal loss and a reduced signal-to-noise ratio, resulting in fading artifacts that usually begin at the plate edges (Çalışkan & Sumer, 2017; Deniz & Kaya, 2019; Gulsahi & Secgin, 2016; Yurttaş, 2024). Depending on the extent of exposure, this may present as generalized brightening or as non-uniform image density when only a portion of the plate is affected. Fluctuating signal loss further contributes to a noisy image (Deniz & Kaya, 2019; Gulsahi & Secgin, 2016). Shining artifacts may appear in the center of radiopaque structures or along the plate edges as a result of complete local signal loss due to excessive energy release (Yurttaş, 2024). When unsheathed plates overlap, partial light exposure generally occurs. In addition to protected areas that maintain their normal density, localized areas

with lower density appear. As a result, different densities can be seen in the same image with the same anatomical structure (non-uniform image density) (Çalışkan & Sumer, 2017; Peker & Yapıcı, 2016; Yurttaş, 2024).

Artifacts related to ambient light generally occur as a result of removing the plate from its protective cover after exposure to prevent contamination (Chiu *et al.*, 2008; Çalışkan & Sumer, 2017; Deniz & Kaya, 2019; Gulsahi & Secgin, 2016). Akdeniz *et al.* (2005) reported that plates begin to release electrons approximately 10 minutes after exposure. Signal loss first appears at the periphery of the image, where photon intensity is lower than at the center (Chiu *et al.*, 2008). High-attenuation areas, such as fixed prostheses and metallic restorations, also suffer photon loss and may appear bright when exposed to ambient light (Deniz & Kaya, 2019).

Çalışkan and Sumer (2017) defined the fading observed in 44.1% of the plates as the most common ambient light artifact. Similarly, delayed scanning artifacts were reported in about 40% of the PSP images by Elkhateeb *et al.* (2022). Yurttaş (2024) reported fading and shining artifacts in 49.3% and 35.9% of PSP images, respectively, while Gulsahi and Secgin (2016) identified non-uniform image brightness as the most frequently encountered artifact.

Several researchers recommended preventive measures to minimize artifacts related to ambient light (Akdeniz, Gröndahl, & Kose, 2005; Ang, Angelopoulos, & Katz, 2006; Ramamurthy, Canning, Scheetz, & Farman, 2004; Wenzel & Møystad, 2010). As image quality decreases in proportion with ambient light intensity and exposure duration, these measures include reducing ambient light intensity, avoiding unnecessary exposure of PSP plates to light during handling and scanning, and inserting the plate into the scanner immediately after exposure (Akdeniz *et al.*, 2005; Ang *et al.*, 2006; Elkhateeb *et al.*, 2022). To prevent light-induced discharge, plates should ideally be scanned in a semi-dark environment within 10 minutes after exposure (Yurttaş, 2024). When immediate scanning is not possible, the plates should be kept at room temperature in a dark environment (Akcicek, Cagirankaya, & Avcu, 2016). Additionally, overlapping plates after exposure should be avoided to prevent the risk of non-uniform image density (Gulsahi & Secgin, 2016).

4. CLINICAL IMPLICATIONS OF PHOTOSTIMULABLE PHOSPHOR SYSTEM ERRORS AND ARTIFACTS

From a clinical perspective, artifacts associated with PSP systems have important implications as they can compromise image quality, may lead to reduced diagnostic accuracy and increased risk of misinterpretation or misdiagnosis. For example, plate-related artifacts such as scratches, cracks, or peeling may resemble root canal fillings or periapical radiolucency, potentially leading to incorrect treatment decisions. Similarly, ambient light-related fading and scanner-induced radiopaque lines may hide or distort anatomical details

critical for diagnosis. Moreover, when unreadable or misleading images need to be repeated, clinicians, patients, and the environment are exposed to unnecessary radiation, which conflicts with the ALADA principle (Yurttaş, 2024).

The clinical impact of PSP system artifacts was further presented by Thang *et al.* (2020), who showed that although even the presence of severe artifacts did not significantly affect diagnostic interpretation negatively, increasing artifact severity markedly reduced clinicians' diagnostic confidence in evaluating periapical inflammatory disease. It was emphasized that this loss of confidence unfortunately often results in image retakes or treatment delays until additional clinical signs, symptoms, and tests become available for a positive diagnosis. Both of these situations may adversely affect patient management and increase radiation exposure (Thang *et al.*, 2020).

CONCLUSION

In conclusion, recognizing PSP-specific artifact types, their underlying causes, and their effects on diagnostic performance, and appropriate preventive strategies is essential for minimizing repeat exposures, reducing costs, improving diagnostic reliability, and maintaining patient safety in routine dental radiology practice. Many artifacts can be reduced through practical measures such as thorough operator training, consistent and gentle plate-handling protocols, regular inspection of plates, limiting plate cleaning to only visible contamination, controlling the scanning environment, keeping software updated, and performing regular scanner maintenance and calibration. Continued research is also needed to optimize PSP use, prolong plate lifespan, and ensure high-quality diagnostic images.

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CHAPTER 31

Chronic Inflammatory Demyelinating Polyneuropathy: Pathophysiology, Clinical Features, and Management

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1. Introduction

Chronic inflammatory demyelinating polyneuropathy (CIDP) is an immune-mediated neuropathy and should be considered in any patient presenting with a progressive polyneuropathy lasting longer than two months. The diagnosis of CIDP is based on a combination of clinical signs and symptoms, electrophysiological findings suggestive of demyelination, and supportive laboratory investigations. Consequently, CIDP is increasingly regarded not as a single disease entity, but rather as a heterogeneous disorder encompassing a broad spectrum of clinical phenotypes, disease courses, and variable responses to immunomodulatory therapies (Joint Task Force of the EFNS and the PNS, 2005; Joint Task Force of the EFNS and the PNS, 2010). This heterogeneity is partly attributable to differences in disease definitions over time. The classical form of CIDP, currently referred to as “typical CIDP,” is clinically characterized by a symmetric polyneuropathy affecting both proximal and distal muscles and represents a relatively homogeneous subgroup. In addition, several other clinical phenotypes are now recognized within the CIDP spectrum, including multifocal acquired demyelinating sensory and motor neuropathy (MADSAM) and distal acquired demyelinating symmetric neuropathy (DADS) (Mathey et al., 2015; Saperstein et al., 1999).

In 2005, the Joint Task Force of the European Federation of Neurological Societies and the Peripheral Nerve Society (EFNS/PNS) proposed a clinical classification of CIDP subtypes. Within this framework, classical CIDP was redefined as “typical CIDP,” while “atypical CIDP” was used to encompass MADSAM (also referred to as asymmetric CIDP), DADS, and pure motor or pure sensory variants of CIDP (Joint Task Force of the EFNS and the PNS, 2005). Although these entities are still considered within the CIDP spectrum, they exhibit distinct clinical and pathological features. Notably, multifocal motor neuropathy (MMN) and anti-myelin-associated glycoprotein (anti-MAG) neuropathy were excluded from CIDP in these criteria. While all CIDP subtypes share the common feature of acquired demyelination of peripheral nerves, the marked phenotypic diversity suggests that the underlying pathophysiological and immunopathogenic mechanisms may differ between subtypes. Previous studies have proposed that variations in the distribution patterns of demyelination play a critical role in determining the observed clinical phenotypes (Kuwabara et al., 2002).

Pathologically, CIDP is characterized by macrophage-mediated inflammatory demyelination, with a predilection for proximal nerve segments compared to distal segments. Inflammatory infiltrates typically accumulate around myelinated nerve fibers or within the epineurium, particularly in perivascular regions (Tracy

et al., 2019). During the early stages of the disease, segmental demyelination predominates; however, with ongoing demyelinating activity over time, repeated and incomplete attempts at remyelination result in the formation of onion bulb structures, ultimately leading to hypertrophic nerves. In CIDP, onion bulbs commonly exhibit a “mixed pattern,” whereby patchy inflammatory demyelination produces large onion bulbs adjacent to normally myelinated fibers. This uneven demyelination contributes to temporal dispersion on electrodiagnostic studies, a hallmark electrophysiological feature of CIDP (Dalakas, 2015). Immune mechanisms involving macrophage-mediated pathways, including the expression of costimulatory molecules such as B7-1 and B7-2, are directed against Schwann cells and myelin epitopes; however, the precise immune targets remain incompletely defined. The identification of specific autoantibodies, including those against neurofascin-155 and contactin-1, has highlighted a subset of patients with nodal or paranodal pathology. Importantly, these cases often demonstrate distinct clinical characteristics and differential responses to treatment. From a therapeutic perspective, intravenous immunoglobulin (IVIg), subcutaneous immunoglobulin (SCIg), corticosteroids, and plasma exchange (PE) have all shown efficacy in typical CIDP, although each modality is associated with specific limitations (Bunschoten et al., 2019).

2. Epidemiology

CIDP is regarded as a rare disorder, with prevalence rates that vary considerably across different geographic regions. A population-based study from the United Kingdom conducted by Mahdi-Rogers and Hughes reported a prevalence of 2.84 per 100,000 individuals (Mahdi-Rogers and Hughes, 2014). In this cohort, male patients outnumbered females by approximately 2:1, and the disease was more frequently observed in older age groups. Previously reported prevalence estimates range from as low as 0.8 per 100,000 in the Tottori region of Japan to as high as 8.9 per 100,000 in Olmsted County, United States (Laughlin et al., 2009; Kusumi et al., 1995). More recent epidemiological data from Ireland indicate a prevalence of 5.87 per 100,000 among adults with CIDP. The underlying reasons for these substantial geographic differences remain unclear (Lefter et al., 2017). Variability in the diagnostic criteria applied across studies may partially account for the observed heterogeneity in reported prevalence and incidence rates.

CIDP can affect individuals across a broad age spectrum, ranging from childhood to beyond the eighth decade of life. Although the highest prevalence has been reported during the seventh decade, disease onset most commonly occurs between the fourth and fifth decades. Consistent with most epidemiological observations, males appear to be affected more frequently than

females. A preceding infectious illness or vaccination has been reported in approximately one-third of CIDP cases, a proportion that is notably lower than that observed in Guillain–Barré syndrome (GBS), where nearly half of patients report an antecedent infection (Laughlin et al., 2009; Shije and Brannagan, 2019).

The reported frequency of CIDP subtypes varies substantially between studies. Analysis of data from 460 patients included in the Italian CIDP Database Study Group revealed that 82% were classified as having typical CIDP, while the remaining 18% were diagnosed with atypical CIDP. The atypical forms included distal acquired demyelinating symmetric neuropathy (DADS) in 7%, Lewis–Sumner syndrome (LSS) in 4%, pure motor CIDP in 4%, and pure sensory CIDP in 3.5% of cases (Allen et al., 2018). In contrast, a Japanese study evaluating 100 consecutive CIDP patients categorized cases as typical CIDP (60%), multifocal acquired demyelinating sensory and motor neuropathy (MADSAM; 34%), DADS (5%), or pure sensory CIDP (1%). These discrepancies may reflect differences in the clinical criteria used to define atypical CIDP across populations. Notably, both studies consistently reported a reduced response to intravenous immunoglobulin (IVIg) therapy among patients with atypical CIDP, supporting the notion of distinct underlying pathophysiological mechanisms within these subgroups (Kuwabara et al., 2015).

3. Pathophysiology

Although the precise pathogenic mechanisms of CIDP have not been fully elucidated, the disorder is widely regarded as immune mediated. Accumulating evidence indicates the involvement of autoreactive T lymphocytes, B lymphocytes, soluble inflammatory mediators such as cytokines and chemokines within neural tissue, autoantibodies directed against a range of neural glycolipid and glycoprotein structures, and increased concentrations of complement components, including C5a and the soluble terminal complement complex (Bunschoten et al., 2019). In a Swiss case–control study examining skin biopsy specimens from 20 patients with CIDP and 17 healthy controls, significant alterations were observed in the expression of genes associated with immune regulation and chemokine signaling in CIDP patients. In treatment-naïve individuals with CIDP, circulating monocytes demonstrated increased expression of the activating FcγRI receptor, whereas inhibitory FcγIIb receptor expression was reduced on both naïve and memory B cells as well as on monocytes (Quast et al., 2015). Importantly, this dysregulated Fcγ receptor balance was partially restored following intravenous immunoglobulin (IVIg) therapy. Clinical improvement observed after IVIg, corticosteroid treatment, or plasma exchange further supports the central role of immune-mediated mechanisms in CIDP pathogenesis (Nagelkerke and Kuijpers, 2014).

Additional pathogenic heterogeneity is suggested by the identification of immunoglobulin G4 (IgG4) autoantibodies targeting nodal and paranodal proteins in a subset of patients exhibiting CIDP-like phenotypes. These autoantibodies are predominantly, though not exclusively, of the IgG4 subclass. IgG4 antibodies are notable for their limited ability to bind FcγIIb receptors, inability to activate the complement cascade, and their generally anti-inflammatory functional profile (Bunschoten et al., 2019; Burnor et al., 2018). Increasing evidence indicates that IgG4 antibodies can directly bind to neural targets, disrupt their physiological function, and thereby contribute to nerve injury. In addition to their presence in CIDP patients, IgG4 antibodies against neurofascin-155 (NF155) have also been detected in selected cases of Guillain–Barré syndrome (GBS) and in genetic or idiopathic neuropathies (Burnor et al., 2018; Delmont et al., 2017).

The immune response in CIDP involves both cellular and humoral components. Antigen-presenting cells (APCs), such as dendritic cells and macrophages, activate autoreactive T cells, which subsequently migrate across the blood–nerve barrier (BNB) into the peripheral nerve. This process is facilitated by chemokines, cellular adhesion molecules, and matrix metalloproteinases. Activated T cells can further stimulate B lymphocytes through the secretion of cytokines such as interleukin (IL)-4 and IL-6 (Rajabally et al., 2017). Within the peripheral nervous system (PNS), T cells are reactivated upon re-encounter with target antigens presented by local APCs, initiating clonal expansion within the nerve. Following activation, CD4⁺ T cells may differentiate into T-helper 1 (Th1) cells, which secrete pro-inflammatory cytokines and amplify inflammatory responses, or into T-helper 2 (Th2) cells, which produce anti-inflammatory mediators and modulate immune activity. Macrophages, primarily activated by Th1 cells, exhibit enhanced phagocytic capacity and release cytotoxic mediators such as nitric oxide, which can directly damage Schwann cells (SCs) and contribute to secondary axonal injury (Burnor et al., 2018; Rajabally et al., 2017).

In addition, pro-inflammatory cytokines locally produced by B cells, including tumor necrosis factor (TNF) and interferon-gamma (IFN-γ), promote demyelination and axonal damage. Autoantibodies may induce demyelination via antibody-dependent cellular cytotoxicity, functionally block epitopes essential for nerve conduction, or activate the complement system through the classical pathway, resulting in the generation of inflammatory mediators and the lytic C5b–9 terminal complement complex. Resolution of inflammation is mediated in part by macrophages that induce T-cell apoptosis and secrete anti-inflammatory cytokines such as IL-10 and transforming growth factor-beta (TGF-β). Several

myelin-associated proteins, including protein zero (P0), peripheral myelin protein 2 (P2), myelin basic protein (MBP), myelin-associated glycoprotein (MAG), connexin 32 (Cx32), and glucagon-like peptide (GLP), have been proposed as putative target antigens in CIDP (Delmont et al., 2017; Rajabally et al., 2017).

An abnormal cytokine profile in the cerebrospinal fluid (CSF) of CIDP patients reflects active intrathecal inflammation and suggests that dysregulated production of pro-inflammatory cytokines is a key component of disease pathogenesis. Compared with individuals with non-inflammatory neurological disorders, CIDP patients negative for anti-NF155 antibodies exhibit significantly elevated CSF levels of IFN- γ and reduced levels of IL-1 receptor antagonist (IL-1ra) and IL-4. In these patients, IFN- γ concentrations correlate positively with CSF levels of CXCL10/interferon gamma-induced protein 10 (IP-10), CXCL8/IL-8, and total CSF protein, supporting a Th1-driven inflammatory response mediated through macrophage activation (Hu et al., 2022; Ogata et al., 2019). In contrast, patients positive for anti-NF155 antibodies demonstrate significantly higher CSF levels of CXCL8/IL-8 and IL-13, along with lower concentrations of IL-1 β , IL-1ra, and IL-6, indicative of a Th2-skewed cytokine profile. Th2 cells promote B-cell activation and antibody production. Recent studies have also highlighted the role of pathogenic Th2 subsets, particularly IL-5-producing Th2 cells, in chronic inflammatory conditions.

Furthermore, the frequency of T-helper 17 (Th17) cells is significantly increased in both the CSF and peripheral blood mononuclear cells of patients with active CIDP compared with those in remission or individuals with non-inflammatory neurological diseases (Chi et al., 2010). Correspondingly, plasma IL-17 levels and the expression of retinoic acid receptor-related orphan receptor gamma t (ROR γ t), a transcription factor essential for Th17 differentiation, are elevated. As a recently recognized immune cell subset in inflammatory disorders, Th17 cells may play a critical role in determining disease activity in CIDP.

Serum cytokine profiles in CIDP further reflect ongoing systemic inflammation. Compared with healthy controls, patients with CIDP exhibit increased serum levels of IL-2, hepatocyte growth factor (HGF), TNF- α , IL-1 β , macrophage inflammatory protein (MIP)-1 α , and MIP-1 β . During active disease phases, serum TNF- α , endothelial leukocyte adhesion molecule-1 (ELAM-1), IFN- γ , and IL-10 are also elevated. Consistent with these findings, expression of TNF- α and IFN- γ has been demonstrated within the endoneurium of affected nerves in CIDP patients (Mathey et al., 1999). Strong mRNA expression of TNF- α and IFN- γ , along with lower levels of IL-2, has been detected in the perineurium. CIDP lesions are frequently infiltrated by V α 24J α Q natural killer T (NK T) cells in association with IL-4 mRNA expression. Reduced serum levels

of granulocyte–macrophage colony-stimulating factor (GM-CSF) have been observed in CIDP patients, potentially impairing SC regeneration; notably, SC dysfunction could not be reversed by exogenous GM-CSF administration. Additionally, CIDP patients demonstrate reduced natural killer (NK) cell numbers, increased monocyte counts, and alterations in regulatory T cell (Treg) function (Sanvito et al., 2009).

4. Clinical Features

CIDP typically presents with sensory symptoms such as numbness and paresthesia, which are followed shortly thereafter by muscle weakness; these manifestations generally progress gradually over time. Patients commonly experience weakness affecting both distal muscles, such as intrinsic hand muscles and ankle dorsiflexors leading to foot drop, and proximal muscle groups, resulting in difficulty with activities such as climbing stairs or reaching overhead objects (Odaka et al., 2003). In most cases, motor impairment represents the most disabling aspect of the disease. Affected individuals may develop gait instability, experience frequent falls, and require assistive devices for ambulation, including walking aids or wheelchairs. Fine motor control, particularly involving the hands and fingers, is often impaired.

Although sensory deficits and sensory ataxia are common clinical features of CIDP, autonomic dysfunction and neuropathic pain occur less frequently. When pain is present, it is usually mild in intensity, although severe pain has been reported in rare cases. The preferential involvement of specific nerve fiber populations—predominantly large myelinated fibers—likely reflects the demyelinating nature of CIDP. Consequently, heavily myelinated motor and proprioceptive fibers are primarily affected, whereas lightly myelinated or unmyelinated fibers, including autonomic and nociceptive fibers, are relatively spared. The clinical severity of CIDP varies widely among patients, ranging from mild forms in which individuals remain ambulatory to severe cases characterized by wheelchair dependence and loss of independent function in activities such as feeding or toileting. Respiratory muscle involvement is uncommon, and the need for mechanical ventilation is rare. Cranial nerve involvement is also infrequent; when present, it most commonly affects the oculomotor and facial nerves (Shije and Brannagan, 2019; Odaka et al., 2003).

The distinction between CIDP and acute inflammatory demyelinating polyneuropathy (AIDP) is primarily based on differences in temporal evolution and long-term disease course. In AIDP, neurological symptoms and deficits develop rapidly over days to weeks, reaching maximal severity within approximately one month. This acute phase is typically followed by gradual recovery, during which patients regain strength, although residual deficits may

persist in some cases. In contrast, CIDP is characterized by a slowly progressive course, with symptom development extending over at least two months. CIDP may also present with a relapsing–remitting pattern marked by acute exacerbations and partial remissions, a presentation that can resemble AIDP. However, unlike AIDP, CIDP is not a self-limiting disorder and tends to recur over time (Bunschoten et al., 2019). This relapsing–remitting pattern is particularly common in children and young adults and may only become distinguishable from AIDP after multiple relapses have occurred. Persistent or progressively worsening neurological deficits favor a diagnosis of CIDP, although long-term residual impairment can also be observed in AIDP. Progressive deterioration on serial neurological examinations further supports CIDP. In general, AIDP is considered a post-infectious neuritis, with a preceding infection often identifiable, whereas CIDP typically lacks a clear antecedent infectious trigger. The insidious onset and gradual progression of CIDP symptoms may contribute to the difficulty in identifying a prior infectious event (Kuwabara and Misawa, 2019).

Neurological examination in CIDP reveals predominantly motor deficits, with weakness affecting the shoulder and hip girdle muscles, as well as distal muscles of the hands and lower extremities. Findings are usually symmetric and involve both upper and lower limbs. Although pathological involvement is most pronounced in proximal nerve segments, including nerve roots and plexuses, clinical deficits are often more evident distally. This apparent discrepancy is likely attributable to the greater vulnerability of longer peripheral nerves supplying distal muscles, which are more susceptible to cumulative damage from inflammatory demyelination. Sensory examination typically demonstrates prominent impairment of proprioception and vibration sense, reflecting involvement of large myelinated sensory fibers. Deep tendon reflexes are usually absent or markedly reduced, representing a characteristic clinical finding in CIDP (Table 2) (Dyck and Tracy, 2018; Odaka et al., 2003).

5. Diagnosis and Differential Diagnosis

CIDP lacks a single disease-specific biomarker; therefore, its diagnosis depends on a combination of clinical evaluation, electrophysiological evidence of demyelination, and supportive ancillary investigations. Over the years, numerous diagnostic criteria have been proposed, reflecting the heterogeneity of the disease and the challenges inherent in distinguishing CIDP from other peripheral neuropathies. Among these, the revised European Federation of Neurological Societies/Peripheral Nerve Society (EFNS/PNS) criteria published in 2010 remain the most widely adopted framework in both clinical practice and research settings (van den Bergh et al., 2010).

The EFNS/PNS criteria integrate clinical phenotypes with electrophysiological findings to stratify CIDP into typical and atypical clinical forms, while classifying electrophysiological certainty as definite, probable, or possible. Motor nerve conduction studies constitute the cornerstone of diagnosis, with demyelination inferred from functional abnormalities such as conduction block, prolonged distal and F-wave latencies, reduced conduction velocities, and temporal dispersion affecting one or more motor nerves (Joint Task Force of the EFNS and the PNS, 2010). However, these electrophysiological features reflect impaired saltatory conduction rather than direct histopathological demyelination and must therefore be interpreted cautiously.

Accurate nerve conduction study interpretation requires strict methodological conditions, including adequate limb temperature control, as hypothermia may falsely exaggerate conduction slowing. In addition, reduced compound muscle action potential (CMAP) amplitudes—particularly below 1 mV—can limit the reliability of demyelinating criteria, as severe axonal loss may mimic demyelinating patterns. This phenomenon represents a major source of diagnostic error and highlights the need for contextual interpretation of electrophysiological data (van den Bergh et al., 2010; Eftimov et al., 2020). Recording from proximal muscles in selected nerves has been proposed as a strategy to enhance diagnostic accuracy in such situations (Van Asseldonk et al., 2005). Importantly, demyelinating features detected at anatomical sites prone to compression or entrapment should not be considered supportive of CIDP, as similar abnormalities may arise from focal mechanical injury rather than immune-mediated pathology (Rajabally & Narasimhan, 2011).

Laboratory investigations primarily serve to exclude alternative or coexisting conditions that may mimic CIDP, including diabetes mellitus, monoclonal gammopathies, infectious neuropathies, and hereditary demyelinating disorders. In this context, exclusion of inherited neuropathies—such as Charcot–Marie–Tooth disease type 1A or transthyretin amyloid polyneuropathy—is particularly important, as these conditions may closely resemble CIDP clinically and electrophysiologically yet require fundamentally different management strategies (Lozeron et al., 2018).

CSF analysis provides supportive but non-specific diagnostic information. Elevated CSF protein concentration with a normal or minimally increased leukocyte count is frequently observed in typical CIDP, although this finding is less consistent in atypical variants (Eftimov et al., 2020). Age-adjusted reference values are essential, as mild protein elevation may occur in older individuals or in other neuropathic conditions; consequently, an upper limit of 0.6 g/L has been suggested for patients over 50 years of age (Breiner et al., 2019). While marked

pleocytosis should prompt evaluation for infectious or malignant etiologies, modest leukocyte elevations do not necessarily exclude CIDP, particularly in patients with subacute onset (Lucke et al., 2018).

Neuroimaging techniques, including magnetic resonance imaging (MRI) and nerve ultrasound, function as adjunctive diagnostic tools, especially in patients who do not fully meet electrodiagnostic criteria. MRI may reveal hypertrophy or contrast enhancement of nerve roots or plexuses, supporting an inflammatory demyelinating process; however, these findings show limited correlation with disease severity or duration and are influenced by variability in imaging protocols (van den Bergh et al., 2010; Jongbloed et al., 2017; Bunschoten et al., 2019). MR neurography studies have suggested a relationship between nerve volume and disease duration, though evidence remains limited by small sample sizes (Ishikawa et al., 2017).

Compared with MRI, nerve ultrasound offers a more accessible and time-efficient modality, allowing real-time assessment of proximal nerve segments. Studies have demonstrated that ultrasound-detected nerve enlargement—particularly within the median nerve and brachial plexus—can reliably differentiate inflammatory neuropathies from axonal neuropathies and motor neuron disease, supporting its growing role in the diagnostic evaluation of CIDP (Goedee et al., 2017a; Goedee et al., 2017b).

Finally, the CIDP diagnosis should be reconsidered in the presence of atypical clinical features, such as marked asymmetry, early muscle atrophy, prominent tremor or ataxia, autonomic dysfunction, severe neuropathic pain, respiratory involvement, or lack of response to established immunotherapies. In such cases, alternative diagnoses should be actively explored and the initial diagnostic assumption critically reassessed (Bunschoten et al., 2019).

6. Treatment

The management of CIDP is primarily based on immunomodulatory therapies, including glucocorticoids, intravenous immunoglobulin (IVIg), subcutaneous immunoglobulin (SCIg), plasma exchange (PE), and immunosuppressive agents. In addition, various supportive and symptomatic treatment strategies are commonly employed to address CIDP-related manifestations (Figure 5) (Ramamoorthy and Cidlowski, 2016; Patel et al., 2010).

Early studies evaluating the efficacy of corticosteroids in CIDP were conducted in relatively small patient cohorts. Subsequent larger investigations compared treatment outcomes in patients receiving prednisone therapy versus those who were untreated over a three-month period, including a study involving 28 patients with CIDP (Dyck et al., 1982). In 2010, van Schaik and colleagues

performed a randomized clinical trial enrolling 41 patients with CIDP to compare two different corticosteroid treatment regimens. One group received oral prednisolone at a daily dose of 60 mg, which was gradually tapered over 32 weeks. The second group was treated with oral dexamethasone at a dose of 40 mg daily for four consecutive days, followed by placebo administration for 24 days; this regimen was repeated monthly for six months. The study demonstrated that dexamethasone therapy did not result in a higher rate of neurological remission compared with the prednisolone-based regimen (Eftimov et al., 2012).

In 2014, Börü and colleagues conducted a retrospective analysis of 20 patients with CIDP who were initially treated with intravenous methylprednisolone at a dose of 1000 mg daily for 10 days, followed by monthly administrations. This treatment protocol was continued for five years, with an additional five-year follow-up period. Clinical improvement was observed in 15 patients during the five years of treatment; however, six relapses were documented during the subsequent follow-up period. Given the relatively low quality of evidence supporting corticosteroid use in CIDP and the potential long-term risks associated with high-dose or prolonged corticosteroid therapy, corticosteroids—whether used as monotherapy or at high doses—should not be considered the sole management strategy for CIDP (Börü et al., 2014).

Intravenous immunoglobulin (IVIg) is widely used in the treatment of CIDP due to its multiple immunomodulatory mechanisms, including inhibition of complement activation, suppression of pro-inflammatory cytokine production, and interference with Fcγ receptor-mediated signaling on phagocytes and B cells. A pivotal clinical trial conducted in 2008 evaluated IVIg therapy in 117 patients with CIDP and demonstrated significant improvements in clinical course and disability within 24 weeks of treatment, along with a low relapse rate (Hughes et al., 2008). More recently, subcutaneous immunoglobulin (SCIg) has been proposed as an alternative or maintenance therapy, particularly for patients with relapsing disease who require ongoing immunoglobulin treatment following IVIg induction.

Individualization of IVIg therapy is essential, as treatment response may be influenced by several immunobiological and pharmacokinetic factors. Key variables include interindividual variability in immunoglobulin G (IgG) pharmacokinetics, differences in IgG catabolic rates, and the optimal serum IgG concentration required to achieve and maintain clinical disease control (van Schaik et al., 2018).

The immunomodulatory effect of plasma exchange (PE) is primarily mediated through the removal of circulating autoantibodies, interleukins, and other pro-inflammatory molecules (Lehmann and Hartung, 2011). Early studies

investigating PE in CIDP reported substantial clinical improvement accompanied by favorable electrophysiological changes (Dyck et al., 1986). In another study involving 18 patients with CIDP, approximately 80% of those who completed the treatment protocol demonstrated a clinical response to plasma exchange (Hahn et al., 1996).

7. Prognosis

Overall, the prognosis of CIDP is generally favorable. In a landmark study published in 1987, McCombe and colleagues evaluated 92 patients with CIDP and reported that after a mean follow-up period of approximately 10 years, 73% of patients demonstrated a good functional recovery and remained independent. However, seven patients either died as a consequence of the disease or became severely disabled and immobilized (McCombe et al., 1987). More recent data from a cohort study involving 38 patients with CIDP revealed that five years after initiation of treatment, 87% of patients were still able to walk independently. Nevertheless, only 26% achieved complete remission, defined as sustained remission lasting longer than two years after discontinuation of therapy. In this study, predictors of complete remission included subacute disease onset, symmetrical clinical manifestations, absence of muscle atrophy at presentation, a favorable initial response to corticosteroid therapy, and the presence of a distal demyelinating pattern on nerve conduction studies (Kuwabara et al., 2006).

In a long-term observational study conducted by Dyck and colleagues, 40 patients with CIDP who were individually followed and treated with intravenous immunoglobulin (IVIg), plasma exchange (PLEX), and corticosteroids were evaluated. All 40 patients exhibited an early clinical response to immunotherapy. However, among the 37 patients for whom long-term outcome data were available, 27 continued to require ongoing immunotherapy, while only 10 achieved remission and were able to discontinue treatment. The authors emphasized that CIDP is often characterized by a marked initial clinical improvement following treatment, which is subsequently followed by a more modest and gradual improvement over time. This biphasic pattern may reflect relatively rapid remyelination, followed by slower and often incomplete axonal regeneration (Dyck et al., 2015).

An important unresolved issue in CIDP concerns whether the clinically defined subtypes represent distinct disease entities or merely different manifestations along a single disease spectrum. In a study by van Dijk and colleagues, seven patients who initially presented with purely sensory symptoms were followed longitudinally, and five of these patients developed muscle weakness after a mean duration of 3.1 years, suggesting clinical evolution over time (van Dijk et al., 1999). A retrospective study examining CIDP subtypes

reported that 64% of patients with classical CIDP achieved clinical remission after five years, whereas remission was observed in only 41% of patients with multifocal CIDP. In the same study, all patients with classical CIDP responded to at least one standard therapy—defined as corticosteroids, IVIg, or PLEX—while 23% of patients with multifocal CIDP were resistant to these treatments (Kuwabara et al., 2006).

Most patients with CIDP require long-term treatment and prolonged clinical follow-up. As with other immune-mediated disorders, the potential benefits of immunosuppressive and immunomodulatory therapies must be carefully weighed against the severity of the underlying disease and the risk of treatment-related adverse effects. The need for treatment and the intensity of therapy may fluctuate over time. The primary therapeutic objective in CIDP management is to gradually taper and, if possible, discontinue immunotherapy while closely monitoring patients through periodic neurological examinations and nerve conduction studies. If no clinical deterioration is observed, treatment intensity may be progressively reduced and ultimately stopped. Conversely, if clinical worsening occurs, treatment dosage or frequency should be increased accordingly. This process requires continuous adjustment, and the notion that there exists a single optimal dose applicable to all CIDP patients is misleading. Instead, treatment regimens should be individualized based on patient-specific responses. Importantly, assessment of treatment efficacy should rely predominantly on objective measures, such as findings from neurological examination and nerve conduction studies, rather than subjective impressions alone (Dyck and Tracy, 2018).

Concluding Remarks

CIDP represents a clinically and biologically heterogeneous immune-mediated neuropathy encompassing a wide spectrum of phenotypes, disease courses, and therapeutic responses. As outlined in this chapter, the diagnosis of CIDP relies on the integration of clinical presentation, electrophysiological evidence of demyelination, and supportive laboratory and imaging findings, rather than on a single definitive biomarker. Advances in immunopathological understanding have highlighted the involvement of both cellular and humoral immune mechanisms, providing a rationale for the use of immunomodulatory therapies. Although most patients respond favorably to treatments such as corticosteroids, intravenous or subcutaneous immunoglobulin, and plasma exchange, long-term disease control often requires individualized and dynamic treatment strategies. Prognosis is generally favorable; however, sustained remission is achieved in only a subset of patients, underscoring the chronic nature of the disease and the need for prolonged follow-up. Recognizing disease

heterogeneity, avoiding misdiagnosis, and tailoring therapy based on objective clinical and electrophysiological assessments remain central to optimizing outcomes in patients with CIDP.

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CHAPTER 32

Sodium and Water Balance Disorders

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1. INTRODUCTION: PHYSIOLOGICAL FOUNDATIONS

1.1. Water and Sodium Homeostasis

The maintenance of water and sodium balance in the human body is a dynamic process essential for cellular functional sustainability and the continuity of tissue perfusion. In an adult, total body water (TBW) constitutes approximately 60% of body weight, though this ratio ranges between 45% and 75% depending on age, sex, and adiposity. Body fluid is organized into two primary compartments: intracellular fluid (ICF) and extracellular fluid (ECF). Sodium serves as the principal osmotic determinant of the extracellular fluid.

While sodium and water balances are closely interrelated, they regulate distinct physiological parameters. Total body sodium content is the primary determinant of extracellular volume, whereas serum sodium concentration is a function of total body water (tonicity). Consequently, abnormalities in sodium concentration reflect a disproportion between sodium and body water—essentially a disorder of water metabolism—rather than a primary problem of absolute sodium quantity. This process is governed by volume regulation via the RAAS and sympathetic nervous system to preserve effective circulating volume (ECV), and by osmoregulation via ADH and the thirst mechanism to maintain plasma tonicity.

1.2. Regulation of Tonicity and Osmolality

Although the terms osmolality and tonicity are often used interchangeably in clinical practice, their physiological distinction is critical. Osmolality refers to the total number of solutes per kilogram of solvent, while tonicity (effective osmolality) represents the concentration of active solutes that cannot freely cross cell membranes and thus exert osmotic pressure. For instance, urea increases plasma osmolality as it diffuses freely across membranes but does not contribute to tonicity or transcellular water movement.

1.2.1. Antidiuretic Hormone (ADH/Vasopressin) Mechanism: ADH is the most sensitive regulatory mechanism for water balance. A mere 1-2% increase in plasma osmolality triggers a robust release of ADH. However, in emergency medicine, "non-osmotic stimuli" are clinically more significant. In states of severe volume depletion or hypotension, baroreceptors override osmotic suppression, stimulating ADH release to prioritize volume preservation over osmotic balance. ADH binds to V2 receptors in the renal collecting ducts, facilitating the translocation of aquaporin-2 channels and increasing free water reabsorption.

1.2.2. Thirst Mechanism: The thirst drive is the body's only means of increasing water intake. This mechanism is typically activated when plasma osmolality exceeds 290 mOsm/kg. In patients with impaired consciousness or restricted access to water, the failure of this mechanism poses a severe risk of dehydration.

2. HYPONATREMIA (Serum Sodium < 135 mEq/L)

2.1. Definition and Pathophysiological Basis

Hyponatremia, defined as a serum sodium concentration below 135 mEq/L, is the most common electrolyte disorder encountered in clinical practice. It generally develops due to impaired renal free water excretion. Three distinct states must be differentiated: hypotonic hyponatremia (true water excess), hypertonic hyponatremia (dilutional effect of effective osmoles like glucose), and isotonic hyponatremia (pseudohyponatremia), which is a measurement artifact caused by elevated lipids or proteins.

Cerebral Adaptation: In acute hyponatremia (less than 48 hours), the influx of water into brain cells leads to cerebral edema. In chronic hyponatremia, the brain adapts by extruding organic osmolytes to lower intracellular osmolality and counteract edema. While this adaptation allows chronic patients to tolerate significantly low sodium levels, it renders them susceptible to Osmotic Demyelination Syndrome (ODS) if correction is performed too rapidly.

2.2. Epidemiology

Hyponatremia is observed in 15-30% of hospitalized patients. Premenopausal women and children are at the highest risk for neurological complications. In the geriatric population, even mild chronic hyponatremia significantly increases the risk of cognitive impairment and bone fractures.

2.3. Classification by Tonicity

Hypotonic hyponatremia (osmolality < 275 mOsm/kg) represents true hyponatremia. If pseudohyponatremia is suspected, the diagnosis should be confirmed using the direct ion-selective electrode method (e.g., blood gas analyzers). In hyperglycemic patients, the measured sodium should be corrected by adding 1.6 mEq/L for every 100 mg/dL increase in glucose above 100 mg/dL (or 2.4 mEq/L if glucose exceeds 400 mg/dL).

2.4. Classification of Hypotonic Hyponatremia by Volume Status

1. **Hypovolemic:** Both sodium and water are lost, but the water deficit is relatively less than the sodium deficit. Urinary sodium below 20 mEq/L suggests extrarenal losses, while levels above 20 mEq/L indicate renal losses.

2. **Euvolemic:** SIADH is the primary cause. Findings include inappropriately high urine osmolality (above 100 mOsm/kg) and urine sodium typically above 40 mEq/L. Differential diagnosis includes primary polydipsia and beer potomania.

3. **Hypervolemic:** Water retention exceeds sodium retention, as seen in heart failure and cirrhosis. A urine sodium below 20 mEq/L reflects the kidneys' appropriate response to systemic hypoperfusion.

2.5. Specific Clinical Syndromes and Management

SIADH is the prototype of euvolemic hyponatremia and is a diagnosis of exclusion; secondary adrenal insufficiency must be ruled out as cortisol deficiency mimics SIADH. Thiazide diuretics can induce severe acute hyponatremia, particularly in elderly women within the first two weeks of therapy.

Management Strategies:

- **Emergency Management:** In the presence of severe symptoms (seizures, coma), the immediate goal is a rapid increase of 5 mEq/L in serum sodium. For this purpose, 100-150 mL of 3% hypertonic saline is administered as a bolus, which may be repeated up to three times every 10-20 minutes until symptoms resolve.

- **Correction Limits:** In chronic cases, the sodium increase should not exceed 8-12 mEq/L in 24 hours (or 6-8 mEq/L in high-risk groups).

- **ODS Prevention:** A sudden increase in urine output (exceeding 100 mL/hour) during correction signals "water diuresis" and an impending uncontrolled rise in sodium. In such cases, 1-2 mcg of Desmopressin should be administered as rescue therapy.

3. HYPERNATREMIA (Serum Sodium > 145 mEq/L)

3.1. Definition and Etiology

Hypernatremia is a state of hypertonicity characterized by a serum sodium concentration exceeding 145 mEq/L, usually resulting from a net loss of free water. It is primarily observed in populations with impaired thirst mechanisms or restricted access to water.

3.2. Classification and Diabetes Insipidus

Hypovolemic hypernatremia involves the loss of both water and sodium, whereas euvolemic hypernatremia (e.g., Diabetes Insipidus) involves isolated water loss. Diabetes Insipidus (DI) is categorized as Central (ADH deficiency) or

Nephrogenic (renal resistance). Central DI responds dramatically to Desmopressin, whereas Nephrogenic DI requires addressing the underlying cause (e.g., hypercalcemia, lithium toxicity).

3.3. Clinical Features and Adaptation

A hypertonic environment draws water out of neurons, causing brain shrinkage. This contraction can lead to the rupture of intracranial veins and subsequent hemorrhage. In chronic cases (over 48 hours), the brain synthesizes "idiogenic osmolytes" to preserve cell volume, necessitating a slow correction rate to avoid cerebral edema.

3.4. Management and Free Water Deficit

The target correction rate is 0.5 mEq/L per hour, not exceeding 10-12 mEq/L in 24 hours. In hypovolemic patients, perfusion should be restored with isotonic saline before transitioning to hypotonic fluids for water deficit replacement.

Free Water Deficit Calculation: The deficit is calculated by multiplying the total body water by the ratio of the measured sodium to the target sodium (typically 140) minus one. The coefficient for total body water is 0.6 for men and 0.5 for women, reduced by 0.1 for geriatric patients. This calculated value must be added to the patient's baseline daily requirements and ongoing losses.

4. CONCLUSION AND CLINICAL PEARLS

1. Hyponatremia is typically a problem of "water excess" rather than "sodium deficiency."
2. To confirm true hyponatremia, serum osmolality and corrected sodium levels (in the presence of hyperglycemia) must be assessed.
3. In symptomatic severe hyponatremia, controlled hypertonic saline boluses are life-saving compared to slow infusions.
4. Sudden polyuria during correction indicates a high risk for overcorrection and ODS; desmopressin should be readily available.
5. In hypernatremia management, "perfusion precedes tonicity"; stabilize the hypovolemic patient with isotonic fluids before addressing the water deficit.

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CHAPTER 33

Acute Pancreatitis

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1. Introduction and Diagnostic Approach

Acute pancreatitis (AP) is an acute inflammatory process of the pancreas with a broad clinical spectrum ranging from mild local tissue injury to Systemic Inflammatory Response Syndrome (SIRS) and multiple organ failure. The diagnosis of AP relies on a synthesis of clinical, biochemical, and radiological findings rather than a single parameter. According to the **2012 Revised Atlanta Classification**, the presence of at least two of the following three criteria is required for diagnosis:

1. **Clinical:** Acute onset, persistent, severe epigastric pain (often radiating to the back).
2. **Biochemical:** Serum lipase or amylase activity at least three times the upper limit of normal ($>3 \times \text{ULN}$).
3. **Radiological:** Characteristic findings of acute pancreatitis on contrast-enhanced computed tomography (CECT), magnetic resonance imaging (MRI), or transabdominal ultrasonography (US).

Clinical and Radiological Nuances: In patients presenting with characteristic abdominal pain and elevated enzymes ($>3 \times \text{ULN}$), routine early CECT is not indicated for confirmation of diagnosis, consistent with "Choosing Wisely" recommendations. Early imaging is reserved for cases of diagnostic uncertainty, atypical clinical presentations (e.g., "painless pancreatitis"), or to exclude other causes of acute abdomen. In biochemical diagnosis, serum lipase is preferred over amylase due to its longer half-life and higher specificity.

2. Etiology and Pathophysiology

Determining the etiology of AP is vital for preventing recurrence and guiding management. Gallstones and alcohol use disorder account for approximately two-thirds of all cases.

A. Major Etiologies

- **Biliary (Gallstone) Pancreatitis (40–70%):** Transient obstruction of the ampulla of Vater by a gallstone or biliopancreatic reflux triggers acinar injury. Abdominal ultrasonography should be performed in all patients to evaluate for biliary etiology.
- **Alcoholic Pancreatitis (25–35%):** Typically develops in the context of long-term (>5 years) and heavy alcohol consumption. Alcohol induces pathology by sensitizing acinar cells to cholecystokinin (CCK) and increasing premature zymogen activation.
-

B. Metabolic and Other Etiologies

- **Hypertriglyceridemia (HTG):** Serum triglyceride levels >1000 mg/dL represent a strong risk factor. Parenchymal injury results from the hydrolysis of triglycerides into toxic free fatty acids by pancreatic lipase (lipotoxicity).
- **Iatrogenic (Post-ERCP):** Post-ERCP pancreatitis (PEP) occurs in approximately 3–5% of procedures. Rectal indomethacin prophylaxis is standard for high-risk patients.
- **Malignancy Alert:** In patients over 40 years of age with unexplained ("idiopathic") etiology, pancreatic or periampullary malignancy must be excluded.

3. Clinical Course and Predicting Severity

The clinical course of AP varies from a mild, self-limiting indisposition to fatal organ failure.

Clinical Presentation and Physical Examination

- **Symptoms:** Epigastric pain is the cardinal symptom (90%), typically radiating to the back in a band-like fashion and relieved by leaning forward (knee-chest position). Intractable nausea, vomiting, and abdominal distension due to paralytic ileus often accompany the pain.
- **Systemic Signs:** Fever, tachycardia, tachypnea, and hypotension are early indicators of developing SIRS and intravascular volume depletion.
- **Rare Signs:** Periumbilical ecchymosis (**Cullen's sign**) or flank ecchymosis (**Grey Turner's sign**) occur in <3% of patients but suggest severe necrotizing pancreatitis and retroperitoneal hemorrhage.

Prognostic Laboratory Markers

The following parameters are more valuable than amylase/lipase levels for predicting severity:

- **Blood Urea Nitrogen (BUN):** An admission BUN >20 mg/dL or a rising trend within the first 24 hours strongly correlates with mortality.
- **Hemoconcentration:** A hematocrit >44% indicates inadequate fluid resuscitation and an increased risk of pancreatic necrosis.
- **C-Reactive Protein (CRP):** Levels >150 mg/L at 48 hours are indicative of severe disease and necrosis.

4. Classification and Local Complications

The Revised Atlanta Classification (2012) categorizes the disease based on Clinical Severity and Morphological Features.

A. Classification by Severity

Severity is defined by the presence and duration of organ failure (Modified Marshall Score ≥ 2):

1. **Mild AP:** No organ failure and no local or systemic complications. Usually resolves in 3–5 days.
2. **Moderately Severe AP:** Transient organ failure (resolves within <48 hours) or local/systemic complications without persistent organ failure.
3. **Severe AP:** Characterized by persistent organ failure (>48 hours). This group carries the highest mortality risk (approx. 35%).

B. Morphological Classification and Local Complications

Local complications are defined based on time (4-week cutoff) and content (presence of necrosis). The formation of a fibrous capsule around collections typically takes about 4 weeks.

1. Interstitial Edematous Pancreatitis (75–80%):

Pancreatic parenchyma is viable; there is no necrosis, only fluid leakage.

- **Acute Peripancreatic Fluid Collection (APFC):** Occurs within the first 4 weeks. Homogeneous fluid adjacent to the pancreas with no definable wall. Usually resolves spontaneously.
- **Pancreatic Pseudocyst:** If an APFC persists beyond 4 weeks, it becomes encapsulated. It has a well-defined non-epithelial wall and contains only fluid.

2. Necrotizing Pancreatitis (20–25%):

Characterized by necrosis of the pancreatic parenchyma and/or peripancreatic tissues.

- **Acute Necrotic Collection (ANC):** Occurs within the first 4 weeks. Contains both fluid and solid necrotic debris. No definable wall.
- **Walled-off Necrosis (WON):** Beyond 4 weeks, the necrotic collection becomes encapsulated. The critical distinction from a pseudocyst is the presence of solid necrotic material. This distinction is crucial as WON may require necrosectomy, whereas pseudocysts can often be drained simply.

5. Management and Treatment Protocols

The management strategy is built upon aggressive supportive care, nutritional support, and a graded approach to complications.

A. Initial Resuscitation

The first 24 hours ("The Golden Hours") are critical for preserving microcirculation and preventing the progression of necrosis.

- **Fluid Selection: Lactated Ringer's (LR)** is preferred over normal saline due to its anti-inflammatory properties and reduced risk of hyperchloremic acidosis (Exception: Hypercalcemia-induced AP).

- **Protocol:** In hypovolemic patients, a bolus of 5–10 mL/kg followed by maintenance at 1.5 mL/kg/hour is recommended.

- **Goals:** Therapy should be goal-directed, aiming for a reduction in BUN, adequate urine output ($>0.5\text{ mL/kg/hr}$), and normalization of hematocrit (35–44%). Over-resuscitation increases the risk of abdominal compartment syndrome.

B. Nutrition and Analgesia

- **Nutrition:** The dogma of "bowel rest" (NPO) has been replaced by "**Early Enteral Nutrition.**" In mild cases, oral feeding is initiated as soon as tolerated. In severe cases, enteral nutrition (preferably via NG tube) within 24–72 hours preserves the gut barrier and prevents bacterial translocation/sepsis.

- **Analgesia:** Severe pain causes hemodynamic instability. Parenteral opioids such as fentanyl or hydromorphone can be used safely.

C. Etiology-Specific and Interventional Treatments

- **ERCP:** Urgent ERCP (<24 hours) is indicated only in the presence of concurrent acute cholangitis or persistent biliary obstruction. Cholecystectomy should be performed in biliary pancreatitis to prevent recurrence.

- **Hypertriglyceridemia (HTG):** The goal is to lower TG levels to <500 mg/dL. Plasmapheresis or IV insulin infusion is used in symptomatic patients.

- **Management of Infected Necrosis (Step-Up Approach):** Infected necrosis is the leading cause of late mortality.

1. Intervention should ideally be delayed until the necrosis becomes encapsulated (WON), typically after 4 weeks.

2. The first step is percutaneous or endoscopic drainage.

3. If drainage fails, minimally invasive necrosectomy (VARD) is performed. Open surgery is the last resort.

4.

6. Conclusion and Clinical Summary

Acute Pancreatitis is a dynamic process, and modern management principles are founded on five pillars:

1. **Diagnostic Discipline:** Diagnosis is clinical/biochemical; avoid routine early CT.

2. **Rational Resuscitation:** Goal-directed fluid therapy with Lactated Ringer's, monitored via BUN.

3. **Nutritional Support:** Early enteral nutrition is the most potent therapeutic tool for reducing infection risk.

4. **Graduated Intervention:** Adopt the "Step-up" approach for necrotizing pancreatitis; delay intervention for infected necrosis until encapsulation (4 weeks) if possible.

5. **Secondary Prevention:** Cholecystectomy is mandatory for biliary etiology.

Successful management relies on early recognition of high-risk patients, strict monitoring of physiological parameters, and a multidisciplinary approach.

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CHAPTER 34

Management and Clinical Approach to Renal Colic

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1. Introduction and Definition

Renal colic is defined as the acute, severe, and paroxysmal pain resulting from sudden obstruction of the upper urinary tract, typically due to the migration of a urolith. It represents one of the most common urological emergencies encountered in emergency departments worldwide. The clinical presentation is primarily driven by an increase in intraluminal pressure and subsequent ureteral smooth muscle spasms as the body attempts to propel the obstructive object (1).

2. Epidemiology and Risk Factors

The prevalence of urolithiasis has shown a significant upward trend globally, currently estimated between 1% and 20% depending on geographic and socioeconomic factors. Men are affected more frequently than women, with a lifetime risk of approximately 12% and 6%, respectively (2). Key risk factors include:

- **Environmental:** High ambient temperatures leading to dehydration and concentrated urine.
- **Dietary:** High sodium intake, excessive animal protein consumption, and low fluid intake.
- **Genetic:** Family history of stone disease, particularly in cases of cystinuria or distal renal tubular acidosis (3).

3. Pathophysiology

The sensation of pain in renal colic is a complex physiological response to acute obstruction. When a stone occludes the ureter, the following sequence occurs:

1. **Proximal Stasis:** Hydrostatic pressure rises proximal to the site of obstruction.
2. **Prostaglandin Cascade:** The stretching of the ureteral wall triggers the release of prostaglandins, specifically PGE2. This causes localized vasodilation and increased renal blood flow, which paradoxically increases intraluminal pressure (4).
3. **Renal Capsule Distension:** The resulting hydronephrosis stretches the renal capsule, stimulating mechanoreceptors that transmit pain signals via the T11-L2 spinal segments (5).

4. Clinical Presentation

4.1. Pain Characterization

The hallmark of renal colic is the "crescendo-decrescendo" nature of the pain. Unlike patients with peritonitis who prefer to remain still, patients with renal colic are typically restless, pacing or constantly shifting positions to find relief (6).

- **Proximal Ureteral Stones:** Pain is localized to the flank and costovertebral angle.
- **Distal Ureteral Stones:** Pain radiates to the lower abdominal quadrants, ipsilateral groin, and external genitalia.
- **Ureterovesical Junction (UVJ) Stones:** May present with irritative voiding symptoms, such as urinary frequency and urgency, mimicking a urinary tract infection (7).

4.2. Physical Examination

While the examination is often non-specific, it is vital for ruling out differential diagnoses. Tenderness at the costovertebral angle (Murphy's punch sign) is common. The absence of peritoneal signs (rebound tenderness or guarding) helps distinguish it from intraperitoneal pathologies (8).

5. Diagnostic Investigations

5.1. Laboratory Analysis

- **Urinalysis:** Microscopic hematuria is present in nearly 90% of cases. However, its absence does not exclude urolithiasis, especially in cases of complete obstruction.
- **Blood Chemistry:** Evaluation of serum creatinine is essential to assess baseline renal function. White blood cell (WBC) count and C-reactive protein (CRP) are monitored to screen for systemic inflammation or infection (9).

5.2. Imaging Modalities

Imaging is the cornerstone of definitive diagnosis, providing information on stone size, location, and the degree of hydronephrosis

Modality	Sensitivity	Specificity	Clinical Utility
Non-Contrast CT (NCCT)	95-100%	94-96%	Gold standard; detects all stone types.
Ultrasonography (USG)	45-70%	90%	Preferred in pregnancy and pediatrics.
KUB Radiography	45-60%	70%	Useful for monitoring radiopaque stones.

6. Differential Diagnosis

Clinicians must remain vigilant for "mimics" of renal colic, some of which are life-threatening:

- **Vascular:** Ruptured Abdominal Aortic Aneurysm (AAA) – *must be ruled out in elderly patients with flank pain.*
- **Gastrointestinal:** Acute appendicitis, diverticulitis, or biliary colic.
- **Gynecological:** Ectopic pregnancy, ovarian torsion, or ruptured ovarian cysts (10).

7. Management Strategies

7.1. Acute Pain Relief

The primary goal is the rapid alleviation of distress.

- **NSAIDs:** Non-steroidal anti-inflammatory drugs (e.g., Diclofenac, Ketorolac) are the first-line treatment. They reduce prostaglandin-mediated ureteral edema and pelvic pressure (11).
- **Opioids:** Reserved for patients with contraindications to NSAIDs or those with refractory pain.

7.2. Medical Expulsive Therapy (MET)

MET is indicated for uncomplicated ureteral stones <10 mm.

- **Alpha-blockers (e.g., Tamsulosin):** These agents inhibit alpha-1 receptors in the distal ureter, inducing smooth muscle relaxation and increasing the rate of spontaneous stone passage by approximately 30% (12).

7.3. Surgical Intervention

Urgent surgical decompression is required if conservative management fails or if complications arise. Common procedures include the insertion of a Double-J (DJ) stent or percutaneous nephrostomy.

Emergency Indications:

1. Obstructed kidney with concomitant infection (Urosepsis).
2. Intractable pain or persistent vomiting.
3. Acute renal failure or an obstructed solitary kidney.
4. Stone size >10 mm (unlikely to pass spontaneously) (13).

8. Special Populations: Pregnancy

Renal colic is the most common non-obstetric cause of abdominal pain requiring hospitalization in pregnant women. Management is challenging due to the risks of ionizing radiation and preterm labor. Ultrasonography is the primary diagnostic tool, and MRI may be used as a second-line option. If intervention is necessary, temporary stenting is preferred until postpartum definitive treatment (14).

9. Conclusion and Prevention

Renal colic requires a multidisciplinary approach focusing on immediate pain control and the prevention of renal injury. Following the acute episode, patients should undergo metabolic evaluation—including stone analysis and 24-hour urine collection—to mitigate the high recurrence rate of urolithiasis (15).

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CHAPTER 35

Paracetamol (Acetaminophen) Toxicity

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1. INTRODUCTION

Paracetamol (N-acetyl-p-aminophenol) is the most widely utilized analgesic and antipyretic agent globally, available both via prescription and over-the-counter. Despite its deceptively benign safety profile at therapeutic dosages, paracetamol overdose remains the leading cause of drug-induced acute liver failure (ALF) in developed nations (1). For emergency physicians, managing toxicity presents both a diagnostic challenge and a time-critical clinical scenario. However, timely intervention and the administration of the specific antidote, N-acetylcysteine (NAC), can effectively reduce mortality rates to below 1%(2).

2. EPIDEMIOLOGY AND RISK FACTORS

Paracetamol toxicity typically manifests in two clinical forms: acute single-dose ingestion and chronic supratherapeutic ingestion.

- **Acute Ingestion:** Generally involves a single, high-dose intake, often with intentional self-harm (suicidal) intent.
- **Chronic Supratherapeutic Ingestion:** Occurs when a patient inadvertently exceeds the maximum daily dose (4g in adults) over several consecutive days for pain management.

Key risk factors for increased toxicity include chronic alcohol consumption, malnutrition (leading to depleted glutathione stores), concomitant use of enzyme-inducing medications (e.g., Rifampin, Phenytoin, Carbamazepine), and genetic polymorphisms (3).

3. PATHOPHYSIOLOGY: MOLECULAR MECHANISMS

The metabolic pathway of paracetamol elucidates the "latent" nature of its clinical toxicity.

3.1. Normal Metabolism

At therapeutic levels, approximately 90% of paracetamol undergoes Phase II conjugation via glucuronidation and sulfation. A minor fraction (5–10%) is metabolized by the Cytochrome P450 system (primarily **CYP2E1**) into a highly reactive electrophilic intermediate: **N-acetyl-p-benzoquinone imine (NAPQI)** (4).

3.2. Toxicity and Necrosis

In overdose scenarios, the primary conjugation pathways become saturated. Consequently, NAPQI production escalates, leading to the exhaustion of protective hepatic **glutathione (GSH)** stores. Unconjugated NAPQI binds to

mitochondrial proteins within hepatocytes, forming "protein adducts". This cascade results in:

- Increased mitochondrial oxidative stress,
- Cessation of ATP production,
- DNA damage and subsequent cell death (centrilobular necrosis)(5).

4. CLINICAL STAGES AND PRESENTATION

The clinical progression is highly time-dependent following ingestion.

- **Stage I (0.5 – 24 Hours):** Often non-specific or asymptomatic. Symptoms may include nausea, vomiting, diaphoresis, and malaise. Liver function tests (LFTs) typically remain within normal limits during this window.

- **Stage II (24 – 72 Hours):** Known as the "Clinical Latent Period." Systemic symptoms may improve, but right upper quadrant pain and hepatomegaly emerge. Elevation of AST and ALT begins, with bilirubin and INR increases becoming prominent toward the end of this stage (6).

- **Stage III (72 – 96 Hours):** The "Hepatic Necrosis Stage." AST/ALT levels frequently exceed 10,000 IU/L. Fulminant liver failure develops, characterized by jaundice, coagulopathy, encephalopathy, hypoglycemia, and acute kidney injury.

- **Stage IV (4 Days – 2 Weeks):** Recovery or mortality. In survivors, hepatic tissue typically regenerates completely within three months.

5. DIAGNOSIS AND LABORATORY MANAGEMENT

5.1. Rumack-Matthew Nomogram

In acute ingestions, the serum paracetamol concentration measured 4 hours post-ingestion is critical for risk stratification. The nomogram utilizes a treatment threshold starting at 150 µg/mL (1000 µmol/L) at the 4-hour mark (7).

Critical Note: Levels obtained prior to 4 hours post-ingestion cannot be plotted on the nomogram as absorption may not be complete.

5.2. Ancillary Laboratory Evaluations

- **LFTs:** AST, ALT, Bilirubin.
- **Coagulation Profile:** PT/INR (the most sensitive indicator of hepatic synthetic function).
- **Renal Function:** Serum creatinine (to monitor for acute tubular necrosis).

- **Blood Gas Analysis:** Lactate and pH levels (essential for prognostic determination).

6. TREATMENT AND MANAGEMENT PROTOCOLS

6.1. Decontamination

Activated charcoal (1 g/kg) effectively reduces absorption if administered within the first 2 hours post-ingestion. This window may be extended to 4 hours in co-ingestions involving anticholinergics or opioids (8).

6.2. N-Acetylcysteine (NAC) Antidote Therapy

NAC functions by facilitating glutathione synthesis and directly neutralizing NAPQI.

- **Indications:** Patients plotting above the treatment line on the nomogram, suspected ingestions >150-200 mg/kg, or any case with evidence of hepatic injury (9).

- **21-Hour Intravenous (IV) Protocol:**

1. **Loading Dose:** 150 mg/kg over 1 hour.
2. **Second Dose:** 50 mg/kg over 4 hours.
3. **Third Dose:** 100 mg/kg over 16 hours.

- **Note:** NAC should not be discontinued if ALT remains elevated or paracetamol is still detectable at the end of the protocol; infusion must continue.

6.3. Emerging Approaches: Fomepizole and Hemodialysis

In massive ingestions (>50-100 g), **Fomepizole** can be used as an adjuvant to inhibit CYP2E1(10). Hemodialysis is indicated for extreme paracetamol concentrations (>900 mg/L) or severe acidosis.

7. PROGNOSIS AND TRANSPLANTATION CRITERIA

The **King's College Criteria** are utilized to identify patients at high risk of mortality. Consultation with a liver transplant center is mandatory if:

- Arterial pH < 7.3 after resuscitation.
- **OR** (concurrently): INR > 6.5, Creatinine > 3.4 mg/dL, and Grade III/IV encephalopathy (11).

8. SPECIAL POPULATIONS

- **Pregnancy:** Paracetamol crosses the placenta. NAC should be initiated immediately to prevent fetal hepatotoxicity and is considered safe for use during pregnancy(12).
- **Chronic Alcoholism:** Due to limited glutathione reserves, these patients require vigilant monitoring for "therapeutic misadventure".

9. CONCLUSION

Paracetamol poisoning is a preventable cause of death with aggressive management and proper timing. Physicians must understand the limitations of the nomogram and focus on both laboratory values and clinical risk factors

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CHAPTER 36

Human Aeromonas Infections: Water and Food Sources

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1. INTRODUCTION

Bacteria of the genus *Aeromonas* are microorganisms widely found in aquatic ecosystems, causing significant infections, especially in fish, and are also considered opportunistic pathogens in humans (Figueras & Beaz-Hidalgo, 2015; Janda & Abbott, 2010). The majority of *Aeromonas* infections reported in humans originate from *A. caviae*, *A. veronii* biovar *sobria*, and *A. hydrophila* in recent years, *A. dhakensis* has become a species that has attracted increasing attention due to its increased virulence and association with severe clinical presentations (Chen et al., 2014; Fernández-Bravo & Figueras, 2020). Transmission mostly occurs through contact with contaminated water sources, oral ingestion of these waters, or consumption of contaminated food, especially of aquatic origin (Figueras & Beaz-Hidalgo, 2015; Parker & Shaw, 2011).

Aeromonas species are Gram-negative, non-spore-forming, facultative anaerobic, rod-shaped bacteria 1–3 µm in size. They are catalase- and oxidase-positive microorganisms that usually have polar flagella, and most species can ferment glucose. According to the current edition of Bergey's Manual of Systematic Bacteriology, the taxonomic position of the genus *Aeromonas* is determined within the Bacteria kingdom by the phylum Proteobacteria, the class Gammaproteobacteria, the order Aeromonadales, and the *Aeromonadaceae* family (Pridgeon & Abeyta, 2013).

Although the number of species belonging to the genus *Aeromonas* varies depending on the classification system used, according to the List of Prokaryotic Names with Standing in Nomenclature (LPSN) data, 34 species have been validated as of 2025 (Table 1); When synonyms are included, the number of validly published subtaxa is reported as 39, and the total number of subtaxa is reported as 49 (LPSN, 2025). The optimum growth conditions for *Aeromonas* species are generally defined as a temperature of 30 °C, a pH range of 7–9, and a water activity (prey) of approximately 0.99 (United States Environmental Protection Agency [USEPA], 2006).

Table 1. Validated *Aeromonas* species by LPSN (n=34) (LPSN, 2025)

Table 1. Validated *Aeromonas* species by LPSN (n=34) (LPSN, 2025)

<i>Aeromonas</i> species name	<i>Aeromonas</i> species name	<i>Aeromonas</i> species name
<i>A. allosaccharophila</i>	<i>A. fluvialis</i>	<i>A. rivipollensis</i>
<i>A. aquatica</i>	<i>A. hydrophila</i>	<i>A. rivuli</i>
<i>A. australiensis</i>	<i>A. ichthiosmia</i>	<i>A. salmonicida</i>
<i>A. bestiarum</i>	<i>A. ichthyocola</i>	<i>A. sanarellii</i>
<i>A. bivalvium</i>	<i>A. jandaei</i>	<i>A. schubertii</i>
<i>A. caviae</i>	<i>A. lacus</i>	<i>A. simiae</i>
<i>A. dhakensis</i>	<i>A. lusitana</i>	<i>A. sobria</i>
<i>A. diversa</i>	<i>A. media</i>	<i>A. taiwanensis</i>
<i>A. encheleia</i>	<i>A. molluscorum</i>	<i>A. tecta</i>
<i>A. enteropelogenes</i>	<i>A. mytilicola</i>	<i>A. veronii</i>
<i>A. eucrenophila</i>	<i>A. piscicola</i>	
<i>A. finlandensis</i>	<i>A. popoffii</i>	

With a widespread distribution in aquatic and terrestrial environments, *Aeromonas* spp. is mainly detected in soil, brackish, fresh, estuary, and marine waters, plants, fruits, vegetables, fish/shellfish, reptiles, amphibians, and birds. *Aeromonas* species, being opportunistic pathogens, typically enter human hosts through contaminated water, seafood, and fresh produce.

1.1. *Aeromonas* infections in humans

Bacteria of the genus *Aeromonas* are Gram-negative microorganisms that are widely distributed in aquatic and semi-aquatic ecosystems and can lead to infections in both animals and humans with their opportunistic pathogenic properties. According to current taxonomic data, approximately 19 of the 36 species identified in this genus have zoonotic potential, but the number of species that cause clinical infections in humans is very limited. *Approximately 95–96.5% of clinically reported human Aeromonas infections are reported to be caused by Aeromonas caviae, Aeromonas veronii (especially biovar sobria), Aeromonas hydrophila, and Aeromonas dhakensis, which have been reported with increasing frequency in recent years* (Barger et al., 2021; Chen et al., 2016; Qu & Liu, 2024). The first identification of *Aeromonas* as a disease agent in humans dates to 1954 (Caselitz, 1996).

Although *Aeromonas infections in humans* are most associated with gastroenteritis, the clinical presentations have a wide range. The species most frequently isolated from gastroenteritis cases include *A. caviae*, *A. veronii*, *A. dhakensis*, *A. hydrophila*, *A. media*, *A. salmonicida* biogroup *salmonicida*, and *A. trota* (Fernández-Bravo & Figueras, 2020; Guerra et al., 2007; Hofer et al., 2006; Huang et al., 2020; Martin-Carnahan and Joseph, 2005; Meng et al., 2020).

However, infections are not limited to the intestinal system, but also to invasive conditions such as wound and soft tissue infections, bacteremia and sepsis; more rarely, they may progress to serious clinical conditions such as pneumonia, peritonitis and meningitis. This diversity and the often limited clinical specificity make *Aeromonas* infections, accompanied by a risk of mortality, a significant diagnostic challenge for both clinicians and laboratory scientists (Qu & Liu, 2024).

In particular, *A. veronii*, *A. caviae*, and *A. hydrophila* have been strongly associated with diarrhea, septicemia, and wound/soft tissue infections following exposure to water after trauma or natural disasters, consumption of contaminated food or water, and medical leech therapy using leeches that are in symbiotic relationship with *Aeromonas* species (Baron et al., 2017). In recent years, the clinical importance of *A. dhakensis*, which is especially associated with purulent and invasive infections, has steadily increased, with accumulating evidence indicating that this species possesses a higher virulence potential (Chen et al., 2016).

1.1.1. Epidemiology and Risk Factors

Bacteria belonging to the genus *Aeromonas* are known to be the main pathogens of fish and other marine animals and are responsible for harmful outbreaks in aquaculture systems due to their wide distribution in various aquatic environments (Beaz-Hidalgo and Figueras, 2013; Figueras and Ashbolt, 2019; Liu, 2015). Moreover, they have been detected in insects, reptiles, amphibians, birds, and other vertebrates (Percival and Williams, 2014; Praveen et al., 2016; Gonçalves Pessoa et al., 2019). Although they were first reported in 1891, *Aeromonas* were only recognized as potential human pathogens when detected about 60 years later (in 1954) in clinical samples from an immunocompromised woman who died suddenly of metastatic myositis (Fernández-Bravo and Figueras, 2020; Liu, 2015; Parker and Shaw, 2011).

The epidemiology of *Aeromonas* infections varies markedly depending on the geographic region, environmental conditions, host characteristics, and diagnostic methods used. In general, although the isolation rate of *Aeromonas* in diarrhea cases has been reported below 5% in most studies, it is emphasized that the role of these pathogens in enteric infections is at a level that cannot be ignored, especially in studies conducted in Asian and African countries. In these studies, *Aeromonas caviae* and *Aeromonas veronii* are isolated at a higher frequency among enteric pathogens compared to *Aeromonas hydrophila* (Fernández-Bravo & Figueras, 2020).

It is noteworthy that the species distribution varies according to the clinical form of the infection. While *A. caviae* and *A. veronii* are predominantly associated with intestinal infections, *A. hydrophila* and, especially, *A. dhakensis* are more frequently implicated in extraintestinal manifestations, such as wound

and soft tissue infections and bacteremia. This pattern is thought to be related to differences in the environmental adaptability and virulence profiles of the respective species (Chen et al., 2016; Qu & Liu, 2024).

Host factors also play a decisive role in the development of *Aeromonas* infections. Conditions such as advanced age, immunosuppression, malignancy, chronic liver disease, and diabetes mellitus are among the primary predisposing factors that increase the risk of infection. However, *Aeromonas* infections can also occur in healthy individuals without any underlying diseases, suggesting that these bacteria have effective virulence mechanisms beyond being opportunistic pathogens. Contact with contaminated water, especially after trauma or natural disasters, is identified as an important risk factor for the development of infection.

The prevalence of *Aeromonas* species in aquatic environments and the relative resistance of some species to standard water treatment processes increase the importance of environmental factors in the transmission of these pathogens. In addition, consumption of poorly cooked fish and shellfish and contaminated drinking and utility water are among the main sources of infection. The fact that the expression of virulence factors in some *Aeromonas* species increases with the increase in global temperature indicates that these pathogens may become even more important for public health in the future (Fernández-Bravo and Figueras, 2020).

It has been reported that antimicrobial resistance rates have increased significantly in *Aeromonas* species in recent years. Increasing resistance to penicillins (including their combination with β -lactamase inhibitors), cephalosporins, and carbapenems has become a major problem complicating clinical treatment (Fernández-Bravo & Figueras, 2020; Lamy et al., 2012). Among the β -lactam group antibiotics, only fourth-generation cephalosporins are reported to largely retain their effectiveness. Alongside this, the increasing frequency of coexistence of genes responsible for resistance to third-generation cephalosporins and carbapenems in the same isolates further limits therapeutic options (Piccirilli et al., 2022). In addition, the presence of mobile genes that confer resistance to colistin has recently been demonstrated, and it has been reported that resistance rates exceed 30% in some studies (Baron et al., 2017). It is thought that this increase in colistin resistance may be related to its use in aquaculture, and when β -lactam resistance is evaluated together, it is considered that *Aeromonas* species become a potential antimicrobial resistance gene reservoir for Enterobacterales, which are more frequently associated with human infections (Baron et al., 2017; Fernández-Bravo & Figueras, 2020; Piotrowska & Popowska, 2015).

1.1.3. Cases

The isolation rate of *Aeromonas* in diarrhea cases has been reported below 5% in most studies, and *A. caviae* and *A. veronii* are more common among enteric pathogens compared to *A. hydrophila*, especially in studies conducted in Asian and African countries (Dallal et al., 2012; Elorza et al., 2020; Mbuthia et al., 2018). In studies conducted in Australia, it has been reported that *A. veronii* biovar *sobria* is dominant in fecal specimens, while *A. dhakensis* is prominent in wound specimens, and in blood cultures, *A. dhakensis* and *A. caviae* together constitute most positive cultures (Sinclair et al., 2020). In a study conducted mainly on extraintestinal samples in Japan, *A. caviae*, it is stated that the *Aeromonas* species distributions reported in different studies to other species may be affected by both geographical factors and the identification methods used. Approaches based on MALDI-TOF MS and *rpoB* and *gyrB* gene sequencing enable reliable identification of species such as *A. dhakensis*, which are difficult to distinguish using classical biochemical methods. In studies using these methods, a marked predominance of *A. dhakensis* has been reported, especially in purulent infections (Chen et al., 2014). This is thought to be related to the fact that *A. dhakensis* has a higher virulence potential compared to other *Aeromonas* species (*A. veronii*, *A. caviae*, and *A. hydrophila*). Experimental studies using cell culture (C2C12 fibroblasts), BALB/c mouse, and *Caenorhabditis elegans* models have demonstrated the increased virulence of this species (Chen et al., 2014; Mosser et al., 2015). Furthermore, the presence of a type VI secretion system (T6SS) is associated with the development of disseminated infection in animal models (Suarez et al., 2008). Exotoxin A has also been identified as a key factor associated with high virulence in both *C. elegans* and mouse models (2025).

It is reported that host factors can also affect species distribution. In studies showing that age may be a determining factor, *A. veronii* is the most isolated species across all age groups, particularly prominent in young adults, while *A. caviae*, *A. hydrophila*, and *A. dhakensis* have been detected more frequently in young children and individuals over the age of 60 (Sing et al., 2025). Similarly, immunosuppressed individuals have a higher prevalence of *Aeromonas* infections, with some studies reporting higher *Aeromonas* isolation rates than *Clostridioides difficile* and significantly higher than *Campylobacter jejuni* (Heydari et al., 2024).

While *Aeromonas* infections are more commonly associated with certain risk factors (advanced age, immunosuppression), they can also occur in individuals without any risk factors and across all age groups (Heydari et al., 2024; Sing et al., 2025). This is explained by the bacteria's ability to bypass host immune mechanisms (Caselitz et al., 2022). The pathogenesis is multifactorial and is associated with the production of numerous virulence factors, including proteases, lipases, hemolysins, cytotoxins, and enterotoxins.

A recent study demonstrated that *A. veronii* predominates in intestinal infections, whereas *A. caviae* and *A. hydrophila* are more prevalent in extraintestinal infections. Furthermore, genes such as *act*, *aexT*, and *ascF–G* were reported to be more frequently detected in intestinal isolates. In contrast, the *alt*, *hlyA*, and *hazel* genes were significantly more common among strains isolated from extraintestinal infections (Ponnusamy et al., 2016).

In a study conducted in India, *Aeromonas* was detected in 50 of the stool samples taken from 1,595 patients with diarrhea; as a result of biochemical tests, it was reported that the most common species out of 35 isolates was *A. caviae* (34%), followed by *A. veronii* biovar *veronii* (29%), *A. veronii* biovar *sobria* (26%), and *A. hydrophila* (9%) (Mohan et al., 2017).

In Kenya, 188 stool samples were examined to identify the etiological agents of diarrhea; among these, *A. hydrophila* was detected in five samples and *A. caviae* in three samples (Mbuthia et al., 2018).

In 16 hospitals in Shanghai, China, 4,529 samples with diarrhea were collected, 193 cases were associated with *Aeromonas* infection: *A. veronii* (42.5%), *A. caviae* (25.3%), *A. aquariorum* (14.5%), *A. hydrophila* (5.7%), *A. enteropelogenes* (4.7%), *A. media* (3.1%), species *A. salmonicida* (2.1%), *A. allosaccharophila* (0.5%) (Li et al., 2015), and isolates of unidentified species (1.6%) were obtained. A study conducted at a hospital in Beijing, China, analyzed stool samples from 1,286 people with acute diarrhea. and 17 strains of *Aeromonas* that cause extraintestinal infections have been detected in blood or bile. Species distribution of *Aeromonas* isolates obtained from intestinal and extraintestinal samples; *A. caviae* (41.7%) was the most frequently isolated species, followed by *A. veronii* (31.3%). The study also identified *A. dhakensis* (13.9%), *A. hydrophila* (5.2%), *A. media* (1.7%), *A. sanarellii* (1.7%), *A. enteropelogenes* (1.7%), *A. bivalvium* (0.9%), and isolates of unknown species (1.7%) (Zhou et al., 2019).

The species distribution of these isolates isolated from the fecal samples of 98 patients in the period from January 2015 to December 2017 at the Galdakao-Usansolo Hospital in Spain; It is estimated that 85 of them were identified as *A. caviae*, 12 as *A. veronii*, and 1 as *A. hydrophila*, and 32 out of 105 cases each year were identified as *Aeromonas* spp. (Elorza et al., 2020).

In Australia, samples obtained from various biological materials, including stool, eye, and sputum, were analyzed for the presence of *Aeromonas* species. Of the 100 isolates examined, 39 were identified as *A. dhakensis*, 21 as *A. veronii*, 20 as *A. hydrophila*, 14 as *A. caviae*, 4 as *A. jandaei*, 1 as *A. bestiarum*, and 1 as *A. sanarellii* (Sinclair et al., 2016). Similarly, in a study of 109 clinical *Aeromonas* isolates obtained from diarrheal patients in Mexico and Spain, the most prevalent species in both countries were *A. caviae*, *A. hydrophila*, and *A. veronii* (Aguilera-Arreola et al., 2007).

Regarding extraintestinal infections caused by *Aeromonas*, multiple anatomical sites may be affected. A range of non-gastrointestinal infections has been attributed to *Aeromonas* species, often associated with exposure to contaminated water. For example, soft tissue infections may develop following injuries sustained in aquatic environments (Lujan-Hernandez et al., 2020), and wound infections may occur after medical leech therapy, as leeches raised in water contaminated with *Aeromonas* species can serve as a source of infection (Masters et al., 2020).

At a medical center in southern Taiwan, 76 *Aeromonas* isolates obtained from wound or pus samples were identified at the species level. These isolates comprised *A. dhakensis* (n = 37), *A. veronii* (n = 14), *A. hydrophila* (n = 13), *A. caviae* (n = 11), and *A. media* (n = 1) (Chen et al., 2014b). In another study conducted at the same medical center, *Aeromonas* species were isolated from 13 of 514 stool samples collected from adults with diarrhea and from only 6 of 167 asymptomatic individuals. The most frequently detected species among these isolates was *A. veronii*, while *A. caviae*, *A. sanarellii*, and *A. dhakensis* were also identified (Chen et al., 2014b). These findings suggest that, even within the same geographic region, *Aeromonas* species distribution varies according to the anatomical site of infection (Pessoa et al., 2022).

The ability of *Aeromonas* species to use their structural components, secreted proteins, and other products for adhesion, colonization, and evasion of host defense has been attempted to fully elucidate the molecular pathogenesis of *Aeromonas* infection, a slow and painful process. Diagnosis of human aeromoniasis involves phenotypic and genotypic techniques, and its treatment is currently based on antibiotic therapy. With antimicrobial resistance becoming an increasingly important issue, it is urgent to find new therapeutics for *Aeromonas* species. Improving our understanding of the molecular mechanisms of *Aeromonas* infection through the application of omics approaches is critical to help achieve this goal (Qu and Liu, 2024).

1.2. Presence of *Aeromonas* in the waters

Water sources are an important carrier of a large microbial community, and *Aeromonas* spp. found in waters has been identified as a potential pathogen that can affect human health by the Environmental Protection Agency's Pollutant Nominees (EPA), as it causes outbreaks to occur, especially in countries with inadequate basic sanitation (United States Food and Drug Administration (FDA) 2012; Chellaiah et al. 2021; Labella et al. 2021; Meng et al. 2020; EPA 2006). In general, outbreaks are more frequently observed in regions with limited economic resources, where untreated water from shallow wells, streams receiving industrial wastewater, springs, and lakes is used directly for drinking or for irrigating vegetables (Nienie et al., 2017).

Several studies have demonstrated the presence of *Aeromonas* species in water sources used for human consumption. These investigations have reported the detection of species such as *A. jandaei*, *A. veronii*, *A. hydrophila*, and *A. caviae* in various water types, including tap water and well water, across different geographical regions, such as China, Spain, and India. A range of identification methods has been employed in these studies, including biochemical testing using the VITEK® 2 system (bioMérieux), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and molecular approaches such as 16S rRNA gene sequencing (Table 2).

Two outbreaks caused by waterborne *Aeromonas* species were reported in the United States in 1989 and 2013. The first outbreak occurred in New Jersey and the second in Virginia, affecting a total of 26 and 5 individuals, respectively; no hospitalizations or deaths were reported in either outbreak (CDC NORS, 2021). In Brazil, *Aeromonas* species—particularly *A. caviae* and *A. veronii* biovar *veronii*—were identified as the etiological agents in 2,170 patients with acute diarrhea, predominantly among children aged 1–5 years, following the consumption of contaminated water in 2004 (Hofer et al., 2006).

Table 2. *Aeromonas* species in water used for human consumption (Carusi et al. 2024)

Source	n		(%)	Method used	Location	Source
Tap water	36	<i>A. veronii</i>	25	16S rDNA and MALDI-TOF-MS Biochemical tests and 16S rRNA gene sequence analysis	China	Meng et al. (2020)
		<i>A. aquariorum</i>	5.6			
		<i>A. hydrophila</i>	2.8			
		<i>A. jandaei</i>	58,3			
		<i>A. salmonicida</i>	2,8			
		<i>A. enteropelognes</i>	2,8			
		<i>Aeromonas</i> spp.	2,8			
In treated waters	53	<i>A. hydrophila</i>	-			
Well water	28	<i>A. caviae</i>	-		China	Chellaiah et al. (2021)

Solamin et al. (2021) investigated the presence of *Aeromonas* in rivers, lakes, farms, and treated waters with PCR of the *gyrB* gene and reported that *A. veronii*, *A. hydrophila*, and *A. jandaei* species were dominant throughout the season. In their study, they detected a larger variety of *Aeromonas* species during the winter months, and the majority of *A. hydrophila* and *A. veronii* species during the summer and autumn months.

Scoaris (2008) from Brazil analyzed the presence of *Aeromonas* spp. in bottled mineral waters and detected the agent at a rate of 12.7% (6/47) in samples belonging to different brands. In water samples collected from well water and tap water, *Aeromonas* species were detected at rates of 8.3% (2/23) and 11% (11/167), respectively. *Aeromonas* species identified with 16S rRNA were reported as *A. hydrophila* and *A. janfaei*.

Aeromonas spp. have also been detected in filtered water. The quality of filtered water is influenced by several factors, including its physical and chemical characteristics, the service life of the filter, filter hygiene, and the potential presence of biofilms, which may impair the ability of reverse osmosis membranes to effectively reject particles and dissolved salts (Elhariry et al., 2012; Oliveira & Pelegrini, 2011).

Based on the findings of previous studies, water has been identified as an important vehicle for *Aeromonas* contamination of fresh fruits and vegetables, fish, meat, and ready-to-eat foods (Latif-Eugenín et al., 2017). More recently, Solaiman and Micallef (2021) confirmed the association between irrigation water and the persistence of *Aeromonas* spp. on lettuce leaves. In their study, 20 *Aeromonas* species were detected in irrigation water collected from the Mid-Atlantic region of the United States. To further assess the enterotoxigenic potential of the isolates, motility and the presence of the adhesion-related gene *flaA* were evaluated, and the adaxial surfaces of lettuce leaves were inoculated with *A. hydrophila*, *A. veronii*, and *A. jandaei*. The authors reported that *A. veronii* exhibited strong adherence to lettuce leaves, whereas *A. hydrophila* and *A. jandaei* showed weaker adherence, with bacterial concentrations decreasing after five days.

1.3. Presence in Foods

In recent years, there has been an increase in the consumption of ready-to-eat food (RTE) and other ready-to-eat meals, including fruits, vegetables, fish, and cooked or raw seafood (Hoel et al. 2019). Table 3 summarizes the studies on these issues.

As shown in Table 3, *Aeromonas* species detected in foods—particularly fish and seafood—exhibit considerable diversity. Among these, *A. hydrophila* is the most extensively studied species and the one most frequently isolated from a wide range of food products, followed by *A. veronii*, *A. caviae*, *A. salmonicida*, and *A. media*. In contrast, species such as *A. enteropelogenes*, *A. jandaei*, *A. sobria*, *A. bivalvium*, *A. dhakensis*, *A. taiwanensis*, *A. allosaccharophila*, *A. bestiarum*, *A. culicicola*, and *A. rivipollensis* were detected less frequently, collectively accounting for approximately 14% of the isolates.

When the incidence of *Aeromonas* spp. in fish and seafood is examined in Table 3, a total of 466 strains were isolated, 220 (47.2%) were identified as *A.*

hydrophila, 80 (17.1%) as *A. veronii*, and 52 (11.1%) as *A. caviae*. *Aeromonas* was found in dairy products in only one of the studies (Hammad et al., 2018), a total of 34 strains were isolated in the study, 17 (50%) were *A. caviae*, 9 (26.5%) were *A. hydrophila*, and 8 (23.5%) were *A. trota*.

Table 3. *Aeromonas* species in various fish, seafood, and other foods

Source	n	Detected Type	%	Insulation Method	Epidemic or disease	Location	Source
Mullet	135	<i>A.hydrophila</i>	37,00	PCR and 16S rDNA gene	Nope	Egypt	Ramadan et al. 2018
Nile freshwater sea bream	93	<i>Aeromonas</i> spp.	94,62	Biochemical tests	Nope	Egypt	Hammad et al. 2018
Fresh Yesso Scallops	105	<i>A.hydrophila</i> <i>A. salmonicidia</i> <i>A. media</i> <i>A. caviae</i> <i>A. veronii</i> <i>A. veronii, A. enteropelogenes</i> and <i>A. sobria</i>	12,40 10.5 0 2,85 1,90 0,95	<i>gyrB</i> gene sequence analysis	Nope	Korea	De Silvia et al. 2018
Abalone	120	<i>A. hydrophila</i> <i>A. enteropelogenes</i> <i>A. veronii</i> <i>A. salmonicida</i> <i>A. sobri</i>	-	PCR and sequence analysis (<i>gyrB</i> -based)	Nope	Korea-Pacific Ocean	Wickramanayake et al. 2019
Fishes	235	<i>A. caviae</i> <i>A. veronii</i> <i>A. bivalvium</i> <i>A. dhakensis</i> <i>A. hydrophila</i> <i>A. enteropelogenes</i> <i>A. taiwanensis</i> <i>A. media</i> <i>A. salmonicida</i> <i>A. jandaei</i>	33,6 25,8 10,2 8,6 7 4,7 4,7 3,1 1,5 0,8	Biochemical tests, VITEK, GNI plus (BioMérieux) and PCR (<i>rpoD</i> gene)	Nope	Taiwan	Wu et al. 2019
Hard-shelled mussel (<i>Mytilus coruscus</i>)	250	<i>A. salmonicida</i> <i>A. veronii</i> <i>A. enteropelogenes</i> <i>A. caviae</i> <i>A. allosaccharophila</i> <i>A. bivalvium</i>	42,4 2 27,2 12,1 2 9,1 6,06 3,03	<i>gyrB</i> sequence analysis	Nope	Korea	Hossain et al. 2019
Snakehead fish	1	<i>A. dhakensis</i>	100	Vitek 2 GN biochemical tests, WGS	Yes Death	China	Huang et al. 2020
Scallops (<i>Pecten maximus</i>)	4	<i>A.hydrophila</i>	100*	Biochemical and PCR (<i>gcat</i> gene)	Nope	Brazil	Roges et al. 2020
Fish (<i>Genidens barbatus</i>)	2 13						

Fish (<i>Rachycentron canadum</i>)	3	1								
Fish (<i>Mugiliza</i>)										
Fish (<i>Oreochromis niloticus</i>)										
Cockle type mussel)	(a of	120	<i>A.veronii</i> <i>A.hydrophila</i> <i>A. media and salmonocidia</i> <i>A. allosaccharophila</i> , <i>A. bestiarum</i> , <i>A. culicicola</i> , and <i>A. rivipollensis</i> <i>A.hydrophila</i>	47 25 6,25 3,12	PCR (gyr gene) and sequence analysis	Nope	Korea	Dahanayake et al. 2020		
Nile Freshwater sea bream		250		19,2	Biochemical testing and PCR (16S rRNA)	Nope	Egypt	Zaher et al. 2021		
Seafood ready to eat		148	<i>A.media</i> <i>A.salmonicida</i> <i>A.bestiarum</i> <i>A.hydrophila</i>	75	PZR (<i>gyrB</i>)	Nope	Norway	Lee et al. 2020		
Oysters chilled		91		57,1	PCR (16S rRNA)	Nope	South Korea	Park et al. 2021		
Frozen oysters	9	62		0						
Sashimi-flatfish		55		54,8						
Sashimi-sea bass	50	26		49,1						
Sashimi-goby		10		32						
Anchovy	30			19,2						
Sashimi				0						
Processed anchovies	33	35								
Frozen tuna	20			3,3						
Sushi flatfish				27,3						
Salmon sushi				51,4						
Shrimp sushi				25						
Mollusks		123	<i>A.veronii</i> <i>A. hydrophila</i>	17 3,3	API (rpoB gyrB) Sequence analysis)	Nope	Taiwan	Chen et al. 2021		
Karish cheese		40	<i>Aeromonas pp.</i>	70,0	Biochemical tests	Nope	Egypt	Hammad et al. 2018		
Ras cheese		40		52,5						
Raw milk		40		0 89,0 0						

Chicken meat	53	<i>Aeromonas</i>	53	High-throughput	Nope	America	Higgins et al.
Vegetables	100	<i>spp.</i>	-	Pyrosequencing			2018
Various foods	21	<i>A.veronii</i> <i>A. hydrophila</i> <i>A. jandei</i> <i>Aeromonas</i> <i>spp.</i> <i>A.aquatorium</i> <i>A.media</i> <i>A.salmonicida</i>	38,1 19 19 9,5 4,8 4,8 4,8	VITEK 2 and MLST (<i>gyrB</i> , <i>groL</i> , <i>gltA</i> , <i>metG</i> , <i>ppsA</i> and <i>andrecA</i>)	Nope	China	Meng et al. 2020
Meat (Bos taurus)	1	<i>A.hydrophila</i>	100 *	Biochemical testing and <i>e-gcat</i>	Nope	Brazil	Roges et al. 2020
Chicken (Gallus gallus)	4						
Cultured fish	37 (79 isolates)	<i>A. molluscorum</i> (n=1), <i>A.caviae</i> (n=1), <i>A.hydrophila</i> (n=4), <i>A. media</i> (n=6), <i>A.veronii</i> (n=7), <i>A. salmonicida</i> (n=7), <i>A. bestiarum</i> (n=24) and <i>A. eucrenophila</i> (n=25), <i>Aeromonas spp</i>	100	MALDI TOFF MS Sanger Sequencing	Nope	Turkey	Onur (2024) Ş.

Conclusion

The genus *Aeromonas* represents a significant water- and foodborne public health concern due to its widespread distribution in aquatic environments and its ability to cause both intestinal and extraintestinal infections in humans. The diversity of species detected in water, food, and clinical samples, together with species-specific differences in virulence and clinical manifestations, highlights the complexity of *Aeromonas* epidemiology. The increasing recognition of highly virulent species, particularly *A. dhakensis*, combined with emerging antimicrobial resistance, underscores the need for improved surveillance, accurate identification, and integrated control strategies to reduce the risk of *Aeromonas*-associated infections.

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CHAPTER 37

Carbonic Anhydrase Isoforms as Preclinical Targets in Gynaecological Disease Biology

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1. Introduction

Carbonic anhydrases (CA) are zinc-containing metalloenzymes that catalyse the hydration of CO_2 (Figure 1) and are involved in fundamental physiological processes such as acid-base balance, respiration, and ion transport. To date, 16 distinct CA isoforms have been identified and classified into the following groups: cytosolic (CA I, II, III, VII, XIII), mitochondrial (CA VA, VB), membrane-bound (CA IV, IX, XII, XIV), secretory (CA VI), and cytoskeleton-associated (CARP VIII, X, XI) (Supuran CT, 2020).

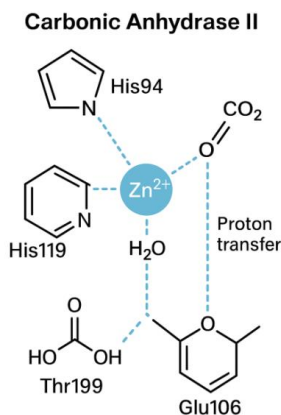


Figure 1. The structural form of the CA enzyme.

CA inhibitors (CAIs) are compounds that reversibly inhibit carbonic anhydrase activity and are clinically used in indications such as glaucoma, epilepsy, and altitude-related disorders (Supuran CT, 2008). Beyond these established applications, increasing attention has been directed toward the biological roles of carbonic anhydrase isoforms in the female reproductive system. In particular, hypoxia- and hormone-responsive isoforms such as CA IX and CA XII are expressed in tissues including the ovary and endometrium, highlighting their relevance to gynaecological disease biology (Alterio et al., 2012).

CA XII exhibits estrogen-dependent expression in endometrial tissue, whereas CA IX displays hypoxia-inducible regulation and is strongly associated with malignant transformation and adverse prognostic features (Ivanov et al., 2001; Pastorekova et al., 2008). The expression patterns of these isoforms have been linked to disease progression and treatment response in gynaecological pathologies. Accordingly, recent research has increasingly focused on carbonic anhydrase biology in conditions such as endometriosis and gynaecological cancers, with most available evidence derived from experimental and preclinical studies (Supuran CT, 2021).

Although carbonic anhydrase activity also contributes to physiological adaptations during pregnancy, including placental development and oxygen transport, evidence supporting therapeutic modulation of CA activity in obstetric settings remains limited and largely experimental. Consequently, current investigations emphasize mechanistic insights into isoform regulation rather than clinical intervention. This review therefore focuses on the pathophysiological roles of carbonic anhydrase isoforms and the preclinical evaluation of their inhibition in gynaecological disease models, with particular emphasis on molecular mechanisms, tumour microenvironment regulation, and translational potential in gynaecological oncology.

2. Carbonic Anhydrase Isoenzymes and Their Physiological Role

CA enzymes are metalloenzymes containing zinc ions that exhibit a very wide phylogenetic distribution from prokaryotes to mammals. Their primary catalytic role is to accelerate the conversion of carbon dioxide into bicarbonate and protons through reaction with water. This reaction supports a number of critical processes in physiological systems, including pH regulation, CO₂ transport, sodium and chloride reabsorption, and buffering of excessive acidosis. CA enzymes have an active site that structurally contains a zinc ion at its centre, which is stabilised by histidine residues (Parkkila S et al., 2000). Enzymatic activity begins with the hydration of the substrate in the zinc-active site, and proton transfer mechanisms are directed by protonic/deprotonic switches throughout the catalytic cycle (Supuran CT, 2020; Alterio et al., 2012). Their stable three-dimensional structures enable mammalian CA isoforms, particularly those in the alpha-CA class, to achieve high substrate conversion rates in specific tissues (Figure 2). These unique physicochemical properties of CAs make them essential for physiological regulation and also serve as a pharmacological target for the design of selective inhibitors. At least 16 CA isoforms have been identified in mammals to date. These isoforms are classified based on their intracellular localisation into cytosolic (CA I, II, III, VII, XIII), mitochondrial (CA VA, VB), membrane-bound (CA IV, IX, XII, XIV), secretory (CA VI), and non-catalytic CA-related proteins (CARP VIII, X, XI) (Supuran CT, 2008; Alterio et al., 2012).

In the physiology of the female reproductive system, the CA IX and CA XII isoforms in particular are of crucial clinical importance. CA IX is an isoform that is activated via HIF-1 α in response to hypoxia and is significantly overexpressed in tumour tissues and in hypoxic regions of the placenta. Due to this property, it is considered a potential biomarker and therapeutic target both in diseases associated with uteroplacental hypoperfusion (such as pre-eclampsia) and in gynaecological malignancies (Pastorekova et al., 2008). CA XII has a hormone-dependent expression profile; it is expressed in response to estrogen and progesterone regulation in endometrial, cervical and ovarian tissue. There is evidence that this isoform plays a role in processes such as endometrial

receptivity and follicular maturation, while its dysregulation is observed in pathologies such as endometriosis and ovarian cancer (Ivanov et al., 2001). Both isoforms play a dynamic role in fundamental physiological functions of the female reproductive system, including oxygenation, cellular pH regulation and cell proliferation. Therefore, the distinct regulatory profiles of CA IX and XII in both physiological adaptations and pathological disorders pave the way for modern pharmacological strategies targeting isoform-selective inhibitors. Carbonic anhydrase isoforms also participate in physiological adaptations during pregnancy, particularly in relation to hypoxia-responsive processes; however, their relevance in this context remains largely descriptive and mechanistic, and falls outside the primary focus of the present review.

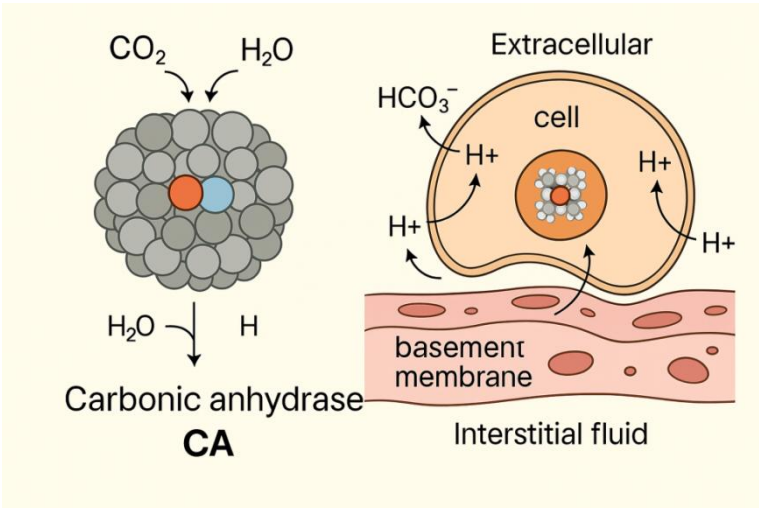


Figure 2. Fundamental catalytic function of carbonic anhydrase in cellular pH regulation.

Carbonic anhydrase catalyzes the reversible hydration of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) and protons (H^+), thereby contributing to intracellular and extracellular acid–base balance. This enzymatic reaction supports essential physiological processes including pH homeostasis, ion transport, and metabolic buffering at the cellular level. The schematic represents the core biochemical function shared by catalytically active carbonic anhydrase isoforms and provides a conceptual framework for understanding isoform-specific roles in tissue physiology and disease. Targeting these isoforms, together with the development of selective inhibitors, represents a promising preclinical strategy for modulating disease-associated carbonic anhydrase activity. Tissue-specific CA expression profiles are particularly important for guiding the development of individualized pharmacological concepts. Isoform-selective

inhibitors may help limit systemic effects while improving target engagement in disease-relevant models (Alterio et al., 2012).

3. Mechanisms of Carbonic Anhydrase Inhibition

CAIs directly target the catalytic cycle of CA enzymes by binding to the zinc-containing active site, most commonly through sulfonamide groups that replace the zinc-bound water molecule essential for catalysis (Supuran CT, 2008) (Figure 3). This interaction reversibly inhibits enzyme activity by disrupting the proton transfer cycle responsible for the rapid interconversion of carbon dioxide and bicarbonate. Consequently, inhibition of CA activity leads to disturbances in pH homeostasis, osmotic balance, and transmembrane ion transport at both cellular and systemic levels.

Among classical inhibitors, sulfonamide derivatives such as acetazolamide exhibit high affinity for CA II but low selectivity for tissue-specific isoforms such as CA IX and XII, which may result in systemic adverse effects including electrolyte imbalance and metabolic acidosis (Supuran CT, 2008). Therefore, the development of isoform-selective inhibitors has become a major research priority, particularly in hypoxia-driven malignancies where CA IX is highly expressed (Supuran CT, 2020).

Structural and crystallographic studies have elucidated detailed interactions between CAIs and the zinc-coordinated active site, revealing key hydrogen bonding networks, van der Waals forces, and π - π interactions that determine binding affinity and isoform selectivity (Alterio et al., 2012). These insights have enabled the rational design of next-generation CAIs with improved therapeutic indices and reduced systemic toxicity, supporting their emerging role in gynecologic oncology and potentially in pregnancy-related vascular stress conditions.

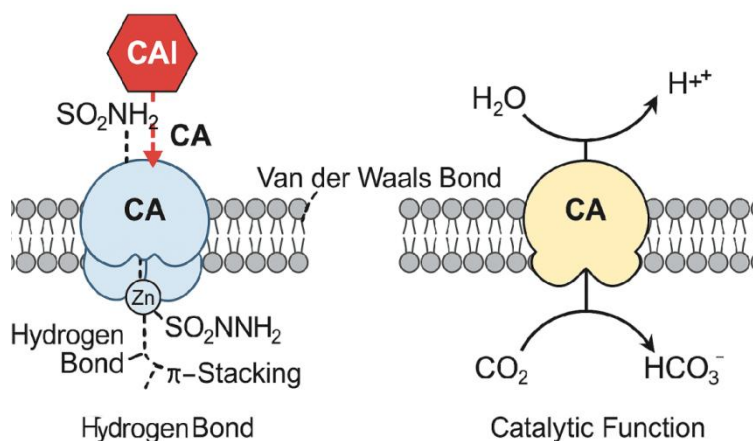


Figure 3. Structural and functional basis of carbonic anhydrase inhibition.

Sulfonamide-based carbonic anhydrase inhibitors (CAIs) inhibit enzyme activity by coordinating with the Zn^{2+} ion in the active site through the SO_2NH_2 moiety, thereby preventing the hydration of carbon dioxide. The right panel depicts the normal catalytic function of carbonic anhydrase, in which CO_2 is reversibly converted to bicarbonate (HCO_3^-) and protons (H^+), a reaction essential for cellular pH regulation.

4. Therapeutic Use of CAIs in Obstetrics and Gynecology

CA Inhibitors and Their Use in Pregnancy

The therapeutic potential of CAIs in obstetric practice has become more evident with the development of targeted treatment approaches for specific pathophysiological conditions encountered during pregnancy. During pregnancy, increased expression of CA isoforms (particularly CA IX and CA XII) in the placenta, decidua, and uterine vascular structures plays a critical role in maternal-fetal oxygen exchange and pH balance (Ivanov et al., 2021) (Figure 4).

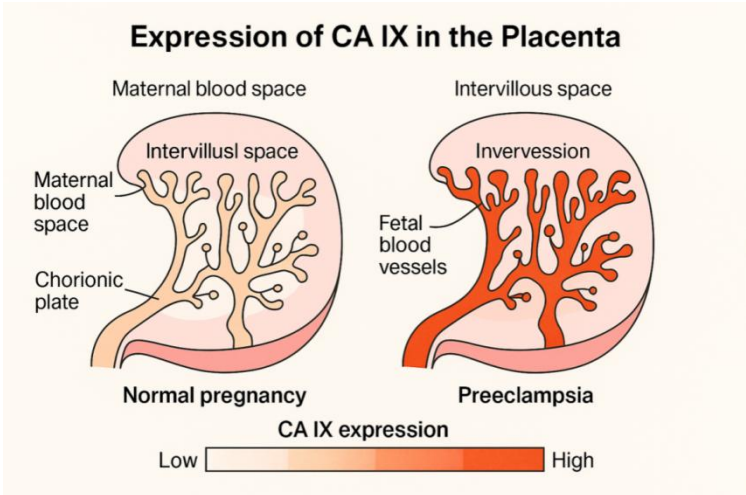


Figure 4. Comparison of CA IX expression in placental tissue in normal pregnancy and pregnancy with preeclampsia.

The upregulation of specific isoforms under hypoxic conditions plays a pivotal role in the pathophysiology of complications such as preeclampsia, intrauterine growth restriction (IUGR), gestational hypertension, and HELLP syndrome. Studies in experimental animal models have demonstrated that CAIs enhance trophoblast cell invasion, thereby supporting spiral artery remodeling. This mechanism leads to significant improvements in uteroplacental blood flow and increased placental oxygenation. These effects are believed to have the potential to improve perinatal outcomes for both the mother and fetus.

CAIs are gaining increasing attention for their potential relevance in endometriosis, a chronic inflammatory disease characterized by hypoxia-driven

metabolic adaptation and aberrant pH regulation within ectopic lesions. Hypoxia-responsive carbonic anhydrase isoforms, particularly CA IX and CA XII, are well-established regulators of extracellular acidification and cellular survival in low-oxygen environments (Parkkila et al., 2000). Given that endometriotic lesions exhibit metabolic features similar to hypoxic tissues, it has been proposed that dysregulation of CA-mediated pH homeostasis may contribute to lesion persistence, inflammatory signaling, and invasive behavior.

Although direct clinical evidence supporting CAI use in endometriosis is currently limited, mechanistic insights derived from hypoxia-associated pathologies suggest that selective inhibition of CA IX and CA XII could potentially interfere with glycolytic reprogramming, extracellular matrix remodeling, and inflammatory microenvironment maintenance. Therefore, CAIs may represent a promising experimental strategy for modulating disease-associated molecular pathways rather than solely providing symptomatic relief. However, further targeted preclinical and clinical investigations are required to substantiate this therapeutic hypothesis (Supuran CT, 2020).

Gestational diabetes mellitus (GDM) is characterized by glucose intolerance developing during pregnancy and represents a major metabolic disorder associated with adverse maternal and fetal outcomes. Insulin resistance, chronic low-grade inflammation, and tissue hypoxia are recognized as central components of GDM pathophysiology. In recent years, increasing attention has been directed toward the role of carbonic anhydrase (CA) isoenzymes particularly CA II, CA IX, and CA XII in the regulation of pH homeostasis, glucose transport, and mitochondrial metabolism (Supuran CT, 2020; Parkkila S, 2000).

Hypoxia-responsive CA isoforms, especially CA IX, are well-established downstream targets of HIF-1 α and contribute to cellular adaptation under low-oxygen conditions through modulation of intracellular and extracellular pH. On this basis, it has been hypothesized that dysregulated CA activity under hypoxic and metabolically stressed conditions may interfere with insulin signaling pathways, promote cellular acidosis, and enhance oxidative and inflammatory responses. Collectively, these mechanisms suggest a potential contributory role for CA-mediated pH regulation in the metabolic disturbances underlying GDM, although direct clinical evidence in pregnancy remains limited and warrants further investigation.

5. Carbonic Anhydrase Isoforms as Preclinical Targets in Gynaecological Disease Models

Hypoxia-responsive carbonic anhydrase isoforms have attracted increasing interest as biological modulators in gynaecological disease models, primarily due to their central role in pH regulation, metabolic adaptation, and cellular survival under low-oxygen conditions. Among these, the transmembrane isoforms CA IX and CA XII are of particular relevance, as they are selectively upregulated in

hypoxic and hormonally responsive tissues and contribute to disease-associated microenvironmental remodeling.

In experimental studies, altered expression of CA IX has been documented in hypoxia-associated gynaecological conditions, where it functions as a downstream effector of hypoxia-inducible factor-1 α (HIF-1 α). In preclinical models, CA IX expression has been associated with changes in extracellular acidification, cellular invasiveness, and stress tolerance. These observations have led to the hypothesis that CA IX represents a biologically relevant marker of hypoxic adaptation rather than a direct therapeutic target in obstetric settings. Consequently, current evidence supports its evaluation at a mechanistic and experimental level rather than as a candidate for clinical intervention during pregnancy.

Beyond hypoxia-related adaptations, dysregulation of carbonic anhydrase-mediated pH homeostasis has also been implicated in chronic inflammatory gynaecological disorders such as endometriosis. Ectopic endometrial lesions exhibit metabolic features characteristic of hypoxic tissues, including enhanced glycolysis and extracellular acidification. Preclinical observations suggest that CA IX and CA XII may contribute to lesion persistence by supporting acid-base balance, inflammatory signaling, and matrix remodeling within the ectopic microenvironment. Although direct clinical evidence remains limited, selective inhibition of these isoforms has been proposed as an experimental strategy to modulate disease-associated molecular pathways rather than to provide symptomatic treatment (Supuran CT, 2020).

Metabolic dysregulation represents another area in which carbonic anhydrase biology has been investigated at the preclinical level. Hypoxia-responsive CA isoforms, particularly CA IX, have been linked to altered glucose metabolism and mitochondrial function through their role in intracellular and extracellular pH regulation (Annunziato et al., 2016). Experimental studies have suggested that such mechanisms may intersect with pathways involved in insulin resistance, oxidative stress, and inflammatory responses. While these findings provide mechanistic insight into disease-associated metabolic adaptation, their relevance remains confined to hypothesis-generating and experimental contexts, as robust clinical data are currently lacking.

Overall, available evidence indicates that carbonic anhydrase isoforms especially CA IX and CA XII play important roles in the pathophysiology of selected gynaecological conditions through regulation of hypoxia-driven pH dynamics and metabolic plasticity. The preclinical evaluation of isoform-selective carbonic anhydrase inhibitors has therefore focused on their capacity to disrupt disease-associated microenvironmental adaptations rather than on direct therapeutic application. Further experimental studies are required to define the translational relevance of these targets and to determine whether modulation of

carbonic anhydrase activity can be integrated into future mechanism-based strategies in gynaecological disease research.

Mechanistic Rationale for Carbonic Anhydrase Inhibitors in Gynaecological Oncology

Preclinical studies have evaluated ureido-sulfamate carbonic anhydrase IX/XII inhibitors in solid tumour models, demonstrating potential antitumour and antimetastatic effects in vitro and in xenograft systems, although clinical translational data remain limited (Williams & Gieling, 2019). Hypoxia is a defining feature of the tumour microenvironment in many gynaecological malignancies, including cervical, ovarian, and endometrial cancers. Under hypoxic conditions, tumour cells activate adaptive mechanisms that promote survival, metabolic flexibility, and resistance to therapy, among which hypoxia-inducible carbonic anhydrase isoforms particularly CA IX and CA XII play a central role.

CA IX and CA XII facilitate tumour-specific pH adaptation by catalysing the reversible hydration of carbon dioxide at the tumour cell surface, thereby maintaining intracellular pH homeostasis while promoting extracellular acidification (Figure 5). This dual regulation enables tumour cells to survive and proliferate in acidic and hypoxic environments and contributes to tumour progression, invasion, and metastatic potential. Ureidosulfamate-based carbonic anhydrase inhibitors have been specifically designed to selectively target these transmembrane isoforms, exhibiting high affinity for the zinc-containing active site of CA IX and CA XII while sparing cytosolic isoforms such as CA I and CA II. This isoform selectivity is critical for enhancing tumour-specific activity while minimizing systemic toxicity (Annunziato et al., 2016).

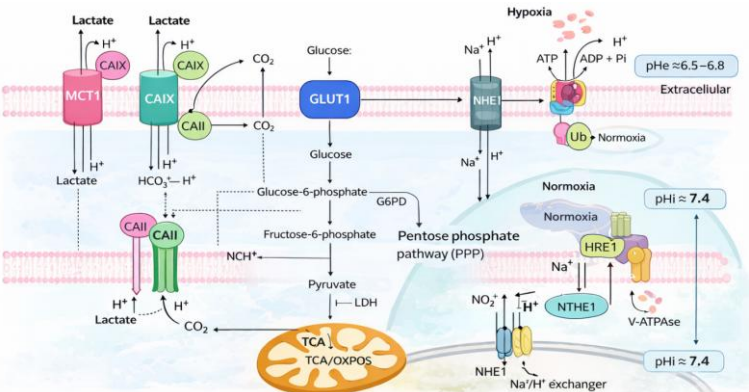


Figure 5. Integrated regulation of tumour metabolism and pH homeostasis by hypoxia-inducible carbonic anhydrase isoforms.

Hypoxia-induced stabilization of hypoxia-inducible factor-1α (HIF-1α) drives transcriptional upregulation of glycolytic and pH-regulatory components, including carbonic anhydrase IX (CA IX), monocarboxylate transporters

(MCT1/MCT4), glucose transporter-1 (GLUT1), and pyruvate dehydrogenase kinase-1 (PDK1). Enhanced glycolysis increases lactate and proton (H^+) production, which are exported via MCTs, promoting extracellular acidification. Carbon dioxide (CO_2) generated by mitochondrial metabolism is converted at the cell surface by CA IX into bicarbonate (HCO_3^-) and protons. Bicarbonate is transported intracellularly via sodium–bicarbonate cotransporters (NBC), where cytosolic carbonic anhydrase II (CA II) contributes to intracellular pH buffering. Additional proton extrusion mechanisms, including Na^+/H^+ exchangers (NHE1) and V-ATPase, further support pH regulation. This coordinated network maintains intracellular alkalinity while sustaining an acidic extracellular microenvironment, facilitating tumour cell adaptation, metabolic plasticity, and therapy resistance under hypoxic conditions. The schematic represents mechanisms characterized in preclinical tumour models.

Mechanistically, inhibition of CA IX and CA XII disrupts bicarbonate recycling and proton extrusion, leading to intracellular acidification and metabolic stress within tumour cells (Angeli et al., 2025). As a consequence, key cellular processes including glycolysis, mitochondrial function, and lactate transport are adversely affected, resulting in reduced tumour cell proliferation, migration, and invasive capacity. Rather than exerting direct cytotoxic effects, carbonic anhydrase inhibitors induce a state of metabolic vulnerability. Importantly, by modulating the acidic tumour microenvironment, CA inhibition has been shown in preclinical models to enhance sensitivity to chemotherapy and radiotherapy, both of which are compromised under hypoxic and acidic conditions.

Collectively, these findings provide a strong biological rationale for the investigation of isoform-selective carbonic anhydrase inhibitors as adjunctive therapeutic agents in gynaecological oncology. Although current evidence remains largely confined to *in vitro* and *in vivo* preclinical models, targeting hypoxia-associated carbonic anhydrase isoforms represents a promising strategy for overcoming therapy resistance and modulating the tumour microenvironment in hormonally and metabolically driven gynaecological cancers (Williams & Gieling, 2019; Supuran CT, 2020).

Clinical relevance (Preclinical implications)

Hypoxia-associated carbonic anhydrase isoforms, particularly CA IX and CA XII, play key roles in tumour microenvironment adaptation and therapy resistance in gynaecological malignancies. Although current evidence is restricted to *in vitro* and *in vivo* experimental models, disruption of carbonic anhydrase-mediated pH regulation has been shown to increase tumour vulnerability to hypoxia-related metabolic stress. From a translational perspective, these findings support further investigation of carbonic anhydrase inhibition as an adjunctive, mechanism-based strategy rather than an immediate

therapeutic application. Careful preclinical validation and biomarker-guided approaches will be essential to define the potential clinical relevance of these targets

Challenges and Future Perspectives in CAI-Based Therapies

Despite the growing body of preclinical evidence supporting the role of carbonic anhydrase inhibitors (CAIs) in targeting tumour-specific metabolic adaptations, several challenges currently limit their translation into routine clinical practice in gynaecological oncology. One of the primary obstacles lies in the complexity and redundancy of tumour pH-regulatory networks. While CA IX and CA XII play central roles in hypoxia-driven pH homeostasis, tumour cells often activate compensatory mechanisms involving proton pumps, ion exchangers, and alternative metabolic pathways. This adaptive plasticity may reduce the long-term efficacy of CAI monotherapy and underscores the need for combination strategies that target multiple components of the tumour microenvironment (Kumar et al., 2022).

Isoform selectivity represents another critical challenge in the development of clinically viable CAIs. Classical carbonic anhydrase inhibitors exhibit limited specificity and may inhibit ubiquitously expressed cytosolic isoforms, leading to off-target effects and systemic toxicity. Although next-generation isoform-selective inhibitors particularly those targeting CA IX and CA XII have demonstrated improved tumour specificity in preclinical models, achieving an optimal balance between potency, selectivity, and pharmacokinetic stability remains a significant hurdle. Advances in structure-based drug design and computational modelling are expected to play an increasingly important role in refining isoform-selective binding profiles and minimizing adverse effects.

Drug delivery also constitutes a major limitation in CAI-based therapies. The hypoxic and heterogeneous nature of solid tumours, including gynaecological malignancies, can impair drug penetration and result in uneven target engagement. Innovative delivery approaches such as nanoparticle-based carriers, antibody–drug conjugates, and tumour-targeted prodrug systems offer promising avenues to enhance intratumoral accumulation of CAIs while reducing systemic exposure. Integration of CAIs with targeted delivery platforms may therefore substantially improve their therapeutic index.

From a translational perspective, the identification of reliable predictive biomarkers remains essential for patient stratification and treatment optimization. While elevated expression of CA IX and CA XII has been associated with aggressive tumour phenotypes and poor prognosis, standardized assays and clinically validated thresholds for patient selection are still lacking. Future clinical development will require robust biomarker-driven trial designs to identify patient subgroups most likely to benefit from CAI-based interventions.

Looking ahead, the most promising applications of CAIs are likely to emerge in combination treatment settings rather than as standalone agents. By modulating tumour pH and metabolic vulnerability, CAIs have the potential to enhance the efficacy of chemotherapy, radiotherapy, and emerging immunotherapeutic approaches, particularly in hypoxia-driven and treatment-resistant gynaecological cancers. As our understanding of tumour metabolism and microenvironmental regulation continues to evolve, CAIs may become integral components of personalized therapeutic strategies aimed at overcoming resistance and improving long-term outcomes.

In conclusion, while significant challenges remain, ongoing advances in isoform-selective inhibitor design, targeted drug delivery, and biomarker-guided therapy provide a strong rationale for continued investigation of CAI-based approaches. With careful translational development, carbonic anhydrase inhibition holds considerable promise as a complementary strategy in the evolving landscape of gynaecological oncology.

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CHAPTER 38

Methicillin Resistant *Staphylococcus aureus* and Candidate Vaccines

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INTRODUCTION

Staphylococcus aureus is a Gram-positive cocci of the *Micrococcaceae* family. The bacterium does not form spores but capsules are observed in some strains (Algammal et al., 2020; Foster, 2002). Asymptomatic colonisation of the skin and mucous membranes, particularly the anterior nostrils, by *S. aureus* is extremely common; approximately 20% of the population is thought to carry *S. aureus* chronically and 60% at various times (Kluytmans, Van Belkum, & Verbrugh, 1997). In addition, *S. aureus* causes various infections in both humans and animals (Leonard & Markey, 2008; McCarthy, Lindsay, & Loeffler, 2012; Peton & Le Loir, 2014). In humans it causes septicaemia, bone and encephalitis, food poisoning, osteomyelitis, polyarthritis, endocarditis, urinary tract infections, pneumonia; in animals it causes skin infections, mastitis, otitis, vaginitis and foot infections. (David & Daum, 2017; Grema, Geidam, Gadzama, Ameh, & Suleiman, 2015; Haag, Fitzgerald, & Penadés, 2019; Nilgün & İstanbulluoğlu, 2009). In animals, it causes many infections including mastitis, wound infections, septic arthritis, otitis and urinary tract infections (Oliveira et al., 2007). *S. aureus* is not host-specific and can be transmitted to humans via the consumption of foods of animal source, including milk, eggs, meat and other animal products. (Pal, Kerorsa, Marami, & Kandi, 2020). *Staphylococcus aureus* is of significant public health importance due to its ability to transmit from humans to animals and from animals to humans (*amphixenoses*) (Attili, Cerquetella, & Rossi, 2018).

The development of resistance of microorganisms against antimicrobial agents is shown globally as one of the most significant challenges of our age. According to scientific estimates, the number of deaths caused by antimicrobial resistance will reach approximately 10 million by the year 2050. The capacity of the target microorganism to proliferate or persist in the presence of the antibiotic employed to impede or eliminate the agent is delineated as antibiotic resistance (O'Neill, 2016; Sabtu, Enoch, & Brown, 2015). *S. aureus* can rapidly adapt to changing environmental conditions. It also has an exceptional ability to develop resistance to a wide range of antibiotics. This process is achieved by mutations leading to changes in the bacterium's genome or by the acquisition of resistance determinants (McCallum, Berger-Bächi, & Senn, 2010). The first reported cases of antibiotic resistance in *S. aureus* strains occurred in the 1940s, when penicillin-resistant *S. aureus* (PRSA), a strain of *S. aureus* resistant to β -lactam antibiotics, was isolated from patients (Khan, 2017). β -lactam antibiotics inhibit cell wall synthesis by acting on enzymes called penicillin binding proteins (PBP). The development of resistance to penicillin by *S. aureus* is mediated by β -lactamases, which are enzymes that hydrolytically degrade β -lactams (Fuda, Fisher, & Mobashery, 2005; Zygmunt, Stratton, & Kernodle, 1992). Methicillin, a semi-synthetic β -lactam, was first produced against PRSA strains in 1959. This antimicrobial agent was found to be highly effective in the early years of its use; however, it was not long before methicillin-resistant *Staphylococcus aureus*

(MRSA) strains were reported in 1961 and the effectiveness of methicillin decreased (Stefani & Goglio, 2010). It has been established that five penicillin-binding proteins (PBPs) are present in methicillin-sensitive *Staphylococcus aureus* (MSSA) isolates. However, MRSA strains have a different PBP, designated 'PBP2a', which has a molecular weight of 78kDa. In contrast to other PBPs, PBP2a exhibits a markedly low affinity for beta-lactam antibiotics. Consequently, in the presence of beta-lactam group antibiotics, it persists in facilitating peptidoglycan synthesis by assuming the role of high affinity PBPs (Hiramatsu, Asada, Suzuki, Okonogi, & Yokota, 1992; Zhan & Zhu, 2018). PBP2a, which plays a role in methicillin resistance in *S. aureus*, is synthesised by *mecA* and *mecC* genes on staphylococcal cassette chromosome *mec* (SCC*mec*) (Aklilu & Chia, 2020; Deurenberg & Stobberingh, 2008). The SCC*mec* elements detected in nearly all MRSA strains belong to a distinct category of staphylococcal mobile genetic elements that encode methicillin resistance. This category is referred to as staphylococcal cassette chromosome *mec* (Katayama, Ito, & Hiramatsu, 2000). To date, 12 distinct SCC*mec* types of varying sizes (ranging from 21 to 67 kilobases) have been identified (Reichmann & Pinho, 2017). SCC*mec* typing is performed by molecular methods (Turlej, Hryniewicz, & Empel, 2011). The expression of the *mecA* gene on SCC*mec*, a mobile genetic element, is regulated by two regulatory genes, *mecRI* and *mecI* (Katayama et al., 2000; Matsushashi et al., 1986). The *mecC* gene, first identified in 2011, is localised on SCC*mec* (type XI), which differs from *mecA*. *mecC* has been designated 'mecALGA251'. At the DNA level, *mecC* is 70% similar to *mecA* (García-Álvarez et al., 2011; Sayın et al., 2016; Shore et al., 2011). It has been hypothesised that the proteins encoded by *mecC* and *mecA* may differ in structure and function. Recent studies have indicated that the homologue encoded by PBP2a and *mecC* (designated PBP2c by EUCAST) exhibits divergent beta-lactam binding properties and temperature-dependent activity (Ballhausen, Kriegeskorte, Schleimer, Peters, & Becker, 2014; Giacinti et al., 2017; Harrison et al., 2013).

Despite the variability in MRSA prevalence across different countries, the threat it poses to global public health remains significant (Garipçin & Şeker). Multi Drug Resistance (MDR) has been demonstrated to reduce the likelihood of successful treatment outcomes for MRSA infections with antibiotics (Kumar, Kala, Dhiman, & Mishra, 2016). Consequently, it has been observed to be a significant cause of mortality in elderly individuals, very young people, and those with compromised immune systems (Andersson, Lindholm, & Fossum, 2011). Initially, MRSA was believed to be confined to hospital settings and those with exposure to such environments were considered to be at risk. However, contemporary epidemiological categorisation of MRSA distinguishes three distinct groups: Hospital Acquired MRSA (HA-MRSA), Community Acquired MRSA (CA-MRSA), and Livestock Associated MRSA (LA-MRSA) (Stefani et

al., 2012). In molecular epidemiology, typing of SCCmec elements is of fundamental importance (Pantosti, Sanchini, & Monaco, 2007).

Preventive measures and the utilisation of a novel antibiotic treatment, either as a monotherapy or in combination with other agents, have been demonstrated to reduce MRSA infections (De Kraker et al., 2013). Cefaroline, daptomycin, oxazolidinones, teicoplanin and telavancin are commonly used antibiotics for the treatment of MRSA infections. It has been documented that linezolid is an effective pharmaceutical agent against MRSA bacteraemia. Vancomycin, a drug that has been used to combat MRSA for the last 50 years, is one of the antibiotic options employed in this effort. It is widely used for the therapy of MRSA bacteraemia and infectious endocarditis. Although vancomycin is widely used in MRSA patients, its clinical efficacy remains limited due to poor tissue penetration, slow bactericidal activity and a number of side effects (Gould, 2008). In addition to antibiotics, alternative therapeutic are usable for the treatment of MRSA infections. These include immunotherapy, phytochemicals, probiotics, nanoparticles and bacteriophages (Lee et al., 2016). Vaccination is of great importance in the present day in the fight against MRSA infections. There are ongoing efforts to develop a potent vaccine against MRSA, with various companies conducting trials (Shoaib et al., 2023).

MRSA and CANDIDATE VACCINE STUDIES

The development of effective treatment and prevention strategies is imperative in addressing the challenges posed by multidrug-resistant and life-threatening MRSA strains. One potential approach to preventing such infections is the utilisation of vaccines (Zuo et al., 2013). Since the 1960s, a significant number of scientists and companies have been engaged in the development of an *S. aureus* vaccine. The initial approach to vaccine development was informed by the success of vaccines for *Streptococcus pneumoniae* and *Haemophilus influenzae*. The vaccine employs antigens from the capsule surrounding the bacterium, which consist of carbohydrate molecules that bind to a protein produced by another bacterium. However, this initial endeavour proved unsuccessful. The reason for this was that *S. aureus* infection developed at the same rate in the blood of the challenge group receiving the candidate vaccine and the placebo group (Fowler Jr & Proctor, 2014).

In the study conducted by Zuo et al., bivalent vaccines were created, based on α -toxin and iron-sensitive surface determinant B (IsdB). The protective efficiency of the bivalent vaccine and vaccines containing individual proteins was compared in murine models infected with systemic *S. aureus* MRSA252. The study reported that the bivalent vaccine showed superior protective immunity compared to vaccines containing individual proteins. The presence of memory T cells producing both IL-17A and IFN- γ , IsdB-specific opsonic antibodies and

neutralising antibodies against α -toxin was found to improve the protective immunity of the bivalent vaccine (Zuo et al., 2013).

Haghighat et al. hypothesised that PBP2a would be a suitable antigen for an MRSA vaccine because it is located on the outer membrane of the bacterial cell wall, could be easily recognised by antibodies in the immune system of the organism and could be eliminated by phagocyte cells. To this end, 242 base pairs (amino acids 370e451) of the *mecA* gene containing transpeptidase activity were amplified by PCR from the genomic DNA of MRSA bacteria using specific primers. Subsequent to this, the PCR products were recovered and purified by DNA extraction from the gel. The next stage of the process involved the cloning and expression of the recombinant bacterium in *Escherichia coli* strains. In mice vaccinated with the recombinant PBP2a vaccine, increased opsonophagocytic activity and antibody response were reported. Consequently, it was reported that the survival rate of MRSA-infected mice increased significantly (Haghighat et al., 2017). *Staphylococcus enterotoxin B* (SEB), an exotoxin, is recognised as one of the most significant MRSA toxins. It plays an important role in CA-MRSA-induced toxic shock syndrome and is produced in high concentrations by most MRSA isolates (Kashiwada et al., 2012). In previous studies, mutant or attenuated SEB has been utilised as a vaccine against lethal SEB challenge and *S. aureus* infection. Nevertheless, a robust immune response against *S. aureus* has not been achieved for two reasons. Firstly, epitope-specific vaccines have been shown to be more potent than vaccines containing all antigens. This is due to the fact that only a few immunodominant epitopes are sufficient to generate a protective response. Secondly, the vaccine was inadequate due to the high ability of *S. aureus* to escape opsonisation. Nevertheless, it has been demonstrated that immunodominant SEB peptide vaccination can elicit a robust humoral immune response, and it has been proposed that this methodology can be employed to optimise MRSA vaccine strategy, although it is inadequate as a vaccine in isolation. Zhao et al. investigated the potential efficacy and immunological mechanisms of an SEB multiple B-cell epitope vaccine for MRSA infection. The researchers mapped B-cell immunodominant epitopes in SEB by synthetic overlapping peptide ELISA and assembled immunogens into a sole SEB-specific multiple B-cell epitope vaccine. The study demonstrated that the vaccine induced synergistic opsonophagocytosis against MRSA and confirmed its ability to protect challenge group to infection with different MRSA isolates (Zhao et al., 2015). In the present study, the effectiveness and immunological mechanisms of a vaccine based on a recombinant glycoprotein antigen (NDV-3) were investigated in a mouse model of MRSA-associated skin and skin structure infection (SSSI). The experimental design involved the administration of only adjuvant to the control group, while the challenge group received the vaccine in various doses. A comparative analysis of the control and challenge groups revealed a decline in MRSA density in the skin, abscess progression, size, and

severity, as well as a reduction in haematogenous spread of the bacteria to the kidneys in the challenge group. NDV-3 was found to enhance neutrophil infiltration and CD3⁺ T-cell, as well as IL-17A, IL-22 and host defence peptide expression in the local environment of SSSI abscesses. The findings suggest that the protective efficacy of NDV-3 against MRSA in SSSI involves a robust and complementary response that integrates innate and adaptive immune mechanisms. These results support further evaluation of NDV-3 vaccine against *S. aureus*-associated diseases in humans (Yeaman et al., 2014).

Cyclic diguanilate (c-di-GMP) which an intracellular signalling molecule, was first discovered in the *Acetobacter xylinum* (Hengge, 2021). Although c-di-GMP can be found in many bacteria, it is absent in eukaryotes. C-di-GMP has been identified as playing a critical role in a variety of pivotal bacterial functions, including survival, adhesion, colonisation, and the formation of biofilms (Condinho et al., 2023; Liu, Cao, Yang, & Gu, 2022). The present study reports the findings of recent research which suggests that the presence of exogenous c-di-GMP may be a regulatory factor in the host cellular responses and the inhibition of *S. aureus* biofilm formation. Consequently, it has been proposed that the presence of exogenous c-di-GMP would enhance infection control and reduce cancer cell proliferation in vitro. More recent studies have demonstrated that c-di-GMP functions as an immunomodulator, thereby stimulating the immune system to prevent and combat deadly bacterial diseases, including *S. aureus* infections (Brouillette, Hyodo, Hayakawa, Karaolis, & Malouin, 2005; Karaolis et al., 2007; Ogunniyi et al., 2008; Rashid, Rajanna, Ali, & Karaolis, 2003). Liang-Hu et al. conducted a study to investigate the potential of c-di-GMP as an adjuvant to stimulate immune responses in mice vaccinated with c-di-GMP and a non-toxic mutant of SEC (mSEC) and ClfA antigens, with the aim of enhancing resistance against systemic *S. aureus* (MRSA) infection. The study's findings indicated that vaccination with c-di-GMP + mSEC or c-di-GMP + ClfA led to substantial antibody production and protection against bacterial infection. These results provide direct evidence that c-di-GMP is an immune stimulant and acts as an effective vaccine adjuvant against systemic MRSA infection (Hu et al., 2009).

DNA vaccines comprise an intact eukaryotic promoter system and a plasmid DNA sequence encoding relevant proteins. These vaccines are administered by direct injection into muscle tissue, with the objective of inducing the synthesis of protective antibodies and cellular immune responses (Cui, 2005). The high immunogenic efficacy of DNA vaccines technology has been demonstrated in a group animal models. However, this immunogenic activity of DNA vaccines cannot be easily extrapolated from non-clinical to clinical use. To date, only one prophylactic DNA vaccine has been authorised for emergency use in humans (Pagliari, Dema, Sanchez-Martinez, Zurbia-Flores, & Rollier, 2023). Senna et al. evaluated the preventive immune response in an experimental group vaccinated with DNA carrying a fraction of the *mecA* gene and subsequently infected with

MRSA. The researchers cloned an inner area from the transpeptidase region of PBP2a into a mammalian expression vector in a mouse model for use as a DNA vaccine. After three sets of DNA vaccinations, the immune response, determined by antibodies counter a segment of PBP2a, was analysed by ELISA and a significant antibody response was reported. The DNA vaccine was evaluated for antibacterial activity, intraperitoneal immunisation and MRSA challenge in mice at sublethal doses over a seven-day period. At the end of the study, the number of bacteria in the kidneys of vaccinated and unvaccinated mice was assessed. The results of the study showed that mice in the challenge group had a 1000-fold reduction in renal bacterial load compared to mice in the control group. The study also showed that the DNA vaccine had no effect against other PBPs of staphylococcal origin and gram-positive bacilli from normal intestine flora. In conclusion, it has been demonstrated that immunisation against PBP2a in MRSA infections using a DNA vaccination method may serve as a new strategy to effectively combat these multi-resistant bacteria (Senna, Roth, Oliveira, Machado, & Santos, 2003).

The role of nanotechnology in the development of vaccine adjuvants is increasing in importance. Nanoemulsion adjuvants have been demonstrated to be reliable, efficient and ideal adjuvants for vaccine. The mean diameter of nanoemulsion adjuvants is 1-100 nm. They consist of surfactants or co-surfactant, water and oil and show high thermodynamic stability. Nanoemulsions have been shown to exhibit potent and extensive antimicrobial, antiviral, and antifungal properties, thereby providing effective adjuvant activity, particularly in vaccines employing intact viruses, such as respiratory syncytial virus (RSV) and influenza (Assis, da Silva, Santiago, Ferreira, & Goulart, 2021; Ren, Jia, Xie, Yu, & Chen, 2023). Sun et al. developed a new bivalent emulsion oil adjuvant vaccine in their study. The vaccine composition included MRSA recombination protein antigen, CremophorEL-35® and propylene glycol. The nanoemulsion vaccine, with a mean diameter of 31.34 ± 0.49 nm, protein particularity and exhibited satisfactory protein structure unity. It also demonstrated satisfactory stability at ambient temperature over a period of one year. The immunological response of this nanoemulsion vaccine was evaluated by intramuscular-systemic and nasal mucosal vaccination of immunocompetent mice. The resultant immune responses in the serum of mice included an increase in specific immune responses of immunoglobulin IgG and associated subclasses, including IgG1, IgG2a and IgG2b, as well as IgA. Moreover, the present study has demonstrated that this nanoemulsion vaccine remarkably boosts interferon- γ and interleukin-17A cytokine cell immune responses, thereby increasing the survival rate and decreasing bacterial colonisation. The collective findings of this study indicate that this novel nanoemulsion vaccine possesses significant potential, eliciting an efficacious immune response within the muscle, system, and nasal mucosa, obviating the necessity for an auxiliary adjuvant. In conclusion, it has been

demonstrated that the new nanoemulsion-adjuvanted vaccine technology can be used to improve both intramuscular, systemic and nasal mucosal immune responses in the prevention of MRSA infections (Sun et al., 2015). In another study, Wang et al. investigated the protective capabilities of nanotoxoid (Hla), a nanoparticle scavenger, against *Staphylococcus Hla*. The study evaluated the ability of the nanotoxoid (Hla) vaccine to protect against MRSA infection and reduce bacterial colonisation and skin lesion formation in a mouse model infected with MRSA skin infection. Furthermore, the immune-enhancing effect of the nanoparticle formulation was investigated by examining the formation of germinal centres in draining lymph nodes (dLNs) of vaccinated mice and then correlating this with anti-Hla titre production. In addition, the impact of nanoparticle vaccination on bacterial invasiveness was examined by quantifying the bacterial load in major organs. The study's findings indicated that the Hla vaccine elicited a more robust protective immune response in the challenge group compared to the micro-vaccine group. The Hla vaccine not only prevented lesion formation at the site of bacterial challenge but also prevented the spread of MRSA to other organs by reducing its invasiveness. This biomimetic nanoparticle-based toxin retention strategy has been reported as a promising method for the design of potent antivirulence vaccines to manage bacterial infections (Wang et al., 2016).

It is hypothesised that the rapid development of antibiotic resistance by MRSA may result in the emergence of resistance to vaccines. The same mechanisms of genetic transduction, conjugation and transformation may result in resistance to vaccines and resistance to vaccines in environments where these therapies can be used due to similar microevolutionary forces causing the spread of the original *mecA* gene (Scully, Mustafa, Hanif, Tunio, & Tunio, 2024). Toxoid vaccines against MRSA are considered promising for further research as they have strong immunogenicity and allow modulation of the MRSA immune response by allowing a strong IFN-gamma, IL-6 and IL-17 response (Angsantikul, Fang, & Zhang, 2017).

CONCLUSION

Staphylococcus aureus is a significant pathogen for both human and animal health, due to its resistance to antibiotics. Until the advent of antibiotics, it was responsible for significant mortality rates in living organisms. With the emergence of PRSA and then MRSA strains, a new problem, defined as antimicrobial resistance, has come to the fore. This resistance has been defined as the ability of the target microorganism to proliferate or survive in the existence of antibiotics with bactericidal or bactericidal properties. This has the consequence of reducing the efficacy of treatment for *S. aureus* infections. Presently, there is a focus on investigating solutions against antimicrobial resistance.

Given the challenges associated with the development of new antibiotics, including the time-consuming and costly process, scientists have directed their attention towards alternative solutions for the therapy and prevention of MRSA infections. Monoclonal antibodies, immunotherapies, bacteriophages and vaccines are being investigated as potential solutions. These include classical vaccines, recombinant vaccines, toxoid vaccines, DNA vaccines, new adjuvants and nanotechnology. The current literature on understanding immune responses and developing vaccines and immunotherapies for MRSA infections is largely based on animal models, despite the fact that these do not have a good predictive value in the evaluation of vaccine efficacy. The global distribution of the agent results in a high degree of antigenic diversity, which has the potential to reduce the efficacy of vaccine candidates. Furthermore, the potential for a vaccine to protect against different forms of MRSA is currently unclear. Consequently, MRSA infections represent a grave threat to human and animal health. Despite the existence of numerous potential vaccines against these infections, none is currently in clinical use. It is imperative to investigate the various antigenic properties of the bacteria to identify the optimal vaccine, necessitating further research in this area.

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CHAPTER 39

One Health Approach to Digital Health Technologies: Integrating Human and Veterinary Medicine

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1.INTRODUCTION

Health sciences have evolved beyond a discipline focused solely on the diagnosis and treatment of diseases and have increasingly embraced a holistic approach that considers human, animal, and environmental health as interconnected components. Globalization, climate change, intensive animal production systems, urbanization, and ecosystem degradation have contributed to the emergence and spread of complex health challenges such as zoonotic diseases, antimicrobial resistance (AMR), and newly emerging infections. Addressing these multifaceted challenges effectively requires strong interdisciplinary collaboration. In this context, the One Health approach has gained a central position within the field of health sciences (World Health Organization [WHO], 2017).

The One Health approach provides a scientific and policy framework emphasizing that human, animal, and environmental health cannot be considered independently. Supported by the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the World Organisation for Animal Health (WOAH), this approach highlights the shared responsibility of human and veterinary medicine, particularly in areas such as zoonotic diseases, food safety, environmental risks, and antimicrobial resistance (FAO, WHO, & WOAH, 2019). Consequently, the development of sustainable and effective solutions in health sciences depends on strengthening interdisciplinary interactions across these domains.

In recent years, the digital transformation of health care has further enhanced the applicability of the One Health approach. Digital health technologies encompass a broad range of tools, including sensors, biosensors, wearable devices, mobile health (mHealth) applications, big data analytics, and artificial intelligence-based decision support systems. These technologies enable continuous monitoring of both human and animal health, facilitate early diagnosis, and support disease prevention strategies (Topol, 2019). Digital health has not only transformed clinical practice but has also fundamentally reshaped research methodologies within health sciences.

In human medicine, digital health applications have improved health care delivery through remote patient monitoring, personalized medicine, artificial intelligence-assisted diagnostic systems, and clinical decision support tools. These technologies offer significant advantages, particularly in the management of chronic diseases and the surveillance of infectious disease outbreaks (Keesara, Jonas, & Schulman, 2020). Similarly, in veterinary medicine, sensor-based monitoring systems have become increasingly prevalent in herd health management, animal welfare assessment, and the early detection of diseases (Neethirajan, 2017).

However, digital health technologies in human and veterinary medicine are often addressed in isolation, limiting the full realization of their potential synergies. Challenges such as zoonotic diseases, antimicrobial resistance, and environmentally driven health risks require integrated data sources and coordinated analytical approaches. At this point, the One Health framework enables digital health technologies to function as a bridge between human and veterinary medicine, supporting integrated health surveillance and intervention strategies (Destoumieux-Garzón et al., 2018).

From a research perspective, addressing digital health technologies through a One Health lens introduces innovative methodological approaches. Sensor-based data collection, real-time monitoring, and artificial intelligence and machine learning algorithms enable comparative analyses of human and animal health data. These approaches support the development of more predictive, comprehensive, and evidence-based models in health sciences (Rashid et al., 2020).

This book chapter aims to examine digital health technologies within the framework of the One Health approach from both human and veterinary medicine perspectives. The chapter first outlines the conceptual foundations of One Health and then introduces the core components of digital health technologies. Subsequently, digital health applications in veterinary medicine and human medicine are discussed under separate sections, followed by an evaluation of shared application areas within the One Health framework. Finally, innovative research methods in digital health, current challenges, and future perspectives are addressed.

In this respect, the chapter seeks to provide an interdisciplinary perspective for researchers, clinicians, and policymakers working in health sciences, highlighting the shared potential of human and veterinary medicine in the field of digital health.

2. CONCEPTUAL FRAMEWORK OF THE ONE HEALTH APPROACH

The One Health approach is based on the understanding that human, animal, and environmental health are interdependent and form a dynamic and integrated whole. This approach argues that addressing health problems through a single discipline or focusing on only one species is insufficient, emphasizing the necessity of multidisciplinary and integrated solutions. In particular, the increasing prevalence of zoonotic diseases, environmental degradation, and global health threats has positioned the One Health approach at the center of health sciences literature (Zinsstag et al., 2011).

The foundations of the One Health concept can be traced back to the nineteenth century, when Rudolf Virchow introduced the concept of “zoonosis”

and emphasized the link between human and animal diseases. However, the development of One Health into an institutionalized and systematic framework accelerated in the early twenty-first century following global outbreaks such as SARS, avian influenza, and Ebola. During this period, the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the World Organisation for Animal Health (WOAH) incorporated the One Health approach into global health policies as a core strategic component (FAO et al., 2019).

One of the key pillars of the One Health approach is the control and prevention of zoonotic diseases. It is estimated that approximately 60% of infectious diseases affecting humans and nearly 75% of emerging infectious diseases originate from animals (Jones et al., 2008). This reality necessitates coordinated intervention strategies, shared data systems, and early warning mechanisms developed collaboratively by human and veterinary medicine. The One Health framework provides a scientific and operational model to support this integration.

Another critical issue addressed within the One Health approach is antimicrobial resistance (AMR). The widespread and often uncontrolled use of antimicrobials in both human and animal health has contributed to the emergence of resistant microorganisms, posing a significant global public health threat. Addressing AMR solely from a clinical or veterinary perspective is inadequate; environmental factors, the food chain, and ecosystem dynamics must also be taken into account (McEwen & Collignon, 2018). The One Health approach promotes integrated surveillance and research strategies to combat antimicrobial resistance more effectively.

Environmental factors constitute the third fundamental component of the One Health approach. Climate change, biodiversity loss, water and soil pollution, and other environmental challenges exert direct and indirect effects on both human and animal health. Environmental changes are known to alter disease ecology, shift the geographical distribution of vector-borne diseases, and create new health risks (Destoumieux-Garzón et al., 2018). Accordingly, the One Health approach underscores the necessity of integrating environmental data into health sciences research.

One of the most significant contributions of the One Health approach to health sciences is the strengthening of a culture of interdisciplinary research. Collaboration among human medicine, veterinary medicine, environmental sciences, epidemiology, bioinformatics, and public health enables the development of more comprehensive and predictive health models. Beyond practical applications, this approach also contributes to the evolution of research methodologies employed in health sciences (Hitziger et al., 2018).

The rapid advancement of digitalization in the health sector has further enhanced the applicability of the One Health approach. Digital health

technologies enable the simultaneous collection, analysis, and interpretation of data related to humans, animals, and the environment. This capability has transformed One Health from a predominantly theoretical framework into a practical and measurable model with tangible outcomes (Rabinowitz et al., 2017).

The conceptual framework presented in this section provides a foundation for understanding how the One Health approach can be integrated with digital health technologies. In the subsequent section, the core concepts of digital health technologies and their role in health sciences will be examined in detail, followed by an assessment of their applications in human and veterinary medicine from a One Health perspective.

3. DIGITAL HEALTH TECHNOLOGIES: FUNDAMENTAL CONCEPTS

Digital health technologies refer to the systematic use of information and communication technologies in the delivery, monitoring, and evaluation of health services. The World Health Organization defines digital health as a broad framework encompassing eHealth, mobile health (mHealth), wearable technologies, telemedicine, big data analytics, and artificial intelligence–based applications (World Health Organization [WHO], 2021). These technologies have become fundamental tools that transform both practice and research methodologies in health sciences.

One of the core components of digital health technologies is sensor and biosensor systems. Sensors are devices that convert physiological, biochemical, or environmental parameters into digital data. Biosensors, in particular, are specialized sensors capable of detecting biological signals such as enzymatic reactions, antigen–antibody interactions, and metabolic changes. These technologies enable continuous and real-time monitoring of parameters including body temperature, heart rate, respiration, physical activity, and metabolic indicators in both human and animal health (Turner, 2013).

Wearable technologies represent a major subcategory of sensor-based digital health applications. Smartwatches, implantable sensors, and body-integrated devices allow uninterrupted collection of health-related data during daily activities. Compared with conventional clinical measurements, wearable devices provide long-term and ecologically valid data, thereby supporting a more comprehensive assessment of health status (Piwek et al., 2016).

Another fundamental element of digital health technologies is the concept of big data. High-volume, high-velocity, and high-variety data generated from sensors, wearable devices, electronic health records, and environmental monitoring systems form complex datasets that are difficult to analyze using traditional statistical methods. Big data analytics enables the extraction of

meaningful patterns from these datasets and supports the development of predictive models in health sciences (Raghupathi & Raghupathi, 2014).

At this point, artificial intelligence (AI) and machine learning algorithms have become integral components of digital health technologies. Artificial intelligence is widely applied in image analysis, pattern recognition, risk prediction, and decision support systems. Machine learning algorithms can learn from historical data and generate predictions for new situations, thereby supporting early diagnosis and proactive health management. AI applications in health sciences hold significant potential for improving diagnostic accuracy and optimizing clinical decision-making processes (Topol, 2019).

Another key component of digital health technologies is clinical decision support systems. These systems integrate clinical, laboratory, sensor-based, and environmental data to provide evidence-based recommendations to health professionals. Decision support systems are particularly valuable in addressing complex, multi-variable health problems, as they help reduce human error and promote standardized approaches to care. Within the One Health context, such systems facilitate the joint evaluation of human and animal health data (Sutton et al., 2020).

Telemedicine and remote monitoring applications also constitute essential elements of digital health technologies. Remote monitoring systems enable the follow-up of individuals' or animals' health status without requiring physical examinations. These applications offer substantial advantages, particularly in rural areas, during pandemic situations, and in conditions requiring continuous monitoring (Keesara et al., 2020).

The significance of digital health technologies in health sciences extends beyond clinical practice. These technologies also transform research methodologies by enabling real-time data collection, long-term monitoring, and multi-source data integration. Such capabilities provide new opportunities for epidemiological studies, risk modeling, and comparative health research. Within the One Health framework, this transformation supports the development of shared research infrastructures addressing both human and animal health (Rashid et al., 2020).

The fundamental concepts outlined in this section provide a theoretical basis for understanding how digital health technologies integrate with the One Health approach. In the following section, the applications of these technologies in veterinary medicine will be examined in detail, focusing on their roles in animal health management, welfare assessment, and disease control.

4. DIGITAL HEALTH APPLICATIONS IN VETERINARY MEDICINE

Veterinary medicine is one of the health disciplines in which digital health technologies have been adopted relatively early and where field-based applications have expanded rapidly. The scalable nature of animal production systems, the concept of herd health, and the limited feasibility of continuous individual clinical monitoring have contributed to the increasing importance of sensor-based monitoring and data-driven approaches in veterinary practice. Digital health applications provide veterinarians with new opportunities for disease prevention, early detection, and the improvement of animal welfare (Neethirajan, 2017).

Sensor-based monitoring systems constitute the foundation of digital health applications in veterinary medicine. Through these systems, physiological parameters such as body temperature, activity level, feeding and drinking behavior, rumen pH, heart rate, and respiratory rate can be continuously monitored. Wearable sensors used particularly in cattle and small ruminants enable the detection of behavioral and physiological changes before clinical signs of disease become apparent, thereby supporting early intervention strategies (Caja et al., 2016).

Herd health management represents one of the most widespread applications of digital health technologies in veterinary medicine. Herd-level data collection and analysis systems allow individual animal data to be evaluated within a holistic framework. This approach facilitates the early identification of conditions that negatively affect productivity, such as mastitis, metabolic disorders, and reproductive problems. Digital monitoring systems contribute to veterinarians assuming not only a therapeutic role but also a preventive and managerial role in animal health (Rutten et al., 2013).

The assessment of animal welfare is another area in which digital health applications are gaining increasing importance in veterinary medicine. Traditional welfare assessments are typically based on observational and periodic measurements, whereas digital systems enable continuous and objective monitoring of animal behavior. Activity sensors, camera-based behavioral analysis systems, and environmental monitoring tools allow the evaluation of indirect indicators related to stress, pain, and comfort levels (Broom, 2011). This development supports the quantification and standardization of animal welfare within the context of health sciences.

Artificial intelligence and machine learning applications are also becoming increasingly prevalent in veterinary medicine. Image processing techniques facilitate automated lameness detection, body condition scoring, and the identification of behavioral abnormalities. In addition, machine learning algorithms can analyze historical herd data to generate disease risk prediction

models and provide early warning systems for veterinarians. Such applications serve as supportive tools in clinical decision-making processes (Kamphuis et al., 2016).

Digital health technologies also offer significant potential for the optimization of antimicrobial use in veterinary medicine. Sensor-based early detection systems contribute to the timely identification of diseases, thereby reducing unnecessary or delayed antimicrobial treatments. This approach strengthens the role of veterinary medicine in combating antimicrobial resistance within the One Health framework (McEwen & Collignon, 2018).

Despite the growing adoption of digital health applications in veterinary medicine, several limitations remain. Issues related to data accuracy, sensor calibration, the influence of environmental conditions on measurements, and insufficient data management infrastructure represent major challenges. Furthermore, the effective use of digital systems requires the development of veterinarians' knowledge and skills related to digital technologies (Eastwood et al., 2019).

In conclusion, digital health technologies are transforming clinical and field practices in veterinary medicine, promoting more proactive and data-driven approaches to animal health, welfare, and herd management. When integrated with human medicine within the One Health framework, these applications create significant synergy for the surveillance and management of shared health threats. In the following section, digital health applications in human medicine will be examined, and similarities and complementary aspects between the two fields will be discussed from a comparative perspective.

5. DIGITAL HEALTH APPLICATIONS IN HUMAN MEDICINE

Digital health applications in human medicine have fundamentally transformed the delivery of health care services, enabling more accessible, continuous, and personalized models of care. Wearable technologies, remote monitoring systems, artificial intelligence–assisted diagnostic tools, and digital decision support systems have contributed to the development of innovative, patient-centered and clinician-oriented approaches in clinical practice. Digital health technologies offer significant advantages, particularly in early diagnosis, preventive medicine, and the management of chronic diseases (Topol, 2019).

Remote patient monitoring systems form the cornerstone of digital health applications in human medicine. These systems allow continuous monitoring of physiological parameters such as heart rate, blood pressure, oxygen saturation, glucose levels, and physical activity during patients' daily lives. Remote monitoring reduces hospital admissions and provides clinicians with real-time data, facilitating timely interventions and improved clinical outcomes (Keesara, Jonas, & Schulman, 2020).

Wearable technologies play a central role in the widespread adoption of digital health applications in human medicine. Smartwatches, biometric sensors, and mobile health applications enable individuals to actively monitor their own health status and share data with health care professionals. Longitudinal data obtained from these devices constitute a valuable resource for assessing disease progression and developing personalized treatment strategies (Piwek et al., 2016).

Artificial intelligence and machine learning applications have driven a major transformation in diagnostic processes in human medicine. Deep learning algorithms used in medical imaging fields such as radiology, pathology, and dermatology have improved the early detection of diseases with high accuracy. In addition, clinical decision support systems analyze patient data to provide evidence-based recommendations, supporting standardized and optimized diagnostic and therapeutic decision-making processes (Esteva et al., 2019).

Digital health applications also play an increasingly important role in public health and epidemic management. Electronic health records, mobile applications, and digital contact tracing systems offer effective tools for monitoring infectious diseases and detecting outbreaks at early stages. The COVID-19 pandemic has clearly demonstrated the critical role that digital health technologies can play in global health emergencies (Whitelaw et al., 2020).

Despite their advantages, the widespread implementation of digital health applications in human medicine presents several challenges. Issues related to data privacy and security, ethical concerns, algorithmic bias, and digital inequalities represent significant barriers to adoption. In particular, the use of artificial intelligence-based systems in clinical decision-making requires careful consideration of transparency, accountability, and regulatory oversight (Char et al., 2018).

Digital health technologies in human medicine share many similarities with applications used in veterinary medicine, including continuous monitoring, early detection, data-driven decision-making, and preventive health strategies. These shared characteristics create opportunities for the integrated use of digital health data within the One Health framework, facilitating the development of comparative research models and coordinated responses to shared health threats.

In conclusion, digital health applications enhance the effectiveness and efficiency of health care delivery in human medicine while supporting a broader shift toward data-driven health sciences. Integrating these applications with veterinary medicine within the One Health framework provides a foundation for addressing complex health challenges through collaborative and interdisciplinary approaches. In the following section, shared digital health applications within the One Health perspective will be examined.

6. SHARED DIGITAL HEALTH APPLICATIONS FROM A ONE HEALTH PERSPECTIVE

One of the areas in which the One Health approach finds its strongest practical application is the shared use of digital health technologies across human and animal health domains. Global challenges such as zoonotic diseases, antimicrobial resistance, and environmentally driven health threats require integrated, data-driven solutions developed through close collaboration between human and veterinary medicine. Digital health technologies have emerged as key tools enabling this integration (Destoumieux-Garzón et al., 2018).

The surveillance and control of zoonotic diseases represent a primary application area for digital health technologies within the One Health framework. The simultaneous collection of clinical, epidemiological, and environmental data from both human and animal populations enables early detection of disease outbreaks and supports predictive modeling of disease transmission dynamics. Sensor-based monitoring systems, geographic information systems (GIS), and digital surveillance platforms are increasingly used as effective tools for tracking zoonotic pathogens (Rabinowitz et al., 2017).

Early warning systems for infectious disease outbreaks constitute another significant contribution of digital health technologies within the One Health perspective. Abnormal health indicators or behavioral changes observed in animal populations may serve as early signals of emerging threats to human health. The analysis of such data through digital platforms allows health authorities to implement timely interventions. These systems have proven particularly valuable in the monitoring of vector-borne diseases, where early detection is critical for effective control (Halliday et al., 2014).

Antimicrobial resistance (AMR) is another central area in which shared digital health applications play a critical role within the One Health framework. Digital recording and monitoring of antimicrobial use in both human and animal health systems contribute to a more comprehensive understanding of resistance development patterns. The integration of clinical, veterinary, and environmental data enables more effective surveillance and supports the design of coordinated intervention strategies to combat AMR (McEwen & Collignon, 2018).

An important component of shared digital health applications in the One Health context is the comparative use of health data. The comparative analysis of similar physiological, biochemical, and behavioral indicators across human and animal populations facilitates a deeper understanding of disease mechanisms. This approach is particularly valuable for translational research and contributes to the development of innovative research models in health sciences (Zinsstag et al., 2011).

Digital health technologies also facilitate the integration of environmental data into health sciences research. Climatic variables, indicators of water and soil pollution, and air quality measurements can be analyzed alongside human and animal health data. This holistic data approach allows for more accurate assessment of the health impacts of environmental changes and strengthens the environmental dimension of the One Health approach (Destoumieux-Garzón et al., 2018).

Despite these advantages, the implementation of shared digital health applications within the One Health framework faces several challenges. Differences in data standards, limitations in cross-sectoral data sharing, and inconsistencies in ethical and legal regulations pose significant barriers. Furthermore, the integration of human and animal health data requires careful consideration of data privacy, security, and governance issues (Rashid et al., 2020).

In conclusion, digital health technologies serve as essential enablers of the One Health approach by supporting the integration of human, animal, and environmental health data. Shared digital health applications enhance the surveillance of zoonotic diseases, enable early warning systems for emerging threats, and strengthen efforts to address antimicrobial resistance. In the following section, innovative research methods in digital health will be discussed in detail within the context of the One Health framework.

7. INNOVATIVE RESEARCH METHODS IN DIGITAL HEALTH

The integration of digital health technologies into health sciences has transformed not only application domains but also research methodologies. In addition to traditional clinical and field-based studies, continuous data streams generated by sensors, big data infrastructures, and artificial intelligence-based analytical methods enable the development of more dynamic, predictive, and holistic research approaches. This transformation is particularly relevant within the One Health framework, as it supports the joint investigation of human and animal health (Rashid et al., 2020).

Sensor-based data collection systems constitute the foundation of innovative research methods in digital health. Wearable devices, environmental sensors, and biosensors generate high-resolution, time-series data encompassing physiological, behavioral, and environmental parameters. Such data provide significant advantages for the early detection of disease-related changes and the long-term monitoring of health status in both human and veterinary medicine (Neethirajan, 2017).

Big data analytics represents another key methodological advancement in digital health research. High-volume, multi-dimensional datasets derived from sensors, electronic health records, and digital monitoring platforms require

analytical approaches beyond conventional statistical techniques. Big data analytics enables the identification of meaningful patterns across large populations and supports more accurate risk stratification and population-level health assessments (Raghupathi & Raghupathi, 2014). Within the One Health context, this approach facilitates comparative analyses of data from human and animal populations.

Artificial intelligence (AI) and machine learning algorithms play a central role in contemporary digital health research. Supervised and unsupervised learning methods enable the identification of complex relationships within health data and support the development of predictive models. Techniques such as image analysis, natural language processing, and time-series modeling enhance diagnostic accuracy and enable proactive health management strategies in both clinical and field settings (Topol, 2019).

The increasing use of real-time monitoring and longitudinal study designs represents another important methodological innovation in digital health research. Continuous data acquisition allows researchers to examine temporal changes in health status with greater precision. Longitudinal digital health studies contribute to a deeper understanding of disease progression and support the development of early intervention strategies (Piwek et al., 2016).

Within the One Health framework, data integration and comparative analytical approaches are of particular importance. The joint analysis of human, animal, and environmental data within a unified analytical framework enables a more comprehensive understanding of complex health challenges. Such integrative methodologies are especially valuable for research on zoonotic diseases and antimicrobial resistance, where multi-sectoral interactions play a critical role (Zinsstag et al., 2011).

Despite their potential, innovative digital health research methods also introduce ethical and methodological responsibilities. Issues related to data privacy, protection of personal health information, algorithmic bias, and the interpretability of analytical models require careful consideration. In particular, ensuring the transparency and validation of artificial intelligence-based models is essential for maintaining scientific rigor and trust in health sciences research (Char et al., 2018).

In conclusion, innovative research methods in digital health contribute to the development of more comprehensive and integrated approaches in health sciences. Sensor-based data collection, big data analytics, and artificial intelligence applications support the joint investigation of human and animal health within the One Health framework. In the following section, the current

challenges and limitations associated with the implementation of these approaches will be discussed.

8. CURRENT CHALLENGES AND LIMITATIONS

Despite the significant opportunities offered by digital health technologies and the One Health approach in health sciences, several challenges and limitations hinder their effective and sustainable implementation. Failure to adequately address these limitations may reduce the impact of digital health applications and lead to methodological weaknesses in scientific outputs. Therefore, the integration of digital health technologies within the One Health framework must be evaluated through a critical and balanced perspective.

One of the primary challenges in digital health applications is the lack of data integration and standardization. Data related to human, animal, and environmental health are often collected using different formats, measurement methods, and objectives. This heterogeneity complicates interdisciplinary data sharing and comparative analyses. For the One Health approach to be effectively implemented, the development of harmonized data standards and interoperable information infrastructures is essential (Rashid et al., 2020).

Another major limitation concerns data quality and accuracy. Sensor-based systems may be affected by environmental conditions, technical malfunctions, and calibration errors. These issues are particularly prominent in veterinary field applications, where data loss and measurement variability occur more frequently. Such limitations can negatively influence data reliability and compromise the validity of research findings (Neethirajan, 2017).

Ethical considerations and data privacy have become increasingly important with the widespread adoption of digital health technologies. In human medicine, the protection of personal health information, patient confidentiality, and data security represent fundamental ethical principles. In veterinary medicine, ethical concerns extend to data related to animal owners, farm management practices, and commercially sensitive information. Within the One Health framework, the integration of human and animal health data further increases the complexity of ethical and legal responsibilities (Char et al., 2018).

The use of artificial intelligence and machine learning-based systems introduces additional challenges related to algorithmic bias and transparency. These systems inherently reflect the characteristics and limitations of the datasets on which they are trained. Incomplete or imbalanced data may result in biased predictions and misleading outcomes. Ensuring the interpretability, validation, and accountability of algorithms is therefore critical, particularly when digital tools inform clinical or field-based decision-making processes (Topol, 2019).

Infrastructure and cost-related constraints represent another important barrier to the widespread adoption of digital health applications. Advanced sensor

systems, data storage platforms, and analytical software may be difficult to access, especially in low- and middle-income settings. Such disparities increase the risk of digital health inequities and limit the global applicability of the One Health approach (World Health Organization [WHO], 2021).

Furthermore, the effective implementation of digital health technologies requires adequate human resources and training. Both human and veterinary health professionals must develop sufficient digital literacy to interpret data accurately and integrate digital tools into decision-making processes. Insufficient training and capacity building may prevent the full realization of the potential benefits offered by digital health technologies (Eastwood et al., 2019).

In conclusion, the effective integration of digital health technologies within the One Health framework depends on recognizing and addressing technical, ethical, methodological, and structural challenges. A transparent acknowledgment of these limitations is essential for improving research quality and developing sustainable, reliable digital health solutions. In the following section, the future perspectives of digital health and the One Health approach will be discussed.

9. FUTURE PERSPECTIVES

The role of digital health technologies and the One Health approach in health sciences is expected to become increasingly prominent in the coming years. Technological advancements, the complex nature of global health threats, and the growing need for interdisciplinary collaboration necessitate a shared future perspective for human and veterinary medicine. In this context, digital health is regarded as a strategic tool that enhances the operational capacity of the One Health approach (WHO, FAO, & WOA, 2019)

In the future, digital health technologies are expected to become more predictive and personalized. Advanced sensor systems integrated with genomic, metabolomic, and other omics-based data will enable earlier identification of disease risks at both individual and population levels. Such developments will contribute to the strengthening of preventive medicine in both human and animal health (Topol, 2019).

The expanded and more reliable use of artificial intelligence and machine learning applications in health sciences will represent a cornerstone of future digital health ecosystems. The development of explainable artificial intelligence (XAI) approaches will improve transparency and acceptability of algorithms used in clinical and field-based decision-making processes. This progress is expected to accelerate the adoption of digital decision support systems in both human and veterinary medicine (Char et al., 2018).

From a One Health perspective, one of the most significant future developments will be the establishment of shared data infrastructures and global

digital platforms. The harmonized collection and exchange of human, animal, and environmental health data will enhance the surveillance of zoonotic diseases, support early warning systems for emerging outbreaks, and strengthen efforts to combat antimicrobial resistance. Ongoing initiatives led by international organizations play a crucial role in reinforcing the institutional capacity of the One Health framework (Rabinowitz et al., 2017).

In the future, the integration of digital health and One Health approaches is expected to extend beyond research and clinical practice into health policy and education. Incorporating digital health, data analytics, and One Health-oriented content into the curricula of human and veterinary medicine will be essential for ensuring sustainability of this transformation. Moreover, policy makers should support digital health investments through a One Health lens to maximize public health impact (Hitziger et al., 2018).

The increasing effects of climate change and environmental degradation on health further underscore the importance of the One Health approach in the coming decades. Digital health technologies facilitate the integration of environmental data with health indicators, enabling more effective management of climate-sensitive health risks. This integration strengthens the link between environmental sustainability and digital transformation in health sciences (Destoumieux-Garzón et al., 2018).

In conclusion, digital health technologies enhance the scientific and practical potential of the One Health approach, offering a new paradigm for health sciences. Coordinated action between human and veterinary medicine within this paradigm enables the development of more effective, integrated, and sustainable responses to global health threats. In the final section, the key findings and implications discussed throughout this chapter will be synthesized in a general conclusion and evaluation.

10. CONCLUSION

This book chapter has examined digital health technologies within the framework of the One Health approach, highlighting the complementary roles of human and veterinary medicine in health sciences through a holistic perspective. As contemporary health challenges become increasingly complex and multidimensional, interdisciplinary approaches that jointly address human, animal, and environmental health have become essential. In this context, the One Health approach, when supported by digital health technologies, offers a powerful and applicable model for health sciences (World Health Organization [WHO], 2017).

The initial sections of the chapter focused on the conceptual foundations of the One Health approach, emphasizing zoonotic diseases, antimicrobial resistance, and the impacts of environmental change on health. It was demonstrated that addressing these challenges within a single disciplinary

framework is insufficient and that collaborative knowledge production and data sharing between human and veterinary medicine are necessary to develop more effective solutions (Zinsstag et al., 2011; FAO et al., 2019).

Subsequent sections discussed the fundamental concepts of digital health technologies, including sensors, biosensors, wearable technologies, big data analytics, and artificial intelligence applications, highlighting their transformative role in health sciences. These technologies serve similar functions in both human and veterinary medicine, particularly in continuous monitoring, early diagnosis, and preventive health strategies. This convergence creates significant opportunities for the development of shared digital infrastructures and research models within the One Health framework (Topol, 2019; Neethirajan, 2017).

Digital health applications in veterinary medicine were shown to provide tangible benefits in areas such as herd health management, animal welfare monitoring, and optimization of antimicrobial use. In human medicine, remote patient monitoring, artificial intelligence–assisted diagnostic systems, and digital decision support tools enhance the effectiveness and efficiency of health care delivery. Integrating these experiences within the One Health perspective enables more effective management of shared health threats (McEwen & Collignon, 2018; Keesara et al., 2020).

The chapter also addressed innovative research methods in digital health, including sensor-based data collection, big data analytics, and artificial intelligence–driven models. These methods support the integrated and comparative analysis of human and animal health data, thereby strengthening the scientific foundation of the One Health approach (Rashid et al., 2020). At the same time, limitations related to data integration, ethical responsibilities, infrastructure constraints, and digital inequalities were acknowledged as critical considerations.

From a future-oriented perspective, the integration of digital health technologies with the One Health approach has the potential to establish a new paradigm in health sciences. The development of shared data platforms, expansion of interdisciplinary education programs, and alignment of policy frameworks with One Health principles are crucial for ensuring the sustainability of this transformation (Hitziger et al., 2018; Destoumieux-Garzón et al., 2018).

In conclusion, this chapter demonstrates that addressing digital health technologies through a One Health lens contributes to the development of more effective, predictive, and sustainable solutions in health sciences. The strategic use of digital tools combined with collaborative knowledge generation between human and veterinary medicine creates a strong synergy in responding to global health threats. This integrated approach is expected to serve as a guiding framework for future research, policy development, and practical applications in health sciences.

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CHAPTER 40

Effects of the Gut Microbiota on the Reproductive System and Gametogenesis: Fundamental Mechanisms and Pathophysiological Relationships

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1. Introduction

The gut microbiome is defined as the collective community of microorganisms, including bacteria, archaea, yeasts, fungi, and protozoa, that inhabit a specific environment within the gastrointestinal tract (Turner, 2018). The terms host microbiome, microflora, and microbiota are frequently used synonymously to refer to all microorganisms residing in a particular ecological niche. Historically, the term "microbiota" originates from the suffix "-biota," denoting the living organisms within an ecosystem. A more nuanced definition distinguishes microbiota as the assemblage of microbial taxa themselves, while the microbiome encompasses the comprehensive catalog of these microorganisms along with their collective genetic material. Both terms are applicable for describing microbial communities, with the "microbiome" explicitly adopting a holistic "-ome" perspective that integrates their genetic information (Bleich & Fox, 2015; Ursell, Metcalf, Parfrey, & Knight, 2012).

While the terms microbiome and microbiota are frequently used interchangeably, they represent distinct concepts. A seminal definition by Berg et al. (2020), clarifies this distinction: microbiota denotes the community of living microorganisms within a defined environment, whereas the microbiome encompasses a broader ecological framework, including the microbiota, their collective genetic material (genomes), their metabolic products, and their environmental habitat (Berg et al., 2020). In the context of the gut, scientific inquiry predominantly focuses on bacteria, as they constitute the source of over 98% of the genetic material isolated from this site. Indeed, the gastrointestinal tract harbors the body's most abundant and complex microbial ecosystem, with estimates exceeding 500 bacterial species, the majority of which are yet to be fully classified. Despite significant research efforts over more than a decade, the comprehensive functional significance of this intricate community remains largely elusive (Turner, 2018). It is also noteworthy that while many microorganisms are viable, only a select few fulfill the stringent criteria required to be classified as probiotics with demonstrated beneficial properties. Various techniques based on immunological, biochemical, molecular biological, and microbiological characteristics have been developed to determine and express the microbiota of the gastrointestinal system from the animal intestine and colon. A thorough and complete elucidation of the genomic functions encoded by the gut microbial community (microbiota) and their collective genetic repertoire (microbiome) is a fundamental prerequisite. This deeper understanding will pave the way for the precise modulation of targeted probiotic strains and facilitate the development of novel or refined strategies for therapeutic microbiome interventions (ÖZTÜRK).

The composition and functional activity of the gut microbiota exert profound and wide-ranging influences on host physiology. The gut microbiome is integral

to host health, with its most fundamental role being the regulation of nutrient and energy harvest through the digestion of otherwise indigestible dietary components. Beyond this primary metabolic function, microbial influence extends to critical processes including the development and maintenance of the immune system, the modulation of behavior, and the regulation of reproductive function. (De Jonge, Carlsen, Christensen, Pertoldi, & Nielsen, 2022).

The gut microbiome (GM) is frequently termed the "second human genome," a designation highlighting its vast genetic capacity—comprising over 3 million genes, which starkly contrasts with the approximately 23,000 genes in the human genome. This complex microbial entity constitutes a vital component of the gastrointestinal system and is increasingly regarded as a metabolic and endocrine organ of the host. By dynamically interacting with dietary inputs and environmental factors, this extensive bacterial community is essential for maintaining the homeostatic equilibrium between the host's internal milieu and the external environment, thereby establishing itself as a cornerstone of overall health. (W. Chen et al., 2024). Comprising a vast array of diverse species, the gut microbiota (GM) engages in intricate crosstalk with multiple host organ systems. This interaction is mediated through a multitude of well-established and emerging molecular mechanisms, thereby contributing to systemic physiological regulation (Y. Chen, Zhou, & Wang, 2021). The gut microbiota (GM) functions as a central regulator of host metabolism, immune responses, and endocrine signaling. Its metabolic activities directly govern essential physiological processes, including lipid and bile acid metabolism, the synthesis of vitamins and short-chain fatty acids (SCFAs), gene expression, and xenobiotic metabolism. (Walter & Ley, 2011). The human gut microbiota is predominantly composed of the Bacteroidetes and Firmicutes phyla. Their relative abundance is not static but undergoes dynamic shifts across different stages of the host's life cycle (Eckburg et al., 2005; Walter & Ley, 2011). The gut microbiota influences gametogenesis (oogenesis and spermatogenesis) through numerous mechanisms. The aim of this section is to describe how the gut microbiota (GM) affects the gametogenesis process through various mechanisms, such as microbial metabolites, epigenetic changes, sex hormones, and the immune system, by affecting the blood-testis barrier.

2. Gut Microbial Metabolites and Mechanisms of Action

The gut microbiota produces a wide variety of bioactive molecules through the fermentation and metabolism of dietary components. These microbial metabolites can reach reproductive organs far from the local gut environment, shaping the host's reproductive physiology by affecting both direct cellular pathways and epigenetic programming.

2.1. Short-Chain Fatty Acids (SCFA) and Their Direct Effects on the Reproductive System

The fermentation of non-digestible carbohydrates by the gut microbiota represents a central metabolic function, with short-chain fatty acids (SCFAs) as the principal end products. These metabolites, including acetate (C2), propionate (C3), and butyrate (C4), play pivotal roles in regulating host glucose and lipid metabolism, immune function, and systemic energy homeostasis. The production of SCFAs through colonic fiber fermentation is a key mechanism by which commensal bacteria contribute to maintaining physiological health (Canani et al., 2011). Short-chain fatty acids (SCFAs) are key microbial metabolites generated in the cecum and colon through the bacterial fermentation of indigestible dietary components. The primary substrates for this process include various forms of dietary fiber (such as resistant starch and cellulose) and, to a lesser extent, proteins and peptides. While numerous members of the gut microbiota contribute to SCFA synthesis, major producers are found within the Firmicutes and Bacteroidetes phyla, which include many commensal and probiotic species essential for maintaining metabolic health (Deleu, Machiels, Raes, Verbeke, & Vermeire, 2021; Fu et al., 2025). These metabolites exert a significant influence on host physiology by modulating glycemic control, immune modulation, and the integrity of the intestinal epithelial barrier. Through the regulation of these fundamental processes, SCFAs play a central role in promoting systemic metabolic health and providing resistance against inflammatory challenges. (Chambers, Preston, Frost, & Morrison, 2018). The effects of short-chain fatty acids (SCFAs) on male reproductive health include: improving sperm quality parameters, primarily sperm motility, concentration, and morphology; supporting testosterone secretion and testicular development by increasing antioxidant enzyme activities (GPx, SOD); and promoting spermatogenesis by regulating impaired gut microbiota and through lipid metabolism. Furthermore, research indicates that SCFAs can ameliorate impaired sperm parameters, such as concentration and viability, in metabolic disorders like type 2 diabetes. This beneficial effect is mediated through a multi-faceted mechanism involving the modulation of gut microbial ecology towards a more favorable profile, the attenuation of systemic inflammation, and subsequent positive influences on testosterone metabolism and overall metabolic homeostasis, which may indirectly support the availability of essential fatty acids (Alhaj et al., 2018; W. Chen et al., 2024; Hao et al., 2022; Yan et al., 2022; Zhou et al., 2023).

Beyond their metabolic roles, short-chain fatty acids (SCFAs) contribute to intestinal barrier integrity by modulating the expression of key structural proteins. Notably, butyrate serves as a principal regulator, directly enhancing the expression of tight junction proteins that seal the paracellular space between epithelial cells (Fu et al., 2025; Mörkl et al., 2018). The functional significance of these cellular junction complexes extends far beyond the gut. Tight and gap

junction proteins are indispensable for the physiology of reproductive organs. Key examples include: the gap junction protein connexin-43 (CX43), essential for follicular development and communication in the ovary; the tight junction proteins claudin-3 (CLDN3) and claudin-4 (CLDN4), critical for endometrial receptivity and successful embryo implantation in the uterus; and claudin-11 (CLDN11), the foundational component of the blood-testis barrier (BTB) that creates an immunoprivileged microenvironment essential for protecting spermatogenesis. Decreases in the expression of these proteins have been shown to result in the cessation of follicular development, decreased implantation rates, and spermatogenesis disorders, respectively. These findings suggest that mechanisms observed in pathologies characterized by intestinal barrier or motility disorders may lead to reproductive system dysfunction via similar junctional proteins (Kanatsu-Shinohara, Ogonuki, Matoba, Ogura, & Shinohara, 2020; Martínez-Peña et al., 2017; Smith & Braun, 2012; Winterhager & Kidder, 2015).

The gut microbiota exerts a significant modulatory influence on the female reproductive system, with notable impacts on ovarian physiology and follicular development. Acting as an environmental sensor, it helps optimize reproductive output when conditions favor offspring viability. A key mechanism involves microbial metabolites, including short-chain fatty acids (SCFAs), which regulate the gene networks controlling the maintenance, activation, and regression (atresia) of the primordial follicle reserve. Disruption of these microbial signals causes aberrant pathway regulation, resulting in premature follicle activation and depletion. Notably, administering SCFAs to germ-free animals can rescue this defective phenotype. Primordial follicles, situated near the ovarian surface and characterized by a metabolically quiescent state, are especially vulnerable to circulating factors of microbial origin and systemic disturbances like oxidative stress. Importantly, a specific postnatal developmental window (e.g., around day 12) exists during which microbial colonization permanently influences ovarian function, coinciding with microbiota maturation. This evidence underscores the microbiota's essential role in maintaining the ovarian follicular reserve and promoting reproductive longevity. Consequently, strategically modifying microbial populations presents a novel therapeutic avenue for enhancing and protecting female fertility (Munyoki et al., 2024).

Beyond their direct actions, short-chain fatty acids (SCFAs) also exert an indirect influence on reproductive capacity by modulating bone metabolism and systemic calcium homeostasis. One primary mechanism is the enhancement of intestinal calcium absorption through the acidification of the gut lumen (D'Amelio & Sassi, 2018). This regulation of calcium availability is vitally important for reproduction, given that calcium ions are essential for key fertilization events, including sperm motility, capacitation, and the acrosome reaction (Vyklícká & Lishko, 2020). Consequently, a state of gut microbial

imbalance (dysbiosis) can impair fertility by simultaneously disrupting the production of SCFAs and the associated metabolic pathways governing calcium utilization (Cai et al., 2022).

2.2. Regulatory Role of Microbial Metabolites via Epigenetic Mechanisms

Gut microbiota (GM) and epigenetic regulation are dynamic processes similarly affected by external factors such as environmental factors and diet. This parallelism suggests that they may share common ground in regulating host physiology and that a functional link may exist between these two systems (Ashonibare, Akorede, Ashonibare, Akhigbe, & Akhigbe, 2024).

Epigenetic mechanisms enable the control of gene expression without permanent changes in the DNA sequence. Commensal microbiota can shape the host's epigenetic profile through various pathways, primarily short chain fatty acids (SCFAs) produced by the fermentation of dietary fibers. Butyrate, one of these acids, acts as a potent histone deacetylase (HDAC) inhibitor, altering chromatin structure, particularly in intestinal cells, and reprogramming gene expression at the epigenetic level (Ashonibare et al., 2024).

Epigenetic regulation occurs primarily through interrelated mechanisms such as histone modifications (e.g., acetylation), DNA methylation, and non-coding RNAs, playing a critical role in cell function and environmental adaptation (Canani et al., 2011; SARI & Ramazan). Acetate, another important metabolite of GM, also acts through a similar HDAC inhibition mechanism. This effect positively supports testicular function, testosterone synthesis, and consequently spermatogenesis by reducing oxidative stress and the NF- κ B-mediated inflammatory response (Ashonibare et al., 2024).

2.3. Effects of Microbiota-Mediated Nutrient and Vitamin Metabolism

The differentiation and maturation of germ cells are critically supported by a specialized microenvironment created by Sertoli cells and their intercellular connections, which coordinate the precise exchange of nutrients and metabolic waste. As the testes lack the capacity for de novo synthesis of most essential nutrients, they depend entirely on systemic delivery. Nutrients, including vitamins and cofactors metabolized or transformed by the gut microbiota (GM), are transported via the bloodstream to the testicular interstitium. From there, they are actively distributed through the intricate network of seminiferous tubules, ultimately reaching the germinal epithelium via Sertoli cells and their junctional complexes. Consequently, a state of gut dysbiosis can directly compromise testicular homeostasis by disrupting this vital, microbiota-dependent nutrient supply (Cai et al., 2022; Dance, Thundathil, Wilde, Blondin, & Kastelic, 2015).

2.3.1. Retinoic Acid and Folic Acid

Vitamins are micronutrients essential for maintaining systemic homeostasis, primarily functioning as enzymatic cofactors. While humans cannot synthesize most vitamins *de novo* and must acquire them through diet, certain gut bacteria contribute to their chemical synthesis and bioavailability (Said, 2013).

Vitamin A and its active metabolite, retinoic acid (RA), are indispensable for reproductive function and embryonic development. In the male reproductive system, RA acts as a critical signaling molecule that induces dormant spermatogonial stem cells to initiate meiosis and differentiate into mature spermatozoa. A deficiency in vitamin A disrupts this process, preventing the differentiation of type A spermatogonia into type A1 cells and thereby impairing spermatogenesis. This dysfunction is further evidenced by pathological changes, such as the abnormal replacement of the epididymal epithelium with a squamous keratinized layer, which contributes to reduced sperm output (Wolbach & Howe, 1925). The gut microbiota (GM) critically regulates vitamin A homeostasis through three interconnected mechanisms: modulating its absorption, metabolism, and bioconversion. First, GM-derived factors can inhibit hepatic cytochrome P450 (CYP) enzymes, which catabolize vitamin A, thereby preserving its systemic levels. Second, GM influences the expression of host proteins involved in the cellular transport and uptake of vitamin A precursors. Third, specific microbial enzymes directly contribute to the conversion of dietary β -carotene into retinoic acid (RA). Collectively, these actions underscore the GM's integral role in determining the bioavailability of active vitamin A metabolites for reproductive and systemic functions (Cai et al., 2022; Stacchiotti, Rezzi, Eggersdorfer, & Galli, 2021). The primary cause of vitamin A deficiency is insufficient vitamin A or β -carotene in the diet. The secondary cause is the breakdown of vitamin A and β -carotene in the digestive system, which prevents their absorption or disrupts the conversion of β carotene to vitamin A, even when sufficient amounts are consumed in the diet (AYAŞ & İNAL, 2025).

Dietary folate plays a crucial role in supporting male reproductive health, particularly in mitigating damage from exposure to reproductive toxicants. It contributes to improved semen parameters and maintains testicular architecture. Folate confers protection upon germ cells against oxidative stress and inflammatory insults, thereby mitigating DNA damage and suppressing apoptotic pathways. Furthermore, it safeguards the processes of germ cell proliferation and differentiation from the deleterious effects of reactive oxygen species (Shalaby, El Zorba, & Ziada, 2010; Swayne et al., 2012).

The body acquires folate through two principal routes: dietary intake (from foods and supplements) and *de novo* bacterial synthesis within the gut. Intestinal bacteria synthesize the active form, tetrahydrofolate (THF), from precursor molecules including GTP, erythrose 4-phosphate, and phosphoenolpyruvate.

This microbially produced THF is subsequently absorbed by colonocytes via the proton-coupled folate transporter (PCFT) and enters systemic circulation. Metagenomic analyses indicate that numerous gut residents, such as *Bacteroides fragilis*, *Lactobacillus plantarum*, and *Streptococcus thermophilus*, possess the genetic machinery for folate synthesis. Notably, genera like *Lactobacillus* and *Bifidobacterium* are considered major contributors to the production and cycling of folate within the gut ecosystem (J. Wu et al., 2022). Low levels of these two vitamins can negatively affect fertility by leading to hyperhomocysteinemia, a disease associated with in vitro fertilization (IVF) failure and reduced sperm concentration, motility, and DNA integrity (Cai et al., 2022).

2.3.2. Nitric Oxide (NO)

The gut microbiota also exerts its effects on reproductive health through the production of signaling molecules such as nitric oxide (NO), in addition to vitamin and hormone metabolism and amino acid conversions. For example, it has been reported that nitrates and their metabolites can cause vitamin A deficiency (AYAŞ & İNAL, 2025). The amino acid arginine serves as a critical substrate for male reproductive physiology, largely due to its role as a precursor for nitric oxide (NO) synthesis. The gut microbiota influences this pathway through the metabolism of arginine. While various intestinal bacteria, including certain *Lactobacillus* and *Bifidobacterium* species, can modulate arginine availability and utilization, it is important to note that *Staphylococcus aureus* is typically characterized as a pathobiont or pathogen rather than a commensal regulator of this metabolic pathway. The microbial modulation of arginine catabolism represents one mechanism by which the gut ecosystem can indirectly affect systemic NO-dependent processes (G. Wu et al., 2009).

Nitric oxide (NO) is a pivotal signaling molecule with essential and diverse functions throughout the male and female reproductive tracts. Within the male system, NO facilitates neuroendocrine regulation by stimulating hypothalamic neurons to secrete gonadotropin-releasing hormone (GnRH). This, in turn, promotes the release of pituitary gonadotropins, leading to increased synthesis of sex steroids such as testosterone and thereby supporting sexual drive and function. In the testis, it contributes to local heat regulation of the organ by dilating blood vessels. It also plays a role in the fundamental mechanism of penile erection ; NO released from cavernous body nerve endings activates the guanylate cyclase enzyme, leading to the production of cyclic guanosine monophosphate (cGMP). This process enables erection by relaxing the smooth muscles in the corpus cavernosum (Cai et al., 2022; Gratzke et al., 2010). In the female reproductive system, NO facilitates egg transport, fertilization, and embryo transport by reducing smooth muscle contractions in the fallopian tubes. In the cervix, which has the highest NO activity among the genital organs, it functions in the cervical maturation process (AYAŞ & İNAL, 2025). These

findings indicate that the gut microbiota plays an active role in regulating various aspects of reproductive physiology via the arginine-NO pathways.

3. Effects of Gut Microbiota on Barrier Function and Systemic Communication Pathways

The gut microbiota affects the reproductive system not only through metabolites but also by regulating the integrity of physical barriers and systemic communication between organs. This section discusses the critical relationships established by the microbiota between the intestinal epithelial barrier and distant reproductive tissues. A healthy microbiota controls intestinal permeability and the local immune response, preventing harmful molecules (such as lipopolysaccharides) from entering the systemic circulation. Furthermore, it transmits genetic and protein-based signals to distant organs via newly discovered transport systems such as extracellular vesicles. Any disruption in these mechanisms (dysbiosis) can lead to increased intestinal permeability, chronic inflammation, and impaired intercellular communication, negatively affecting reproductive processes from spermatogenesis to implantation.

3.1. Regulation of Blood-Testis Barrier (BTB) Integrity by the Gut Microbiota

The testes possess a unique immunoprivileged status, meaning they are protected from the body's own immune surveillance to safeguard the developing germ cells. This protection is crucial because male haploid germ cells (spermatozoa) express antigens that are not present during the establishment of immune self-tolerance in early life. If recognized, these "new" antigens could trigger an autoimmune attack. To prevent this, the blood-testis barrier (BTB) creates a physical and immunological shield around the seminiferous tubules, isolating the post-meiotic germ cells from the systemic immune system and thereby maintaining the immunoprivileged microenvironment essential for spermatogenesis (Qu et al., 2020).

The seminiferous tubules, where spermatogenesis occurs, are enclosed by a specialized basement membrane. Crucially, the blood-testis barrier (BTB) is not this membrane itself but a dynamic structure formed by tight junctions between adjacent Sertoli cells near the tubule's base. This BTB subdivides the tubule into basal and adluminal compartments, creating the unique, immunoprivileged microenvironment essential for meiosis and spermatid development (Cheng & Mruk, 2002; Guo et al., 2024; Morita, Furuse, Fujimoto, & Tsukita, 1999).

The gut microbiota (GM) is a key modulator of systemic immunity and the specialized immunoprivileged microenvironment of the testes. This crosstalk is bidirectional: a healthy GM contributes to testicular immune homeostasis, while dysbiosis can precipitate its breakdown.

Mechanistically, the GM influences systemic immune tone through metabolites like short-chain fatty acids (SCFAs) and microbial components such as Polysaccharide A (PSA) from *Bacteroides fragilis*. These factors help maintain a balance between pro-inflammatory and regulatory immune cells. Immune cells educated in the gut-associated lymphoid tissue can subsequently traffic to distant sites, including the testes, via systemic routes such as the mesenteric lymphatics and bloodstream, thereby influencing the local immune milieu.

Under conditions of dysbiosis, this regulatory balance is lost. An increase in gut-derived pro-inflammatory signals can lead to the activation of testicular resident immune cells (e.g., macrophages). Furthermore, a loss of immune tolerance mechanisms may allow innate immune cells to attack sperm antigens in the epididymis, impairing sperm viability and compromising fertility (Zheng et al., 2021).

The blood-testis barrier (BTB) is composed of Sertoli cells interconnected by adhesion junctions (AJ) and tight junctions (TJ) that involve proteins like occludin, claudins (CLDN), and zonula occludens proteins (ZO-1, ZO-2, ZO-3) (Mruk & Cheng, 2015). Development of Sertoli cells and the assembly of tight junctions between them are supported by the gut microbiota (GM). This contribution facilitates the structural organization of the seminiferous tubules and helps maintain the homeostatic balance of the spermatogenic microenvironment. The significance of this role is particularly clear in germ-free mouse models, which exhibit a substantial decrease in the expression of adhesion and tight junction proteins, including occludin, ZO-2, and E-cadherin (Al-Asmakh et al., 2014). Normal GM colonization, on the other hand, positively supports the development of the blood-testis barrier (BTB) by regulating androgen secretion. In this process, the binding of secreted testosterone to receptors on Sertoli cells increases the expression of the claudin-3 protein, which is critical for the integrity and tightness of the BTB (Meng, Holdcraft, Shima, Griswold, & Braun, 2005). Therefore, the effect of GM on the BTB occurs both directly at the cellular level and indirectly through hormone mediated pathways. Shifts in the composition of the gut microbiota (GM) can modify intestinal barrier function and systemic immune tone. This occurs via microbial metabolites, endotoxins, and pro-inflammatory mediators, which collectively influence the immune milieu of the testis and can lead to impairment of the reproductive system (Cai et al., 2022).

3.2. Effects of Dysbiosis, Lipopolysaccharide Translocation, and Chronic Inflammation on Reproductive Organs

Numerous studies have shown that disruption of the gut microbiota can promote the overgrowth and proliferation of harmful bacteria, which can lead to increased lipopolysaccharide (LPS) content and, as a result, increased permeability due to damage to the intestinal mucosal barrier, allowing enteric LPS to enter the bloodstream (Guo et al., 2024; Hussain et al., 2021). LPS that

enters the systemic circulation reaches peripheral organs such as the testes and triggers a pro-inflammatory response via Toll-like receptor 4 (TLR4) found on cells there. This signaling pathway leads to increased production of potent inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1 β) in testicular tissue (Guo et al., 2024). This resulting chronic, low-grade inflammatory state directly disrupts spermatogenesis by causing atrophy in the spermatogenic tubules and apoptosis (programmed cell death) in germ cells. The effects of dysbiosis and LPS translocation on the reproductive system are not limited to direct inflammation. This process can lead to inflammation (orchitis) in the testes and epididymis due to excessive activation of the immune system (T. Chen et al., 2024; Guo et al., 2024; Sarkar et al., 2011). At the same time, systemic inflammation increases insulin resistance and resistance to gastrointestinal hormones such as leptin and ghrelin. These metabolic and hormonal imbalances disrupt the secretion balance of key reproductive hormones that regulate spermatogenesis, such as follicle-stimulating hormone (FSH), luteinizing hormone, and testosterone (T. Chen et al., 2024; Y. Wang & Xie, 2022).

The process of spermatogenesis proceeds continuously within the seminiferous epithelium of the adult testis. This process is subject to stringent regulation, principally by steroid hormones and gonadotropins (Hess & De Franca, 2009; Ramaswamy & Weinbauer, 2014; Sofikitis et al., 2008). An imbalance in these hormonal functions can compromise spermatogenesis and lead to infertility. Furthermore, obesity and microbial dysbiosis resulting from a high-fat diet (HFD) are demonstrated to directly impair sperm motility, mediated through changes in the gut microbiota profile (Deshpande et al., 2019; Guo et al., 2024). Consequently, dysbiosis-induced increased intestinal permeability and LPS translocation contribute to spermatogenesis damage and male infertility through both direct testicular inflammation and systemic hormonal imbalances. (Ding et al., 2020; Guo et al., 2024).

In conclusion, increased intestinal permeability and LPS translocation caused by dysbiosis contribute to spermatogenesis damage and male infertility through both direct testicular inflammation and systemic hormonal imbalances.

3.3. Effects of Microbiota-Derived Extracellular Vesicles (Exosomes) on the Reproductive System

Exosomes, a class of extracellular vesicles ranging from 30 to 150 nanometers in diameter, are secreted from cells via a specific exocytosis pathway. These nanovesicles transport bioactive cargo—including lipids, proteins, and nucleic acids—that varies according to their cell of origin, and they are recognized as significant facilitators of intercellular communication (C. Chen et al., 2023; Doyle & Wang, 2019; Kalluri & LeBleu, 2020). In the context of reproductive biology, exosomes have been reported to play a role in regulating processes such

as gametogenesis, embryogenesis, and embryonic development (Machtinger, Laurent, & Baccarelli, 2016). Notably, exosomes present in seminal plasma play a vital role in key processes of male fertility, such as sperm maturation, capacitation, the acrosome reaction, and fertilization (C. Chen et al., 2023). Many functions of exosomes are mediated by the microRNAs (miRNAs) they carry and transfer to target cells, which are approximately 19-22 nucleotides in length [25]. It has been demonstrated that an imbalance in the gut microbiota (dysbiosis) has a direct effect on the content profile of gut derived and circulating exosomes. Dysbiosis significantly alters the expression levels of miRNAs carried in these exosomes; for example, it increases miR-211-5p levels while decreasing miR 122-5p levels (T. Chen et al., 2024). Studies have shown that miR 211-5p, which abnormally increases in circulating exosomes originating from the gut after dysbiosis, is transported to the testes via the bloodstream. In the testes, this miRNA was found to suppress the expression of Meioc, a protein necessary for the progression of meiosis in spermatogenesis. This mechanism reveals that dysbiosis-induced impaired gut exosomes can carry harmful signals to distant testicular tissue and cause spermatogenesis dysfunction by interrupting the meiotic process through Meioc inhibition (T. Chen et al., 2024). This finding suggests that exosome-mediated miRNA transport may represent a novel communication pathway linking gut microbiota and reproductive health.

4. Effects of Gut Microbiota on the Reproductive Endocrine System

cholesterol serves as the fundamental precursor for the biosynthesis of sex steroid hormones across animal species. Both male (testosterone) and female (estrogen and progesterone) sex hormones share a largely common biosynthetic pathway (Miller & Auchus, 2011). Estrogen regulates numerous reproductive functions, including estrus behavior, increased serum concentrations, ovulation, uterine proliferation, and endometrial gland secretion (Hussain et al., 2021; Nelson & Habibi, 2013). The regulation of male reproductive functions is fundamentally governed by gonadotropin-releasing hormone (GnRH) and the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These hormones act primarily on Leydig and Sertoli cells within the testes to direct the processes of steroidogenesis and spermatogenesis. Testosterone, the most predominant androgen, is essential for maintaining the functional integrity of the testes and the male accessory sex organs. Additionally, the stimulation of testosterone synthesis involves other reproductive hormones such as prolactin, while estrogen plays a significant role in the negative feedback loop within the hypothalamic-pituitary-gonadal (HPG) axis (Dutta, Sengupta, & Muhamad, 2019).

4.1. Effects on Testosterone Metabolism and Action

Emerging research indicates that the gut microbiota serves as a significant modulator of reproductive physiology, influencing the secretion of reproductive hormones as well as sperm motility and concentration (Ding et al., 2020; Guo et al., 2024; Mayneris-Perxachs et al., 2020). Analyses have demonstrated that the gut microbiota plays a pivotal role in the intestinal metabolism of testosterone, particularly through a process known as deglucuronidation. In this process, microbial enzymes, primarily hydroxysteroid dehydrogenases (HSD), reconvert testosterone that has been conjugated with glucuronic acid in the liver for detoxification and thereby rendered biologically inactive back into its free and biologically active form. As a result of this microbial reactivation, the concentration of potent androgens is elevated in the colonic contents of healthy young male individuals. This finding underscores the direct impact of microbial metabolism on hormonal activity within the colonic environment, where bacterial density is at its highest. (Hussain et al., 2021). In one experimental model, a microbiota-dysbiosis group exhibited significantly lower serum testosterone concentrations and reduced mRNA expression of genes involved in testosterone biosynthesis relative to controls (Guo et al., 2024). It is thought that GM may be a primary regulatory factor in testosterone production and metabolism. The study conducted by Tang et al. (2024) elucidates a novel mechanism through which the gut microbiota regulates host sex hormone levels. The research demonstrated that the administration of colistin, a narrow-spectrum antibiotic, induced a relatively selective reduction in the abundance of *Akkermansia* bacteria within the gut. This alteration led to the inhibition of bacterial-derived inosine production, which subsequently resulted in the downregulation of serum testosterone levels in male mice. The observed decrease in testosterone was further associated with a disruption of the immunosuppressive microenvironment in the testes. Moreover, the research demonstrated that inosine supplementation was able to restore testosterone secretion. This effect is likely attributable to the enhancement of the intestinal mucus barrier and the attenuation of circulating lipopolysaccharide (LPS) levels. Together, these results identify a novel signaling pathway through which a specific gut microbiota-derived metabolite, inosine, can modulate host endocrine function (Tang et al., 2024).

4.2. Role in Bile Acids and Steroid Hormone Metabolism

The gut microbiota exerts a direct and substantial influence on steroid hormone metabolism via the enzymatic conversion of bile acids. Specific bacteria, including *Clostridium scindens*, transform host-derived primary bile acids into secondary bile acids, which possess distinct biological properties. More importantly, these microbial enzymatic processes can directly contribute to testosterone synthesis by catalyzing the cleavage of side chains of steroids such as glucocorticoids (Ridlon et al., 2013). Consequently, bile acids and microbial

enzymes such as hydroxysteroid dehydrogenase (HSDH) are regarded as key metabolites that, although host-produced, are functionally modified by the microbiota to modulate endocrine signaling (W. Chen et al., 2024; Lv et al., 2024). The impact of this microbial bile acid metabolism is evident in the reproductive systems of both sexes. Notably, bile acids are reported to be present in human ovarian follicular fluid at concentrations exceeding those in serum (Ellwood, Kramer, & Dutta, 2025; Nagy et al., 2019). This suggests their entry into the follicular microenvironment, either through local synthesis by granulosa cells or via active transport from the circulation.

Bacteria specialized in bile acid metabolism, such as *Turicibacter*, can produce products that increase vitamin D absorption by deconjugating these acids. Increased vitamin D bioavailability may positively affect the levels of hormones associated with ovarian function and folliculogenesis, such as anti-Müllerian hormone (AMH) and melatonin (Ellwood et al., 2025; Li et al., 2023; L. Wang et al., 2024). In conclusion, a healthy microbiota supports steroid hormone homeostasis and local signaling in reproductive tissues through bile acid transformations. Conversely, dysbiosis (e.g., disruption of *Turicibacter* balance) can negatively affect fertility by leading to disturbances in bile acid metabolism, reduced vitamin D absorption, and ultimately irregularities in critically important reproductive hormones such as AMH and testosterone. All these mechanisms demonstrate that GM has a decisive effect on sperm production and motility by modulating BTB permeability and hormone levels.

4.3. Effects on Estrogen Metabolism (Estrobolome)

The gut microbiota and estrogens engage in a reciprocal interplay, each significantly modulating the activity and homeostasis of the other. The microbiota is not only affected by estrogen levels, but also actively regulates estrogen metabolism and, consequently, systemic estrogen levels (Qi, Yun, Pang, & Qiao, 2021). The molecular basis for this reciprocal interaction is the "estrobolome," a collection of genes encoding bacterial enzymes capable of metabolizing estrogens (Ashonibare et al., 2024; Baker, Al-Nakkash, & Herbst-Kralovetz, 2017). The key mechanism in the functioning of the estrobolome is the microbially derived β -glucuronidase enzyme. This enzyme converts estrogens that are conjugated (bound) for detoxification in the liver into their deconjugated (free) and biologically active forms (Ervin et al., 2019). The released estrogens enter the systemic circulation and exert their effects by regulating gene expression in target tissues via estrogen receptor alpha ($ER\alpha$) and beta ($ER\beta$) (Plottel & Blaser, 2011). Thus, a healthy gut microbiota contributes to maintaining homeostasis in distant sites, including the intestinal mucosa (Ashonibare et al., 2024). However, disruption of gut microbial balance (dysbiosis) perturbs this finely tuned metabolic regulation, including the activity of β -glucuronidase. Such disturbance can result in abnormal levels of circulating free estrogen, which

ultimately exerts adverse effects on reproductive health. The interaction between estrogen metabolism and the gut microbiota is central to female reproductive health and overall physiology. The expression of estrogen receptor beta (ER β) and serum concentrations of steroidal hormones like estradiol change dynamically across the lifespan. The gut microbiota is instrumental in modulating these hormones, as evidenced by the decline in estrogen levels observed following antibiotic treatment (Adlercreutz, Pulkkinen, Hämäläinen, & Korpela, 1984; Qi et al., 2021). The fundamental mechanism of this regulation is governed by microbial genes responsible for estrogen metabolism, termed the "estrobolome." The microbially derived enzyme β -glucuronidase converts conjugated (inactive) estrogens in the liver into their biologically active, unconjugated forms in circulation (Plottel & Blaser, 2011). A healthy microbiota maintains systemic estrogen homeostasis through this process. A microbial imbalance, or dysbiosis, accompanied by reduced gut microbiota diversity, diminishes β -glucuronidase activity. This situation reduces the conversion of estrogen and phytoestrogens into active forms, leading to decreased circulating estrogen levels (Plottel & Blaser, 2011; Qi et al., 2021).

Certain bacterial species play specific roles in this process. For example, the genus *Ligilactobacillus* can potentially reduce circulating levels through estrogen conjugation and breakdown (Aragón, Jurado, Jara, Rodríguez, & Orgaz, 2024). The *Akkermansia* genus has been reported to be directly linked to steroid biosynthesis and to exhibit high sensitivity to estrogen (Sakamuri et al., 2023). These observations indicate that certain bacterial genera, such as *Acinetobacter*, may impair reproductive processes, whereas others like *Ligilactobacillus* and *Akkermansia* could serve protective or beneficial functions (Ellwood et al., 2025). Moreover, the microbiota's composition is itself shaped by steroid hormones. In murine models, for instance, the relative abundance of specific phyla—including *Lactobacillales*, *Proteobacteria*, *Bacteroidetes*, and *Firmicutes*—has been shown to vary in association with ER β status. (Menon et al., 2013; Qi et al., 2021).

5. Conclusion

In conclusion, the gut microbiota profoundly affects both hormonal homeostasis and reproductive health as a key regulator of estrogen metabolism; dysbiosis, on the other hand, can pave the way for various pathologies by disrupting this delicate balance. The relationship between the gut microbiota and reproductive health emerges as an interdisciplinary and multi-layered network of interactions. As discussed in this review, the microbiota directly regulates fundamental processes such as spermatogenesis, folliculogenesis, hormone homeostasis, and epigenetic programming through metabolites such as short-chain fatty acids, bile acids, and vitamins. At the same time, it supports the integrity of protective structures such as the intestinal epithelial barrier and the

blood-testis barrier, preventing harmful antigens and pro-inflammatory signals from reaching the reproductive organs; it can even communicate with distant organs by carrying genetic information via exosomes . An imbalance (dysbiosis) in these systems can contribute to the pathophysiology of both male and female infertility by causing malabsorption, chronic low-grade inflammation, hormonal irregularities, and disruptions in cellular communication. In light of these findings, the gut microbiota emerges as a new target and regulatory system to be considered in the preservation of reproductive health and infertility treatments. The development of microbiota-based therapeutic approaches (pre/pro/postbiotics, dietary modulation) and the evaluation of microbial profiles in personalized treatment protocols offer a promising perspective for the future of reproductive medicine.

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CHAPTER 41

The Weighted Functional Sound Teeth (WFS-T) Model:A Conceptual and Methodological Advancement over the FS-T Index

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INTRODUCTION

According to the World Health Organization (WHO), approximately 60%–90% of adults experience at least one episode of dental caries during their lifetime (1). Dental caries remains one of the most prevalent chronic diseases worldwide and continues to directly affect quality of life, masticatory function, and the economic burden on health care systems.

Reliable and biologically consistent measurement tools are essential for monitoring population oral health, identifying epidemiological trends, and planning effective preventive strategies. For nearly a century, the Decayed, Missing, and Filled Teeth (DMFT) index has served as one of the principal indicators in global oral health research (2). In public health planning and policy development, dental public health professionals have relied predominantly on composite epidemiological measures such as DMFT to communicate oral health status. Consequently, DMFT has been extensively applied in epidemiological surveys, national oral health assessments, and clinical studies (3).

The underlying assumption of the DMFT index is that dental status can be adequately represented by the cumulative number of decayed (D), missing (M), and filled (F) teeth (T) or surfaces (S) (1). Despite its widespread use, DMFT has several well-documented limitations (4–6). It combines disease experience and treatment history within a single score, assigns equal weight to decayed, missing, and filled teeth, and excludes sound teeth from functional consideration. Moreover, DMFT fails to reflect the biological depth of carious lesions, the functional importance of individual teeth, the quality of restorative treatment, and the underlying reasons for tooth loss. As a result, individuals with identical DMFT scores may present markedly different clinical and functional conditions.

Because of these shortcomings, various alternative indices and indicators have been proposed to better describe oral health status. Measures that selectively weight DMFT components have been suggested as more sensitive tools for detecting differences between population groups. Featherstone emphasized that caries assessment should incorporate biological and behavioral factors (7), while Marthaler described DMFT as a “measurable but interpretatively limited” index (8).

Within this context, Sheiham and colleagues introduced alternative composite measures, including the Functional and Sound Teeth (FS-T) index (9). The FS-T approach assigns equal functional value to sound and restored teeth based on the assumption that they are functionally comparable. Previous studies have shown that FS-T provides a more realistic representation of dental status than DMFT and may better reflect functional oral health outcomes (10–13). Findings derived

from indices developed from FS-T, such as the Simplified Functional Sound Teeth (SFS-T) index, have also been consistent with these observations (14).

However, advances in clinical imaging techniques, restorative materials, and the understanding of caries progression have revealed important structural limitations of the FS-T index. Both DMFT and FS-T fail to differentiate between varying levels of tissue destruction and restoration extent. Early enamel lesions and extensive dentinal lesions approaching the pulp are assigned identical scores, despite their markedly different biological severity and treatment requirements. This limitation is particularly relevant in adult populations, in whom restorative burden and disease complexity may therefore be underestimated.

Functional tooth presence is a critical determinant of masticatory efficiency, oral integrity, and quality of life. Although FS-T has been preferred in some studies due to its conceptual simplicity, it does not account for restoration quality, endodontic burden, biological progression, or radiographic findings as described in the literature. Consequently, small enamel restorations, extensive dentinal restorations, and endodontically treated teeth are assigned the same functional value.

To overcome these limitations, the Weighted Functional Sound Teeth (WFS-T) model has been developed as a novel conceptual framework. The WFS-T model introduces a weighted scoring system that distinguishes restorative levels (f1–f3) while incorporating biological depth and functional contribution. Unlike FS-T, WFS-T aims to provide a more biologically and clinically realistic assessment of functional oral health.

The present study conceptually analyzes the structural and biological limitations of the FS-T index and evaluates how the proposed WFS-T model addresses these shortcomings.

MATERIALS AND METHODS

This study was designed as a conceptual–analytical investigation aimed at theoretically comparing the Functional Sound Teeth (FS-T) approach with the proposed Weighted Functional Sound Teeth (WFS-T) model. No quantitative field data were used. Instead, the evaluation was based on a systematic conceptual analysis of the structural, biological, and functional characteristics of both indices. No clinical examinations, radiographic evaluations, or human data were collected or analyzed.

The methodological framework consisted of four main stages.

1. Literature Review and Development of the Conceptual Framework

A comprehensive review of the literature was conducted focusing on functional tooth presence, restoration biology, weighted oral health indices, and the application principles of the FS-T approach. The review primarily addressed the following themes:

- Epidemiological relevance of functional tooth presence
- Biological and functional effects of dental restorations
- Relationship between endodontic burden, function, and biological integrity
- Strengths and methodological limitations of the FS-T index
- The necessity of weighted models in oral health assessment

This stage established the theoretical framework defining the parameters used for comparing the FS-T and WFS-T models .

2. Theoretical Analysis of the FS-T Model

The FS-T index was evaluated in terms of its calculation method, component structure, level of biological sensitivity, and capacity to represent functional status. The analysis focused on the following criteria:

- Ability to represent enamel–dentin–pulp progression
- Differentiation of restoration quality
- Inclusion of indicators related to functional capacity
- Compatibility with radiographic findings
- Potential utility as a clinical decision-support tool

This analysis conceptually demonstrated why the FS-T index fails to fully represent functional oral health, particularly in adult populations with high restorative and endodontic treatment burden .

3. Theoretical Construction of the WFS-T Model

The WFS-T model was developed as a weighted scoring system designed to account for both biological depth and functional value of restorations. The model is based on three restorative levels (f1–f3):

- **f1:** Restorations limited to the enamel level
- **f2:** Moderate restorations involving dentin

- **f3:** Extensive restorations approaching the pulp or endodontically treated teeth

The mathematical structure of the model is summarized by the following formula:

$$\text{WFS-T} = (\text{FS-T} - \text{F}) \times 3 + \text{F\_point}$$

Where:

- **FS-T** represents the number of functional sound teeth
- **F** represents the number of restored teeth
- **F_point** represents the weighted sum of restorations according to f1–f3 levels

Unlike the FS-T index, this structure explicitly incorporates biological phase, restorative depth, and functional compromise into the measurement process.

4. Theoretical Comparison Strategy

The FS-T and WFS-T models were compared across four principal dimensions:

- **Biological sensitivity:** Capacity to represent enamel, dentin, and pulp-level involvement
- **Evaluation of restoration quality:** Consideration of restoration extent and biological depth
- **Representation of functional capacity:** Association with masticatory efficiency, clinical stability, and endodontic burden
- **Conceptual compatibility with modern clinical practice:** Integration of digital radiography, detection of secondary caries, and advances in restorative material technology as described in the literature

This multidimensional comparative approach was used to identify the conceptual advantages of the WFS-T model over the FS-T index.

RESULTS

This theoretical analysis examined the structural limitations of the Functional Sound Teeth (FS-T) index and the conceptual advantages of the proposed Weighted Functional Sound Teeth (WFS-T) model across four major dimensions. Overall, the findings indicate that the WFS-T model offers a more biologically sensitive and functionally consistent conceptual representation of oral health compared with the FS-T approach.

1. Structural and Biological Limitations of the FS-T Index

1.1. Inability to Differentiate Restoration Quality

The FS-T index classifies teeth with minor enamel restorations, extensive dentinal restorations, and endodontically treated teeth within the same category. Consequently, the biological burden associated with restoration extent and tissue loss is not reflected, which may lead to an underestimation of disease severity.

1.2. Limited Representation of Functional Capacity

In the FS-T approach, the mere presence of a tooth in the oral cavity is treated as equivalent to functional value. Endodontic burden, restoration size, occlusal integrity, and long-term clinical stability are not incorporated into the scoring system.

1.3. Exclusion of Biological Stage

The progression from enamel to dentin and ultimately to pulpal involvement is not represented in the FS-T index. As a result, biologically distinct conditions are assigned identical functional scores.

1.4. Overgeneralization of Functional Contribution

All teeth are assigned the same coefficient regardless of their actual contribution to masticatory efficiency. This uniform weighting tends to obscure functional differences among teeth with varying restorative and biological status.

1.5. Inability to Incorporate Radiographic Findings

Radiographic indicators such as secondary caries, dentinal shadowing, and pulpal proximity of restorations cannot be quantitatively represented within the FS-T framework.

2. Theoretical Advantages of the WFS-T Model

2.1. Quantification of Restoration Quality

The f1–f3 categorization allows differentiation of restorations based on biological depth and functional risk. This structure enables restoration extent and tissue involvement to be numerically represented.

2.2. More Accurate Representation of Functional Capacity

Through weighted scoring, teeth with extensive restorations or endodontic treatment are assigned proportionally lower functional values. This approach suggests an assessment that more closely approximates theoretical masticatory capacity.

2.3. Integration of Biological Progression

The WFS-T model incorporates enamel-, dentin-, and pulp-level involvement into the scoring process, thereby reflecting differences in biological severity.

2.4. Compatibility with Modern Imaging Techniques

Findings described in the literature from digital radiography, including early dentinal invasion, secondary caries, and restoration-related radiolucencies, can be conceptually weighted within the WFS-T framework.

2.5. Increased Epidemiological Sensitivity

Weighted scoring produces a variance structure that more closely aligns with biological diversity. This balanced distribution may enhance the sensitivity of epidemiological comparisons.

2.6. Clinical Decision-Support Potential

By indirectly conveying information related to restorative burden, biological severity, and functional risk, the WFS-T model may provide clinically relevant insights that support decision-making processes.

3. Comparative Findings on Biological Representation

FS-T Index: The FS-T index does not distinguish between enamel, dentin, and pulpal involvement. Teeth with substantial biological compromise may be classified as functionally sound, resulting in an underrepresentation of disease burden.

WFS-T Model: Biologically critical conditions, such as deep dentinal lesions, extensive restorations, and endodontically treated teeth, are represented with higher weighted values, reflecting their relative biological and functional impact.

Result:

The WFS-T model suggests superior sensitivity in representing biological severity and caries progression compared with the FS-T index.

4. Ability to Reflect Functional Capacity

FS-T Index: All teeth present in the oral cavity are considered functionally equivalent, irrespective of restoration success, occlusal stability, or endodontic status.

WFS-T Model: Restoration type functions as a determining variable of functional value, and endodontic burden is reflected through reduced weighted scores.

Result:

The WFS-T model appears to provide a more nuanced representation of functional capacity than the FS-T approach.

5. Variance and Distribution Characteristics

The FS-T index exhibits an expanded variance and an imbalanced distribution, which may obscure meaningful differences between individuals or population groups. In contrast, the WFS-T model demonstrates a variance structure that is more consistent with biological diversity, offering conceptual advantages for epidemiological analyses.

6. Integration of Radiographic Findings

The WFS-T model conceptually incorporates radiographic indicators related to restorative depth and biological involvement, whereas the FS-T index lacks the capacity to numerically represent such findings.

Summary of Findings

Overall, the WFS-T model integrates biological progression, restoration quality, and functional capacity into a unified conceptual framework, whereas the FS-T index remains limited by its unidimensional structure. These findings suggest that the WFS-T model offers a clear theoretical advantage in the assessment of functional oral health.

DISCUSSION

This theoretical study demonstrates that the Functional Sound Teeth (FS-T) approach, although widely used to assess functional tooth presence, exhibits significant limitations when evaluated against contemporary clinical and epidemiological requirements. While FS-T was originally developed as a practical index to meet the epidemiological needs of its time, advances in restorative dentistry, imaging technologies, and the understanding of caries biology have rendered its unidimensional structure increasingly insufficient.

The core assumption underlying FS-T—that the mere presence of a tooth equates to functional value—is particularly problematic in adult populations. Teeth restored with extensive dentinal restorations, those requiring endodontic treatment, or those with compromised clinical stability cannot be considered functionally equivalent to minimally restored or sound teeth. The inability of FS-T to distinguish among these conditions may result in an overestimation of functional oral health and an underestimation of biological disease burden.

In contrast, the Weighted Functional Sound Teeth (WFS-T) model introduces a multidimensional conceptual framework that aligns more closely with modern

clinical paradigms. By differentiating restorative levels through the f1–f3 categorization, incorporating biological progression, and accounting for endodontic burden, WFS-T provides a biologically consistent and functionally meaningful assessment. This weighted structure allows functional value to decrease proportionally with increasing biological and restorative compromise, thereby reflecting clinical reality in a more nuanced manner.

Unlike FS-T, which focuses solely on anatomical presence, WFS-T integrates restoration extent, biological integrity, and functional stability into a single conceptual metric. This integration is particularly relevant in adult populations with high restorative loads, where functional capacity may differ substantially despite similar tooth counts. Teeth with restorations approaching the pulp or those that have undergone endodontic therapy are theoretically assigned lower functional values, whereas minimally restored enamel-level lesions exert minimal impact on functional capacity. Consequently, WFS-T translates biological severity into a quantitative functional framework.

Contemporary dental practice increasingly emphasizes digital imaging, minimally invasive restorative strategies, evaluation of restoration longevity, and assessment of endodontic prognosis. The FS-T index lacks the conceptual capacity to accommodate these parameters, limiting its relevance within modern diagnostic and treatment paradigms. In contrast, the WFS-T model is conceptually compatible with radiographic indicators and treatment-planning considerations as described in the literature, extending its potential relevance beyond descriptive epidemiology toward clinical decision-support contexts.

The theoretical nature of this study represents a limitation, as the findings are not derived from empirical field data. Nevertheless, the conceptual advantages identified strongly support the need for future validation studies. Comparative field-based investigations assessing FS-T and WFS-T at individual and population levels are required to evaluate the model's biological sensitivity, functional accuracy, and epidemiological performance.

Overall, the WFS-T model not only addresses the inherent limitations of FS-T but also advances functional oral health assessment toward a biologically grounded, multidimensional conceptual framework. These characteristics position WFS-T as a promising alternative for future epidemiological research and hypothesis-driven clinical investigations.

CONCLUSION

This theoretical study demonstrates that the Functional Sound Teeth (FS-T) approach has important limitations in meeting contemporary clinical and epidemiological requirements for the assessment of functional tooth presence. The unidimensional structure of FS-T fails to incorporate key parameters such as restoration quality, biological progression, endodontic burden, and functional stability. As a result, functional capacity—particularly in adult populations—may be overestimated, while biological disease burden may be inadequately represented.

The proposed Weighted Functional Sound Teeth (WFS-T) model addresses these shortcomings by integrating multidimensional parameters into the assessment process. By incorporating biological depth, restoration extent, functional contribution, and radiographic indicators as described in the literature, the WFS-T model offers a more comprehensive and biologically consistent conceptual evaluation of functional oral health. The f1–f3 weighted structure enables numerical differentiation of restorative stages from enamel-level involvement to pulpal proximity, allowing functional capacity to be represented in a more realistic manner.

The strong conceptual alignment of WFS-T with modern clinical practice, its ability to reflect biological severity, and its balanced variance structure provide a clear theoretical advantage over FS-T. These characteristics enhance its potential utility in epidemiological comparisons, population-based analyses, and hypothesis-driven clinical decision-making processes.

In conclusion, the WFS-T model emerges as a more comprehensive, biologically coherent, and clinically meaningful conceptual index for assessing functional oral health compared with the FS-T approach. Future validation using field data is essential to confirm its applicability in real populations and to support its potential adoption as a standard tool in functional oral health assessment.

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CHAPTER 42

The Importance of Preventive Oral Health Care Services in Protection Against Non-Communicable Diseases*

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1. Introduction:

Non communicable diseases (NCDs) also known as chronic diseases are one of the major challenges for health and sustainable human development. Among the different types of NCDs, the major ones – cardiovascular diseases, cancer, diabetes, chronic respiratory diseases and oral diseases, remains burden of health challenges globally. These diseases account for 41 million deaths each year, which is equivalent to 71% of global mortality, common among those aged 30-69, >15 million people die from NCDs annually. More than 85% of these premature deaths occur in low- and middle-income countries (Thwin, 2022).

Oral health is a key indicator of general health and well-being to attain a qualitative life. This is why oral diseases should be prioritized as part of the NCDs responses as they share common modifiable risk factors in one way or the other thereby, resulting in the interlink between both diseases. Oral health is integral to overall health and shares many of the same risk factors as tobacco use, unhealthy diet consumption, physical inactivity and alcohol intake (Wolf et al., 2021). For instance, excessive consumption of sugars contributes to intermediary risk factors, such as obesity, high blood glucose concentrations, and high blood pressure, that pose challenges across the entire life course. Sugars are thus a pivotal risk for diabetes, insulin resistance, and cardiovascular disease (Benzian, et al., 2023).

Excessive alcohol consumption predisposes individuals to cardiovascular diseases, cancer and contributes to poor oral hygiene status. Inadequate essential nutrients containing diets results in the decrease of systemic immunity as well as effects on oral health tissues. Dietary counseling or nutrition guidance are essential in dental practice because sugar consumption is linked to the onset of dental caries, and a high fat and high calory diet, associated with the onset of diabetes, increases the risk of exacerbation of periodontitis (Ariizumi et al., 2023). Poor oral hygiene due to inadequate brushing, use of fluoride containing toothpaste, antiseptic mouth wash can all contribute to bacterial induced oral infections. These behavioral risk factors can directly or indirectly influence the development of NCDs. Oral health behaviors (e.g., interdental cleaning, tongue brushing) were correlated with the daily intake of vitamins B12, K, and folic acid (Ariizumi et al., 2023).

The most common oral conditions favoring the predisposition of NCDs is Periodontitis- a chronic inflammatory disease which is highly prevalent worldwide, interacts with a variety of NCDs. It is a risk factor in the complex pathogenesis of diabetes mellitus and cardiovascular disease and plays a role in the development of endocarditis and recurrent pneumonia in elderly people (Dörfer et al., 2017).

Preventive oral and dental health is vital for healthy living which, however is compromised among many individuals due to high cost of treatment. Unequal

distribution of oral health professionals and a lack of appropriate health facilities to meet population needs in most countries means that access to primary oral health services is often low. Out-of-pocket costs for oral health care can be major barriers to accessing care (WHO, 2025). In an effort to reduce oral disease burden which can result in systemic inflammatory conditions, there is need for an early intervention and management of NCDs on the ground and achieving good oral hygiene status among individuals. Emphasis should be given to disadvantage groups of individual, where low socio-economic background remains the highest reason behind poor oral health care. The integration of oral health services into general health standard and universal health coverage must be emphasized in the global health system. Provision of oral health care services should not be undermined to individuals that have limited access to oral and dental health facilities including - geriatric oral health care, school oral and dental services, remote areas, middle urban groups, refugee camps and orphanages.

2. Pathophysiology Oro-Dental NCDs:

In the presence of poor oral hygiene, bacterial collections major- (*Staphylococcus aureus*, *Streptococcus viridians*, *S. sanguis*, *actinomycescomitans*, *P. intermedia*, *p. gingivalis*) induce plaque – A sticky colorless film of colonized bacteria that releases acids destroying tooth hard substance. If not hygienically removed, it becomes mineralized hard substance causing inflammation of the surrounding tissue supporting the teeth. This bacterium metabolizes and release by products which results in halitosis, gum recession and gradual tooth loss depending on the disease severity.

The inflammatory tissues harbor group of bacteria that transgress along area of least resistance disseminating through areas around head and neck regions. This simplifies their route to incorporate into blood circulation raising systemic inflammatory conditions. For instance, periodontitis induced bacterial cardiovascular inflammatory responses - Pathogens escape into bloodstream, attach to damaged cardiac tissue, and initiate an inflammatory response, ultimately leading to the characteristic lesions of Infective endocarditis (Falconer et al, 2024).

Since inflammation is a critical factor in the development of atherosclerosis, it is a major risk factor for heart disease, stroke, and peripheral artery disease. Elevated C-reactive protein (CRP) levels are linked to an increased risk of cardiovascular diseases, including heart attacks and stroke. Higher CRP levels are often seen in individuals with periodontal disease blood clotting takes place along the vasculature which affects other vital organs – heart; blood pressure, bacterial infective endocarditis, heart failure and stroke (Kurtzman., et al. 2025).

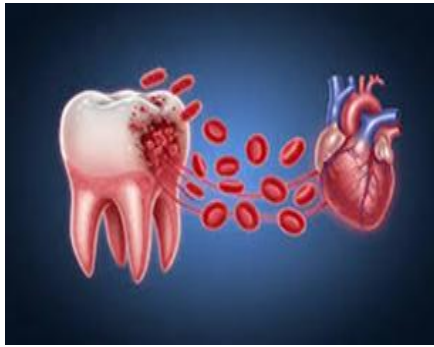


Figure 1. Pathogenic oral bacteria effects on heart

Poor oral health may also have effects on brain to some extent, causing diseases such as Alzheimer's and dementia. Pathogenic oral bacteria called spirochetes have been studied as a potential cause of the disease, with their presence being significantly higher in the brains of Alzheimer's patients. Emerging clinical and experimental studies indicate that these two conditions share overlapping risk factors and may be interconnected. One notable finding is the detection of specific periodontal pathogens, such as *Porphyromonas gingivalis* in the brains of individuals with Alzheimer's (Seyedmoalemi and Saied-Moallemi, 2025).

Oral bacterium is strongly interlinked with the prevalence of respiratory diseases. The relationship between oral microecosystem and respiratory diseases have been proved by plenty of studies. Indigenous oral microorganisms take a part in the occurrence and development of respiratory diseases via their pathogenicity and virulence factors when inhaled into respiratory tract or interact with respiratory pathogens (Dong et al., 2022). This is as a result of bacterial loads present in the saliva, inter dental spaces and throat secretions or aspirated during dental procedures which potentially lead to respiratory infections such as bronchitis, pneumonia, Chronic Obstructive Pulmonary Disease (COPD) and lung disease. Ventilator- associated Pneumonia (VAP) can also develop within 48hrs after mechanical ventilation where oral dental plaque and secretions can harbor bacteria that migrate along the endotracheal tube into the lungs. - Endotracheal intubation represents a significant risk factor for VAP. It affects the body's airway defenses, hinders the ability to cough and clear mucus, and causes the accumulation of secretions containing bacteria above the inflatable cuff of the endotracheal tube that are aspirated (Huang et al., 2025).

From the perspective of interlinkage between diabetes and oral diseases, poor oral health contributes to rise in blood glucose level due to bacterial growth in oral cavity. The prevalence of this disease is increasing as a public health problem and cause of death worldwide, and the number of people with diabetes is expected to double by 2030 (Khoshbakhti et al., 2025). Oral tissue infections, dry

mouth, opportunistic infections, excessive accumulation of plaque, changes in taste, candidiasis are some of the consequences of diabetes on oral health. In uncontrolled cases of diabetes in association with poor oral hygiene, fungal infections can be appreciated (candidiasis, lichen planus) and delayed healing process is encountered after dental procedures.

Among the most complicated oral inflammatory responses, periodontitis is the most common symptom indicating diabetic response in an individual. The underlying mechanisms of this interrelationship involve complex pathways, including inflammatory responses, altered immune functions, and microbial dysbiosis. The mechanistic insights into the interrelationship between periodontitis and diabetes mellitus revolve around the role of inflammation as a common link between the two diseases (Yang et al., 2024). Diabetic patients who lack information about treatment or whose blood sugar levels are not controlled are more likely to develop periodontitis. In addition, diabetic patients present with symptoms of periodontal diseases - periodontal pocket depth > 4, gingival inflammation, recession and gradual tooth loss.

Poor oral health is also significantly associated with the prevalence of cancers of the head and neck regions and to some extent those affecting distant body organs. This is well evidenced in many studies conducted by researchers with regards to the considerable efforts of eradicating the burden of this deadly disease. Oral and dental health disease shared common modifiable risk factors with NCDs contributing to the occurrence of cancers in the head and neck region. This includes cancers of the oral cavity, oropharynx, hypopharynx, and larynx. It is the seventh leading cancer in the world, with approximately 710000 new cases diagnosed worldwide annually (Bai et al., 2022). Oral cancer is a multifactorial lesion and the risk factors include tobacco and alcohol, chronic inflammation, ultra violet radiation, human papilloma virus (HPV) or Candida infections, immunosuppression, genetic predisposition, and diet (Irani., 2020).

Pathogenicity of the synergistic and interactive effect of combined alcohol and tobacco use is believed to be the highest risk combination causing deterioration of the oral mucosa, leading to the development of oral cancer. Leukoplakia, erythroplakia, lichen planus which if not tackled at earlier stage, progress to oral cancer that can be fatal in many cases (Bai et al., 2022). Locally, alcohol acts as a solvent that increases the absorption of carcinogens into mouth mucosa. Ethanol facilitates the uptake of environmental carcinogens, especially from tobacco smoking, through the cell membrane of oral keratinocytes made more permeable by its direct effect (Irani., 2020).

Among the modifiable risk factors, tobacco and alcohol are considered the major risk factors in the development of malignancies of oral cavity. Periodontal infection can lead to the spreading of inflammation far off the oral cavity and result in the increase of inflammatory markers of systemic circulation.

Compelling evidence has suggested that periodontally diseased patients are more likely to have pulmonary diseases, such as COPD and pneumonia (Verma., 2023). Oral radiation therapy in cancer patients has negative impacts on oral health. This is profoundly seen in case of radiotherapy of Oro-pharyngeal cancer, pancreatic, lung, and blood cancers (Irani., 2020). Smoking and chewing tobacco further increase the risk of oral cancer. Individuals infected with Human Papilloma Virus, especially (HPV 16) are at highest risk of developing HPV induced Oral cancer

3. Common Modifiable Risk Factors in the Prevalence of NCDs and Poor Oral Health:

Oral and systemic health shared common modifiable risk factors that alter the normal condition health. Excessive sugar consumption, all forms of tobacco use, alcohol consumption, unhealthy diets, poor oral hygiene and their underlying determinants contribute significantly to the burden of this diseases. This risk factors are also implicated in the etiology of other systemic NCDs, thereby suggesting shared molecular and immunological pathways (Chaffee et al., 2020).

3.1. Smoking Effects on Oral Health and NCDs: The hard substance in tobacco (nicotine) suppresses vascular blood supply to the gums and weaken immunity of the oral cavity making it prone to bacterial attack. Tobacco smoke disrupts the functioning of nearly every human organ system, causing most deaths through cancer, heart disease, and non-cancer respiratory diseases and metabolic disorders. The medical evidence of harm caused by tobacco is well established. First in relation to cancers of the lip and mouth, and then in relation to vascular diseases and cancer of the lung (Chaffee et al., 2020).

Use of tobacco in association with poor oral hygiene, induced infectious transgression into systemic circulation causing inflammatory responses. This leads to the formation of vascular clots or infections that result in cardiovascular diseases, chronic pulmonary respiratory diseases, and triggers metabolic disorders as in case of insulin resistance in diabetes.

3.2. Alcohol Effects on Oral health NCDs: Harmful alcohol consumption has negative effects on oral, cardiac and general health. Globally, alcohol consumption accounted for 2.4 million deaths and 93 million disability-adjusted life years (DALYs) in 2019, most of them due to NCDs attributable to alcohol consumption (Vegi et al., 2024). It causes mouth dryness (Xerostomia) with low salivary secretions affecting self-oral cleansing effect, thus making the oral cavity prone to bacterial infections. Alcohol is among the leading causes of periodontitis and may indirectly impact periodontal disease through systemic health deterioration such as obesity, diabetes, and liver abnormalities (Kusu., 2025). This combined modifiable risk factors also contributes to delayed healing process after dental procedures in immuno-compromised individuals as well as early deterioration of both Oral and systemic health.



Figure 2. Exposure to modifiable risk factors

3.3. Poor Oral Hygiene and NCDs: Poor oral health condition, account the highest risk factor in the prevalence of carious lesions and diseases of the oral cavity. Food remnants that stick between teeth for longer duration in the form of carbohydrates and sugar, attracts the normal oral bacterial flora to ingest from it which if ignored, become pathogenic to the oral cavity. Dietary habits account for the major reasons in the prevalence of poor oral hygiene. (Highly refined sugary foods, insufficient vitamins and minerals) Modifiable behavior – All forms of tobacco smoking, harmful alcohol consumption leads to poor oral hygiene status in individuals. Lack of self-oral care – Regular toothbrushing, flossing and mouth rinsing post consumption of sugary foods are all contributory factors to poor oral health.

Among oral inflammatory conditions, periodontitis still remain burden of concern as a contributory factor in the prevalence of systemic diseases- diabetes, cardiovascular diseases and cancers (oral, lung and colorectal). Common oral diseases, especially periodontitis, interact with a variety of NCDs and different systemic disorders and conditions have the potential to affect a risk of developing oral diseases. An oral inflammation, as it happened during periodontitis process, can contribute to the systemic inflammatory burden and may have effects on general health (Wolf et al., 2023).

Poor oral hygiene has also been linked to socio economic background. Low and middle-class groups account the highest percentage in the poor oral hygiene index worldwide. Due to economic crisis, individuals find it difficult to practice dental care with adequate brushing, flossing and rinsing. This makes it a challenging for disadvantaged groups. The magnitude of the burden of oral disease is unparalleled by any other disease group, despite its low mortality (Benzian, et al., 2023). Global case numbers increased by a staggering 1 billion over the past 30 years. The high average prevalence is topped by high rates in poor, disadvantaged, and vulnerable groups, in which the prevalence of dental

caries among children and adolescents can reach more than 90% (Benzian, et al., 2023).

Patients with NCD's associated with poor oral hygiene are at more risk of developing both oral and systemic infections due to low immunity count. Oral complications are among the many complications suffered by diabetic patients, including periodontal disease, dental caries, oral infections, salivary dysfunction, taste dysfunction, halitosis, and lichen planus. Diabetes mellitus and oral complications are interrelated, and in conditions like periodontal diseases, the relationship is. On the other hand, control of blood glucose in Type 2 diabetic patients deteriorates due to periodontal disease. It induces systemic inflammation through pro-inflammatory cytokine release that causes insulin resistance and bacteremia. Periodontal disease treatment will improve glycemic control in diabetic patients (Ahmad and Haque, 2021).

Poor oral hygiene has been linked to many respiratory disorders. This is observed in case of dental procedures using high speed aerosols in patients with poor oral health. Pathogenic bacteria particles escape through the throat or secretion to reach lungs, resulting in aspirated pneumonia, or blockage of lung outflow. Poor oral hygiene is also common among individuals with mental problems. They lost confidence to obtain proper oral health, therefore are prone to develop oral and systemic diseases. Evidence based studies suggests that individuals who have experienced a mental health disorder underutilize dental services. Reasons such as depression, helplessness, lack of health insurance, dental fear and restlessness in the dental waiting environment contribute to underutilization (Tiwari et al., 2022).

3.4. Diet - Oral health and NCDs: The correlation between dietary habits and its alteration in oral health is proportional to the level of quality and quantity of the type of diet intake. The effect of refined carbohydrates on teeth and surrounding oral cavity is well established. Dental caries, the most common oral disease occurs as a result of demineralization of enamel and dentine by organic acids formed by bacteria in dental plaque through the anaerobic metabolism of dietary sugars. Adequate nutrition preserves healthy teeth and gums, while nutritional deficiencies may cause cavities, gum disease, halitosis and enamel erosion (Shakir et al., 2025). On the contrary, poor oral health can also affect systemic ailments, including majors heart diseases and diabetes.

Vitamin C- is an antioxidant that helps in neutralizing free radicals in the mouth and repair damages by supporting the integrity of gums. Vitamin D is important for tooth mineralization while vitamin A helps with saliva production, that aid in washing away bacteria and food remnants from teeth. Calcium helps build strong bones and teeth. Similarly, the health benefits of nutrients in the prevention of prevalence of non- communicable diseases have been evidence based and treated accordingly. A higher intake of fruits and vegetables has

additionally been linked to a lower likelihood of stroke. Several investigations have also hinted at a protective effect of fruits and vegetables against cancer. A healthy diet in association with physical activity is considered an essential in the preventive phenomena of both NCDs as well as boosting immunity against oral infections. Lack of physical activity has been attributed to consumption of unhealthy diet such as junk food, which predisposes many young adults and middle- aged people to develop obesity a modifiable risk factor in the prevalence of Diabetes and Cardiovascular diseases (Shakir et al., 2025).

The interlink between obesity and poor oral health was evidenced with OHI records. (Oral hygiene index). It includes the evaluation of oral hygiene habits, presence of periodontal diseases, carious teeth, filled teeth, number of missing teeth and the amount of mastication forces. Frequent consumption of ultra-processed foods, salty snacks, processed meats, and sugar-sweetened beverages was consistently associated with increased NCD risk (Modjadji et al., 2025). This indicate the types of diet exposure of an individual hence the risk of developing obesity can be highlighted. A high intake of sugary drinks is common across diverse populations therefore, the interconnection between unhealthy diet, poor oral hygiene and prevalence of NCDs is well established and requires special considerations and intervention with multidisciplinary approach.



Figure 3. The effects of junk food on oral health

4. Preventive Oral and Dental Health Care Services in Protection Against NCDs:

Oral diseases are among the most common and preventable NCDs worldwide. Major oral diseases and NCDs are closely linked and shared modifiable risk factors including tobacco use, harmful alcohol consumption, poor nutrition and the same socio-economic determinants. Accordingly, a common risk factor approach is desirable and more rationale compared to those disease specific (Thwin, 2022). The approach towards preventive dental health care services aimed in the protection against non- communicable diseases while providing positive impact in the reduction and management of the diseases. It requires a multidisciplinary health approach including oral healthcare professionals, general physicians, nutritionist, psychologists and others in counseling related to

modifiable risk factors. It involves systematically addressing social, environmental and economic determinants of health through health promotion.

The latest developments in dentistry provide organizations and policymakers with the opportunity to critically reflect on the current and future orientation toward the oral health promotion and oral disease prevention (D'Aiuto et al., 2025). Promoting the general health of an individual by controlling a number of risk factors will aid in the reduction of the disease burden. Most of the Oral-NCDs related diseases are high in the low- and middle-income countries. This is due to low socio-economic status of the society, poor nutrition, limited access to dental care and NCDs services, lack of Medical and Dental facilities, high cost of treatment, oral hygiene and medical health status awareness. Poor and disadvantaged populations in all countries have a higher burden of oral diseases than wealthier populations, together with less access to oral health care, prevention, and insurance coverage (Benzian, et al., 2023).

Oral health is an integral part of general health. Integrated oral health preventive care against NCDs progressions is an utmost important. WHO highlights the need to focus not only on reducing disease, but also on tackling its root. This involves systematically addressing social, environmental and economic determinants of health (Wolf et al., 2021). Preventive Oral and Dental health care services begin initially from childhood. Diagnosis of any signs of NCDs through oral examination can be preventive at earlier stage to reduce/prevent further health challenges as a result of poor oral health. Since most of the signs of NCDs can be manifested orally, integrating oral health care services into primary healthcare system will aid in early diagnosis of some underlined medical conditions with prompt preventive action. Oral health care services are mostly ignored among elderly groups with highest number of NCDs.

4.1. Oral and Dental Health Care in NCDs: Provision of health awareness at public level regarding importance of oral health care to avail general health is essential for a productive society. Population's oral health needs are often not aligned with availability, location, and dental care provided by public services (Wolf et al., 2021). In most of the low- and middle-income countries, threat of poor oral hygiene still remains a challenge with high rate of associated NCDs in most of the adult and elderly groups.

4.1.1. Oral Health Care Promotion: Public health counseling with regards to modifiable risk factors, conducting free tests for diabetes, hypertension, and other related NCDs should all be encouraged and organized at some time intervals. Vulnerable groups should be informed about the importance of preventive oral health care in the prevention and management of NCDs. The services should be applied at multi sectorial levels through establishing standard dental care services in populated sectors- industries, schools. Psychiatric patients are known to be among those groups diagnosed with frequent oral infections due

to ignorance. Incorporating dental care services with antenatal care in an effort to ensure better pregnancy health outcomes is also highly recommended. Adequate Health education materials for interpretation, demonstration and explanation of the educational motive for oral health to the vulnerable group along with provision of free dental kits, and one - one demonstration of oral hygiene.

4.1.2. Oral Health Care Accessibility: Barriers to seeking regular dental care, including the availability of dental services, dental fear, cost, income the distance a person had to travel to get care, and preference for preservation of teeth (FDI World Dental Federation, 2023). These overall barriers serve as a junction between access to dental care and proneness of the vulnerable groups to NCDs. Elderly groups of patients account the highest number with NCDs. They find it difficult to go and seek dental treatment due to their condition with low immunity count. Majority are disabled and from low- socio economic background. The need for Dental professionals in providing home dental services will aid in reducing the burden and managing the condition positively. Providing mobile dental services to remote areas, refugee camps and community in need, will aid in both reduction of NCDs burden and improving oral hygiene status of the community.

4.1.3. Integration of Oral and Systemic Health Care: Since the implication of poor oral hygiene is well established in the prevalence of non-communicable diseases, the approach towards prevention of the diseases burden is essential for both, economic, social and personal development. To achieve this, a joint approach of medical and Oral health professionals along with other related cadres is required to implement integrated vision towards reduction and counseling on shared modifiable risk factors. The concept of incorporating Oral healthcare into primary health care settings will give sound base in the prevention and management of NCDs. Benefits of integration includes, early detection of disease, saving double or multiple treatment costs, oral screening, better quality of life, and straightening collaborative management among professions. Oral health professionals must be empowered to identify early signs of systemic diseases, creating a bidirectional flow of referrals between oral healthcare professionals and physicians. Ultimately, prioritizing oral health not only enhances individual well-being but also serves the greater public health good (D'Aiuto et al., 2025).

Reduction of healthcare costs is an essential portion of consideration. In general, oral healthcare treatment is costly worldwide. This contributes to the ignorance in seeking dental treatment among the society. Integrating oral and dental health care with general medical universal health coverage will help save the treatment costs for the patient and subsequent benefit in the reduction of disease burden and improvement of the quality of life. WHO's vision of UHC for oral health for all individuals and communities by 2030 is aligned with FDI's

Vision 2030. However, this will require the development and implementation of comprehensive evidence-based policies and benefit packages that integrate oral health promotion and oral health care into NCDs and UHC responses (FDI World Dental Federation, 2023).

4.1.4. Managing NCDs in Dental Settings: NCDs patients are delicate to handle in dental clinical settings. This is due to their conjoined low immunity count making them susceptible to infections and shock. Medical emergencies are common in dental practice. This is why, dentist provide special treatment schedule and plan for NCDs patients to avoid medical complications before, during, and after dental procedures. It is the responsibility of the dental professional to properly review medical and drug history of a patient before the commencement of any dental procedure. Develop strategies to identify patients at risk of medical problems, to assess the severity of those risks and, seek for the advice from a colleague with special competence in the relevant fields (Srivasta and Pradhan, 2025). Early morning appointment is scheduled for NCDs patients to avoid dental queue and ensure a stress-free treatment. Long dental procedure is limited in case of NCDs patients. It is mandate for every oral and dental health clinic setting to possess dental emergency kit- containing essential drugs, sprays, apparatus for safe management of NCDs complications. Patients may need to be kept in an upright sitting position in the dental chair to enable them to better handle their commonly copious sputum/mucous (Fatima et al., 2021).

5. Discussion:

For many years, studies has been conducted and still ongoing with regards to association of NCDs and oral infections due to its high prevalence among individuals especially elderly groups. Emphasis is given to provision of dental services in managing and preventing rate of NCDs. Many scholars have been sharing different ideas, findings and recommended methods of management and prevention at inter professional level. Many suggested the incorporation of oral healthcare into primary healthcare sectors with the aim of reducing burden of the diseases among vulnerable group and also adresssing the common risk factors involved. For instance, in a study conducted by Benzian et al. (2023) the scholar indicated, detrimental role of sugars as a risk factor for oral diseases and other NCDs has also been well documented. Despite the evidence, oral diseases and sugars are not part of the current NCD framing, which focuses on five diseases and five risk factors, (5×5). Oral diseases and sugars remain sidelined, disproportionately affecting poor and disadvantaged populations. With these evidence, they suggested that, an expanded 6×6 framework would recognize growing evidence and would reiterate the need to strengthen action, resource allocation, and policy development for NCDs.

Considering the fact that, both Oral and NCDs shared common modifibale risk factors, the need for integrated preventive intervention is important.

Kutikuppala, et al. (2022) the astonishing link between periodontal disease and heart health is considered crucial, where the dental profession can be regarded as a key assessor of not just the oral health, but also the heart health. The researchers have also found that people with existing periodontal disease are almost twice as likely to develop heart disease, and this risk can be even greater for people with higher levels of cholesterol. Provision of patients awareness with regards to Oral-Systemic connection between poor oral hygiene leading diseases and NCDs can eventually help prevent and save lives.

From many reviewed studies, it is well evidenced that, the regional distribution of chronic diseases and poor oral health is more appreciated in low-middle income countries with much concern to Asia and African regions. A systemic review few studies on oral health in relation to the slum environment with most data from Asia and sub-Saharan African countries. Accordingly, the commonest oral diseases reported were dental caries (prevalence: 13%-76%), and periodontal diseases (prevalence: 23%-99%) (Osuh et al., 2024). These were higher in slum settings and showed differences across age groups, gender, and socioeconomic classes. Further reported that, most participants in the studies perceived their oral health status as satisfactory, a belief commoner among younger people, males, those in higher socio-economic classes, and employed. There was widespread engagement in home remedies for oral disease cure or prevention, while the use of professional dental care facilities was generally low and problem-driven (Osuh et al., 2024). This can be attributed to the lack of sufficient and availability of dental facilities in most of the affected low-middle income countries, which signifies set back in the prevention of oral diseases among factors induced NCDs.

In view of accessibility to oral healthcare care in prevention against NCDs, Wolf et al. (2021) reported - in high- and middle-income countries, young children from low-income families, people with disabilities, institutionally elderly people, are often excluded from dental services while healthy adults might be receiving unnecessary treatments. This is building a barrier against receiving good oral health care. Compared with other health care professionals, oral healthcare services are rarely integrated within the different health care systems.

6. Global Vision in the Preventive Oral and Dental Care Services against NCDs:

Considering the rapid prevalence of NCDs in conjugation with poor oral hygiene which, still remains a dilemma among elderly and low, middle-income communities, world health organization and other related bodies, has put great concern with the vision of reducing and eliminating the burden of the disease to the vulnerable groups. According to WHO, approximately 3.5 billion people are affected by oral diseases among which highest are from low-middle income countries. On 26th May 2025, the WHO released its global strategy and action

plan for oral health for the period 2023 – 2030. The foremost target in the strategical plan is set towards the vision of universal health coverage (UHC) for oral health, reaching all individuals and communities by 2030. This will reduce oral disease burden on disadvantage groups with subsequent prevention of NCDs. WHO Oral health work aligns with other program areas within the Management of Non-communicable Diseases (MND) unit through focusing on strengthening cost-effective population-wide oral health promotion and oral health care within the primary health care system to achieve UHC for oral health by 2030 (Osuh et al., 2024).

Similarly, FDI (World Dental Federation) - Policy Statement on - Oral Health and NCDs was adopted in September 2024 at General Assembly held at Istanbul, Turkey. Some of the policy actions and recommendations includes: Suggestion on implementation of relevant articles in the Framework Convention on Tobacco Control and policies and reduction of harmful alcohol use and sugars intake, including tax increment and restrictions on advertisements. FDI also emphasizes the importance of improving access to health care services which will ensure more supportive social conditions for disadvantaged group. With the recognition of shared modifiable risk factors of Oral and NCDs as well as common social determinants, integrating action can both improve oral health and reduce the NCD burden. Further suggestion includes, the unity of national Dental Associations with other NCDs organizations to promote common solutions. Integrating oral and NCD care into broader efforts to achieve UHC, emphasis is given more to low - middle income countries for the policy of integrating essential oral and NCD care into primary health care (PHC) (FDI World Dental Federation, 2023).

The Bangkok Declaration of 2024, entitled "No Health Without Oral Health," which aims for universal oral health insurance by 2030, reported that oral diseases affect 3.5 billion people worldwide (Wolf et al., 2023). They describe the threat as a situation that poses significant public health challenges for all WHO Member States and highlights the critical need to address oral diseases and conditions as part of the broader burden of NCDs. The WHO emphasizes the promotion of dental research to strengthen evidence regarding oral health inequalities and the link between oral diseases and other NCDs (WHO, 2025).

7. Conclusion:

The study clearly demonstrates significance and bi-directional relationship between oral hygiene and NCDs. Poor oral hygiene characterized by conditions such as periodontal diseases, and dental caries, not only affects the right to quality of oral functions but also demonstrate effects on general health for both individuals and vulnerable groups at risk. Oral inflammatory conditions, especially periodontitis is the most common condition signaling underline medical conditions of NCDs and in turn, NCDs prevalence shows association with poor oral hygiene. Oral health education should not just raise the awareness

of promoting good oral hygiene practice but consider it as integral part of comprehensive health strategies aimed at reducing the burden of chronic diseases globally. For effective prevention, it is essential to extend the prevention care system to the vulnerable populations as outlined by WHO in its mission of ensuring UHC. Oral health professionals can be allies of tobacco control and food policy advocates for the regulation of NCD risk factors. National policies should discourage highly sugar intake and discourage tobacco use will serve as a key pillar in prevention of bi-directional effects on both NCDs and oral health.

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CHAPTER 43

Tetrahydroquinoline (THQ) Derivatives in Cancer Research: Small-Molecule Design, Mechanisms of Action, and Therapeutic Potential

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Cancer displays marked molecular heterogeneity at genetic, epigenetic, and phenotypic levels. These differences exist both between tumors and within the same tumor. This heterogeneity leads to variable responses to treatment and promotes the development of resistance to single-agent therapies.[1][2][3].

Malignant tumors represent highly heterogeneous structures, varying not only between patients but also within distinct regions of the same tumor. This complexity is largely driven by genomic instability, dynamic gene expression profiles, and the continuous evolution of cancer cells [1]. These biological variations have profound clinical implications, often culminating in therapeutic resistance. By allowing resistant clones to survive and expand despite targeted interventions, molecular heterogeneity serves as a key determinant of therapy failure and adaptive resistance [1]. Although understanding heterogeneity supports precision medicine, it also requires strategies that target multiple pathways to manage tumor complexity.

Over the past decade, biologic therapies, particularly monoclonal antibodies targeting EGFR, VEGF, and immune checkpoints such as PD-1/PD-L1, have reshaped the oncological landscape. Despite this clinical success, these macromolecules face intrinsic limitations. These include restricted tissue penetration, a lack of oral bioavailability, and high costs [4][5]. Their long half-life and immune mechanism of action can lead to prolonged *immune-related adverse events* (irAEs) such as autoimmunity and cytokine-release syndromes[4][6]. For example, checkpoint-blocking antibodies can trigger systemic inflammation (pneumonitis, colitis, etc.) due to overactivation of T-cells[4][6]. Furthermore, antibodies are generally restricted to extracellular targets because their size prevents them from efficiently crossing the cell membrane to access intracellular oncogenic proteins.

These factors support the development of small-molecule therapeutics that can complement antibody-based therapies or serve as alternatives in settings where antibody approaches are less effective [6][5]. Specifically, small molecules are often favored because they can often be given orally and are capable of penetrating both tumor tissue and the intracellular space. Since they are generally less immunogenic and possess shorter half-lives, they enable greater control over dosing due to the quick reversibility of their actions [5]. In addition, small molecules are typically less costly to manufacture and their chemical structure is readily modified. This facilitates the optimization of both pharmacokinetic properties and target specificity.

Small-molecule drugs have been widely used in targeted cancer therapy, such as kinase inhibitors including imatinib and erlotinib, and they continue to be important alongside newer biologic therapies [4][5]. They can engage a wider range of molecular targets, including intracellular enzymes and protein–protein interactions that are inaccessible to antibodies. Furthermore, their typically

shorter systemic persistence can reduce prolonged immunosuppression or off-target effects, an important consideration in immuno-oncology where on-target and off-tumor toxicities, such as immune-related adverse events (irAEs) from checkpoint antibodies can be severe[4][5]. For example, small-molecule immune checkpoint inhibitors are being explored as alternatives to antibodies, with the potential for oral administration and fewer systemic immune side effects. Small-molecule inhibitors can overcome several limitations of monoclonal antibodies, such as poor oral bioavailability, high cost, limited tissue and tumor penetration, and long half-life [5]. This potential to mitigate the shortcomings of biologics has prompted intense research into small-molecule anticancer agents that could either replace or synergize with antibody therapeutics.

Tetrahydroquinoline as a Privileged Scaffold

Among anticancer small molecules, the 1,2,3,4-tetrahydroquinoline (THQ) scaffold is considered a privileged structure in medicinal chemistry. Privileged scaffolds are molecular frameworks that can bind to multiple biological targets with high affinity. The THQ nucleus is an example of such a scaffold and has given rise to many bioactive compounds across different disease areas. The THQ structure is a partially saturated quinoline consisting of an aromatic benzene ring and a hydrogenated pyridine ring containing a basic amine. This bicyclic structure is found in various natural products and synthetic compounds with antibacterial, antimalarial, and neuroactive activities[7]. In cancer research, THQ derivatives have shown notable cytotoxic and antiproliferative activities, making THQ a versatile platform for designing novel antitumor agents[8]. Small modifications to the tetrahydroquinoline core can markedly alter target specificity and biological activity. This allows the same scaffold to be explored against different biological targets. THQ-based compounds have been described to inhibit kinases, interfere with oncogenic protein–protein interactions, and modulate pathways involved in cell survival. [8][9] [10].

Chemical and Structural Features of Tetrahydroquinolines

The tetrahydroquinoline scaffold is composed of a fused benzene ring and a partially saturated nitrogen containing six membered ring, forming a bicyclic system that combines aromatic character with enhanced three-dimensionality relative to fully aromatic quinoline derivatives. In 1,2,3,4 tetrahydroquinolines, partial hydrogenation of the heterocyclic ring reduces overall planarity while preserving the aromatic π system of the benzene ring. This structural arrangement allows tetrahydroquinolines to engage both flat and nonplanar binding sites and can support a broader range of molecular interactions than fully aromatic analogues. The presence of a secondary or tertiary amine further contributes to favorable physicochemical properties, including improved solubility and the capacity for hydrogen bonding with biological targets [8].

Partial saturation of the quinoline core has also been associated with improved drug-like behavior. Compared with fully aromatic quinoline or quinazoline systems, tetrahydroquinolines often display reduced nonspecific binding and improved metabolic stability, likely due to decreased planarity and altered electronic distribution[8]. In several medicinal chemistry studies, replacement of aromatic heterocycles with tetrahydroquinoline cores preserved biological activity while improving selectivity or pharmacokinetic profiles, supporting the use of tetrahydroquinoline as a viable alternative scaffold in anticancer drug design.

Another important feature of tetrahydroquinolines is their stereochemical complexity. Substituted tetrahydroquinolines may contain one or more stereocenters, and individual enantiomers can differ substantially in biological activity or metabolic fate. Because most protein targets represent chiral environments, stereochemical control is a key consideration in tetrahydroquinoline-based medicinal chemistry. Advances in asymmetric synthesis, including enantioselective cyclization reactions and asymmetric hydrogenation, have enabled reliable access to optically enriched tetrahydroquinoline derivatives suitable for biological evaluation [11].

The tetrahydroquinoline scaffold also provides multiple positions for chemical modification, allowing systematic tuning of molecular properties. Substitution on the aromatic ring, the saturated ring, or the nitrogen atom can be used to adjust electronic character, lipophilicity, and solubility. Common modification strategies include the introduction of electron-donating or halogen substituents, as well as solubilizing functionalities such as amines or heterocycles. These features allow systematic modification of the tetrahydroquinoline scaffold through aromatic substitution and nitrogen or peripheral functionalization, including the introduction of sulfonamides or morpholine groups, to modulate electronic properties, lipophilicity, pharmacokinetics, and target interactions in anticancer drug design.

Comparison with Other Heterocyclic Anticancer Scaffolds

Tetrahydroquinoline can be compared with other heterocyclic scaffolds frequently used in anticancer drug discovery. Fully aromatic quinolines and quinazolines are well established, as exemplified by EGFR inhibitors such as gefitinib and erlotinib. Their planar structures favor binding to flat active sites, including kinase adenine binding pockets, and enable aromatic stacking interactions. However, this planarity may also increase the risk of DNA intercalation, off-target binding, and metabolic deactivation through aromatic oxidation.

In contrast, the partially saturated tetrahydroquinoline scaffold introduces three-dimensional character and reduced planarity. This feature can lower nonspecific interactions while preserving effective engagement with enzymatic

targets. Compared with indole-based scaffolds, tetrahydroquinoline lacks a fully conjugated bicyclic system but contains a basic amine, which can be protonated to enhance solubility or participate in ionic interactions within binding sites.

Tetrahydroquinoline is closely related to tetrahydroisoquinoline scaffolds, which differ mainly in ring fusion but share many favorable properties. Tetrahydroisoquinolines have been described as privileged structures in anticancer research, with reported activity across multiple targets and progression of some derivatives toward clinical evaluation [12]. Many structure-activity relationship trends are therefore transferable between tetrahydroquinoline and tetrahydroisoquinoline series.

Tetrahydroquinolines represent a balanced scaffold that combines key binding features of aromatic heterocycles with enhanced flexibility for optimizing drug-like properties. These characteristics support their continued use in the development of small anticancer molecules.

Synthetic Strategies for Tetrahydroquinoline Derivatives

The synthesis of tetrahydroquinolines has been investigated for more than a century, and several classical methods remain in use. Among these, the Povarov reaction is one of the most versatile approaches for constructing highly functionalized tetrahydroquinoline frameworks. In this transformation, an aniline and an aldehyde form an imine in situ, which undergoes a cycloaddition with an activated alkene to generate a dihydroquinoline intermediate. Subsequent reduction affords the corresponding 1,2,3,4 tetrahydroquinoline. This reaction and its intramolecular variants enable access to diverse substitution patterns through appropriate choice of anilines and aldehydes, allowing substitution on the aromatic ring and at the C4 position [11]. Traditional Povarov reactions typically rely on protic or Lewis acid catalysts and generally produce racemic mixtures. More recent developments have focused on enantioselective variants, which are particularly relevant for medicinal chemistry applications. The use of chiral Brønsted acids, including BINOL-derived phosphoric acids, or chiral Lewis acid complexes has enabled asymmetric cycloadditions that deliver tetrahydroquinolines with high enantiomeric excess [11][13][14][15]. These methods provide efficient access to enantioenriched tetrahydroquinolines bearing multiple stereocenters in a single synthetic step.

In addition to Povarov chemistry, tetrahydroquinolines can be prepared by reductive cyclization strategies, such as partial reduction of quinoline derivatives, although regioselectivity and stereocontrol can be limited. Other approaches involve cyclization-based reactions followed by reduction steps, including cascade processes that combine multiple transformations in a single sequence [14]. Such cascade strategies illustrate how modern catalysis can simplify THQ synthesis. Overall, a range of classical and contemporary methods is available for the construction of tetrahydroquinoline cores, including highly substituted and

chiral derivatives. These classical and modern methods offer a broad toolkit for the synthesis of tetrahydroquinoline cores, including highly substituted and chiral derivatives.

Enantioselective Synthesis (e.g., Povarov Reaction)

One important development in THQ synthesis is the introduction of enantioselective catalytic methods. In particular, the Povarov reaction has been widely adapted to asymmetric versions over the last decade[16]. The first asymmetric Povarov example was reported in the mid-1990s with chiral Lewis acids[14], and since then numerous catalyst systems have been developed to improve stereocontrol and substrate scope. Both metal based catalysts and organocatalysts have been applied to asymmetric tetrahydroquinoline synthesis. Among these, chiral phosphoric acids have proven particularly effective in controlling stereoselectivity in Povarov reactions and often deliver high enantioselectivity across diverse tetrahydroquinoline derivatives[13]. These methods enable access to chiral tetrahydroquinolines substituted at C2 and C4, corresponding to two stereocenters.

Asymmetric hydrogenation of quinoline precursors represents another strategy for preparing enantioenriched tetrahydroquinolines. Using suitable chiral catalysts, quinolines can be reduced stereoselectively to give partially or fully hydrogenated products with defined configurations. Although additional functionalization or reduction steps may be required, this approach has been applied to the preparation of chiral intermediates relevant to tetrahydroquinoline-based drug candidates. Resolution methods and chiral pool strategies are also used, but catalytic asymmetric synthesis is generally favored in modern drug discovery due to its efficiency and scalability. Overall, these enantioselective approaches provide reliable access to chiral tetrahydroquinoline derivatives, supporting their continued application in medicinal chemistry.

Molecular Targets of THQ-Based Small Molecules in Cancer

Tetrahydroquinoline derivatives have been investigated against a broad array of molecular targets in oncology, reflecting the scaffold's versatility. Key target classes include protein kinases, immune checkpoints, apoptosis regulators, and components of the tumor microenvironment. Here we discuss prominent examples and classes of targets modulated by THQ-based compounds.

Kinase Signaling Pathways

Kinase signaling pathways represent a major area of interest for tetrahydroquinoline-based anticancer agents, particularly dysregulated systems such as EGFR and the PI3K–AKT–mTOR axis. Although many ATP competitive kinase inhibitors rely on fully aromatic heterocycles, THQ scaffolds have also been successfully incorporated into this class. THQ-based mTOR inhibitors have shown strong cellular activity and broader mTOR complex inhibition compared

with earlier rapamycin analogs. [8]. In these systems, the THQ core has functioned as a bioisosteric replacement for quinoline or quinazoline motifs, with reported improvements in metabolic stability [48][16].

Beyond mTOR, THQ derivatives have also been explored as inhibitors of receptor tyrosine kinases such as EGFR. Several studies report low micromolar inhibition of EGFR signaling and antiproliferative effects in EGFR-overexpressing cancer cell models, supporting the ability of THQ frameworks to engage kinase ATP-binding sites when appropriately substituted [8][17][18]. Additional investigations have extended THQ-based kinase inhibition to other regulators of cancer cell growth, including cyclin-dependent kinases, Aurora kinases, and angiogenesis-related pathways [8][17]. Collectively, these findings indicate that tetrahydroquinoline derivatives can function as effective kinase inhibitors across multiple signaling pathways relevant to tumor growth and survival.

Immune Checkpoint–Related Targets (PD-1/PD-L1 Axis)

The PD-1/PD-L1 immune checkpoint is a prime target in immuno-oncology, traditionally drugged by antibodies. Recently, efforts to find small-molecule inhibitors of the PD-1/PD-L1 interaction have increased in recent years [19]. The PD-1/PD-L1 interface is large and relatively flat, which makes it difficult to target with small molecules. However, some chemical scaffolds, including partially saturated ring systems similar to THQ, have shown activity by inducing PD-L1 dimerization or through allosteric mechanisms. Bristol-Myers Squibb’s seminal work identified biphenyl derivatives that bound PD-L1 and caused it to dimerize, thereby blocking PD-1 binding[19]. While those initial compounds did not contain a THQ core, subsequent patent literature and studies indicate that tetrahydroquinoline and tetrahydroisoquinoline motifs have been explored as part of PD-L1 inhibitors. The 1,2,3,4 tetrahydroisoquinoline scaffold has been identified as a suitable framework for the design of small molecule PD-L1 inhibitors, supporting de novo development of compounds with immunomodulatory activity [12]. By analogy, tetrahydroquinolines may function in a similar manner. Their bicyclic structure can act as a rigid framework for positioning hydrophobic substituents toward the PD-L1 surface, while the amine may contribute polar interactions or influence aqueous solubility. Some N-substituted THQ derivatives have been patented as PD-L1 inhibitors, often bearing two aryl arms to mimic the biphenyl orientation of the BMS compounds[20]. This clinical progress has reinforced interest in small-molecule approaches to the PD-1/PD-L1 axis and provides a broader context for the exploration of related heterocyclic scaffolds.[4].

Although detailed information on THQ-based PD-L1 inhibitors remains limited, design principles established for small-molecule PD-L1 antagonists, such as stabilization of inactive PD-L1 dimeric forms, provide a relevant

framework.[4]. This could certainly be applied using a THQ as a central scaffold bearing the appropriate substituents to span the interface. The potential advantage of THQ in this context is its use as a privileged scaffold to support effective binding while maintaining favorable drug-like properties, such as oral bioavailability. This appears feasible given that multiple small-molecule PD-L1 inhibitors are currently in clinical development [4]. In summary, alongside antibody-based approaches to the PD-1/PD-L1 axis, THQ-based small molecules are being explored as alternative immune checkpoint modulators with features such as oral delivery and suitability for combination strategies.

Apoptosis, Proliferation, and Survival Signaling Pathways

Many tetrahydroquinoline derivatives do not act through a single molecular target but instead influence central cellular pathways regulating apoptosis, cell cycle progression, and survival signaling. These effects often arise indirectly from upstream target engagement, such as kinase inhibition or modulation of signaling networks, although some THQ compounds display broader mechanisms of action. Several THQ derivatives have been reported to activate DNA damage or oxidative stress responses, leading to cell cycle arrest and induction of apoptosis or autophagy.

In particular, some THQ analogues increase intracellular reactive oxygen species levels or disrupt mitochondrial function, thereby promoting programmed cell death. For example, a THQ derivative was shown to suppress PI3K AKT mTOR signaling through oxidative stress, resulting in autophagic cell death and apoptosis [21]. Other studies report that THQ-based compounds can alter the balance of pro- and anti-apoptotic regulators, including members of the BCL-2 family and p53, leading to enhanced apoptotic responses in cancer cells [22]. In addition, selected THQ hybrids have been explored for their ability to interfere with broader survival pathways implicated in cancer progression.

These findings indicate that tetrahydroquinoline scaffolds can modulate multiple pathways governing cancer cell survival and death, either through direct inhibition of signaling proteins or by inducing cellular stress responses. Such multi pathway effects may contribute to cytotoxic activity and provide an advantage in settings where single target inhibition is insufficient.

Tumor Microenvironment Related Mechanisms (Inflammation and Immune Modulation)

Cancer growth and response to therapy are strongly influenced by the tumor microenvironment, which includes surrounding stromal cells, immune cells, and tumor-associated blood vessels. Small molecules that modulate the TME are of great interest, and THQ derivatives have begun to find roles here as well. One approach involves modulating tumor-associated inflammation. Certain THQ compounds show inherent anti-inflammatory activity[8], for example by

inhibiting pro-inflammatory enzymes or signaling (like COX-2 or NF- κ B pathways). An anti-inflammatory THQ might reduce the levels of cytokines and growth factors in the TME that typically support tumor progression. THQ derivatives have been explored as inhibitors of inflammatory mediators. In cancer, reducing chronic inflammation can limit tumor progression and metastasis, as inflammatory signaling often supports tumor growth and angiogenesis.

Another microenvironmental target is hypoxia and acidosis prevalent in tumors. Carbonic anhydrase IX (CAIX) is a pH-regulating enzyme that is overexpressed in many tumors and supports survival under hypoxic and acidic conditions. THQ sulfonamide derivatives have been specifically designed to inhibit CAIX[23]. By inhibiting CAIX, such compounds cause an intracellular acid build-up in tumor cells, leading to decreased proliferation and making the tumor microenvironment less immunosuppressive (a less acidic TME is more amenable to T-cell activity).

Beyond immune checkpoints, small molecules can also modulate immune cell recruitment and activity within the tumor microenvironment. For example, inhibitors of indoleamine 2,3-dioxygenase (IDO1), an enzyme involved in tumor-mediated T-cell suppression, have been developed, although not yet based on THQ scaffolds. In principle, THQ structures could be adapted to target IDO1 or other metabolic enzymes in the tumor microenvironment that regulate immune responses.

Finally, physical barriers within the tumor microenvironment, such as dense stroma or abnormal vasculature, can also be addressed with small molecules. Although THQs have not been widely reported as direct stromal modulators, compounds that reduce fibrosis or normalize tumor vasculature can enhance drug delivery and immune cell infiltration. In this context, some THQ analogues have shown anti-angiogenic activity, suggesting indirect effects on the tumor microenvironment. By inhibiting angiogenesis, such compounds may transiently normalize tumor vasculature or limit nutrient supply to the tumor [14].

Multidrug Resistance Mechanisms

Beyond direct inhibition of oncogenic targets, tetrahydroquinoline-based compounds have also been explored as modulators of multidrug resistance in cancer. In these studies, THQ derivatives were shown to interfere with drug efflux processes, thereby increasing intracellular drug accumulation and restoring sensitivity to chemotherapeutic agents in resistant cancer cells [24].

Mechanistic investigations suggest that THQ hybrids can interact with efflux systems and suppress their transport activity, contributing to improved treatment response [24][25]. By addressing resistance mechanisms rather than a single molecular target, these compounds act in a supportive role alongside

conventional chemotherapy. This application further highlights the versatility of the tetrahydroquinoline scaffold and supports its classification as a privileged structure capable of engaging diverse biological pathways relevant to anticancer therapy.

Comparison with Antibody-Based Checkpoint Inhibitors

Small molecule immune checkpoint inhibitors, including those based on tetrahydroquinoline scaffolds, differ fundamentally from monoclonal antibodies in their mode of action and pharmacological properties. Monoclonal antibodies targeting PD-1 or PD-L1 act by directly blocking the protein-protein interaction between immune cells and tumor cells and typically produce sustained checkpoint inhibition due to their long circulation half-lives. While this prolonged activity supports durable immune activation, it is also associated with limited dose flexibility and an increased risk of persistent immune-related adverse effects [4].

In contrast, small molecules interact with checkpoint proteins in more diverse ways and generally have shorter half-lives, allowing for reversible and adjustable modulation of immune signaling. Their effects can be more readily controlled through dosing, and treatment can be discontinued if toxicity arises, with relatively rapid clearance[26]. In addition, small molecules exhibit superior tissue penetration compared with antibodies, enabling more uniform distribution within solid tumors and access to poorly vascularized regions, as well as potential access to immune privileged sites such as the central nervous system [4][5].

Another distinguishing feature is the target scope. Antibodies are typically monospecific, whereas small molecules can be designed to engage multiple immune checkpoints or signaling pathways simultaneously. This multi-target potential may be advantageous in overcoming redundant immunosuppressive mechanisms within the tumor microenvironment. Collectively, these characteristics highlight why small-molecule checkpoint inhibitors, including THQ-based compounds, are being actively explored as complementary or alternative approaches to antibody-based immunotherapies.

In Silico Approaches in THQ-Based Drug Discovery

Computational approaches are widely used in contemporary drug discovery and have also been applied in the development of THQ-based anticancer compounds. In silico tools guide the design and optimization of THQ derivatives by predicting binding modes, estimating binding affinities, and helping prioritize which analogues to synthesize. Key approaches include molecular docking, molecular dynamics simulations, free-energy calculations (like MM-GBSA), and virtual screening campaigns. Here, we discuss how these have been applied to THQ scaffold optimization.

Molecular Docking and Binding Mode Analysis

Molecular docking is commonly used in tetrahydroquinoline (THQ)–based drug discovery to examine how this scaffold interacts with protein structures. Docking analyses of THQ derivatives are typically performed to propose plausible binding orientations, assess steric and electronic compatibility, and identify stabilizing noncovalent interactions, including hydrogen bonding, hydrophobic contacts, and aromatic stacking. Evaluation of how the bicyclic THQ core fits within three-dimensional binding regions provides insight into substitution patterns that are compatible with binding and into structural features that appear critical for maintaining favorable interaction geometries.

In several THQ-centered studies, molecular docking has been combined with quantitative structure–activity relationship (QSAR) analysis to interpret experimental activity data and guide scaffold optimization. Within this framework, docking results are used to relate physicochemical descriptors to specific interaction features observed in modeled binding modes. Docking has also been applied to systems characterized by structural flexibility or less-defined binding regions, where it serves to explore potential interaction sites beyond classical active-site models. Although docking does not provide quantitative predictions of biological activity, it remains useful for prioritizing THQ analogues and informing rational design when interpreted alongside experimental data [27][28].

MM-GBSA and Free-Energy Calculations: Beyond molecular docking, more detailed computational methods are used to better estimate ligand binding affinity and account for solvation and energetic contributions. MM-GBSA is commonly applied as a post docking approach to rescore tetrahydroquinoline complexes and refine the ranking of candidate compounds by approximating their binding free energies. In tetrahydroquinoline-based drug design, MM-GBSA calculations have been used to compare substituted analogues and prioritize those predicted to form more energetically favorable complexes with targets, capturing interaction features that may not be fully reflected in docking scores [29][30][31].

In cases where tetrahydroquinoline derivatives target protein-protein interfaces, more advanced free energy approaches such as free energy perturbation or thermodynamic integration can be applied to quantify the energetic impact of specific substituent changes, although these methods are computationally demanding. Molecular dynamics simulations are also frequently combined with docking and MM-GBSA analyses to assess the stability of tetrahydroquinoline ligand binding over time. The integration of docking, MM-GBSA, and molecular dynamics provides a more comprehensive and reliable framework for evaluating tetrahydroquinoline binding behavior in silico.

Preclinical Evaluation of Tetrahydroquinoline-Based Anticancer Agents

In vitro and preclinical evaluations provide essential support for the anticancer potential of tetrahydroquinoline-based compounds. Across multiple studies, THQ derivatives have been tested in a wide range of cancer cell models using standard viability and proliferation assays. Many of these compounds demonstrate low micromolar to nanomolar antiproliferative activity, indicating strong cellular potency. Importantly, several THQ-based agents display preferential activity toward cancer cells compared with nonmalignant cells, suggesting the presence of a therapeutically relevant selectivity window. In addition to short term viability assays, longer term clonogenic studies have shown that THQ treatment can markedly suppress the ability of cancer cells to sustain proliferative growth.

Mechanistic in vitro experiments further indicate that THQ derivatives can modulate key cellular processes involved in tumor progression. Depending on their molecular targets, THQ compounds have been reported to induce cell cycle arrest, activate apoptotic pathways, and suppress cell migration or invasive behavior. These effects are supported by biochemical and molecular analyses showing alterations in signaling proteins associated with proliferation, survival, and metastasis. In the context of immuno oncology, some THQ-based small molecules have also been evaluated in functional cellular systems relevant to immune checkpoint regulation, where they demonstrate the ability to restore immune cell activity by modulating inhibitory signaling pathways.

Although in vivo data remain limited for many THQ series, available preclinical findings collectively support the translational potential of this scaffold. The consistency between cellular efficacy, mechanistic readouts, and selectivity profiles suggests that tetrahydroquinoline-based compounds represent promising candidates for further optimization and evaluation in animal models. Overall, these in vitro and early preclinical studies reinforce the role of tetrahydroquinoline as a versatile and pharmacologically relevant scaffold in anticancer drug discovery.

Current experimental models support the anticancer potential of tetrahydroquinoline-based compounds, but their reliance on simplified in vitro systems and limited in vivo evaluation restricts how well these results reflect true tumor behavior and therapeutic performance.

Resistance Mechanisms

As with other targeted anticancer agents, tetrahydroquinoline-based compounds are expected to encounter resistance during prolonged treatment. Potential mechanisms include target modification through mutation, reactivation of compensatory signaling pathways, and adaptive changes in tumor cell survival networks. In addition, resistance may arise from increased drug efflux or altered intracellular drug handling, particularly given the physicochemical properties common to small molecule therapeutics. For THQ based immune modulators, adaptive immune escape mechanisms, such as upregulation of alternative

inhibitory pathways, may also limit long term efficacy. These considerations highlight the importance of continued scaffold optimization, rational combination strategies, and early evaluation of resistance pathways during the development of tetrahydroquinoline-based anticancer agents.

Comparison with Existing Clinical Anticancer Agents

Tetrahydroquinoline derivatives can be positioned alongside existing anticancer agents as emerging small-molecule alternatives within established therapeutic classes. Compared with approved mTOR inhibitors and antibody-based immune checkpoint therapies, THQ-based compounds have demonstrated strong cellular activity and may offer advantages such as alternative binding modes, oral bioavailability, and improved tissue penetration. Although their clinical performance remains to be established, these comparisons suggest potential niches in which THQ analogs could complement current anticancer treatments. Continued progress in synthetic methodology, early integration of developability considerations, and careful selection of clinical indications are expected to facilitate further advancement. In parallel, increasing experience with small-molecule targeted therapies and immunomodulators is helping to clarify regulatory expectations for novel scaffolds such as tetrahydroquinolines, supporting their potential progression into clinical evaluation when efficacy, safety, and manufacturability are adequately balanced.

Future Perspectives

Future research on tetrahydroquinoline based anticancer agents is expected to progress along multiple directions as advances in cancer biology. The structural versatility of the tetrahydroquinoline scaffold allows its application across diverse molecular targets and supports combination strategies designed to address compensatory and overlapping signaling pathways. Integration with immuno oncology, including combination approaches and selection of patients based on molecular biomarkers, represents a promising direction. Advances in synthetic methods will play an important role in the development of tetrahydroquinoline based anticancer agents. Scalable, robust, and reproducible synthetic routes are required to enable systematic structure modification, efficient access to analog series, and reliable preparation of material for biological evaluation. Overall, continued improvements in synthetic methodology are expected to support efficient optimization and further development of tetrahydroquinoline based anticancer agents.

Conclusion

Tetrahydroquinoline derivatives are a class of small molecules investigated in anticancer drug research. This chapter has examined their development from chemical design through biological activity and preclinical evaluation. The tetrahydroquinoline core allows systematic structural modification and can be prepared using established synthetic methods, enabling exploration across different biological targets.

Cellular and molecular analyses show that tetrahydroquinoline-based compounds act through multiple anticancer mechanisms, including inhibition of signaling kinases, modulation of immune checkpoint pathways, induction of cell death, and interference with drug resistance processes. Preclinical data demonstrate consistent cellular activity and, in some cases, selectivity toward cancer cells. Most available evidence remains at the *in vitro* or early *in vivo* stage.

As with other small molecule candidates, tetrahydroquinoline derivatives face challenges related to pharmacokinetics, resistance development, and safety evaluation. Addressing these issues requires careful optimization, appropriate experimental models, and clear clinical context. Overall, current findings place tetrahydroquinoline derivatives within ongoing efforts to develop targeted and combination based anticancer therapies.

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CHAPTER 44

Harnessing the Power of Artificial Intelligence for Oral and Maxillofacial Surgery

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1. Introduction

Artificial intelligence is a discipline of computer science focused on creating systems capable of performing tasks that typically require human intelligence, such as learning, decision-making, and problem-solving (Sillmann et al., 2025). Machine learning (ML), a subfield under this broad umbrella, enables systems to mimic intelligent behavior by learning from data without being explicitly programmed for each task (Sillmann et al., 2025). Deep learning (DL), on the other hand, represents a more sophisticated subset of machine learning that uses multi-layered artificial neural networks to process complex data models and perform hierarchical feature extraction (Hung et al., 2023; Sillmann et al., 2025).

The volume and complexity of data generated in the healthcare sector have reached a level that challenges the limits of human cognitive capacity. In this context, machine learning and deep learning algorithms support physicians by processing large patient datasets at unprecedented speeds in the analysis of medical images such as computed tomography (CT) and X-rays (Sillmann et al., 2025). AI algorithms analyze pixel and voxel-level relationships in radiological images, revealing quantitative features (radiomics) that are imperceptible to the human eye, thereby paving the way for personalized medicine applications (Hung et al., 2023). These technologies play a critical role not only in increasing diagnostic accuracy but also in the early detection of diseases and treatment planning processes (Sillmann et al., 2025).

In contemporary medical practice, the exponential increase in computer performance and data processing capacity has triggered a fundamental paradigm shift in surgical disciplines. Particularly in the field of Oral and Maxillofacial Surgery (OMS), the integration of artificial intelligence (AI)-based algorithms has the potential to increase diagnostic accuracy, optimize surgical planning, and maximize postoperative predictability (Sillmann et al., 2025). AI is broadly defined as a set of systems capable of simulating cognitive tasks that require human intelligence, while machine learning (ML) is a subdiscipline that enables these systems to recognize patterns in data without explicit programming (Sillmann et al., 2025). Deep learning (DL), on the other hand, represents a more sophisticated subset of ML that processes complex data sets using multi-layered artificial neural networks (ANN) and can perform hierarchical feature extraction (Hung et al., 2023).

Surgical decision-making processes are subject to physicians' experience, fatigue levels, and cognitive biases; this situation makes it difficult to achieve standardization in diagnostic and therapeutic processes (Rasteau, Ernenwein, Savoldelli, & Bouletreau, 2022). AI algorithms aim to eliminate these subjective variables by offering “superhuman” accuracy across a broad spectrum, ranging

from pixel/voxel-level analysis of radiological images to histopathological classification (Hung et al., 2023). This section will examine the current applications of AI in the subspecialties of ADC, its algorithmic infrastructure, and its impact on clinical outcomes in light of the literature.

2. The Use of Deep Learning Algorithms in Oral, Dental, and Maxillofacial Surgery

A series of studies have been conducted to determine in which branches of dentistry the use of artificial intelligence is currently more prevalent. Between 2011 and 2021, there has been a significant increase in the number of publications on artificial intelligence in dentistry, particularly since 2015. Over the past decade, the number of academic publications in this field has increased by an average of 21.6% per year, while in the last five years, this increase has risen to 34.9%. When compared to the single-digit annual growth rates observed in the early 2000s, these findings demonstrate that AI-focused dental literature has accelerated dramatically (Thurzo et al., 2022).Son yıllarda yapay zeka (YZ) teknolojilerinin Ağız, Diş ve Çene Cerrahisi (ADÇC) alanındaki uygulamaları önemli bir ivme kazanmış; Şekil 1’de görüldüğü üzere tanısal süreçlerden sefalometrik analizlere, preoperatif planlamadan postoperatif takibe kadar uzmanlık alanının birçok boyutuna katkı sağlamıştır (Miragall ve diğerleri, 2023; Sillmann ve diğerleri, 2025).

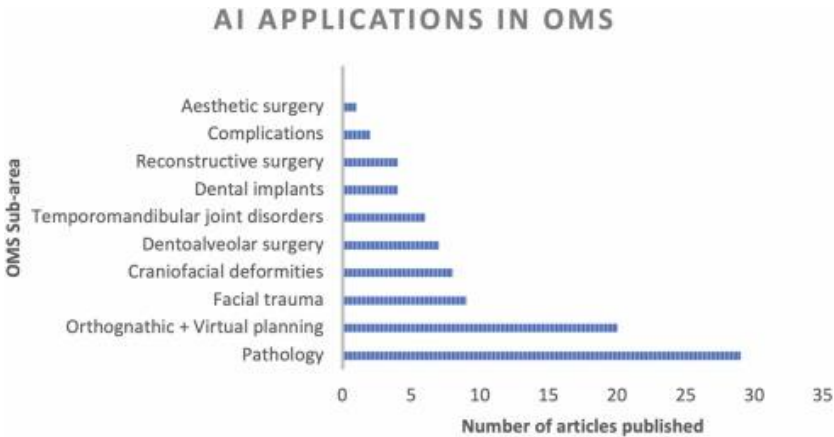


Figure 1: Number of publications on the areas of OMFS where artificial intelligence is most commonly used (Sillman et al. 2025)

Especially within the scope of dentoalveolar surgery, optimizing the management of impacted teeth is one of the main areas where AI algorithms show promise. Developed models help determine the indication for tooth extraction by predicting potential risks associated with tooth eruption and can estimate the

difficulty level of planned surgical procedures prior to surgery (Miragall et al., 2023; Rasteau, Ernenwein, Savoldelli, & Bouletreau, 2022). Similarly, in implantology, algorithms trained with clinical data can predict osseointegration success and guide treatment planning by optimizing implant design prior to surgery (Miragall et al., 2023).

One of the most tangible reflections of artificial intelligence in surgical practice is its integration with robotic surgical systems. AI-supported robotic systems have demonstrated successful outcomes in procedures requiring precision, such as dental implant placement, tumor resection, biopsy procedures, and temporomandibular joint surgery (Miragall et al., 2023; Sillmann et al., 2025). These approaches are illustrated in Figure 2.

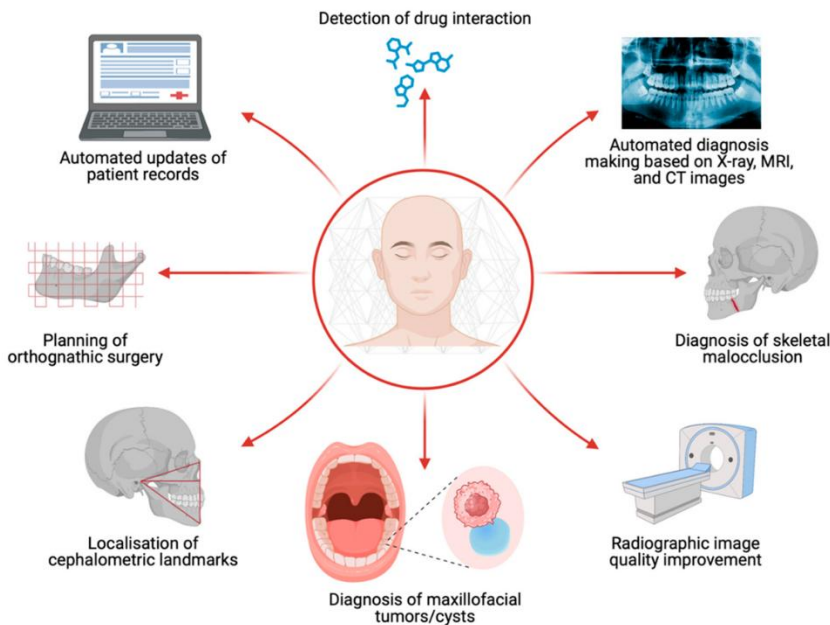


Figure 2: Current use of artificial intelligence algorithms in Oral and Maxillofacial Surgery (Miragall et al. 2023)

Studies have demonstrated that AI-assisted surgical approaches improve the accuracy and safety of procedures compared to traditional freehand techniques (Miragall et al., 2023). By minimizing errors in implant positioning, thereby reducing the need for revision surgery, and providing more precise margins in cyst or tumor resection, thereby eliminating the need for additional procedures, this technology clearly demonstrates its contribution to clinical efficiency (Miragall et al., 2023).

2.1. Orthognathic Surgery and Deep Learning Integration

Although virtual surgical simulation has become the cornerstone of preoperative preparation in orthognathic surgery planning, the segmentation of anatomical structures remains a labor-intensive and time-consuming procedure when performed using traditional methods (Deng et al., 2023). In this context, deep learning (DL) algorithms offer revolutionary speed and accuracy, particularly in the automatic segmentation of the mandible, maxilla, and dentition from cone-beam computed tomography (CBCT) images. For example, multi-stage convolutional neural network (CNN) models such as “SkullEngine” can complete the segmentation process, which takes hours for an expert operator, in minutes and demonstrate accuracy comparable to manual segmentation with a Dice similarity coefficient of 96% (Deng et al., 2023). This technological automation optimizes workflow by allowing surgeons to focus on clinical decision-making mechanisms rather than technical drawing processes (Sillmann et al., 2025).

Beyond segmentation, DL models also provide clinicians with a significant support mechanism in the automatic detection of cephalometric landmarks and the classification of treatment requirements. Minimizing observer-dependent variability in traditional cephalometric analyses, these systems can predict the necessity of surgical intervention with over 90% accuracy by analyzing the patient's skeletal malocclusion degree (Dong et al., 2024; Rasteau, Ernenwein, Savoldelli, & Bouletreau, 2022). Furthermore, during the optimization phase of surgical planning, the automatic positioning of anatomical landmarks in 3D space increases the reliability of surgical simulation and contributes to the standardization of the treatment plan (Dong et al., 2024).

In evaluating the clinical success of orthognathic surgery, aesthetic outcomes and soft tissue changes play a critical role alongside skeletal stability. While traditional mass-spring models or finite element analysis (FEM) have certain biomechanical limitations in predicting soft tissue changes, deep learning-based algorithms can simulate postoperative facial appearance with higher accuracy by learning from past patient data (Almarhoumi, 2024; Sillmann et al., 2025). It has been reported that, particularly in predicting changes in lip and chin tip morphology after mandibular advancement surgery, DL models reduce the average error to 1.0 mm levels and thus provide realistic visualization that enhances communication between the patient and the physician (Almarhoumi, 2024; Dong et al., 2024).

2.2. Identification of Anatomical Landmarks and Automated Cephalometric Analysis

Cephalometric analysis, the cornerstone of cranio-maxillofacial surgery and orthodontic treatment planning, relies on the accurate and reproducible identification of anatomical reference points. Traditional manual marking methods exhibit high intra-observer and inter-observer variability depending on the clinician's experience and fatigue level, which compromises diagnostic consistency (Rasteau, Ernenwein, Savoldelli, & Bouletreau, 2022). In this context, artificial intelligence (AI), particularly deep learning (DL) and convolutional neural networks (CNN), is being deployed for the automatic detection of anatomical points on radiographic images; it offers speed and efficiency that can reduce analysis time to as little as one percent of manual methods by standardizing these processes (Sillmann et al., 2025). The literature reports that models detecting landmarks on lateral cephalograms provide accuracy comparable to experts, unaffected by changes in image brightness and contrast, and minimize human-induced errors (Miragall et al., 2023; Sillmann et al., 2025).

With the increasing use of three-dimensional imaging modalities (CT and CBCT), research has shifted from two-dimensional planes to three-dimensional spatial analysis. Particularly in orthognathic surgery simulations, skeletal structure segmentation and landmark detection can be performed simultaneously. For example, “SkullEngine,” a multi-stage CNN framework, can complete both bone segmentation and the detection of 68 different cephalometric landmarks on CBCT images in approximately 4 minutes (Deng et al., 2023). Such systems use landmarks as “anchor” points to separate anatomical structures (midface, mandible, teeth), thereby improving segmentation quality and automating the measurements required for surgical planning (Deng et al., 2023; Yan, Liu, & Li, 2021).

However, there are still some technical limitations that need to be overcome regarding the clinical accuracy of current algorithms. Although segmentation processes achieve high success rates with Dice similarity coefficients of up to 96%, it has been reported that the average error during automatic landmark detection can vary between 2.0 mm and 3.0 mm, and in some cases (e.g., at indistinct points such as the antegonial notch), deviations can push the limits of clinical acceptance (Deng et al., 2023). Nevertheless, AI models show promise not only for bone structures but also for detecting facial soft tissue landmarks. HRNet-based models have been developed that can accurately detect superficial landmarks even in the presence of pathological conditions such as scar tissue,

defects, or edema, demonstrating the potential of AI in the follow-up of trauma or reconstructive surgery patients (Sillmann et al., 2025).

2.3. Maxillofacial Trauma and Its Integration into Deep Learning

Maxillofacial traumatology occupies a unique position among surgical disciplines due to the complexity of anatomical structures and their close relationship with adjacent vital organs. Particularly in emergency department settings, the masking of fracture lines due to superimpositions or edema creates diagnostic challenges for inexperienced clinicians and increases the risk of morbidity (Pham, Holmes, & Coulthard, 2024). In this context, artificial intelligence (AI)-based systems have the potential to detect fracture lines that are imperceptible to the human eye by performing pixel-level analysis of radiological images and providing a “second opinion” (Sillmann et al., 2025). Deep learning (DL) algorithms, particularly when trained on conventional radiographs and computed tomography (CT) slices, can autonomously detect the presence, localization, and classification of fractures (Pham et al., 2024).

A significant portion of the studies in the literature have focused on mandibular fractures, which have the highest incidence. Developed to overcome the limitations inherent in the two-dimensional nature of panoramic radiographs, Faster R-CNN and Swin-Transformer-based models have demonstrated performance comparable to that of expert radiologists in detecting mandibular fractures, achieving an F1 score of 94.7% and an AUC (Area Under the Curve) value of 0.977 (Vinayahalingam et al., 2022). However, hybrid models have been developed to increase diagnostic accuracy in anatomically complex areas such as the condylar head, which carries a risk of dislocation. For example, approaches combining the object detection capability of the YOLOv4 (You Only Look Once) algorithm with the segmentation success of the U-Net architecture have significantly contributed to minimizing missed cases by increasing the detection rate of mandibular fractures from 79% to 87% (Son, Yoon, Kwon, & Lee, 2022).

Midface and orbital trauma present a more challenging area for AI applications due to the complex structure of the zygomaticomaxillary complex. However, convolutional neural networks (CNNs) developed in recent years have shown promising results in the automatic detection of zygomatic fractures, orbital blowout fractures, and nasal bone deformities from CT images (Sillmann et al., 2025). Advanced algorithms such as “SkullEngine” and YOLO variants, in particular, can not only detect the fracture line but also analyze the amount of displacement of bone segments, providing clinicians with predictive data regarding surgical requirements (operative or non-operative approach) (Pham et al., 2024). These systems can also quantitatively report post-traumatic

asymmetries and volumetric losses by analyzing CT images in three-dimensional space.

Despite all these technological advances, there are still some barriers to overcome in the clinical integration of AI models in traumatology. Current algorithms may experience poor performance, particularly in pediatric trauma or rare complex panfacial fractures, due to insufficient and imbalanced datasets (Pham et al., 2024). Furthermore, the detection of small cracks or nondisplaced fractures remains a “challenge” area for deep learning models. Future studies focusing on developing highly explainable models trained on multicenter and heterogeneous datasets will solidify this technology's place in emergency trauma management (Sillmann et al., 2025).

2.4. Deep Learning Integration in Oral Pathology and Oncology

The differential diagnosis of cystic and neoplastic lesions in the oral and maxillofacial region poses a significant diagnostic dilemma for clinicians due to the similarity in the radiographic appearance of these lesions and the asymptomatic nature of some pathologies (Yang et al., 2020). In this context, artificial intelligence (AI)-based image processing algorithms analyze pathological changes in panoramic radiographs and cone beam computed tomography (CBCT) images at the pixel level, acting as a “digital pathologist” in pre-biopsy decision-making processes. Deep learning (DL) architectures, in particular, show promising results in the detection and classification of odontogenic lesions such as ameloblastoma, odontogenic keratocyst (OKC), and dentigerous cyst. For example, a large-scale study using the YOLO v2 algorithm reported that AI demonstrated diagnostic performance equivalent to that of expert surgeons (70.7% accuracy and 68.0% sensitivity, respectively) in the detection and classification of these lesions (Yang et al., 2020). Furthermore, KIBT-based studies using the GoogleLeNet Inception v3 architecture achieved 96.1% sensitivity and 77.1% specificity in detecting cystic lesions, proving that the integration of 3D imaging with AI increases diagnostic accuracy (Rahmanzadeh, Rustamzadeh, Gorgich, Mehrbani, & Aghakouchakzadeh, 2025).

Within the spectrum of oncological surgery, the management of oral squamous cell carcinoma (OSCC), the most common type of head and neck cancer, depends on early diagnosis of the disease and accurate staging of cervical lymph node metastasis (LNM). While traditional imaging methods may be insufficient for detecting microscopic metastases or occult lymph node involvement, U-Net-based DL models trained on contrast-enhanced computed tomography (CT) images have demonstrated success in segmenting and detecting metastatic lymph nodes, surpassing the performance of radiologists (Ariji et al., 2022; Rahmanzadeh et al., 2025). By analyzing the morphological features,

boundary irregularities, and internal heterogeneity of lymph nodes, these algorithms can predict the presence of metastasis with AUC (Area Under the Curve) values reaching 95%, thus providing surgeons with critical data for preventing unnecessary neck dissections or planning necessary surgical interventions (Rahmanzadeh et al., 2025; Sillmann et al., 2025).

The study evaluated the performance of deep learning-based multi-class classification and object detection models for the early detection of oral squamous cell carcinoma (OSCC) and oral potential malignant disorders (OPMD) using oral photographic images (Warin et al., 2022). Using a total of 980 images (OSCC=365, OPMD=315, normal=300) collected from two centers between January 2009 and December 2020 and verified by histopathology; DenseNet-169, ResNet-101, SqueezeNet, and Swin-S were used for classification, while Faster R-CNN, YOLOv5, RetinaNet, and CenterNet2 were used for detection. These models were trained and tested using 5-fold cross-validation. The best results in classification were obtained with DenseNet-169; AUC=1.00 for OSCC and AUC=0.98 for OPMD were reported, and this performance was shown to be comparable to experts and better than practitioners. For object detection, the best AUC (precision–recall curve) for OSCC was achieved with CenterNet2 (AUC=0.91), while the best result for OPMD detection was reported with Faster R-CNN (AUC=0.64). The authors emphasized the potential for a decision support tool that could support practitioners' visual examination-based screening; noting limitations such as the scope of the dataset and the labeling burden, they suggested development with broader data (mobile application/clinical workflow integration) including different lesion types. Images where different architectures correctly identified oral lesions as positive are listed in Figure 3.

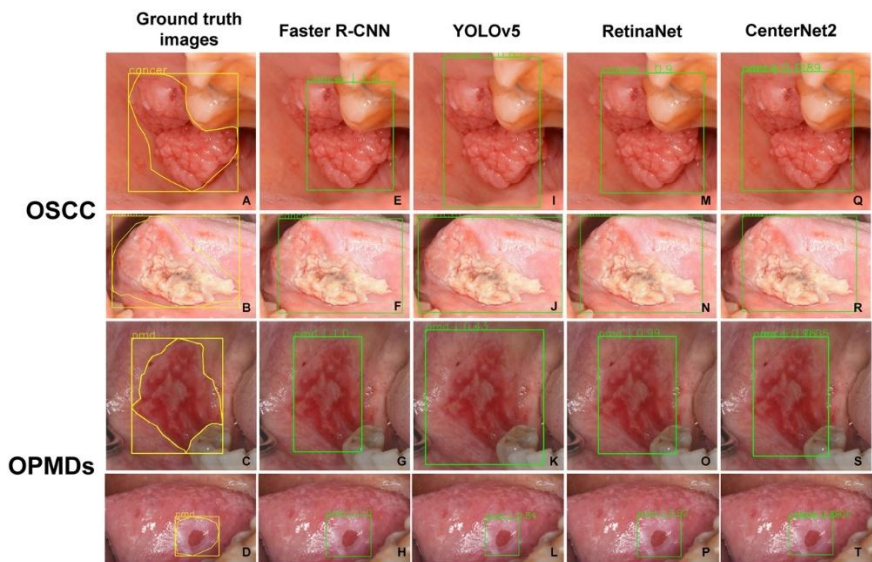


Figure 3: (A-B) Ground truth bounding boxes based on surgeons' annotations for images of a patient with OSCC in the retromolar trigon and lateral tongue region; (C–D) Ground truth bounding boxes based on surgeons' annotations for images of a patient with OPMD in the retromolar trigon and lateral tongue region; (E–H) True positive outputs obtained from the Faster R-CNN detection model; (I–L) True positive outputs obtained from the YOLOv5 detection model; (M–P) True positive outputs obtained from the RetinaNet detection model; (Q–T) True positive outputs obtained from the CenterNet2 detection model. (Warin et al. 2022)

One of the most revolutionary contributions of artificial intelligence in oncology is the prediction of tumor biology and prognosis through “radiomic” analyses. Radiomics is based on the principle of performing high-dimensional data mining by extracting quantitative data (tissue, shape, density histograms, etc.) from medical images that cannot be perceived by the human eye (Hung et al., 2023). This method is considered a “glass box” approach that provides clinicians with more explainable and biologically grounded data, unlike the “black box” nature of deep learning (Hung et al., 2023). Studies have shown that radiomic signatures and machine learning (ML) algorithms (e.g., Support Vector Machines - SVM) provide high accuracy in predicting occult cervical lymph node metastasis, tumor histological grade, and disease prognosis in oral cancer patients (Yan, Liu, & Li, 2021; Sillmann et al., 2025). These predictive models play a vital role in creating personalized treatment protocols and determining the risk of recurrence after treatment.

Finally, the evaluation of salivary gland pathologies has also benefited from the capabilities of AI. DL and radiomic models are used in the differential diagnosis of salivary gland tumors (e.g., pleomorphic adenoma and Warthin tumor) and in the diagnosis of autoimmune diseases such as Sjögren's syndrome

(Hung et al., 2023). These algorithms, trained on CT and Magnetic Resonance Imaging (MRI) data, can distinguish benign and malignant lesions with high accuracy by analyzing the boundary characteristics of fatty degeneration or tumor masses in the gland parenchyma (Hung et al., 2023; Sillmann et al., 2025). All these developments demonstrate that artificial intelligence is progressing toward becoming not just an imaging assistant, but also the surgeon's most powerful ally throughout the process, from pathological diagnosis to treatment planning and prognosis prediction.

2.5. Integration of Deep Learning in Dental Implantology

Clinical success in dental implant rehabilitation depends on achieving osseointegration and maintaining long-term stability; this process requires a careful assessment of bone quality, quantity, and anatomical relationships. Today, deep learning (DL) algorithms and cone beam computed tomography (CBCT) integration serve as a “digital assistant” in implant planning, increasing surgical precision (Hung et al., 2023). Particularly in the preoperative planning phase, the automatic segmentation of critical anatomical structures such as the mandibular canal and maxillary sinus minimizes the risk of iatrogenic complications such as inferior alveolar nerve damage (Hung et al., 2023). Furthermore, DL models trained on 3D CBCT images can perform implant planning with a level of accuracy comparable to manual measurements in the maxillary molar/premolar and mandibular premolar regions and guide clinicians in determining the appropriate implant size for the bone morphology (Yan, Liu, & Li, 2021).

Another significant challenge encountered in implantology practice is the diversity of existing implant systems; the numerous implant brands and systems available on the market make identifying the implant type from radiographic images a challenging process for clinicians (Sillmann et al., 2025). At this point, convolutional neural networks (CNN) come into play, enabling the classification of implant brands, types, and even treatment stages (healing cap, abutment, etc.) with high accuracy (over 90%) from panoramic and periapical radiographs (Sillmann et al., 2025; Yan, Liu, & Li, 2021). These automated identification systems provide vital convenience, particularly in revision surgeries or the procurement of prosthetic parts for patients who have developed complications and whose records are inaccessible. Furthermore, AI-supported systems have shown promising results in the detection and classification of fractured dental implants through periapical radiographs (Yan, Liu, & Li, 2021).

The role of artificial intelligence in implantology is not limited to diagnosis and planning, but also extends to predicting treatment success and managing complications. Machine learning (ML) algorithms, particularly “Random Forest” models, are used to predict implant failure and peri-implantitis risk by analyzing variables such as the patient's systemic condition, surgical technique, and implant position (e.g., mesiodistal position) (Sillmann et al., 2025; Yan, Liu, & Li, 2021). These predictive models enable clinicians to create personalized treatment protocols and optimize patient follow-up by identifying risk factors that affect osseointegration success or survival rates in advance (Rasteau, Ernenwein, Savoldelli, & Bouletreau, 2022).

Finally, artificial intelligence-based approaches are also attracting attention in implant design and biomechanical optimization processes. Studies combining artificial neural networks (ANN) and genetic algorithms with finite element analysis (FEA) help determine the most suitable implant design (porosity, length, diameter) to minimize stress at the implant-bone interface (Rasteau et al., 2022; Yan, Liu, & Li, 2021). However, the majority of current studies still rely on two-dimensional (2D) imaging data, which does not fully reflect the three-dimensional nature of implantology; moreover, the continuous introduction of new implant systems poses a challenge in keeping algorithms up-to-date (Sillmann et al., 2025).

2.6. Temporomandibular Joint Disorders and Artificial Intelligence-Supported Analytical Approaches

Temporomandibular joint (TMJ) disorders represent a spectrum of pathologies that pose significant challenges for clinicians in diagnosis and treatment planning due to their multifactorial etiology and the complexity of clinical symptoms. Artificial intelligence (AI)-based systems in this field, particularly through artificial neural networks (ANN) and machine learning (ML) algorithms, are used to analyze clinical signs and symptoms to predict subtypes of TMD (e.g., internal derangements) and create decision support mechanisms (Sillmann et al., 2025). Furthermore, the processing of semantic and radiomic features obtained from infrared thermography images using ML classifiers has been established in the literature as an innovative approach that increases diagnostic accuracy in the early diagnosis of TME pathologies and the monitoring of inflammatory processes (Sillmann et al., 2025)

In terms of imaging analytics, deep learning (DL) models have made revolutionary advances in the evaluation of both soft tissue (disc, ligaments) and hard tissue (condyle, fossa) components of TME. Convolutional neural networks (CNNs) trained on magnetic resonance imaging (MRI) slices can perform automatic segmentation of the joint disc and detection of anterior disc

displacement with high accuracy (Rahmanzadeh, Rustamzadeh, Gorgich, Mehrbani, & Aghakouchakzadeh, 2025; Sillmann et al., 2025). Similarly, U-Net-based architectures working on cone-beam computed tomography images automate labor-intensive processes such as measuring cortical bone thickness at the condylar head and performing quantitative analysis of morphological changes by automatically segmenting the mandibular condyle verumus (Hung et al., 2023).

The early diagnosis and grading of osteoarthritis is one of the most promising application areas for artificial intelligence. It has been reported that deep learning models achieve 91% agreement with expert opinion in classifying osteoarthritic changes (erosion, osteophyte formation, flattening) from KIBT images (Hung et al., 2023). As a more advanced approach, “radiomic” analyses that go beyond image data enable the creation of hybrid models by integrating biomolecular, clinical, and demographic data. For example, in multimodal studies using advanced ML algorithms such as XGBoost and LightGBM, diagnostic accuracy (AUC > 0.80) exceeding 80% was achieved in the early diagnosis of TME osteoarthritis, providing a strong foundation for developing personalized treatment protocols (Hung et al., 2023; Sillmann et al., 2025).

However, there are some limitations to the full integration of current AI applications into clinical routine. Despite the dynamic nature of TME disorders, most existing algorithms analyze static images or a single sagittal plane, which limits the detection of pathophysiological changes during functional movements of the joint (opening and closing the mouth) (Sillmann et al., 2025). Furthermore, the fact that most studies in the literature have been case-control designs and rely on small data sets is considered a factor that limits the ability of algorithms to distinguish rare TME pathologies (e.g., posterior or mediolateral disc displacement) and their generalizability (Czako et al., 2024; Sillmann et al., 2025).

3. Conclusion

The discipline of Oral and Maxillofacial Surgery is undergoing a historic transformation through the integration of artificial intelligence (AI) and deep learning (DL) algorithms; the conventional “intuitive” surgical approach is increasingly giving way to a data-driven, predictable, and precision-focused “computational” paradigm (Sillmann et al., 2025). Accelerated by the digitization of radiological imaging modalities (CT, PET-CT, MRI), this process represents a quest for “error-free” perfection by offering data processing and analysis capabilities that exceed human capacity in diagnosis and treatment planning, the most fundamental components of surgical practice (Minnema et al., 2022). A cumulative analysis of the literature reveals that AI is not merely an isolated

technological tool but a holistic ecosystem that reshapes the backbone of the surgical workflow.

Within the spectrum of clinical applications; in orthognathic surgery, the automatic segmentation of anatomical structures and the high-accuracy simulation of soft tissue changes standardize preoperative planning (Deng et al., 2023; Almarhoumi, 2024); in maxillofacial traumatology, automated detection of fracture lines maximizes patient safety by minimizing missed diagnoses, especially in emergency department settings (Pham, Holmes, & Coulthard, 2024). A similar revolutionary impact is observed in oncology and pathology; deep learning models and radiomic analyses provide clinicians with critical data as a pre-biopsy decision support mechanism, ranging from the differential diagnosis of cystic lesions to the prediction of cervical lymph node metastasis (Rahmanzadeh et al., 2025; Yang et al., 2020). Advances in areas such as preserving vital structures and predicting implant success in implantology (Yan, Liu, & Li, 2021) and early diagnosis of TME disorders (Hung et al., 2023) demonstrate that AI has achieved versatile competence covering the entire surgical spectrum.

However, despite this promising picture presented by the current literature, there are still epistemological and technical barriers to overcome in clinical translation. The generalizability of algorithms across different devices and populations, the standardization of datasets, and the explainability issues arising from the “black box” nature of deep learning are priority areas awaiting solutions for the universal acceptance of the technology (Zhang et al., 2025; Dong et al., 2024). Looking ahead, it is essential to develop AI models that are trained on multi-centered and heterogeneous data and are transparent and compliant with ethical norms. In this context, artificial intelligence is not a rival threatening the surgeon's professional autonomy; rather, it will solidify its place in the surgical theater as an indispensable “digital assistant” that lightens the surgeon's cognitive load, expands their sensory limits, and strengthens the final clinical decision (Sillmann et al., 2025; Czako et al., 2024).

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CHAPTER 45

Dating Violence and Nursing

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Introduction

Violence is defined as the intentional use of force or threats that cause or may cause psychological harm, injury, death, or developmental impairment to oneself, another person, a group, or a community (WHO, 2025). A dating relationship is a relationship in which a person establishes romantic and/or sexual intimacy with another person but is not engaged, married, or in a similar binding relationship (Murray and Kardatzke, 2007). It is characterised by attitudes and behaviours that manifest themselves in the desire for power and control, and in suppressing, restricting, and obstructing one's partner. When acts of violence occurring in the early stages of a dating relationship are accepted by the parties as feelings of love/jealousy, these acts can intensify over time and become constant, turning into violence (Özdere and Kürtül, 2018). Dating violence is defined as "any type of violent incident, such as physical, sexual, or verbal abuse, threats, or stalking, that occurs from the beginning of a romantic relationship until it becomes official (Quinlan, 2011). Dating violence mostly occurs during adolescence (17-29 years), when dating relationships are common, and this period is considered a risky period in terms of dating violence (Yörüktepe and Çalık, 2021). Studies indicate that both men and women can experience dating violence, but women are found to be more affected by it than men (Cloutier and Barling, 2025; Castillo-González et al., 2024). Dating violence causes many negative consequences for the person exposed to it (Toplu-Demirtaş and Fincham, 2020). Considering its prevalence and negative consequences, addressing, preventing, reducing and eliminating dating violence is an urgent need. Health professionals play a key role in combating this widespread problem. Violence is a preventable problem. Raising awareness among young people about dating violence, educating them on how to recognise dating violence and how to protect themselves are important nursing steps in combating violence (Koş and Karacan, 2023; Esen et al., 2018).

1. The Definition of Violence

Violence is an act that has been perpetrated or endured since ancient times. There is no clear information regarding the source, causes, and dimensions of violence; therefore, it is difficult to establish a standard definition that would be applicable to all societies and all times (Kepir-Savoly et al., 2014). According to the Turkish Language Association (2025), it is defined as '1. The degree, intensity, or severity of a movement or force. 2. Speed. 3. The force generated by a movement. 4. The use of brute force against those with opposing views. 5. Brute force. 6. Excessiveness in emotion or behaviour.' According to the World Health Organisation (2025), violence is defined as behaviour that causes injury, death, physical harm, or mental or developmental impairment to oneself, another person, a community, or a group through the use of physical force.

2. Dating Violence

Dating is defined as ‘a relationship between two people who fully and openly participate in social life together, lasting until marriage or until the relationship ends’ (Sezer, 2008). Dating fulfils individuals' need to form emotional closeness, seek social support, love and be loved. The experience of establishing a dating relationship is important not only in supporting young individuals' emotional maturity but also because their future spouse selection preferences will be shaped by their dating experiences during this period (Galdo Castiñeiras et al., 2023). Although perpetrators of dating violence are mostly men, it is known that women also perpetrate it. Dating violence can be one-sided or mutual (Carlos et al., 2017). It is known that factors such as age, ethnic group, gender, and sexual orientation do not create differences in the emergence of dating violence and that it can occur in any group (Zimmer, 2017). The fundamental intention behind perpetrating dating violence is the desire to exert power and control over one's partner (Özdere and Kürtül, 2018). Dating violence is defined as the restrictive and coercive actions of individuals in a dating relationship towards each other or one towards the other, whether physical, psychological, sexual, or social in nature (Öztürk et al., 2021). The risk of experiencing dating violence persists throughout life; however, the most risky period is adolescence and young adulthood, covering the age range of 17-29 (Cangür et al., 2021). During this period, in addition to physical and psychological changes, inexperience in romantic relationships, cultural characteristics, and the influence of media factors contribute to greater exposure to dating violence (Çoban et al., 2020).

2.1. Prevalence of Dating Violence

It is difficult to determine the prevalence rates of dating violence with certainty for various reasons. Some reasons for this difficulty include individuals' attitudes towards violence, differences in types of violence, definitions of violence, research methods, sampling techniques, interviewer skills, and individuals' desire to conceal their experiences due to reasons such as shame/guilt, as well as cultural differences (Fraga, 2016). According to World Health Organisation data, 26–34 per cent of women over the age of 15 have experienced dating violence (WHO, 2014). According to data from the European Union Agency for Fundamental Rights (FRA), 33% of women have experienced some form of physical or sexual violence during their youth, and this problem recurs in the university environment (Neilson et al., 2023). According to research data conducted with 500 female university students in Turkey, it was concluded that 88% of students were victims of emotional violence, 21.4% were victims of economic violence, 16.4% were victims of physical violence, and 7.2% were victims of sexual violence (Dikmen et al., 2018). In a study conducted by Gök and Sözbir (2025) with female university students, it was reported that 81% of 195 students were in dating relationships and 32% of these had been subjected to

dating violence. Another study conducted with 727 university students indicated that 25.3% of students were victims of dating violence (Demir and Biçer, 2017). Dating violence is significant not only because of its prevalence or negative effects on health, but also because it can be learned and increase the risk of becoming a victim or perpetrator in adulthood (Taquette and Monteiro, 2019). The concept of violence is often perceived by society as physical violence that causes tangible harm, but violence is evaluated within a broader spectrum.

2.2. Types of Dating Violence

The presence of discourse and attitudes related to a type of violence during dating, the existence of the behaviour, does not sharply distinguish it from other types of violence but feeds into another (Aslan et al., 2008). However, in a dating relationship where one type of violence is present, other types of violence are also likely to occur (Adejumo et al., 2022). Dating violence, a multidimensional concept, is classified in various ways according to its forms of application. These include physical violence, psychological violence, sexual violence, gender-based violence, economic violence, social violence, digital violence, and stalking.

2.2.1. Physical Violence

Physical violence is ‘the use of physical force, ranging from causing injury to even death, by using brute force as a means of intimidation, coercion, or punishment’. Physical violence is fundamentally based on consciously using force to harm another person's body, and some of its effects are clearly visible. Although the reason, purpose, legitimacy, or scope of resorting to physical violence may not be known for certain, physical violence is a relatively easier form of violence to identify compared to other types of violence (Ekinçi et al., 2024). Focusing on physical dating violence can prevent other violent behaviours from being noticed. However, physical violence is often the final stage of escalating violence (Akcan, 2020).

Punching, slapping, biting, mauling, kicking, crushing, twisting the arm, tying up, choking, pulling hair, throwing objects, pulling hair or ears, pinching, injuring with cutting or piercing instruments, burning with boiling water or acid, extinguishing cigarettes on the body, maiming, forcing someone to live in unhealthy conditions, torturing, threatening with knives and weapons, and causing physical harm by preventing access to healthcare are examples of non-accidental injuries that constitute physical violence (Morrison et al., 2023). More generally, any form of contact that the person does not consent to is physical violence (Lazarus et al., 2019). According to the Association for Combating Sexual Violence (2018), physical violence does not necessarily require the person to suffer direct harm, pain, or marks or wounds on their body; it also includes indirect behaviours such as damaging property, possessions, or living beings (animals, plants, children) and forcing someone to consume alcohol or drugs. Any situation that causes a person to doubt their safety without physical contact is also

considered physical violence (Zimmer, 2017). Studies indicate that physical dating violence is the final form of dating violence where concrete evidence is directly produced; that is, if physical violence is present, other forms of violence have been or are being experienced (Bhatta et al., 2021).

2.2.2. Psychological/Emotional Violence

Psychological violence is divided into six subheadings (Follingstad et al., 1990). The first type of psychological violence is verbal abuse; violent behaviours such as mocking, taunting and swearing. Isolation (social or economic) involves the abusive partner suppressing and/or preventing the other person from accessing their social support system. Jealousy is characterised by the abusive person restricting and controlling their partner's interactions with other people in their life. Friends, family, and even pets can be objects of jealousy. Behaviours such as dictating what the partner should wear or who they can see, or accusing the partner of infidelity, fall within this category. Threats are another dimension of psychological violence. This involves the abuser engaging in behaviours or making statements that will put the partner in a difficult situation, frighten them, or cause them anxiety if their demands are not met. Damaging or destroying the partner's personal belongings is the last type of psychological violence. It includes behaviours that cause material and moral damage to the victim, such as scratching their car, cutting their clothes, or tearing up a photo they cherish (Şenol-Durak and Ayvaşık, 2010).

Psychological violence, on the other hand, involves ignoring or failing to meet the partner's needs for love, attention, and affection; neglect; pressure; swearing and insulting; mocking; ridiculing; constant criticism; shouting; belittling, giving orders, intimidating, threatening, accusing, manipulating to make the victim doubt themselves, restricting their behaviour, making them feel worthless and inadequate, threatening to commit suicide/harm themselves. It involves creating constant points of conflict to disrupt the emotional state of the victim of violence, attempting to isolate them by disrupting their relationships with their social circle and work environment, ignoring them, preventing them from feeling safe, making statements that damage their reputation, preventing them from developing themselves, constantly monitoring their phone and messages, restricting their access to the outside world, and holding them responsible for tasks beyond their capacity are among the numerous actions involved (Boyacıoğlu, 2019).

The purpose of psychological violence is to weaken the individual's self-esteem and independence, to isolate them from social support systems, family and friends, and to make them feel abnormal by constantly questioning their decisions. As with other types of violence, it is to gain control over the partner, keep them under pressure and prove one's power. The person subjected to psychological violence is made to feel guilty for their behaviour by the perpetrator (Zimmer, 2017).

Psychological dating violence, which is often seen alongside other types of violence, progresses slowly over the long term and is more difficult to recognise and identify than other types of violence because there is no direct, concrete evidence. It diminishes the individual's self-esteem by making them feel bad and undermining their self-confidence, negatively affecting their health (Karadayı, 2020).

2.2.3. Sexual Violence

Sexual violence is when a person is forced into any sexual activity against their will, even if they are married to the other person. Sexual violence is not limited to physical contact; it encompasses a wide range of acts, from touching to forcing someone to watch sexually explicit films, from verbal sexual assault to coercion into sexual intercourse. According to the World Health Organisation (2025), sexual violence is any sexual act, attempted sexual act, or other act committed by any person against another person's sexuality using force, regardless of the relationship between the perpetrator and the victim, in any setting. This includes rape, defined as the physical penetration of the vulva or anus with a penis, other body part, or object, or forcible penetration by other means. According to World Health Organisation data, the rates of exposure to any form of sexual violence vary by region. Based on recorded rates, the African region is the region with the highest incidence of sexual violence at 27%, while the Western Pacific region is the region with the lowest incidence of sexual violence at 6% (WHO, 2025). Sexual violence also brings with it numerous health problems such as mental and neurological disorders, infectious diseases, unhealthy risky behaviours, a decline in quality of life, termination of pregnancy, injury, and sexual and reproductive health problems (Biçer, 2022). Sexual violence includes forcing someone to have sexual intercourse against their will, at an unwanted time, place, or in an unwanted manner (rape), comparing one's sexuality with that of others, harming or potentially harming sexual organs, using sexuality as a method of punishment, forcing someone to have or not have children, preventing someone from using birth control methods, forcing someone into prostitution, forced marriage, or engaging in sexually harassing behaviour via telephone, email, or verbally are acts of sexual violence. In addition, genital examinations performed without the person's consent and without following legal procedures for the purpose of virginity checks, which violate bodily integrity, also fall under the scope of sexual violence. (Demirtaş and Sümer, 2022). The most important criterion in assessing sexual dating violence is that neither partner has given 'consent' for the sexual behaviour (Toplu-Demirtaş and Aracı-İyiyaydın, 2021). Consent is a one-time act that implies that partners are conscious when giving consent for sexual acts and are able to exercise their free will (Toplu-Demirtaş and Aracı-İyiyaydın, 2021). The process of not accepting a person's refusal to consent to an act and attempting to turn that refusal into consent, and behaving in a manner intended to achieve this (giving gifts, persistent demands,

lying, playing the victim, etc.), is referred to as 'consent construction' and falls within the scope of sexual violence (Toplu-Demirtaş and Aracı-İyiyaydın, 2021). Without obtaining the person's consent, by constructing consent or in situations where the other party's consent is not possible (if the person is under the influence of alcohol or drugs, under the age of 18, physically and/or mentally incapable of giving consent, under coercion, pressure or threat, or if the animal is exposed to the behaviour in question, consent is not possible) constitutes sexual violence (Gürhan et al., 2020). Sexual violence does not involve sexual gratification or honour-related behaviour. It is fundamentally driven by a desire to humiliate or dominate. Due to society's perception of sexuality, most cases of sexual violence go unreported because victims fear further harm from the perpetrator and the risk of stigmatisation. Compared to other types of violence, sexual violence is more difficult to talk about, and it is even believed that it should not be discussed and should remain hidden (Mangat and Gill, 2024).

2.3.4. Economic Violence

Economic violence is the use of financial resources as a means of power, threat and control over another person (Alkan et al., 2021). The fundamental aim of this type of violence is the perpetrator's attempt to make the victim dependent on them. Economic violence involves controlling a woman's ability to obtain, use, and maintain financial resources, thereby threatening her economic security and potential for self-sufficiency (Schrag and Ravi, 2020). Although economic dating violence is normalised in patriarchal societies and therefore less frequently discussed, its significance remains unchanged. Economic dating violence negatively affects the victim's health and paves the way for other types of violence (Sırım, 2021). Both women and men can be subjected to economic violence, but due to the influence of gender stereotypes and the resulting limitations on education and employment opportunities, women, who earn less than men, are at greater risk of facing economic violence (UN Women, 2019).

Denying access to the economic resources necessary for sustaining one's life, seizing all income, demanding items that cannot be purchased with the small amount of money provided, withholding money or providing only very limited amounts, forcing the partner to work or having them dismissed from work, preventing them from being promoted, controlling or seizing bank accounts, forcing the other party to pay their own debts are examples of economic dating violence (Alkan et al., 2021).

2.4.5. Digital Violence

This refers to violent acts committed using information technologies. Often stemming from the malicious use of media platforms, digital violence is also referred to in the literature as 'cyber violence, cyberbullying, cyber harassment, cyber assault, virtual violence, virtual bullying, online violence, and electronic bullying' (Abınık, 2021). Digital dating violence refers to violent behaviour

perpetrated by one partner against the other, or by both partners against each other, using technological tools (telephone, computer, tablet, etc.) and/or virtual environments (social media, email, instant messaging, text messaging, etc.) with the aim of gaining power and control or maintaining pressure (Toplu-Demirtaş and Fincham, 2020). There are two sub-dimensions of digital dating violence. The first dimension involves direct aggression, such as threatening, humiliating, insulting the victim, and disseminating or threatening to disseminate the victim's private information online. The other dimension is control and monitoring; behaviours that violate privacy by spying on the victim, such as using their personal passwords without their permission, checking their social media accounts, restricting their posts/followers, calling/texting frequently and expecting quick responses, and coercing the partner into sending pictures or videos (Sarışın, 2022). The shift of interpersonal communication to digital platforms via the internet; the ability to reach many people beyond time and space constraints, the perpetrator's ability to conceal their identity, the ability to reach a large number of people in a short time, the permanence of content in the virtual environment, the possibility of unlimited viewing or copying of images and messages, and applications such as social media location sharing and instant messaging has increased the scope of violence (Seçgin and Selçuk, 2023; Sırım, 2021).

3. Effects and Consequences of Dating Violence

Victims of dating violence sometimes ignore it, while at other times they hesitate to seek support or help due to reasons such as shame, guilt, feelings of inadequacy, fear of exclusion, and fear of exposure (Aslan and Arslantaş, 2024). Violence is behaviour that subjugates the other party, causes physical or psychological harm, and threatens their physical and mental integrity. When violence occurs, it is not only the victim who is affected. The perpetrator of the violence is also negatively affected. The perpetrator of violence against their partner is likely to have a fear of not being loved or abandoned, and a lack of self-confidence. Dating violence causes individuals to suffer physical, psychological, economic, and social disadvantages in the short and long term (Özkan, 2022). In cases of dating violence, which can result in injuries and even death; physical disorders (urinary tract infections, vaginal bleeding, uterine fibroids, multiple sexual partners, unprotected sex at an early age, sexually transmitted diseases, miscarriages, unwanted pregnancies, etc.), psychosomatic disorders (anxiety, anger management problems, decreased self-confidence and self-esteem, low self-esteem, post-traumatic stress disorder, depression, eating disorders, increased smoking, alcohol and substance abuse, suicide attempts or thoughts, appetite, sleep disorders, low life satisfaction, mood disorders) and social health (low academic achievement, social isolation) may be observed (Akı, 2022; Set, 2020).

4. Ways to Prevent Dating Violence and Nursing

Although there are many programmes with different theoretical foundations and techniques aimed at reducing and preventing dating violence, intervention programmes are categorised into primary, secondary, and tertiary prevention approaches (Wong et al., 2023). Primary prevention programmes aim to prevent dating violence by targeting the entire population of a school directly or by identifying at-risk groups. Primary prevention programmes are considered successful when dating violence does not occur (De La Rue et al., 2014). Secondary prevention programmes are designed to prevent violence that already exists. Secondary prevention programmes are considered successful when the dating violence victim protects themselves from violence by separating from the perpetrator or when the perpetrator stops committing violence (Cornelius and Resseguie, 2007). Tertiary prevention aims to treat and rehabilitate victims of violence and perpetrators. These programmes focus on long-term post-violence care, such as rehabilitation and reintegration, and involve trauma-reducing interventions (Butchart and Burrows, 2023). Governments also play a key role in preventing violence and protecting against violence by establishing the necessary legislation, guidelines, and health policies to protect victims of violence and reduce perpetrators (Gündüz, 2019). Routine screening for violence and abuse by healthcare professionals should become a standard of care (Oram et al., 2022). Women's health and nursing play a role in preventing violence within the scope of their roles and responsibilities. Within this role, their fundamental responsibilities include recognising signs of violence; planning medical diagnosis and treatment; providing information about violence, legal rights, security and social support systems, and communication networks; informing and empowering the community about all forms of violence against women, including cyber violence; and conducting research on violence against women (Women's Health Nursing Association, 2025).

Conclusion

Regardless of the dynamics involved, no emotion (anger, rage, irritation, depression, jealousy, etc.) can legitimise violence. The prevalence of dating violence and its negative effects on health parameters are alarming. Its transmission to later stages of life and its continuation throughout life are significant problems that threaten public health. Dealing with health problems arising after an act of violence is quite costly in terms of resources (labour, time, economy). Dating violence prevention programmes can be a valuable resource in helping young people confront the difficult tasks involved in forming romantic relationships and encouraging them to form respectful relationships, thereby supporting the positive development of partners in romantic relationships (CDC, 2018). Combating violence requires every member of society to develop awareness against violence and healthy behavioural development as fundamental

steps. It would be beneficial for nurses to provide accessible counselling services and evidence-based intervention programmes to raise awareness against violence. Programmes that teach safe and healthy relationship skills and the creation of protective environments in interactions established by adolescents are important for the prevention of dating violence, as is the nursing care provided to young people who are victims of dating violence. It is anticipated that the prevention of dating violence and the establishment of healthy relationships will reduce increasing divorce rates.

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CHAPTER 46

Preeclampsia: A Comprehensive Review for Clinical Practice

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Background

Preeclampsia constitutes a pregnancy-specific syndrome driven by placental dysfunction and manifested through new-onset hypertension developing after 20 weeks of gestation with evidence of maternal end-organ damage or uteroplacental insufficiency [1, 2]. Contemporary understanding has evolved beyond the traditional "high blood pressure plus proteinuria" paradigm; proteinuria, while common, is no longer mandatory for diagnosis [2, 3]. The clinical presentation can progress rapidly, potentially endangering both maternal and fetal wellbeing, necessitating heightened vigilance and prompt intervention.

Modern management frameworks rest upon two fundamental principles: first, maternal stabilization and protection from severe complications such as stroke, seizures, and multiorgan failure; second, pregnancy prolongation only when safe for the mother and beneficial for the fetus [2, 4]. Guidelines from leading professional organizations—including the American College of Obstetricians and Gynecologists (ACOG), the Society for Maternal-Fetal Medicine (SMFM), the National Institute for Health and Care Excellence (NICE), the International Society for the Study of Hypertension in Pregnancy (ISSHP), and the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG)—demonstrate broad consensus on diagnostic and management principles, though practical differences exist in testing algorithms and surveillance intervals [2, 4, 5, 6].

Epidemiology and disease burden

Preeclampsia complicates approximately 2–8% of pregnancies globally, with substantial geographic and demographic variation [1, 22, 23]. The condition remains among the leading causes of maternal morbidity and mortality, particularly in resource-limited settings where access to prenatal care, laboratory diagnostics, and critical care interventions may be constrained [1]. Early-onset preeclampsia, typically defined as disease presenting before 34 weeks of gestation, occurs less frequently than late-onset disease but carries disproportionately higher risks of fetal growth restriction (FGR), preterm delivery, and serious maternal complications including hepatic rupture, renal failure, and cerebrovascular accidents [12, 23].

The burden extends beyond immediate pregnancy outcomes. Women with a history of preeclampsia face elevated lifetime cardiovascular risk, including premature coronary artery disease, stroke, and chronic hypertension [22, 23]. This association suggests that pregnancy may serve as a physiologic stress test, unmasking underlying vascular susceptibility and metabolic dysfunction that predispose to both pregnancy complications and long-term cardiometabolic disease [22].

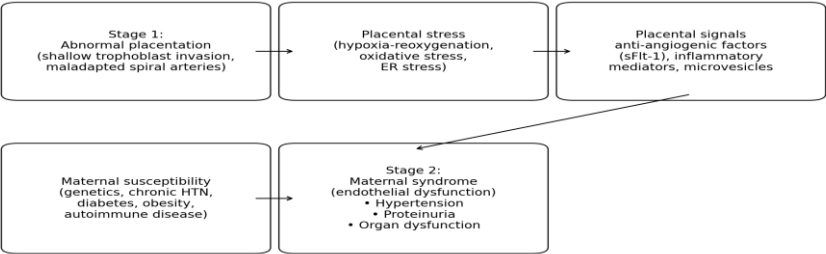
Normal placentation and maternal cardiovascular adaptation

Understanding preeclampsia's pathogenesis requires familiarity with normal pregnancy physiology. During early gestation, extravillous cytotrophoblast cells invade the decidua and penetrate maternal spiral arteries, replacing the endothelial lining and smooth muscle walls [12, 24]. This remodeling transforms small, high-resistance vessels into dilated, low-resistance conduits, establishing the high-flow, low-pressure perfusion system essential for adequate placental function. Concurrently, maternal blood volume expands by 40–50%, cardiac output increases, and systemic vascular resistance decreases, creating the characteristic hemodynamic profile of normal pregnancy [22, 23].

In preeclampsia, this adaptive remodeling is incomplete or dysfunctional [12, 22]. Shallow trophoblast invasion leaves spiral arteries unremodeled, maintaining high resistance and limiting placental perfusion. The resulting placental ischemia and oxidative stress trigger release of anti-angiogenic factors, inflammatory mediators, and other soluble products into the maternal circulation, culminating in widespread endothelial dysfunction [12, 14, 22]. This endothelial injury provides a unifying mechanism linking the diverse clinical manifestations: hypertension from impaired vascular tone, proteinuria from glomerular endotheliosis, thrombocytopenia from platelet activation and consumption, hepatic dysfunction from periportal necrosis, neurologic symptoms from cerebral edema and vasospasm, and fetal growth restriction from placental insufficiency [22, 24].

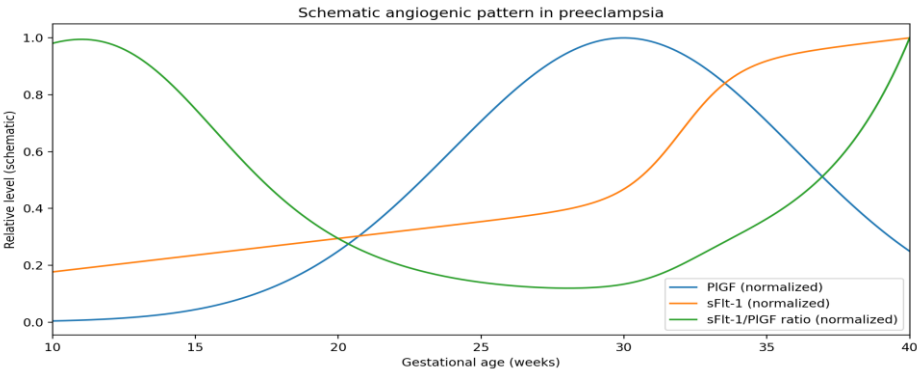
Pathophysiology: the two-stage model

A widely accepted conceptual framework describes preeclampsia as evolving through two sequential stages (Fig. 1) [12, 22, 24]. Stage 1, the placental stage, occurs during early-to-mid gestation when abnormal placentation establishes the foundation for disease. Defective trophoblast invasion and spiral artery remodeling lead to chronic uteroplacental ischemia. The stressed placenta responds by releasing excess anti-angiogenic proteins, particularly soluble fms-like tyrosine kinase-1 (sFlt-1), which binds and neutralizes vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), shifting the balance toward an anti-angiogenic state [8, 14, 22].



Stage 2, the maternal syndrome, emerges as circulating anti-angiogenic factors and inflammatory mediators overwhelm maternal compensatory mechanisms, producing systemic endothelial dysfunction [12, 22]. Clinical manifestations become apparent, typically after 20 weeks when placental mass and metabolic demands peak. The severity and timing of maternal disease reflect the interplay between placental dysfunction magnitude and maternal constitutional factors, including baseline cardiovascular health, genetic predisposition, and immune adaptation [19, 22, 24].

This model explains why angiogenic biomarkers often precede clinical disease [8, 14]. Serial measurements of sFlt-1/PlGF ratio have demonstrated utility in predicting subsequent preeclampsia development, particularly in symptomatic women requiring evaluation before 34 weeks (Fig. 2) [8, 14]. While not universally adopted, such biomarkers can aid risk stratification and inform surveillance intensity in specialized centers [3, 8].



Classification of hypertensive disorders of pregnancy

Hypertensive disorders in pregnancy encompass a spectrum of conditions distinguished by timing of onset, presence of proteinuria or organ dysfunction, and progression pattern [2, 5, 18]. Most guidelines define hypertension as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg measured on two occasions at least 4 hours apart using standardized technique [2, 5]. Severe-range blood pressure, defined as systolic ≥ 160 mmHg and/or diastolic ≥ 110 mmHg, represents a medical emergency requiring urgent pharmacologic intervention to reduce stroke risk [2, 9, 20]. Table 1 provides a practical classification framework synthesized from ISSHP and ACOG guidance [2, 5, 18].

Table 1. Classification of Hypertensive Disorders of Pregnancy

Category	Definition	Clinical Significance
Chronic hypertension	Hypertension present before pregnancy, diagnosed before 20 weeks' gestation, or persisting >12 weeks postpartum	May be complicated by superimposed preeclampsia, which carries higher morbidity than either condition alone
Gestational hypertension	New-onset hypertension after 20 weeks without proteinuria or end-organ dysfunction	Approximately 15–25% progress to preeclampsia; requires close surveillance
Preeclampsia	New-onset hypertension after 20 weeks plus proteinuria OR maternal organ dysfunction OR uteroplacental dysfunction	Proteinuria is not mandatory for diagnosis; organ dysfunction or fetal compromise suffices
Preeclampsia with severe features	Preeclampsia plus severe-range BP and/or severe maternal organ dysfunction (see Table 4)	Typically mandates delivery at ≥ 34 weeks after maternal stabilization
Eclampsia	New-onset generalized tonic-clonic seizures in a woman with preeclampsia, without alternative explanation	Prevented and treated with magnesium sulfate; can occur antepartum, intrapartum, or postpartum
HELLP syndrome	Hemolysis, Elevated Liver enzymes, Low Platelets	Severe variant overlapping with preeclampsia; may present without classic hypertension/proteinuria

Abbreviations: BP blood pressure, HELLP hemolysis elevated liver enzymes low platelets

Risk factors and recurrence

Risk stratification guides both preventive interventions and surveillance intensity [2, 7, 21]. Risk factors can be conceptually divided into placental/immunologic factors (reflecting novel paternal antigen exposure or aberrant maternal-fetal immune interactions) and maternal constitutional factors (reflecting underlying vascular or metabolic susceptibility) [21, 22]. Table 2 summarizes established risk factors with their approximate strength of association.

Table 2. Risk Factors for Preeclampsia

High-Risk Factors	Moderate-Risk Factors	Notes
History of preeclampsia (especially early or severe)	Nulliparity (first pregnancy)	Recurrence risk increases with earlier gestational age at prior onset; severe preeclampsia before 34 weeks confers 25–65% recurrence risk
Multifetal gestation	Maternal age ≥ 35 years	Twin pregnancies carry 2–3 fold increased risk; higher-order multiples have proportionally greater risk
Chronic hypertension	Body mass index ≥ 30 kg/m ²	Obesity contributes through chronic inflammation, insulin resistance, and endothelial dysfunction
Pregestational diabetes (type 1 or 2)	Family history (mother or sister with preeclampsia)	Suggests genetic susceptibility; shared environmental factors may also contribute
Chronic kidney disease	Assisted reproductive technology	Particularly elevated with oocyte donation; may reflect immune maladaptation to foreign conceptus
Antiphospholipid syndrome	Interpregnancy interval >10 years	Prolonged interval may allow regression of maternal immune tolerance to paternal antigens
Systemic lupus erythematosus		Active SLE increases risk substantially; requires preconception optimization

Abbreviation: SLE systemic lupus erythematosus

Women with a history of preeclampsia face variable recurrence risks depending on severity and gestational age at diagnosis [1, 21]. Early-onset disease (before 34 weeks) recurs in approximately 25–65% of subsequent pregnancies, while late-onset disease recurs in approximately 5–20% [21]. Recurrence tends to occur at similar or later gestational ages compared to the index pregnancy.

Screening and prevention

First-trimester risk assessment

Several first-trimester screening algorithms combine maternal characteristics, biophysical measurements (mean arterial pressure, uterine artery Doppler pulsatility index), and biochemical markers (PIGF, pregnancy-associated plasma protein-A) to predict preeclampsia risk [7, 17]. The Fetal Medicine Foundation (FMF) algorithm has been extensively validated and can identify approximately 75% of early-onset preeclampsia cases with a 10% false-positive rate [7, 17].

However, implementation requires standardized protocols and quality assurance, limiting widespread adoption outside specialized centers [6, 17].

Evidence-based prevention strategies

Table 3 summarizes interventions with robust evidence for preeclampsia prevention.

Table 3. Evidence-Based Prevention Strategies

Intervention	Target Population	Recommended Approach	Level of Evidence
Low-dose aspirin	Women with ≥ 1 high-risk factor or ≥ 2 moderate-risk factors	81–150 mg daily, initiated between 12–16 weeks (ideally before 16 weeks), continued until delivery	Strong; multiple RCTs and meta-analyses demonstrate 10–15% relative risk reduction for preeclampsia, with greater effect ($>60\%$ reduction) for preterm preeclampsia when initiated early [7, 16, 21]
Calcium supplementation	Women with low dietary calcium intake (<800 mg/day)	1.5–2.0 g elemental calcium daily in divided doses	Strong in calcium-deficient populations; approximately 55% relative risk reduction [15]
Lifestyle modification	All women, ideally preconception	Weight optimization, exercise, smoking cessation, glycemic control	Indirect evidence; benefits maternal health broadly but direct effect on preeclampsia prevention unclear [21]
Anticoagulation	Selected thrombophilias (antiphospholipid syndrome with obstetric history)	Per hematology consultation; typically LMWH	Not for routine preeclampsia prevention; specific to thrombophilia management [5]

Abbreviations: RCT randomized controlled trial, LMWH low-molecular-weight heparin

The ASPRE trial demonstrated that early initiation (before 16 weeks) of aspirin 150 mg daily in women at high risk by FMF screening reduced preterm preeclampsia by 62% [7]. Current ACOG and SMFM recommendations endorse aspirin 81–162 mg daily initiated between 12–28 weeks (ideally before 16 weeks) in women with high-risk factors [2, 16].

Diagnosis and initial evaluation

Blood pressure measurement

Accurate blood pressure assessment is foundational [2, 5]. Patients should be seated or in left lateral recumbent position (if supine measurement is necessary) for at least 5 minutes before measurement. An appropriately sized cuff (bladder width 40% of arm circumference, length 80%) should encircle at least 80% of the arm [2]. Automated devices used in obstetric settings should be validated for use in preeclampsia, as some devices underestimate severely elevated pressures [5].

Diagnostic criteria

Once hypertension is confirmed after 20 weeks, the key diagnostic question is whether maternal organ dysfunction or uteroplacental compromise is present [2, 5]. Table 4 provides a comprehensive diagnostic framework aligned with current ACOG and ISSHP criteria [2, 5].

Table 4. Diagnostic Criteria for Preeclampsia and Features of Severity

Diagnostic Findings			
Domain	Supporting Preeclampsia	Assessment Method	Severe Features
Blood pressure	SBP ≥140 mmHg or DBP ≥90 mmHg on two occasions ≥4 hours apart OR SBP ≥160 mmHg or DBP ≥110 mmHg (confirmable within minutes)	Standardized measurement with appropriate cuff; repeat elevated readings	SBP ≥160 mmHg or DBP ≥110 mmHg on two occasions minutes apart
Proteinuria	≥300 mg per 24-hour collection OR Protein/creatinine ratio ≥0.3 mg/dL OR Dipstick ≥2+ (only if quantitative methods unavailable)	Spot urine protein/creatinine ratio preferred for rapid assessment	Not a marker of severity by itself
Hematologic	Platelet count <100,000/μL	Complete blood count	Thrombocytopenia <100,000/μL
Hepatic	Serum transaminases >2× upper limit of normal	Comprehensive metabolic panel	ALT or AST >2× upper normal; severe persistent RUQ or epigastric pain
Renal	Serum creatinine >1.1 mg/dL OR doubling of baseline creatinine (absent other renal disease)	Serum creatinine, assess baseline if available	Creatinine >1.1 mg/dL or doubling; acute kidney injury
Pulmonary	Pulmonary edema	Clinical examination, oxygen saturation,	Pulmonary edema with hypoxia

Domain	Diagnostic Findings Supporting Preeclampsia		Assessment Method	Severe Features
Neurologic	New-onset severe headache unresponsive to medication; visual disturbances (scotomata, photophobia, blurred vision); altered mental status		chest imaging if indicated Focused neurologic examination; ophthalmoscopy if indicated; imaging if atypical	Severe headache, visual symptoms, hyperreflexia with clonus, seizure (eclampsia)
Uteroplacental	Fetal growth restriction (typically <10th percentile); abnormal umbilical artery Doppler; oligohydramnios; stillbirth		Ultrasound biometry, amniotic fluid assessment, Doppler velocimetry	Severe FGR, absent or reversed end-diastolic flow

Abbreviations: SBP systolic blood pressure, DBP diastolic blood pressure, RUQ right upper quadrant, ALT alanine aminotransferase, AST aspartate aminotransferase, FGR fetal growth restriction

Initial workup

Upon diagnosing preeclampsia, initial evaluation should include [2, 5]:

- Complete blood count with platelet count
- Comprehensive metabolic panel (renal and hepatic function)
- Quantitative proteinuria assessment (spot protein/creatinine ratio or 24-hour collection)
- Lactate dehydrogenase (if hemolysis suspected)
- Coagulation studies (if severe thrombocytopenia or hepatic dysfunction)

Maternal and fetal surveillance after diagnosis

Management of established preeclampsia centers on structured monitoring and readiness for delivery [2, 4, 6]. Surveillance intensity scales with disease severity and gestational age. Four key questions guide ongoing assessment [2]:

1. Is blood pressure controlled?
2. Are laboratory parameters stable or deteriorating?

3. Are maternal symptoms evolving?
4. Is the fetus showing signs of compromise?

Maternal monitoring

Outpatient management (gestational hypertension or preeclampsia without severe features, stable patient, reliable follow-up) [2, 4]:

- Blood pressure assessment twice weekly
- Symptom education (headache, visual changes, right upper quadrant pain, dyspnea)
- Laboratory evaluation (platelets, creatinine, transaminases) weekly to twice weekly
- Prompt transition to inpatient care if severe features develop

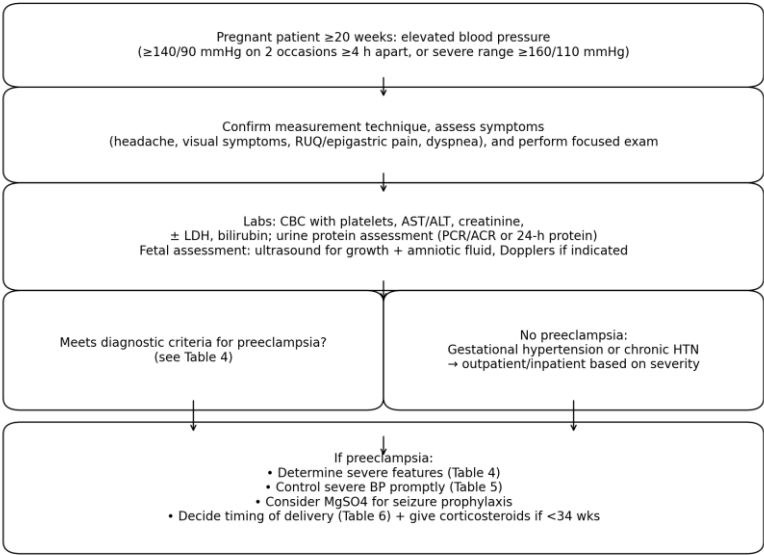
Inpatient management (preeclampsia with severe features or concern for compliance) [2, 4]:

- Blood pressure monitoring every 4 hours minimum, more frequently if unstable
- Daily laboratory assessment (complete blood count, comprehensive metabolic panel)
- Strict intake and output monitoring
- Daily symptom assessment
- Continuous fetal monitoring depending on gestational age and clinical stability

Fetal surveillance

- **Fetal movement counts:** Daily by patient [6]
- **Nonstress testing or biophysical profile:** Frequency determined by severity; typically twice weekly for preeclampsia without severe features, daily for severe disease or fetal compromise [6]
- **Ultrasound for growth:** Every 3–4 weeks; more frequent assessment if growth restriction identified [6]
- **Umbilical artery Doppler velocimetry:** When fetal growth restriction suspected or confirmed; abnormal Doppler findings (absent or reversed end-diastolic flow) indicate severe placental insufficiency and typically prompt delivery at viable gestational ages [6]

Figure 3 outlines a simplified evaluation algorithm for hypertension after 20 weeks.



Pharmacologic management

Acute management of severe-range blood pressure

Severe hypertension ($\geq 160/110$ mmHg) requires urgent treatment to reduce maternal cerebrovascular accident risk [2, 9, 20]. Target blood pressure for acute management is $<160/110$ mmHg within 30–60 minutes, with care to avoid precipitous decreases that may compromise uteroplacental perfusion [2, 9]. Table 5 outlines first-line agents.

Table 5. Pharmacologic Agents for Preeclampsia Management

Medication	Indication	Typical Dosing	Onset	Key Considerations
Acute Blood Pressure Management				
Labetalol IV	First-line for acute severe HTN	20 mg IV over 2 min; if inadequate response after 10 min, give 40 mg IV; then 80 mg IV every 10 min (max cumulative 220 mg)	5–10 min	Contraindicated in asthma with active bronchospasm, greater than first-degree heart block, or acute heart failure; monitor for bradycardia
Hydralazine IV	First-line for acute severe HTN	5–10 mg IV over 2 min; repeat every 20 min as needed	10–20 min	May cause reflex tachycardia, headache, or flushing; monitor

Medication	Indication	Typical Dosing	Onset	Key Considerations
Immediate-release nifedipine PO	First-line for acute severe HTN	(typical max 20–30 mg) 10 mg PO; if inadequate after 20 min, give 20 mg PO; may repeat (max 50 mg in first hour)	10–20 min	closely for hypotension Concurrent use with magnesium sulfate safe but monitor BP closely; avoid sublingual route
Maintenance Antihypertensive Therapy Labetalol PO	Chronic BP control	200–800 mg twice or three times daily	1–2 hours	Compatible with breastfeeding
Nifedipine extended-release PO	Chronic BP control	30–90 mg once daily	Variable	Compatible with breastfeeding
Methyldopa PO	Chronic BP control	250–1000 mg two to three times daily	4–6 hours	May cause drowsiness; extensive safety data in pregnancy
Seizure Prophylaxis and Treatment Magnesium sulfate IV	Eclampsia prevention and treatment	Loading: 4–6 g IV over 20–30 min; Maintenance: 1–2 g/hour continuous infusion	Immediate	Monitor deep tendon reflexes, respiratory rate (>12/min), urine output (>25 mL/hour); calcium gluconate is antidote (1 g IV over 3 min for toxicity)
Fetal Lung Maturation Betamethasone IM	Fetal benefit before preterm delivery	12 mg IM, repeat in 24 hours (complete course)	N/A	Administer between 24–34 weeks when delivery anticipated within 7 days; maximal benefit 24 hours to 7 days after completion

Abbreviations: IV intravenous, PO oral, IM intramuscular, HTN hypertension, BP blood pressure

Magnesium sulfate for seizure prophylaxis

Magnesium sulfate remains the standard for eclampsia prevention and treatment, superior to phenytoin and benzodiazepines in randomized trials [20]. Indications include [2, 20]:

- Preeclampsia with severe features (to prevent progression to eclampsia)
- Eclampsia (for seizure control)

Standard regimen involves a 4–6 g intravenous loading dose over 20–30 minutes followed by continuous infusion at 1–2 g/hour [2, 20]. Duration varies by protocol but typically continues for 24 hours postpartum or 24 hours after last seizure (eclampsia) [20]. Magnesium is renally excreted; dose adjustment may be necessary in renal insufficiency [20].

Monitoring includes [2, 20]:

- Deep tendon reflexes (diminished reflexes precede respiratory depression)
- Respiratory rate (goal >12 breaths per minute)
- Urine output (goal >25–30 mL/hour)
- Serum magnesium levels not routinely necessary if clinical monitoring adequate, but therapeutic range is 4–7 mEq/L

Signs of toxicity include loss of deep tendon reflexes (8–12 mEq/L), respiratory depression (>12 mEq/L), and cardiac arrest (>25 mEq/L) [20]. Calcium gluconate (1 g IV over 3 minutes) serves as antidote [2, 20].

HELLP syndrome and catastrophic complications

HELLP syndrome—characterized by hemolysis (H), elevated liver enzymes (EL), and low platelet count (LP)—represents a severe variant of preeclampsia with distinct complications and management considerations [1, 20]. Diagnosis requires [1, 20]:

- Hemolysis: elevated LDH (>600 U/L), elevated bilirubin (>1.2 mg/dL), decreased haptoglobin, or schistocytes on peripheral smear
- Elevated liver enzymes: AST or ALT >2× upper limit of normal
- Low platelets: <100,000/μL

HELLP syndrome occurs in approximately 10–20% of cases of severe preeclampsia and may present without marked hypertension or proteinuria, potentially delaying recognition [1, 20]. Maternal complications include [1, 20]:

- **Hepatic complications:** Subcapsular hematoma, hepatic infarction, or catastrophic hepatic rupture (rare but often fatal)
- **Hematologic complications:** Disseminated intravascular coagulation (DIC), requiring blood product support
- **Renal complications:** Acute kidney injury, which may necessitate dialysis
- **Pulmonary complications:** Pulmonary edema, acute respiratory distress syndrome
- **Neurologic complications:** Cerebral hemorrhage, posterior reversible encephalopathy syndrome (PRES)

Differential diagnosis includes acute fatty liver of pregnancy, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, and acute viral hepatitis [1, 20]. Distinguishing features and specific therapies guide management.

Delivery is the definitive treatment for HELLP syndrome [1, 2, 20]. Expectant management is generally inappropriate once diagnosis is confirmed, regardless of gestational age, due to rapid deterioration risk. Corticosteroids (typically betamethasone or dexamethasone) may improve laboratory parameters and facilitate safer delivery, though evidence for maternal benefit is mixed [20].

Timing of delivery

Delivery timing represents the most critical management decision, balancing maternal risk against fetal immaturity [2, 4, 11]. General principles include [2, 11]:

- Delivery is the only definitive cure for preeclampsia
- Expectant management benefits the fetus through maturation but exposes the mother to complications
 - Severe features shift the balance toward earlier delivery
 - Gestational age thresholds guide decision-making

Table 6 synthesizes evidence-based recommendations for delivery timing [2, 4, 11].

Table 6. Delivery Timing in Hypertensive Disorders of Pregnancy

Clinical Scenario	Recommended Delivery Timing	Rationale
Gestational hypertension or preeclampsia without severe features, ≥ 37 weeks	Deliver (induction of labor or cesarean per obstetric indications)	Progression risk and maternal morbidity exceed benefits of prolongation; HYPITAT trial demonstrated improved outcomes without increased cesarean delivery [11]
Gestational hypertension or preeclampsia without severe features, 34–36+6 weeks	Individualized; many experts recommend delivery at 37 weeks if stable	Close surveillance may be reasonable; deliver earlier if progression to severe disease or fetal compromise [2, 4]
Preeclampsia with severe features, ≥ 34 weeks	Deliver after maternal stabilization (typically within 24–48 hours)	Maternal risks escalate rapidly; fetal outcomes at ≥ 34 weeks are favorable [2, 4]
Preeclampsia with severe features, < 34 weeks	Consider expectant management in tertiary center IF: BP controllable, No persistent severe symptoms, Laboratory abnormalities non-progressive, Fetal status reassuring	Goal is prolongation for fetal benefit (corticosteroids, maturation); requires intensive maternal and fetal surveillance; many experts recommend delivery at 34 weeks regardless of stability [2, 4]
ANY gestational age with: Eclampsia, Pulmonary edema, Disseminated intravascular coagulation, Placental abruption, Non-reassuring fetal status, Fetal demise	Deliver promptly (often urgently)	These represent absolute or near-absolute indications for delivery; maternal and/or fetal compromise outweighs any benefit from delay [2, 4]

Abbreviation: HYPITAT Hypertension and Preeclampsia Intervention Trial At Term

Expectant management considerations

When expectant management is pursued for preeclampsia with severe features before 34 weeks, strict eligibility criteria and intensive monitoring are essential [2, 4]:

Inclusion criteria:

- Maternal condition stable and BP controllable
- Laboratory abnormalities present but not rapidly deteriorating

- Fetal status reassuring
- Availability of tertiary care resources (maternal-fetal medicine specialists, neonatal intensive care unit)

Exclusion criteria (immediate delivery indicated):

- Uncontrollable severe hypertension
- Eclampsia
- Pulmonary edema
- DIC or severe thrombocytopenia with bleeding
- Placental abruption
- Non-reassuring fetal status
- Fetal demise

Monitoring:

- Inpatient hospitalization mandatory
- Blood pressure every 4 hours minimum
- Daily laboratory assessment
- Daily or twice-daily fetal testing
- Delivery at 34 weeks even if stable (prolongation beyond 34 weeks not recommended)

Postpartum management and long-term implications

Immediate postpartum period

Preeclampsia does not resolve immediately at delivery [2, 20]. Blood pressure often peaks 3–6 days postpartum, and severe complications—including eclampsia, stroke, and pulmonary edema—can occur after birth [2, 20]. Postpartum management includes [2]:

Blood pressure management:

- Continue or initiate antihypertensive therapy for BP $\geq 150/100$ mmHg
- Severe-range BP ($\geq 160/110$ mmHg) requires urgent treatment postpartum just as antepartum

- Most antihypertensives are compatible with breastfeeding (labetalol, nifedipine, enalapril preferred; avoid atenolol, ACE inhibitors with caution in first week)

Magnesium sulfate:

- Continue for 24 hours postpartum in women with severe features or eclampsia
- Monitor for toxicity as renal function and volume status change postpartum

Symptom education:

- Instruct patients to seek urgent care for severe headache, visual changes, right upper quadrant pain, or dyspnea
- Most postpartum eclampsia occurs within first week but can occur up to 4 weeks postpartum

Follow-up:

- Blood pressure check within 72 hours of discharge
- Repeat assessment at 7–10 days and 6 weeks postpartum
- Many women can discontinue antihypertensives by 6–12 weeks; persistent hypertension suggests underlying chronic hypertension

Long-term cardiovascular risk

A history of preeclampsia marks women as having elevated lifetime cardiovascular risk [22, 23]. Epidemiologic studies demonstrate 2–4 fold increased risk of future hypertension, 1.5–2 fold increased risk of ischemic heart disease, 1.8 fold increased risk of stroke, and increased risk of premature cardiovascular death [22, 23]. Risk is highest among women with early-onset preeclampsia, recurrent preeclampsia, or preterm delivery [22, 23].

The American Heart Association recognizes pregnancy complications, including preeclampsia, as sex-specific risk factors for cardiovascular disease and recommends incorporating obstetric history into cardiovascular risk assessment [22]. Clinicians should:

- Document preeclampsia history prominently in medical records
- Screen for traditional cardiovascular risk factors (hypertension, diabetes, dyslipidemia) within 3–6 months postpartum and periodically thereafter
- Encourage lifestyle modification (weight management, exercise, smoking cessation, healthy diet)

- Consider early or more aggressive management of hypertension and metabolic risk factors
- Refer to cardiology or internal medicine for comprehensive risk assessment if early preeclampsia or multiple risk factors

Counseling for future pregnancy

Table 7 summarizes key counseling points for women planning future pregnancies after preeclampsia [2, 7, 16, 21, 22].

Table 7. Postpartum Counseling After Preeclampsia		
Topic	Recommendation	Key Counseling Points
Recurrence risk	Risk stratification based on severity and timing	Late-onset preeclampsia: 5–20% recurrence; Early-onset (<34 weeks): 25–65% recurrence; Recurrence typically at similar or later gestational age [21]
Interpregnancy interval	Optimize health before next conception	Address modifiable risk factors (weight, blood pressure, glycemic control); ensure adequate interpregnancy interval for recovery (at least 6–12 months) [21]
Aspirin prophylaxis	81–162 mg daily starting 12–16 weeks in subsequent pregnancy	All women with history of preeclampsia qualify as high-risk for aspirin prophylaxis [2, 7, 16]
Preconception counseling	Maternal-fetal medicine consultation before next pregnancy	Discuss individualized recurrence risk, prevention strategies, and monitoring plan [2, 4]
Long-term health surveillance	Primary care follow-up for cardiovascular risk assessment	Screen for chronic hypertension, diabetes, dyslipidemia; encourage heart-healthy lifestyle [22]
Breastfeeding	Encourage if desired and feasible	Most antihypertensives compatible with breastfeeding; discuss specific medications with provider [2]

Special populations and considerations

Preeclampsia in women with chronic hypertension

Chronic hypertension complicates approximately 3–5% of pregnancies and substantially increases preeclampsia risk (up to 25–30%) [10, 18]. Distinguishing superimposed preeclampsia from worsening chronic hypertension can be challenging [10, 18]. Diagnostic clues include:

- New-onset or worsening proteinuria
- New-onset thrombocytopenia
- New-onset or worsening hepatic transaminase elevation

- New-onset severe headache, visual symptoms, or right upper quadrant pain
- Fetal growth restriction or abnormal Doppler studies

The CHAP trial (2022) demonstrated that treating mild chronic hypertension (target <140/90 mmHg) improved pregnancy outcomes compared with reserving treatment for severe-range pressures, reducing the composite of preeclampsia with severe features, preterm birth <35 weeks, placental abruption, or fetal/neonatal death [10]. Current guidelines support treating chronic hypertension to maintain BP <140/90 mmHg during pregnancy [2, 10].

Preeclampsia in multiple gestations

Twin and higher-order multiple pregnancies carry 2–3 fold increased preeclampsia risk compared to singletons [21, 23]. Management principles are similar, but:

- Earlier onset is more common
- Delivery timing requires additional consideration of multiplicity (many deliver twins at 37–38 weeks regardless of preeclampsia)
- Fetal surveillance may be more complex
- Low-dose aspirin prophylaxis is appropriate for all multifetal gestations

Obesity and preeclampsia

Obesity (BMI ≥ 30 kg/m²) increases preeclampsia risk through multiple mechanisms: chronic inflammation, insulin resistance, endothelial dysfunction, and baseline hypertension [21, 23]. Management challenges include:

- Blood pressure measurement (requires appropriate cuff size)
- Clinical assessment (symptom detection may be more difficult)
- Ultrasound visualization (fetal assessment may be limited)
- Anesthetic considerations for delivery

Weight optimization before conception provides the greatest benefit, though even modest weight loss improves metabolic parameters and may reduce risk [21].

Future directions and research priorities

Biomarker development

While the sFlt-1/PlGF ratio has demonstrated clinical utility in prediction and diagnosis, particularly in preterm presentations, broader implementation faces

challenges including cost, lack of universal cutoffs, and need for integrated clinical algorithms [3, 8, 14]. Ongoing research explores additional markers including:

- Pregnancy-specific glycoproteins
- Cell-free fetal DNA
- Metabolomic and proteomic profiling
- MicroRNAs

Ideal biomarkers would enable early risk stratification and targeted prevention, predict disease progression to guide surveillance intensity, and inform delivery timing [3, 8, 13].

Mechanistic insights

Emerging research areas include [12, 13, 19, 22, 24]:

- **Immunologic mechanisms:** Abnormal maternal-fetal immune tolerance may underlie shallow trophoblast invasion; understanding these pathways could enable immunomodulatory therapies
- **Genetic susceptibility:** Genome-wide association studies have identified candidate loci; polygenic risk scores may eventually enhance prediction
- **Epigenetic modifications:** Placental DNA methylation patterns differ in preeclampsia and may serve as early biomarkers or therapeutic targets
- **Vascular dysfunction:** Detailed characterization of endothelial injury mechanisms may suggest protective interventions

Novel therapeutics

Most current management remains supportive (blood pressure control, seizure prophylaxis) rather than disease-modifying [21, 22, 24]. Promising investigational approaches include:

- **Pravastatin:** Preclinical data suggest benefit through nitric oxide pathway; phase 2/3 trials ongoing
- **Metformin:** Potential to reduce placental oxidative stress and inflammation; early-phase trials in progress
- **Antioxidants:** Prior trials of vitamins C and E were negative, but more targeted antioxidant approaches may have benefit
- **Extracorporeal removal of sFlt-1:** Apheresis to reduce circulating anti-angiogenic factors has shown promise in small studies but requires further validation

Conclusions

Preeclampsia remains a major obstetric challenge with significant implications for maternal and fetal health. Optimal management requires early risk identification, evidence-based prevention with low-dose aspirin initiated between 12–16 weeks in high-risk women, accurate diagnosis recognizing that proteinuria is not mandatory, recognition of severe features mandating urgent evaluation and often prompt delivery, individualized delivery timing balancing maternal risk against fetal benefit based on gestational age and disease severity, postpartum vigilance as most maternal complications can occur after birth, and long-term counseling about increased lifetime cardiovascular risk.

By understanding the pathophysiology, recognizing risk factors, implementing evidence-based prevention strategies, diagnosing disease promptly, and managing with severity-appropriate interventions, clinicians can substantially improve outcomes for mothers and babies affected by this complex disorder. Continued research into biomarkers, mechanistic pathways, and disease-modifying therapeutics promises to further advance care and reduce the burden of preeclampsia worldwide.

Abbreviations

ACOG: American College of Obstetricians and Gynecologists; AHA: American Heart Association; AKI: Acute kidney injury; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BP: Blood pressure; CHAP: Chronic Hypertension and Pregnancy Trial; CHIPS: Control of Hypertension in Pregnancy Study; DIC: Disseminated intravascular coagulation; FGR: Fetal growth restriction; FMF: Fetal Medicine Foundation; HELLP: Hemolysis, elevated liver enzymes, low platelets; HTN: Hypertension; HYPITAT: Hypertension and Preeclampsia Intervention Trial At Term; ISSHP: International Society for the Study of Hypertension in Pregnancy; ISUOG: International Society of Ultrasound in Obstetrics and Gynecology; LDH: Lactate dehydrogenase; LMWH: Low-molecular-weight heparin; NICE: National Institute for Health and Care Excellence; PlGF: Placental growth factor; PRES: Posterior reversible encephalopathy syndrome; RCT: Randomized controlled trial; RUQ: Right upper quadrant; sFlt-1: Soluble fms-like tyrosine kinase-1; SLE: Systemic lupus erythematosus; SMFM: Society for Maternal-Fetal Medicine; VEGF: Vascular endothelial growth factor

Declarations

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Authors' contributions

CB conceived the review, performed the literature search, synthesized the evidence, and wrote the manuscript. The author read and approved the final manuscript.

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Figure Legends

Figure 1. Two-stage model of preeclampsia pathophysiology. Stage 1 (placental stage): Abnormal placentation with shallow trophoblast invasion and failed spiral artery remodeling results in placental ischemia and release of anti-angiogenic factors. Stage 2 (maternal syndrome): Circulating factors induce widespread endothelial dysfunction, manifesting as hypertension, proteinuria, and multiorgan involvement.

Figure 2. Representative patterns of angiogenic biomarkers across gestation. In normal pregnancy, PlGF rises through mid-pregnancy then declines toward term, while sFlt-1 remains low until late gestation. In preeclampsia, PlGF levels are suppressed and sFlt-1 concentrations increase markedly, with the sFlt-1/PlGF ratio rising weeks before clinical disease onset.

Figure 3. Clinical decision algorithm for evaluation of new-onset hypertension after 20 weeks' gestation. Initial assessment establishes blood pressure severity and presence of maternal organ dysfunction or uteroplacental compromise. Management intensity and delivery timing depend on gestational age and severity classification.

CHAPTER 47

Bond Strength and Adhesive Performance of 3D-Printed Dental Materials

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1. Introduction

In recent years, with the advancement of technology, Additive Manufacturing (AM) in dentistry has become the subject of various laboratory and clinical studies. This technology is defined by the American Society for Testing and Materials (ASTM) as "the process of joining materials to make objects from 3D model data, usually layer upon layer." 3D printing resins contain various dental materials in their structures, ranging from polymers to composite resins, and ceramics to metals. Particularly with the development of resin-based photopolymers, techniques such as stereolithography (SLA), digital light processing (DLP), and material jetting (MJ) have become usable in dental applications, and have found a wide range of applications, from fixed and removable prostheses to surgical guides(1). The clinical success of 3D printing technologies is closely related to factors such as production accuracy, surface quality, and bond strength between materials(2). This book chapter provides information on the interfacial bonding between 3D-printed materials and dentin, metal, ceramic, or other polymeric substrates, and the parameters closely related to this bonding, such as production techniques, surface pretreatments (e.g., sandblasting, silanization, acid applications, tribochemical coating); light polymerization properties, layer structure, and pre- and post-curing processes.

2. Factors Affecting Bonding

Surface properties and preparation techniques play a critical role in the clinical success of dental materials produced with 3D printing technologies. Layer lines, oxidation, and microporosities formed on the material surface after printing directly affect both esthetic outcomes and bonding efficiency(1). Therefore, controlling the morphological, chemical, and physical properties of the surface is of great importance for the longevity of the restoration. Parameters such as surface roughness (R_a , S_a), wettability, contact angle, and surface energy are the fundamental factors determining the retention potential of adhesive systems to the material. Proper surface preparation aims to increase micromechanical retention and chemical interaction at the bonding interface through procedures such as air-particle abrasion, etching, tribochemical coating, silanization, or plasma treatment(3–5). Consequently, the selection of the surface preparation method should be carefully planned according to the structure of the material used, the structure of the substrate to be bonded, the printing technology, and the targeted bond type. Research aimed at increasing bond strength in 3D-printed materials indicates that modifying the micromorphological and chemical surface properties is crucial. There are many studies in the literature examining the effect of post-printing surface treatments on the retention strength of adhesive systems. Bourgi et al. (2025), in their published review and meta-analysis, systematically evaluated the effects of surface treatments applied to 3D printed resins on adhesion strength. According to the meta-analysis results examining

nine studies, compared to control groups without surface treatment, applications of sandblasting, silane, and hydrofluoric acid (HF) treatments provided significantly higher bond strength both initially and after aging. While sandblasting increased micromechanical retention on the surface, silane supported chemical bond formation. The application of adhesive agent alone did not create a significant difference. The study emphasizes that proper surface preparation is critical for the clinical success of 3D printed resins, with sandblasting + silane or sandblasting + HF combinations being the most effective protocols(6). Dederichs et al. (2023) examined the effect of different surface treatments on the adhesive bond strength of 3D printed permanent resins. In samples produced by the DLP method, sandblasting, tribochemical silica coating, and silane applications were compared. The highest shear bond strength (SBS) values were obtained in the sandblasting + silane group, followed by samples subjected to tribochemical coating. Bond strength was lower in groups subjected only to silane or only to mechanical treatment. SEM examinations showed distinct micro-roughness formation after sandblasting. The authors emphasized that the combined application of mechanical roughening and chemical activation is the most appropriate approach for bonding success in 3D printed resins(7). Alanazi et al. (2023) examined the effect of different surface treatments by comparing the bonding of 3D printed and milled resin posts to composite core material. In the study, sandblasting, silanization, and tribochemical silica coating were applied to both post types; bond strength was evaluated via the shear bond strength (SBS) test. The results revealed that 3D printed posts showed higher SBS values compared to milled posts, especially in the sandblasting + silane combination. Researchers stated that this effect stems from the increased chemical bonding due to the micropore structure formed on the 3D printing surface. Furthermore, it was emphasized that silane application alone did not significantly strengthen the bond. The study demonstrates that 3D printed resins can provide clinically reliable bonding when used with chemical activation following surface roughening(8). Mao et al. (2024) investigated the effect of different surface treatments on bond strength in hybrid crown materials produced by additive (3D printing) and subtractive (milled) methods. In the study, sandblasting, silanization, tribochemical silica coating, and laser surface modification was applied to samples prepared by both production methods. Bond strength was evaluated via the shear bond strength (SBS) test. Results showed that the combination of silica coating and silane provided the highest SBS values in both 3D printing and milling groups. Although laser treatment improved bond performance by improving surface morphology, sufficient strength could not be obtained without chemical activation. Researchers emphasized that chemical processes that increase surface energy in additive and subtractive methods are decisive for bonding success(9). Ersöz et al. (2024) evaluated the effect of different surface treatments on shear bond strength (SBS) in 3D printed permanent resins. Sandblasting, etching, silanization, and plasma applications

were applied to resin samples produced with DLP technology; results were analyzed with the SBS test. The highest bonding values were obtained in the plasma + silane combination. Although bond strength increased in sandblasting and etching groups, microcrack formation was observed on the surface. SEM examinations showed that the surface became more homogeneous and microretentive in the plasma process. The authors stated that chemical bonding is strengthened by plasma activation and that this method could improve the bonding performance of 3D printed resins(10). Kim et al. (2023) examined the effect of different surface treatments on shear bond strength (SBS) in 3D printed resins used in permanent restorations. Sandblasting, silanization, etching, and laser surface treatments were applied to resin samples produced by the DLP method, results were evaluated via the SBS test. The highest bond strength was seen in the sandblasting + silane group, while the lowest values were seen in samples where only silane was applied. It was observed that surface energy increased in the laser process, but microcracks formed in some regions due to overheating. Researchers concluded that the combined application of mechanical roughening and chemical activation significantly strengthens the adhesive bond in 3D printing materials(11). Literature findings indicate that bonding success in 3D printing materials is directly dependent on surface modification. In most studies (Dederichs et al., 2023; Alanazi et al., 2023; Mao et al., 2024; Ersöz et al., 2024; Kim et al., 2023), the highest shear bond strength (SBS) values were obtained in groups where sandblasting and silanization or tribochemical silica coating were applied together. Bonding is significantly lower in samples subjected only to mechanical or only chemical treatment. Plasma and laser applications support bonding by increasing surface energy. These results reveal that the combination of mechanical roughening and chemical activation is the most effective surface preparation strategy for 3D printed dental materials.

Post-processing steps, namely washing, post-curing, and light intensity, directly affect the material's degree of polymerization, surface energy, and consequently, its bonding capacity. Katheng et al. (2024) examined the effect of different post-rinsing times (5, 10, and 15 min) and washing methods (manual, ultrasonic, automatic) on surface properties and degree of polymerization in 3D printed photopolymer resins. Although direct bond strength was not measured in the study, the data obtained show that the post-processing time and method have significant effects on surface roughness, hardness, and polymerization rate. It was particularly noted that longer and more effective washing protocols contribute to the reduction of residual monomers on the surface and make the surface chemically more stable. These findings suggest that post-printing processes may influence the surface interaction with adhesive systems, although further studies are required to confirm their impact on bond strength(12). In another study conducted by Büyükpölat M. et al. (2024), the effect of post-curing time and the type of device used (e.g., LED or xenon light sources) on the mechanical and

optical properties of the material was examined in two different 3D printed resin systems. Research findings show that the post-printing polymerization time and light source can lead to changes in the material's hardness, color stability, and surface properties. It was stated that inadequate curing applications could cause residual monomer residue on the surface and an increase in the oxygen inhibition layer, which would negatively affect interaction with adhesive systems. Conversely, post-curing processes performed for the appropriate time and with the appropriate device contribute to the formation of more homogeneous and fully polymerized surfaces. Although direct bond strength was not measured in the study, the data obtained suggest that post-printing polymerization conditions may affect the behavior of the surface regarding cementation procedures to be applied in later stages(13).

Print orientation and layer direction are important parameters affecting the internal bond integrity and interface behavior of 3D printed structures. Khanlar et al. (2023) compared the surface roughness and shear bond strength (SBS) values with composite resin of temporary DLP resins printed in different orientations 0°, 45°, 90°. Although print direction significantly affected surface roughness in the study, no significant difference was observed in bond strength. This result shows that print direction may have an indirect effect on bonding but is not decisive on its own(14). de Castro et al. (2022) examined mechanical properties such as accuracy, flexural strength, and elastic modulus in resin specimens produced in different print orientations. As the layer angle increased, changes were observed in surface roughness and flexural resistance, suggesting that orientation may influence surface energy and potential adhesive retention(15). Lu et al. (2024) evaluated the effect of print layer orientation (parallel, perpendicular, and cross) and subsequent surface polishing on the mechanical performance of the material. It was reported that parallel layered prints created a more homogeneous surface and this situation could provide advantages in load distribution and adhesion interfaces(16).

The chemical structure of the material forms the basis of the bonding mechanism. Literature emphasizes that the chemical composition of 3D printing materials plays a decisive role in adhesive bonding behavior. Jockusch and Özcan (2020) reported that most 3D printing polymers used in dentistry consist of acrylate and methacrylate-based photopolymers; these structures may retain residual monomers and form an oxygen-inhibition layer on the surface due to the layer-by-layer polymerization process. It was stated that this layer reduces surface energy, lowering the wettability of adhesive systems(1). Revilla-León and Özcan (2019) emphasized that the chemical structure of these materials, along with the degree of polymerization and cross-link density, affects bonding quality; inadequate curing conditions can limit chemical bond formation(17). These findings show that the monomer type, cross-link density, and amount of free radicals of the material have a direct effect on the permanence and durability

of the adhesive bond. Therefore, the selection of appropriate post-curing and surface treatment protocols for each material contributes to increasing bonding success while preserving the chemical integrity of the 3D printed structure.

Aging and thermal cycling tests are performed to ensure the bond remains stable over time. Mao et al.(9) applied 5,000 thermal cycles to hybrid materials produced by additive and subtractive methods and observed an approximately 20% decrease in SBS values. The chemical bond weakened primarily due to the hydrolysis of silane groups. Conversely, in a study by Kim et al. (2023) on 3D printing resins for permanent restorations, thermal aging was not applied; only the effects of different surface treatments (e.g., sandblasting, silane, acid etching) on immediate bond strength were evaluated. This indicates a significant limitation in the literature: many 3D printing material studies measure only initial bonding performance rather than long-term durability of the bond(11).

The type of adhesive system stands out as one of the important factors affecting the bonding performance of 3D printing resins in the literature. A systematic review by Bourgi et al. (2025) evaluated the effects of adhesive systems used in conjunction with surface treatments on bond strength in 3D printing resins and reported that universal adhesives containing MDP and silane applications, especially when combined with sandblasting, significantly strengthened the bond. Researchers emphasized that adhesive application after mechanical roughening enhances chemical bond formation in addition to micromechanical retention(6). Mao et al. (2024) compared different adhesive systems and surface treatment combinations in additive (3D printing) and subtractive hybrid crown materials; they showed that adhesives containing 10-MDP provided higher bond strength even after aging (5,000 thermal cycles). This demonstrates that the chemical bond is more stable than the mechanical bond even under thermal stress conditions(9). A similar trend is observed in studies conducted on removable denture bases. Park et al. (2022) evaluated the bond strength between 3D printed denture bases and reline materials and showed that primer use alone was not sufficient; sandblasting + silane or primer combinations provided much higher bond strength(18). When these findings are evaluated together, it is seen that the effectiveness of the adhesive system type depends on the material structure and surface treatment protocol. In particular, mechanical (sandblasting) + chemical (MDP or silane) combinations tend to provide stronger and more permanent bonding in 3D printed resins. However, it is also emphasized in the literature that results cannot be fully standardized due to the diversity of adhesives used and differences in test conditions.

Bonding quality in 3D printing materials is closely related not only to surface treatment and the adhesive system but also to the *type of cement used*. Resin cements are classified as chemical, light, or dual-polymerization (dual-cure), and the monomer type in their content (e.g., Bis-GMA, UDMA, TEGDMA) and

functional components (e.g., MDP, silane, phosphate groups) directly affect the bonding mechanism. Ersöz et al. (10) compared different cement types in DLP-printed permanent resins and reported that dual-cure resin cements provided the highest SBS values thanks to both light and chemical curing mechanisms. Dual-cure cements supported by sandblasting and plasma applications were found to significantly increase bond strength. Mao et al. (2024) reported that dual-cure resin cements used with different surface treatments in additively and subtractively produced hybrid crown materials provided higher bond strength compared to systems polymerized only by light. Dual polymerization increases bonding integrity by providing more homogeneous curing in areas with limited light transmission of 3D printing materials(9). Kim et al. (2023) showed that MDP-containing resin cements (e.g., Panavia V5, RelyX Universal) formed stronger chemical bonds in different 3D printing resins compared to other conventional resin cements. This effect was associated with the ability of phosphate groups to react with the methacrylate chains of 3D printing resins(11). Additionally, Park and Lee (2022) stated that in bonding with liner materials applied to 3D printed denture bases, acrylic-based autopolymerizing resins showed better bond strength compared to silicone-based soft liners. This finding indicates that the chemical compatibility of the polymer matrix and cement type used plays a critical role in bonding(18). In general, the literature shows that dual-cure resin cements containing MDP or chemically compatible self-adhesive systems provide more stable bonding for 3D printing materials, especially in resins whose surfaces are activated after post-curing. However, more long-term studies are needed to clarify the relationship between cement type and bond durability under aging conditions.

The clinical success of these materials depends not only on their mechanical properties but also on their ability to *bond reliably to different substrates*. Bonding to dentin, titanium, and PEEK requires a specific adhesive approach for each of these surfaces, which differ significantly in terms of structure, surface energy, and chemical properties. In the study by Donmez et al., the bonding of two different 3D printed composite materials—CrownTec (CT) and VarseoSmile Crown Plus (VS)—to dentin and titanium was evaluated, and these results were compared with a PICN material named Enamic (EN) produced by milling. According to the findings, Enamic showed significantly higher bond strength on both dentin and titanium surfaces compared to the other two 3D printing materials. It was reported that the type of resin cement used on the dentin surface (RelyX Universal, Panavia V5, Variolink DC) did not significantly change the results, whereas on the titanium surface, Variolink DC specifically caused the lowest bonding values in all three materials. When fracture types were examined, mostly mixed/cohesive fractures were observed in dentin, while mostly adhesive character separations were observed in titanium. Overall, the study shows that 3D printed composites such as CrownTec and VarseoSmile Crown Plus can bond to

both dentin and titanium, but their bonding performance is not as high as Enamic and varies depending on the surface type and the cement used(19). A study by Montenbruck et al. evaluated the bond strength to dentin of two different 3D printed composite resins—3Delta Crown (DeltaMed GmbH) and saremco print CROWNTEC (SAREMCO Dental AG). Two different mechanical treatments (air abrasion / no treatment) and three chemical pretreatments (Adhese Universal, Heliobond, Monobond Plus) were applied to the specimens. All samples were cemented to dentin with Variolink Esthetic LC. The findings of the study showed that the applied pretreatment types did not significantly change the tensile bond strength. TBS values remained in the range of approximately 6.5–10.5 MPa, with no statistical difference found between groups. Although air abrasion significantly increased surface roughness, this increase was not reflected in bond strength. Additionally, the degree of polymerization of the two materials was found to be quite similar (approximately 70%). The vast majority of fracture types were adhesive in nature, and it was seen that the weakest link of the bond was usually the cement layer. Overall, the findings suggest that mechanical or chemical treatments aimed at increasing bonding in 3D printed composites have limited effect and that the high monomer content of the material may be more decisive in chemical bonding(20). Another study was conducted to evaluate the bond strength of different CAD-CAM resin-based materials to PEEK and titanium surfaces. Three additive manufacturing materials (CrownTec – AM_CT, FREEPRINT temp – AM_FP, VarseoSmile Crown Plus – AM_VS) and two subtractive manufacturing materials (G-CAM – SM_GC, breCAM.HIPC – SM_BC) were tested. All samples were subjected to air abrasion with 50 μm Al_2O_3 ; Visio.link was used on PEEK surfaces, and Clearfil Ceramic Primer Plus (MDP-containing) was used on titanium surfaces. Cementation was performed with the same resin cement, PANAVIA SA Cement Universal, in every group. Results revealed that additive manufacturing materials showed higher bond strength compared to subtractive materials, regardless of the abutment type. The highest value was observed in the AM_VS–PEEK combination, whereas the lowest values were recorded in the SM_GC–PEEK and SM_BC–PEEK groups. However, the effect of the abutment material is quite limited; the difference between PEEK and titanium is not statistically significant. In all samples, the majority of fractures were adhesive in character; cohesive fracture was not observed. Overall, the study indicates that 3D printed composites (especially VarseoSmile Crown Plus) offer clinically acceptable and higher bonding on PEEK and titanium surfaces compared to subtractive materials, while subtractive PMMA-based materials (G-CAM and breCAM.HIPC) have lower bonding values(21).

When all these findings are brought together, it is seen that bond strength in 3D printing materials has a multifactorial structure. The highest bonding is generally obtained under conditions where optimum post-curing time,

appropriate print orientation, a combination of mechanical roughening and silanization, and the combined use of MDP-containing universal adhesives and dual-cure cements are present. Optimizing these parameters is the fundamental element determining the long-term clinical success and bonding stability of 3D printed dental restorations.

3. Advanced Techniques and Current Studies

One of the most current approaches to improving the mechanical strength and bonding properties of 3D printing resins is nano-scale surface and structural modifications. These techniques aim to create a stronger interaction with adhesive systems by changing both the internal structure and surface energy of the material. One of the innovative studies in this field was carried out by Darbandi and Amin (2024). Researchers enriched 3D printing resins with zirconia nanoparticles (ZrO_2) and halloysite nanotubes modified with silver nanoparticles (Ag-HNTs). In samples prepared at various concentrations (ZrO_2 : 1–16%; Ag-HNT: 1–7.5%), flexural strength, fracture toughness, elastic modulus, and microhardness parameters were evaluated. According to the findings, the highest mechanical performance was obtained particularly with ZrO_2 4% and Ag-HNT 5% additions. Although a direct bonding test was not performed in the study, the observed increase in surface hardness and structural homogeneity indicates that such nano-reinforcements could indirectly strengthen the interaction of adhesive systems with the surface. The ceramic-like structure of zirconia nanoparticles and the nanoscale topography created by halloysite nanotubes can produce a surface morphology that supports adhesive wettability and mechanical micro-retention(21). Addressing these findings more directly in terms of bonding, ElMalah et al. (2025) examined the bond strength of nano- TiO_2 -reinforced 3D-printed resins to rough and smooth titanium surfaces. Three resin formulations based on VarseoSmile Crown Plus (control, 0.2% TiO_2 , 0.4% TiO_2) were prepared; air abrasion and citric acid were applied to all samples. Surface roughness increased significantly in both titanium and resins; high Ra values were obtained especially on the titanium surface after sandblasting. According to shear bond strength results, rough titanium surfaces provided higher bond strength in all groups. After thermal aging, the highest SBS was observed in the bond of the resin containing 0.4% TiO_2 to rough titanium (≈ 12.8 MPa). The increase in bonding success is attributed to mechanical roughening, chemical interactions created by citric acid on the surface, and the functional monomer effect of the GPDM/4-META containing resin cement used. The study shows that the combined application of nano- TiO_2 addition and surface treatments strengthens the composite-titanium interface in implant-supported temporary restorations. These results contribute to the development of optimized bonding protocols that will increase long-term durability(23). A review published by Duarte and Phark (2025) addressed machinable and 3D printed ceramic-reinforced composites (CRC), comparing their mechanical and bonding

performances. The authors stated that 3D printed CRCs yield variable results in terms of both mechanical strength and adhesive bonding depending on parameters such as ceramic particle content, filler-matrix ratio, and the production process. In the review, it was emphasized that as the ceramic ratio increases, the surface becomes more inert, so surface activation with adhesive systems containing silane or MDP is of critical importance. Furthermore, it was stated that layer lines and microporosities specific to the 3D printing process could affect the continuity of the bond interface. Therefore, it was stated that bonding success depends not only on surface treatment but also on production quality and post-curing conditions. Overall, the study indicates that 3D printed CRCs are promising in terms of adhesive bonding, but long-term stability requires further investigation(24).

4. Conclusion

3D printing technologies have enabled the production of patient-specific, high-accuracy, and biocompatible dental restorations. However, the clinical success of these materials depends not only on aesthetic or mechanical properties but also on the durability of the interfacial bonding. Studies conducted reveal that there are numerous factors affecting bond strength. Surface modification, post-processing parameters, adhesive and cement selection, print orientation, and the chemical composition of the material are the fundamental determinants of this strength. The highest bonding values have been obtained in groups where mechanical (sandblasting, tribochemical coating) and chemical (silanization, MDP-containing adhesive) treatments are used together (Alanazi et al., 2023; Kim et al., 2023; Mao et al., 2024; Ersöz et al., 2024) (8-11). Post-printing polymerization time and light-source intensity directly affect both surface energy and the degree of polymerization. Büyükpolat (2024) and Katheng (2024) reported that post-curing processes performed at the appropriate time and intensity positively affect bonding quality by increasing surface homogeneity. Inadequate curing reduces adhesive retention by increasing the thickness of the oxygen-inhibition layer(12, 13). The chemical basis of bonding relies on the interaction between the monomer structure and functional groups (e.g., MDP, silane, phosphate). Dual-cure cements provide long-term stability, especially in materials with low light transmission, thanks to both chemical and light curing mechanisms (Mao, 2024; Park, 2022)(9, 18). Approximately 20% bond loss has been reported after thermal cycling; this indicates the necessity of protecting chemical bonds against hydrolytic degradation. In recent years, nano-scale modifications have also become a promising area for increasing bonding performance. Resins reinforced with zirconia, silica, or silver-based nanofillers may enhance bonding potential by increasing surface energy (Darbandi & Amin, 2024)(22). However, further studies are needed on the long-term stability and biocompatibility of these additives. Additionally, the establishment of standard

protocols evaluating the effect of print orientation, layer thickness, and surface polishing processes on bond strength is recommended.

In conclusion, the following strategies stand out for high bond strength in 3D printing dental materials:

- * Optimum post-curing and full polymerization,
- * Combined application of sandblasting and silanization,
- * Use of MDP-containing dual-cure resin cements,
- * Material-specific surface protocols,
- * Careful application of nano-modifications.

Optimizing these elements together will ensure the provision of more reliable and sustainable restorative solutions in digital dentistry by increasing the long-term bond durability of 3D printing materials.

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CHAPTER 48

Cytokine Storm and Dysregulated Host Response in Critical Illness

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Abbreviations

AKI: Acute kidney injury; ARDS: Acute respiratory distress syndrome; ATP: Adenosine triphosphate; CpG: Cytosine–phosphate–guanine; CRP: C-reactive protein; DAMP: Damage-associated molecular pattern; DNA: Deoxyribonucleic acid; EAA: Endotoxin activity assay; ECMO: Extracorporeal membrane oxygenation; HLA-DR: Human leukocyte antigen–DR; HMGB1: High-mobility group box 1 protein; ICAM-1: Intercellular adhesion molecule 1; iNOS: Inducible nitric oxide synthase; ICU: Intensive care unit; IL-1 β : Interleukin 1-beta; IL-1Ra: Interleukin 1 receptor antagonist; IL-6: Interleukin 6; IL-10: Interleukin 10; JAK–STAT: Janus kinase–signal transducer and activator of transcription; LPS: Lipopolysaccharide; MODS: Multiple organ dysfunction syndrome; NET: Neutrophil extracellular trap; NF- κ B: Nuclear factor kappa-B; NLR: NOD-like receptor; PAMP: Pathogen-associated molecular pattern; PCT: Procalcitonin; PRR: Pattern recognition receptor; RIG-I: Retinoic acid–inducible gene I; SIRS: Systemic inflammatory response syndrome; TGF- β : Transforming growth factor beta; TLR: Toll-like receptor; TNF- α : Tumor necrosis factor alpha; VCAM-1: Vascular cell adhesion molecule 1

Conceptual Foundations of the Cytokine Storm

The term "cytokine storm" is extensively utilized in critical care medicine to denote a condition of uncontrolled systemic inflammation, characterized by significantly elevated circulating levels of pro-inflammatory mediators. Despite its frequent mention in scientific literature and clinical discussions, the cytokine storm is not a formally defined disease entity, nor does it represent a uniform or mechanistically distinct syndrome. Instead, it serves as a descriptive construct encompassing a range of hyperinflammatory states observed in various clinical contexts, including sepsis, severe trauma, acute pancreatitis, ischemia–reperfusion injury, and inflammation associated with extracorporeal circulation (Bhaskar et al., 2020; Pelaia et al., 2020; Zanza et al., 2022).

Conceptually, the cytokine storm signifies a failure of immune regulation, wherein the delicate balance between pro-inflammatory and anti-inflammatory signaling collapses. Under physiological conditions, cytokine release is spatially and temporally regulated, facilitating effective pathogen clearance or tissue repair while minimizing collateral damage. However, in critical illness, this regulatory framework becomes disrupted, resulting in excessive mediator release, systemic spillover, and self-propagating inflammatory cascades. Importantly, the cytokine storm should not be perceived merely as an exaggeration of normal inflammation. Rather, it reflects a qualitative shift in immune behavior, characterized by loss of compartmentalization, altered receptor signaling, endothelial activation, and dysregulated feedback inhibition. This distinction is crucial, as it elucidates why therapeutic strategies that simply block or neutralize individual cytokines have

largely failed to improve outcomes in critically ill patients (Bhaskar et al., 2020; Hiti et al., 2025; Pourgholaminejad, Pahlavanneshan, & Basiri, 2022).

In intensive care medicine, the significance of the cytokine storm lies not only in its association with disease severity but also in its direct contribution to hemodynamic instability, organ dysfunction, and treatment resistance. Vasoplegic shock, capillary leak syndrome, microvascular thrombosis, and mitochondrial dysfunction can all be attributed, at least in part, to unchecked inflammatory signaling. Therefore, understanding the cytokine storm as a pathophysiological process, rather than a laboratory abnormality, is essential for rational therapeutic decision-making (Hiti et al., 2025; Mitzner et al., 2023).

Physiological Role of Cytokines in Host Defense

Cytokines are small, soluble proteins that serve as pivotal regulators of immune communication, orchestrating the initiation, amplification, and resolution of host defense mechanisms. Under physiological conditions, cytokine signaling is meticulously regulated both temporally and spatially, ensuring that inflammatory responses remain proportionate to the inciting insult and confined to the affected tissue compartment. Innate immune activation constitutes the initial line of cytokine-mediated defense. Following tissue injury or microbial invasion, resident immune cells -most notably macrophages, dendritic cells, and endothelial cells- rapidly recognize danger signals and initiate cytokine release (Liu et al., 2023; McIntire, Yeretssian, & Saleh, 2009). Pro-inflammatory mediators such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6) are produced early and function to increase vascular permeability, promote leukocyte recruitment, and activate antimicrobial effector functions. This early cytokine burst is adaptive, facilitating rapid containment of infection or tissue damage. Simultaneously, anti-inflammatory cytokines are generated to counterbalance pro-inflammatory signaling. Interleukin-10 (IL-10), interleukin-1 receptor antagonist (IL-1Ra), and transforming growth factor- β (TGF- β) play critical roles in attenuating excessive immune activation, limiting tissue injury, and promoting resolution of inflammation (Donniacuo et al., 2025; Iordache et al., 2025).

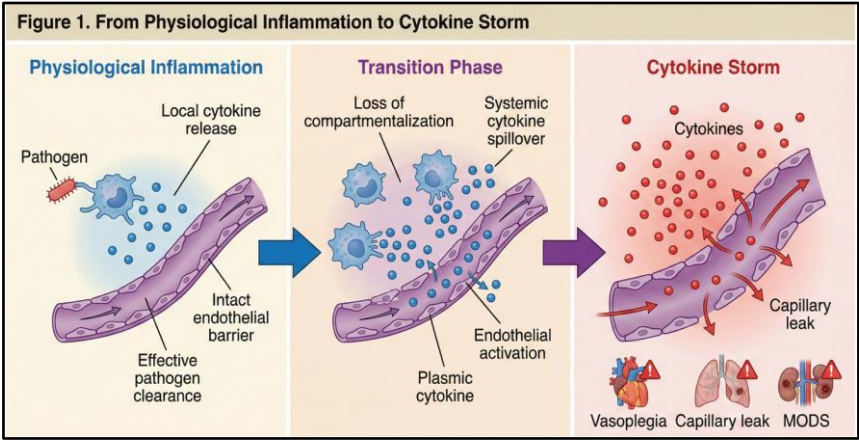
The coexistence of pro- and anti-inflammatory mediators is not paradoxical; rather, it reflects a dynamic regulatory equilibrium that permits inflammation to proceed while preserving host integrity. A defining feature of physiological cytokine signaling is compartmentalization. Cytokines are predominantly produced and act locally within tissues, exerting paracrine and autocrine effects. Systemic spillover into the circulation is minimal and transient, owing to short cytokine half-lives, rapid receptor binding, and efficient clearance mechanisms. Circulating cytokine concentrations under normal conditions are low or undetectable, even during robust local inflammatory responses (Cavaillon & Annane, 2006; Liu et al., 2023).

Cytokines also function as essential modulators of the adaptive immune system. Antigen presentation by innate immune cells triggers cytokine-dependent differentiation of T lymphocytes into effector and regulatory subsets. This process shapes immune memory, determines pathogen-specific responses, and contributes to long-term immune competence. Importantly, cytokines not only activate immunity but also encode signals for immune contraction, apoptosis of activated cells, and restoration of homeostasis once the threat has been eliminated (Heidarian, Griffith, & Badovinac, 2023; Lauvau & Goriely, 2016). For intensivists, it is critical to recognize that cytokines are not inherently harmful. They are indispensable for survival in the face of infection, trauma, and surgical stress. The pathological consequences observed in critical illness arise not from cytokine presence per se, but from loss of regulatory control, inappropriate systemic dissemination, and sustained activation beyond physiological thresholds. Understanding the normal role of cytokines clarifies why indiscriminate suppression or removal of inflammatory mediators carries theoretical risks. It also explains why therapeutic strategies aimed at modulating, rather than abolishing, cytokine activity have been proposed in critical care. The transition from physiological inflammation to pathological hyperinflammation represents a tipping point, one that is poorly defined clinically but central to the development of shock and organ failure (Kuypers, 2022; Molinaro et al., 2020; Pearce, Davidson, & Yellon, 2020).

From Protective Inflammation to Pathological Hyperinflammation

The progression from physiological inflammation to pathological hyperinflammation constitutes a pivotal juncture in the trajectory of severe illness. While the initial inflammatory response is crucial for host defense and tissue repair, its persistence or dysregulation converts a protective mechanism into a catalyst for organ injury and hemodynamic collapse. This transition is neither abrupt nor uniform; rather, it signifies a gradual loss of regulatory control over immune signaling pathways. In the early stages of critical illness, inflammatory activation is typically proportional to the magnitude of the insult. Surgical trauma, infection, ischemia–reperfusion injury, or extracorporeal circulation all provoke predictable cytokine responses that subside as the inciting stimulus is managed (Molinaro et al., 2020; Neher, Stahel, Weckbach, & Huber-Lang, 2012; Zhou, Wang, & Aziz, 2021). Pathological hyperinflammation arises when this resolution phase fails. Continued cytokine production, impaired clearance, and sustained cellular activation lead to amplification loops that are no longer governed by physiological feedback mechanisms. Several factors contribute to this maladaptive shift. Ongoing tissue injury and microbial persistence perpetuate cytokine release through continuous stimulation of immune cells. Simultaneously, damage to endothelial barriers and microcirculatory beds facilitates the escape of locally produced cytokines into the systemic circulation (Figure 1). What was once a compartmentalized response

becomes a systemic inflammatory state, exposing distant organs to mediators intended for local defense (Hasan et al., 2021; Zhang et al., 2023).



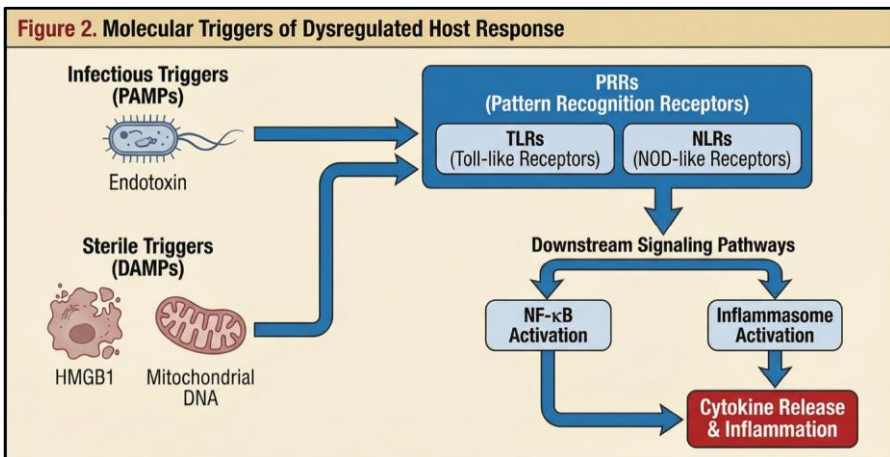
At the molecular level, pathological hyperinflammation is characterized by altered receptor signaling and intracellular transcriptional reprogramming. Nuclear factor κ B (NF- κ B), Janus kinase–signal transducer and activator of transcription (JAK–STAT), and inflammasome pathways remain persistently activated, driving sustained cytokine synthesis even in the absence of ongoing pathogen load. This phenomenon explains why inflammatory markers may remain elevated despite adequate source control and antimicrobial therapy (Terrell et al., 2006). Clinically, the consequences of this dysregulated response are profound. Excessive cytokine signaling induces widespread vasodilation, increased vascular permeability, and myocardial depression, culminating in distributive shock. Simultaneously, leukocyte activation and endothelial injury promote microvascular thrombosis, impairing tissue oxygen delivery. Mitochondrial dysfunction and altered cellular metabolism further exacerbate organ failure, rendering conventional supportive therapies progressively less effective (Hawkins et al., 2018; Zhang et al., 2023). Importantly, pathological hyperinflammation does not imply a uniformly “hyperactive” immune system. Paradoxically, markers of immune exhaustion and cellular apoptosis often coexist with elevated cytokine levels. This coexistence underscores that hyperinflammation is not merely excessive immunity, but rather disordered immunity, in which destructive inflammatory signaling persists alongside impaired pathogen clearance and adaptive immune dysfunction. For anesthesiology and intensive care practice, recognizing this transition has direct therapeutic implications. Escalating vasopressor requirements, refractory metabolic acidosis, and progressive organ dysfunction despite guideline-based management should prompt consideration of ongoing immune dysregulation as a contributing factor. These clinical features have driven interest in adjunctive

therapies aimed at modulating the inflammatory milieu rather than solely supporting failing organs (S. Liu et al., 2023; McIntire, Yeretssian, & Saleh, 2009).

Molecular Triggers: PAMPs, DAMPs, and Pattern Recognition Receptors

At the core of cytokine dysregulation in critical illness is the innate immune system's recognition machinery, which is designed for rapid detection of danger but can become maladaptive when excessively or persistently activated. This recognition is mediated through conserved molecular motifs -pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs)- and their interaction with pattern recognition receptors (PRRs) expressed on both immune and non-immune cells. PAMPs are structural components of microorganisms absent from host tissues, such as lipopolysaccharide (LPS) from Gram-negative bacteria, peptidoglycans, flagellin, and unmethylated CpG DNA. Their detection is essential for early host defense, enabling immune cells to distinguish infection from sterile injury. In sepsis, however, sustained or overwhelming exposure to PAMPs, particularly endotoxin, drives continuous activation of innate immune pathways, even after partial microbial clearance (Hasan et al., 2021; H. Zhang et al., 2023). In contrast, DAMPs originate from the host itself. They are released by stressed, injured, or necrotic cells and include molecules such as high-mobility group box 1 protein (HMGB1), mitochondrial DNA, histones, heat shock proteins, and extracellular ATP. DAMPs serve as endogenous alarm signals, alerting the immune system to tissue damage. In critical illness, extensive cellular injury, ischemia-reperfusion, and endothelial disruption result in persistent DAMP release, effectively sustaining inflammation in the absence of viable pathogens (Murao, Aziz, Wang, Brenner, & Wang, 2021; Roh & Sohn, 2018).

Both PAMPs and DAMPs are detected by PRRs, a diverse family of receptors that include Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I-like receptors, and C-type lectin receptors. These receptors are expressed not only on professional immune cells such as macrophages and dendritic cells but also on endothelial cells, epithelial cells, and even cardiomyocytes. Consequently, PRR activation produces system-wide inflammatory signaling, extending beyond classical immune compartments (Figure 2). Upon ligand binding, PRRs initiate intracellular signaling cascades that converge on transcription factors responsible for cytokine gene expression. While these pathways are indispensable for mounting an effective immune response, their persistent activation leads to self-reinforcing inflammatory loops. Cytokines produced downstream of PRR signaling further amplify PRR expression and sensitivity, lowering activation thresholds and promoting exaggerated responses to otherwise minor stimuli (Jang et al., 2020; Kumar & Stewart Iv, 2024; Lai & Gallo, 2008).

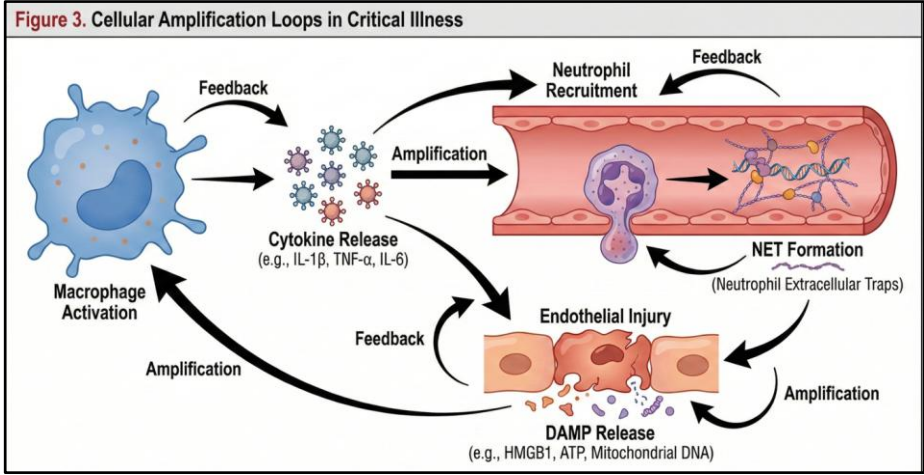


A critical feature of this process is the loss of discrimination between infectious and non-infectious triggers. In advanced critical illness, DAMP-driven signaling may dominate, meaning that inflammation continues despite microbiological control. This phenomenon explains why some patients exhibit worsening inflammatory and hemodynamic profiles even after appropriate antimicrobial therapy and source control have been achieved. From the perspective of intensive care, several common clinical scenarios act as potent sources of DAMPs. Major surgery, cardiopulmonary bypass, extracorporeal membrane oxygenation, prolonged hypoperfusion, and massive transfusion all induce cellular stress and tissue injury. These sterile insults activate the same PRR pathways as infection, blurring the distinction between septic and non-septic inflammation and contributing to cytokine-mediated organ dysfunction. Understanding PAMP- and DAMP-driven immune activation provides critical insight into why purely antimicrobial or supportive strategies may be insufficient in advanced critical illness. It also clarifies the mechanistic rationale behind therapeutic approaches that aim to reduce the circulating burden of inflammatory mediators or their upstream triggers, rather than targeting individual cytokines (Eppensteiner, Kwun, Lee, Davis, & Barbas, 2018; Horner, Hazeldine, & Lord, 2023; Saavedra-Torres et al., 2025).

Cellular Amplification Loops and Loss of Immune Homeostasis

Upon initiation of innate immune activation via PAMP- and DAMP-mediated signaling, the progression towards a cytokine storm is facilitated by a series of cellular amplification loops that progressively disrupt immune homeostasis. These loops emerge from reciprocal interactions among immune cells, endothelial cells, and parenchymal tissues, transforming an initially appropriate response into a self-sustaining pathological process. Macrophages and monocytes play a central role in this amplification. Following PRR activation, these cells rapidly produce pro-inflammatory cytokines such as $\text{TNF-}\alpha$, $\text{IL-1}\beta$, and IL-6 .

Under physiological conditions, cytokine release is followed by receptor downregulation, cellular deactivation, or apoptosis. In critical illness, however, continued exposure to inflammatory stimuli maintains macrophages in a persistently activated state. Cytokines released by these cells act in an autocrine manner to reinforce their own activation while also stimulating neighboring immune cells, thereby creating positive feedback loops. Neutrophils further intensify this process. Excessive cytokine signaling promotes neutrophil recruitment, delayed apoptosis, and the formation of neutrophil extracellular traps (NETs). While NETs contribute to pathogen containment, they also damage endothelial surfaces, activate coagulation pathways, and release additional DAMPs (Figure 3). This cycle links inflammation directly to microvascular injury and propagates immune activation beyond the original site of insult (Denning et al., 2019; Pelaia et al., 2020; Vanderbeke et al., 2021).



The adaptive immune system, rather than restoring balance, often becomes dysregulated as well. Persistent cytokine exposure leads to lymphocyte apoptosis, impaired antigen presentation, and altered T-cell differentiation. Regulatory T-cell function may be insufficient to counteract inflammation, while effector T-cell responses become ineffective or exhausted. The result is a paradoxical state in which hyperinflammation coexists with immunosuppression, undermining pathogen clearance while sustaining tissue injury (Eppensteiner, Kwun, Lee, Davis, & Barbas, 2018; Horner, Hazeldine, & Lord, 2023; Saavedra-Torres, Pinzón-Fernández, Ocampo-Posada, et al., 2025). Endothelial cells are not passive bystanders in this process. Cytokine exposure transforms the endothelium into an active inflammatory organ, characterized by increased expression of adhesion molecules, procoagulant factors, and inflammatory mediators. Activated endothelium amplifies leukocyte recruitment and perpetuates vascular permeability, contributing to capillary leak syndrome and interstitial edema. Endothelial injury also exposes subendothelial structures, promoting thrombosis

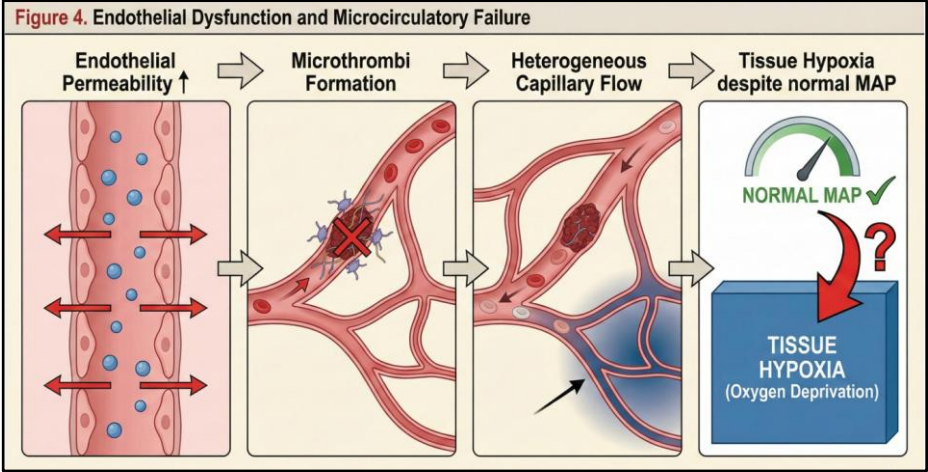
and further tissue ischemia, which in turn generates additional DAMPs. Collectively, these cellular interactions dismantle the normal checks and balances of the immune system (Dolmatova, Wang, Mandavilli, & Griendling, 2020; Theofilis et al., 2021). Anti-inflammatory pathways become insufficient or mistimed, and feedback inhibition fails to restore equilibrium. Rather than progressing toward resolution, the immune response becomes locked in a dysregulated state, with ongoing mediator release independent of the original trigger. For intensivists, this loss of immune homeostasis explains several characteristic features of advanced critical illness: persistent vasoplegia, refractory hypotension, progressive organ dysfunction, and vulnerability to secondary infections. It also highlights why late-stage interventions targeting single inflammatory mediators are unlikely to succeed, as the pathological process is maintained by interconnected cellular networks rather than isolated signaling pathways (Burgdorff, Bucher, & Schumann, 2018; Gutierrez et al., 2018).

Endothelial Dysfunction and Microcirculatory Failure

Endothelial dysfunction represents a pivotal intermediary between cytokine dysregulation and the development of organ failure in severe illness. The vascular endothelium, which ordinarily maintains barrier integrity, regulates vascular tone, and modulates coagulation, undergoes substantial alterations under persistent inflammatory stress. During a cytokine storm, the endothelium transitions from a homeostatic interface to a pathologically activated organ system. Pro-inflammatory cytokines, such as TNF- α , IL-1 β , and IL-6, directly disrupt endothelial tight junctions, increasing vascular permeability and facilitating plasma leakage into the interstitial space. This compromise in barrier function results in intravascular volume depletion, tissue edema, and impaired oxygen diffusion at the cellular level. Clinically, these alterations manifest as hypotension, reduced responsiveness to fluid resuscitation, and worsening tissue hypoxia despite seemingly adequate macrocirculatory parameters (Dolmatova et al., 2020; Gallo & Savoia, 2024; Six et al., 2022).

Concurrently, cytokine-mediated endothelial activation induces the expression of adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). These changes promote leukocyte adherence and transmigration, while also facilitating endothelial injury through direct cellular contact and oxidative stress. Activated leukocytes release proteases, reactive oxygen species, and additional inflammatory mediators, further compromising endothelial integrity and perpetuating inflammation. Endothelial dysfunction also significantly alters the balance between coagulation and fibrinolysis. Cytokine signaling favors a procoagulant phenotype characterized by tissue factor expression, suppression of anticoagulant pathways, and impaired fibrinolysis. Microvascular thrombosis

ensues, obstructing capillary blood flow and contributing to regional ischemia (Figure 4). Notably, this process occurs at the microcirculatory level and may not be detected by standard hemodynamic monitoring, leading to a disconnect between systemic blood pressure and effective tissue perfusion (Ammollo, Semeraro, Colucci, & Semeraro, 2015; Dolmatova et al., 2020; Theofilis et al., 2021).



The resultant microcirculatory failure is a defining characteristic of advanced critical illness. Capillary flow becomes heterogeneous, with areas of shunting adjacent to regions of no-flow. Oxygen delivery is impaired not only by reduced perfusion but also by diffusion limitations imposed by interstitial edema. Mitochondrial dysfunction further exacerbates these effects, as cells become unable to utilize available oxygen efficiently, a phenomenon often described as cytopathic hypoxia. From an intensive care perspective, endothelial dysfunction elucidates why aggressive fluid administration may worsen outcomes by exacerbating tissue edema, and why escalating vasopressor doses can fail to restore organ perfusion. Vasoplegia in this context reflects not merely smooth muscle relaxation but a fundamental loss of vascular responsiveness due to nitric oxide overproduction, receptor desensitization, and endothelial injury. Importantly, endothelial damage also serves as a source of additional DAMPs, reinforcing innate immune activation and perpetuating a vicious cycle between inflammation and vascular injury (Chappell, Westphal, & Jacob, 2009; Cusack, Leone, Rodriguez, & Martin-Loeches, 2022; Roy & Secomb, 2020).

Hemodynamic Consequences: Vasoplegia and Shock

The hemodynamic manifestations of cytokine-induced endothelial dysfunction culminate in vasoplegic shock, a clinical condition characterized by severe hypotension, decreased systemic vascular resistance, and reduced responsiveness to conventional vasoactive therapies. In critical illness, this form

of shock represents not merely a failure of circulatory support but the physiological expression of widespread inflammatory and microvascular derangement. At the molecular level, excessive cytokine signaling induces overproduction of nitric oxide through upregulation of inducible nitric oxide synthase (iNOS) in vascular smooth muscle cells and endothelial cells. Elevated nitric oxide levels lead to sustained vasodilation and diminished vascular tone. Concurrently, inflammatory mediators downregulate adrenergic receptors and disrupt intracellular calcium handling, rendering vascular smooth muscle increasingly insensitive to catecholamines. This phenomenon explains the escalating vasopressor requirements frequently observed in advanced septic and inflammatory shock (Joffe et al., 2021; B Levy et al., 2010; Bruno Levy et al., 2018).

Myocardial function is also affected. Cytokines such as TNF- α and IL-6 exert direct negative inotropic effects, impairing both systolic and diastolic performance. Although cardiac output may initially be preserved or even elevated in distributive shock, myocardial depression becomes increasingly relevant as inflammation persists. The combination of vasodilation and cardiac dysfunction further compromises effective tissue perfusion. Capillary leak syndrome amplifies these effects by reducing effective circulating volume. Despite aggressive fluid resuscitation, intravascular volume remains insufficient due to ongoing plasma extravasation. This contributes to the clinical paradox in which hypotension coexists with generalized edema and elevated filling pressures. From a practical standpoint, this explains why additional fluid administration often fails to restore hemodynamic stability and may instead worsen pulmonary and tissue edema. Importantly, shock in the context of cytokine storm is heterogeneous and dynamic. Patients may transition between distributive, cardiogenic, and hypovolemic components over time, reflecting the evolving interplay between inflammation, endothelial injury, and organ dysfunction (Nouveau et al., 2021; Papamichail et al., 2023).

Organ Crosstalk and Multiple Organ Dysfunction Syndrome

Multiple organ dysfunction syndrome (MODS) constitutes the cumulative clinical manifestation of persistent cytokine dysregulation, endothelial damage, and microcirculatory failure. Rather than resulting from isolated organ insults, MODS embodies a process of pathological organ interaction, wherein dysfunction in one system exacerbates injury in others through inflammatory, metabolic, and neurohumoral pathways. Cytokines are pivotal in facilitating this inter-organ communication. Once released into the systemic circulation, inflammatory mediators exert pleiotropic effects across distant tissues, altering cellular metabolism, mitochondrial function, and vascular regulation. Organs with high metabolic demand or dense microvascular networks -such as the lungs, kidneys, liver, and brain- are particularly susceptible to these effects. Notably,

organ injury itself generates additional DAMPs, reinforcing immune activation and perpetuating systemic inflammation (Gourd & Nikitas, 2019; Lehr, Kirkpatrick, & Bittinger, 2000; Moriyama & Nishida, 2021).

The lung frequently serves as an early target and amplifier of systemic inflammation. Increased pulmonary capillary permeability leads to interstitial and alveolar edema, impaired gas exchange, and the development of acute respiratory distress syndrome (ARDS). Hypoxemia and mechanical ventilation-induced lung stress further exacerbate systemic inflammation, contributing to cytokine release into the circulation. This lung–systemic inflammatory axis exemplifies how local organ injury can propagate global immune dysregulation (Belperio, Strieter, Keane, & Lynch, 2006; Rocha, Silva, Battaglini, & Rocco, 2024). Renal dysfunction similarly plays a dual role as both a consequence and driver of systemic illness. Acute kidney injury (AKI) impairs clearance of inflammatory mediators, alters acid–base and electrolyte balance, and disrupts fluid homeostasis. Uremic toxins and retained cytokines further depress immune and endothelial function, while renal ischemia promotes additional DAMP release. This kidney–immune interaction is particularly pertinent in critically ill patients requiring renal replacement therapy, where extracorporeal circuits themselves may modulate inflammatory responses (Fani et al., 2017; Kinsey & Okusa, 2012). The liver functions as a central immunometabolic hub and is profoundly affected during cytokine storm. Hepatic dysfunction impairs synthesis of acute-phase proteins, coagulation factors, and immunomodulatory molecules. Altered hepatic clearance of cytokines and endotoxins contributes to their accumulation in the circulation, while cholestasis and mitochondrial injury further amplify systemic inflammation. Hepatic failure thus weakens both immune regulation and metabolic resilience (Strnad, Trautwein, Koch, & Tacke, 2016).

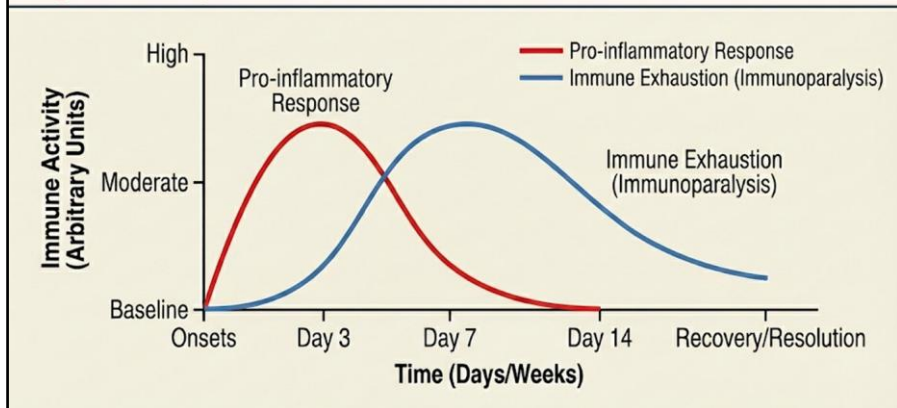
Neurological involvement, often manifesting as delirium or encephalopathy, reflects the vulnerability of the central nervous system to systemic inflammation. Cytokines disrupt the blood–brain barrier, alter neurotransmitter balance, and impair cerebral autoregulation. These effects not only contribute to acute neurological dysfunction but also influence long-term cognitive outcomes in ICU survivors. Crucially, MODS should not be perceived as a linear accumulation of independent organ failures. Instead, it represents a network phenomenon, in which dysfunction in one organ accelerates failure in others through shared inflammatory and metabolic pathways. This interconnectedness elucidates why isolated organ support strategies may be insufficient once MODS is established, and why early modulation of systemic inflammation has been proposed as a means to prevent irreversible organ injury (Colombari et al., 2025; Gourd & Nikitas, 2019; Kamphuis et al., 2015; Yang et al., 2022).

Temporal Phases: Hyperinflammation and Immunoparalysis

The immune response in critical illness is dynamic, evolving through distinct yet overlapping phases over time. Comprehending this temporal progression - from initial hyperinflammation to subsequent immunoparalysis- is crucial for interpreting clinical trajectories and for the rational timing of therapeutic interventions. A lack of appreciation for this dynamic nature has significantly contributed to the disappointing outcomes of many immunomodulatory strategies in critical care. The initial phase of critical illness is often dominated by hyperinflammation, characterized by robust activation of innate immune pathways and widespread cytokine release. During this phase, pro-inflammatory mediators drive vasodilation, endothelial activation, and early organ dysfunction. Clinically, patients present with shock, elevated inflammatory markers, and rapid progression of disease severity. This phase is often the most visible and has historically been the primary target of anti-inflammatory therapies (Mithal et al., 2021; Saavedra-Torres, Pinzón-Fernández, Ocampo-Posada, et al., 2025; Valsamaki, Vazgiourakis, Mantzaris, Manoulakas, & Makris, 2025).

However, hyperinflammation rarely exists in isolation or persists indefinitely. As critical illness progresses, many patients enter a phase of immune exhaustion or immunoparalysis, wherein adaptive immune function becomes profoundly impaired. This state is characterized by lymphocyte apoptosis, reduced antigen presentation, and diminished cytokine production capacity. Anti-inflammatory mediators such as IL-10 may predominate, and immune cells exhibit features of functional anergy rather than hyperactivity. Importantly, these phases are not strictly sequential. Hyperinflammatory and immunosuppressive features frequently coexist, both systemically and within different tissue compartments (Figure 5). A patient may display elevated circulating cytokine levels while simultaneously exhibiting profound lymphopenia and impaired pathogen clearance. This coexistence challenges simplistic therapeutic paradigms that assume a uniform immune state across time and space (Hasan et al., 2021; Ju et al., 2021; Kuypers, 2022).

Figure 5. Temporal Immune Phases in Critical Illness



From a clinical standpoint, immunoparalysis carries significant consequences. Patients become highly susceptible to secondary infections, viral reactivations, and poor wound healing. Seemingly minor infectious insults may precipitate severe deterioration, and response to antimicrobial therapy may be blunted. In this context, indiscriminate suppression or removal of inflammatory mediators may exacerbate immune dysfunction rather than restore balance. For intensive care specialists, the temporal phase of immune response has direct implications for patient management. Early in critical illness, interventions aimed at attenuating excessive inflammation may theoretically mitigate organ injury. Later, however, strategies that further dampen immune signaling risk deepening immunosuppression. This temporal mismatch between treatment and immune state is a key reason why timing has emerged as a critical determinant of therapeutic efficacy. Recognition of immune phase transitions remains challenging in daily practice. Traditional biomarkers provide limited insight into functional immune competence, and clinical signs often lag behind underlying immunological changes. Nonetheless, appreciating the dynamic nature of the host response underscores the need for phase-specific and patient-specific approaches, rather than uniform protocols applied indiscriminately (Nomellini, Caldwell, Kaplan, & Sims, 2018; Silva, Skon-Hegg, Badovinac, & Griffith, 2023).

Biomarkers of Cytokine Dysregulation in Critical Care

Inflammatory biomarkers are routinely employed in critical care settings to evaluate disease severity, inform therapeutic decisions, and monitor treatment responses. In the context of cytokine dysregulation, these biomarkers offer indirect insights into immune activity, although none fully encapsulate the complexity or temporal dynamics of the host response. Consequently, their interpretation necessitates careful clinical contextualization. Among circulating cytokines, IL-6 has emerged as the most frequently measured mediator in critically ill patients. IL-6 levels correlate with disease severity, the extent of

organ dysfunction, and mortality risk in sepsis and other inflammatory conditions. Its relatively longer half-life compared to other cytokines renders it detectable in systemic circulation, enhancing its clinical utility. However, elevated IL-6 indicates ongoing immune activation, not necessarily pathological hyperinflammation, and declining levels may occur with or without clinical improvement (J. Liu et al., 2021; Y. Liu & Chen, 2024; Sivakorn et al., 2021).

Acute-phase reactants, such as C-reactive protein (CRP) and ferritin, are widely available and commonly used as surrogate markers of inflammatory burden. CRP reflects the hepatic response to IL-6 signaling and provides a global measure of systemic inflammation, while ferritin may rise dramatically in hyperinflammatory syndromes associated with macrophage activation. Despite their accessibility, these markers lack specificity and may be influenced by non-inflammatory factors, including liver dysfunction and disturbances in iron metabolism. Procalcitonin (PCT) is often utilized to distinguish infectious from non-infectious inflammation and to guide antimicrobial stewardship. In cytokine-mediated critical illness, elevated PCT may reflect bacterial infection-driven immune activation, but it does not reliably quantify the degree of cytokine dysregulation. Moreover, PCT kinetics may be altered in patients receiving extracorporeal therapies or experiencing renal dysfunction, limiting its interpretive value (Gervaix et al., 2001; Kiya, Asefa, Abebe, & Mekonnen, 2024; Tjendra et al., 2020).

Beyond soluble markers, cellular biomarkers provide insight into immune competence. Lymphocyte counts, monocyte HLA-DR expression, and neutrophil phenotypes have been studied as indicators of immune suppression or exhaustion. Reduced monocyte HLA-DR expression, for example, has been associated with immunoparalysis and increased susceptibility to secondary infections. However, such measurements are not routinely available in many intensive care units and lack standardized thresholds for clinical decision-making (Conway Morris et al., 2018; Venet, Lepape, & Monneret, 2011). The commonly used biomarkers of inflammation and immune dysregulation, together with their principal clinical utilities and limitations, are summarized in Table 1 and should be interpreted in the context of overall clinical trajectory rather than as isolated indicators of cytokine storm. A fundamental limitation of biomarker-based assessment is compartmentalization. Circulating cytokine levels may not reflect tissue-level inflammation, where much of the pathological process occurs. Cytokines exert their effects locally, and systemic concentrations may underestimate or misrepresent inflammatory activity within organs. Furthermore, the short half-lives and pulsatile release of many cytokines complicate single-point measurements. Trends over time, integration with clinical trajectory, and correlation with organ function are more informative than isolated values (Molinaro et al., 2020; Neher, Stahel, Weckbach, & Huber-Lang, 2012; M. Zhou, Wang, & Aziz, 2021).

Table 1. Biomarkers in Cytokine Dysregulation: Clinical Use and Key Limitations

Biomarker	Clinical Utility	Major Limitations / Cautions
Interleukin-6 (IL-6)	Correlates with inflammatory burden and disease severity; useful for trend monitoring	Poor reflection of tissue-level inflammation; single measurements are unreliable; declines do not ensure clinical recovery
C-reactive protein (CRP)	Widely available marker of systemic inflammation; useful for longitudinal follow-up	Non-specific; slow kinetics; influenced by surgery, trauma, and liver dysfunction
Procalcitonin (PCT)	Supports identification and follow-up of bacterial infection; antimicrobial stewardship aid	Not a direct marker of cytokine dysregulation; altered by AKI and extracorporeal therapies
Ferritin	Suggests macrophage-driven hyperinflammatory states when markedly elevated	Very low specificity; influenced by liver disease, transfusions, and iron metabolism
Lactate	Strong prognostic indicator of circulatory and metabolic failure	Reflects downstream physiological derangement rather than immune activity
Absolute lymphocyte count	Readily available indicator of immune suppression or exhaustion	Non-specific; affected by stress response and corticosteroids
Monocyte HLA-DR expression	Marker of immune competence and immunoparalysis	Limited availability; lack of standardized thresholds
Endotoxin activity (EAA)	Indicates Gram-negative endotoxin-mediated immune activation	Not informative for sterile or DAMP-driven inflammation; limited access

Implications for Intensive Care Practice

For specialists in intensive care, the clinical significance of cytokine dysregulation is rooted not in its theoretical framework but in its practical implications for patient management, therapeutic prioritization, and prognostic reasoning. The sections demonstrate that a cytokine storm is neither a discrete diagnosis nor a uniform therapeutic target; rather, it is a dynamic manifestation of immune failure that intersects with hemodynamics, organ support, and perioperative care. Firstly, cytokine-mediated pathology should be perceived as a contextual modifier of critical illness, rather than its sole cause. In patients experiencing shock or progressive organ dysfunction, escalating inflammatory markers should prompt clinicians to reassess the adequacy of source control, perfusion, ventilation, and metabolic support before considering adjunctive immunomodulatory interventions. Cytokine dysregulation often reflects unresolved physiological stress rather than an independent process amenable to isolated treatment (Hiti et al., 2025; Jain, Singam, & Mudiganti, 2024; Reddy et al., 2024).

Secondly, the timing of intervention is crucial. Early phases of hyperinflammation may theoretically present a window during which modulation of circulating mediators could attenuate downstream organ injury. In contrast, later phases characterized by immune exhaustion and susceptibility to secondary infection necessitate caution, as further reduction of immune signaling may exacerbate immunosuppression. Intensivists must therefore integrate temporal disease trajectory into decision-making rather than relying solely on static biomarker thresholds (Brands et al., 2020; Z. Liu et al., 2024; Pfortmueller, Fux, Schefold, & Meisel, 2017). In summary, cytokine storm represents a pathophysiological lens through which severe illness can be understood, but not a standalone target that supersedes established principles of critical care. Its greatest value lies in sharpening clinical insight into the interconnected nature of inflammation, shock, and organ dysfunction, an insight that must guide, rather than dictate, therapeutic decisions (Fenner et al., 2021; Gao, Xu, Liu, & Wang, 2020).



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CHAPTER 49

Liquid Biopsy and Minimal Residual Disease (MRD)

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Liquid biopsy has become a powerful advancement in modern oncology, offering a minimally invasive means to detect and track minimal residual disease (MRD). By analyzing circulating tumor DNA (ctDNA) present in biological fluids—most commonly blood—it enables real-time evaluation of tumor burden, identification of residual malignant cells after therapy, and estimation of recurrence risk (Pandey & Yadav, 2025).

Liquid biopsy involves the detection and analysis of tumor-derived biomarkers within body fluids—including blood, urine, and saliva—to obtain clinically relevant insights into tumor presence and behavior without performing conventional tissue biopsies. In this framework, circulating tumor DNA (ctDNA) is the most widely utilized marker, consisting of fragmented DNA shed by malignant cells into the circulation (Siravegna, Marsoni, Siena, & Bardelli, 2017). The minimally invasive nature of liquid biopsy enables serial sampling, making it highly suitable for tracking disease dynamics, therapeutic response, and progression over time (Ignatiadis, Sledge, & Jeffrey, 2021).

ctDNA has emerged as a valuable tool for identifying MRD—the small population of residual cancer cells that may persist following therapy and potentially trigger recurrence. Its utility as an MRD biomarker lies in its ability to capture tumor-specific genomic alterations in real time, providing a molecular snapshot of disease status (Garcia-Murillas et al., 2015). In support of this, Tie et al. reported that ctDNA testing successfully identified residual disease in individuals with early-stage colorectal cancer, achieving a detection sensitivity of 48% (Tie et al., 2016). Such evidence highlights the clinical potential of ctDNA for refining relapse risk assessment and guiding personalized therapeutic decisions.

Furthermore, ctDNA profiling has enabled the detection of resistance-associated mutations, offering valuable information about tumor heterogeneity and the evolutionary changes that occur under therapeutic pressure (Zhu et al., 2023). In this context, Newman and colleagues developed the Cancer Personalized Profiling by deep Sequencing (CAPP-Seq) method, a high-sensitivity sequencing approach that markedly improves ctDNA detection breadth and accuracy, thereby enhancing MRD identification across multiple malignancies (Newman et al., 2014).

The incorporation of liquid biopsy into routine clinical workflows has opened the door to numerous clinical applications. In early-stage breast cancer, for example, postoperative detection of ctDNA has been shown to be a strong indicator of future metastatic recurrence (Garcia-Murillas et al., 2015). Likewise, in colorectal cancer, liquid biopsy enables longitudinal molecular monitoring throughout and after therapy, providing valuable guidance for selecting subsequent treatment strategies (Liatou et al., 2025). In addition, emerging evidence suggests that liquid biopsy is effective in detecting MRD across a

variety of tumor types beyond colorectal and breast cancer, emphasizing its growing relevance in oncology (Malik & Zaheer, 2025). By leveraging ctDNA assessment, clinicians can not only monitor minimal residual disease but also detect early signs of therapeutic resistance, ultimately supporting more individualized and adaptive treatment planning (Yang et al., 2023).

1. Integration into Clinical Practice

The adoption of circulating tumor DNA (ctDNA) testing in clinical oncology has become a major milestone, particularly for the detection and surveillance of minimal residual disease (MRD). ctDNA is now being utilized across multiple clinical areas, including the development of standardized analytical approaches, early cancer detection, informed therapy selection, assessment of treatment response, and monitoring for recurrence.

1.1. Standardization of ctDNA Analysis: Ensuring standardized ctDNA testing is a critical requirement for its successful adoption in everyday clinical workflows. At present, numerous approaches are used for ctDNA extraction and analysis, which contributes to inconsistencies in outcomes across laboratories and published studies. To enable ctDNA to function as a routine clinical biomarker, harmonization of analytical platforms, handling procedures, and processing protocols is necessary (Garcia-Murillas et al., 2015). Regulatory interest is also increasing—Breakthrough Device designation by the FDA for emerging MRD detection assays reflects recognition of their clinical promise (Parikh et al., 2021). In addition, establishing standardized sampling time points and conducting large-scale validation studies will be essential to confirm clinical utility and ensure reproducible, trustworthy results across settings (Telekes & Horváth, 2022).

1.2. Application in Early Diagnosis and Screening; ctDNA-based testing is increasingly recognized as a valuable approach for early cancer detection and screening, particularly in colorectal cancer (CRC). Evidence indicates that ctDNA can identify malignant changes earlier than conventional imaging modalities and is capable of detecting disease even at early stages—an advantage that may significantly improve prognosis (Dasari et al., 2020). Furthermore, assays designed for minimal residual disease (MRD) assessment have successfully detected early recurrence risk following surgical tumor removal, allowing for timely adjustment of treatment strategies (Siravegna et al., 2019). As analytical platforms continue to evolve, the feasibility of integrating ctDNA into standard diagnostic and screening workflows is steadily increasing.

1.3. Treatment Selection and Therapy Guidance; ctDNA analysis also contributes significantly to therapeutic decision-making and personalized treatment planning. By revealing the genomic landscape of a tumor, ctDNA enables clinicians to match patients with targeted treatments that are most likely to be effective for their specific molecular alterations (Nagayama, Low, Kiyotani, & Nakamura, 2021). In lung cancer, for example, ctDNA profiling has become

an important tool for identifying actionable mutations and determining suitability for targeted agents, thereby shaping precision-based treatment pathways (Zameer, Jou, & Middleton, 2024). Additionally, clinical data highlight its utility in guiding adjuvant therapy, particularly when post-surgical ctDNA positivity indicates persistent residual disease and the need to intensify treatment to reduce recurrence risk (Parikh et al., 2021).

1.4. Monitoring Treatment Response; The use of ctDNA for tracking treatment response has become an important aspect of clinical management. Research demonstrates that variations in ctDNA concentration over time reflect how well a patient is responding to therapy, enabling real-time evaluation of treatment effectiveness (Chakrabarti, Xie, Urrutia, & Mahipal, 2020). This approach is particularly valuable when monitoring the impact of emerging therapeutic agents, as treatment adjustments can be guided by observed ctDNA trends. As a surrogate biomarker, ctDNA offers predictive insight into therapeutic outcomes and supports timely modification of treatment strategies when necessary (Desai et al., 2024).

1.5. Recurrence Surveillance and Early Relapse Detection; One of the most notable advantages of ctDNA analysis is its ability to signal relapse at an early stage. The presence of ctDNA after therapy is strongly associated with increased recurrence risk in multiple cancer types (L. Li & Sun, 2024). This makes ctDNA a clinically valuable tool, as early molecular detection of recurrence enables timely therapeutic intervention and may ultimately enhance patient survival. Clinical applications in breast and colorectal cancer have demonstrated its effectiveness in recurrence surveillance, supporting more informed decision-making regarding subsequent treatment planning (Vacante, Ciuni, Basile, & Biondi, 2020) .

1.6. MRD Evaluation in Routine Oncology; Minimal residual disease (MRD) assessment using ctDNA has introduced a significant advancement in modern cancer management. Detecting traces of tumor DNA after treatment offers valuable prognostic insight, helping clinicians estimate the likelihood of recurrence and tailor ongoing therapeutic strategies accordingly (Liatsou et al., 2025). As clinical evidence increasingly highlights the strong predictive capacity of ctDNA in the MRD setting, its incorporation into routine oncology practice is gaining momentum (W. Li et al., 2019). Even so, further studies and protocol refinements are still needed to strengthen validation and streamline its application in day-to-day patient care (Hassan, 2025).

Overall, ctDNA holds considerable promise across the continuum of oncology care—from early detection and therapy selection to real-time treatment monitoring and MRD evaluation. Realizing its full clinical impact, however, will depend on continued efforts to standardize laboratory procedures, establish robust

evidence frameworks, and enhance clinician awareness regarding its effective implementation.

2. Multi-Analyte Approaches

Recent advancements in liquid biopsy have significantly improved cancer detection and monitoring strategies. Multi-analyte approaches, which enable the simultaneous analysis of multiple biomarkers, are gaining increasing attention for their ability to deliver a more holistic understanding of tumor behavior (Velpula & Buddolla, 2025). Current applications incorporate circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), extracellular vesicles (EVs), along with complementary biomarkers such as microRNAs and proteins. These combined strategies offer greater diagnostic depth than single-marker assays, though they also introduce analytical and technical challenges. Multi-omics innovations continue to evolve, presenting new opportunities to refine liquid biopsy platforms and enhance their clinical utility.

2.1. Combined Assessment of ctDNA, CTCs, and Exosomes; The combined exploration of ctDNA, circulating tumor cells (CTCs), and exosomes forms a robust foundation for improving diagnostic precision and guiding patient management in cancer care. ctDNA is composed of tumor-derived DNA fragments that circulate in the bloodstream and reflect the genetic characteristics of the malignancy, whereas CTCs represent viable tumor cells shed from the primary mass into circulation (Nikanjam, Kato, & Kurzrock, 2022).

Exosomes—small extracellular vesicles secreted by both healthy and malignant cells—contain diverse molecular cargo including proteins, lipids, mRNAs, and microRNAs. These vesicles facilitate intercellular signaling and are strongly implicated in cancer progression and metastasis (Maia, Caja, Strano Moraes, Couto, & Costa-Silva, 2018). When analyzed together, ctDNA, CTCs, and exosomes offer valuable insight into tumor heterogeneity, support real-time evaluation of treatment outcomes, and contribute to the detection of minimal residual disease (Kosmachevskaya, Novikova, & Topunov, 2025).

The integration of these biomarkers can leverage the unique advantages of each: ctDNA provides genomic mutation data, while RNA and protein content from CTCs and exosomes reveals information about tumor behavior, cellular interactions, and microenvironmental status (Dubrovsky, Ross, Jalali, & Lotze, 2024). By adopting a multi-analyte perspective rather than relying on a single biomarker, the complexity of tumor biology can be captured more comprehensively, helping to overcome limitations associated with one-dimensional analyses.

2.2. Integration of Additional Biomarkers (miRNA, Proteins, EVs); Expanding beyond ctDNA, CTCs, and exosomes, the incorporation of additional molecular indicators such as microRNAs (miRNAs) and proteins further

broadens the analytical capability of liquid biopsy platforms. miRNAs, which are short non-coding RNA molecules involved in post-transcriptional gene regulation, can function either as oncogenes or tumor suppressors depending on their expression patterns (Cha et al., 2015). The identification of cancer-specific miRNA profiles has created new opportunities for their application in both diagnostic and prognostic settings.

Protein-based markers, whether isolated from exosomes or detected within CTCs, provide complementary information about tumor biology, including aggressiveness and therapeutic response mechanisms (Palieri et al., 2025). In colorectal cancer, for instance, exosomal analyses have revealed distinct miRNA signatures with diagnostic potential, reinforcing the significance of these molecules in tumor characterization and clinical decision-making (Galbiati et al., 2021).

2.3. Multi-Omics Liquid Biopsy Strategies; The emergence of multi-omics methodologies has become instrumental in elevating liquid biopsy research and application. By combining genomic, transcriptomic, proteomic, and metabolomic layers of analysis, multi-omics platforms enable a far more comprehensive understanding of tumor biology (Koo et al., 2025). This integrated perspective supports the characterization of complex cancer phenotypes and aids in the discovery of clinically relevant biomarkers across several biological levels.

Despite their advantages, merging such diverse data streams introduces considerable analytical challenges. High data volume, biological variability, and inconsistencies in processing techniques can complicate interpretation and standardization (Koo et al., 2025). To address this, machine learning and computational modeling are increasingly being employed to unify and interpret multidimensional datasets, improving the efficiency and accuracy of biomarker identification (Xiao, Bi, Guo, & Li, 2022). Ultimately, the advancement of precision oncology is expected to depend heavily on the successful adoption of multi-omics systems, paving the way for highly individualized therapeutic strategies.

2.4. Analytical Sensitivity and Detection Challenges; Although multi-analyte approaches offer substantial potential, a number of analytical barriers continue to limit their broader implementation. Detecting biomarkers present at low concentrations—including ctDNA and specific exosomal components—requires highly sensitive technologies, making advancements in detection modalities essential (W. Li et al., 2019). Traditional isolation and enrichment techniques often fail to capture the full diversity of tumor-derived material circulating in biofluids, thereby reducing the depth of information obtained (Palieri et al., 2025).

Emerging microfluidic and nanotechnology-based solutions may help overcome these limitations by improving both sensitivity and specificity. For

example, 3D-nanopatterned microfluidic platforms have demonstrated enhanced performance in detecting circulating exosomes (Zhang et al., 2019). Nonetheless, standardization remains a major challenge, as inconsistencies in isolation procedures and analytical workflows continue to generate variable results across different laboratories (Mitchell, Ma, Carter, & Loudig, 2022).

2.5. Advantages of Multi-Analyte Versus Single Biomarker Testing; Multi-analyte testing offers several advantages compared with single biomarker-based assays. By assessing multiple biomarkers simultaneously, these approaches can improve diagnostic precision, inform treatment selection, and enable closer surveillance of disease evolution in real time (Hofmann et al., 2020). Single-marker methods often fail to represent the full scope of tumor heterogeneity and may overlook clinically relevant alterations, which can result in less effective therapeutic decision-making (Palieri et al., 2025). In contrast, integrating diverse biomarker types provides a more detailed and multidimensional view of tumor biology—capturing aspects such as angiogenic activity, metastatic potential, and drug resistance mechanisms (Marrugo-Ramírez, Mir, & Samitier, 2018). Such an approach supports the principles of precision oncology by enabling more personalized and data-rich treatment strategies based on comprehensive molecular profiles.

Multi-analyte liquid biopsy platforms represent an expanding and highly promising direction in modern cancer diagnostics and therapeutic decision-making. The integration of ctDNA, CTCs, exosomes, and complementary biomarkers—including miRNAs and proteins—enables a more comprehensive molecular overview of tumor behavior than single-analyte methods alone (Velpula & Buddolla, 2025). Although issues such as sensitivity in detecting low-abundance markers and protocol standardization continue to pose challenges, rapid advancements in sequencing technologies, nanotechnology, and multi-omics integration are steadily addressing these limitations and moving the field closer to clinical routine. With continued progress, multi-marker approaches are anticipated to significantly improve diagnostic accuracy, guide individualized treatment strategies, and ultimately enhance patient outcomes in the era of precision oncology (Jiang, Zhang, Gao, & Sun, 2025).

3. MRD-Based Treatment Modification

Minimal Residual Disease (MRD) describes the presence of a small population of cancer cells that persist in the body following treatment, remaining below the detection threshold of standard clinical and imaging techniques. MRD evaluation has become increasingly important in the management of many cancers, particularly in hematological disorders such as leukemia and multiple myeloma, where treatment decisions increasingly rely on molecular evidence rather than solely on clinical remission (Song et al., 2025). Current discussions surrounding MRD focus on therapy escalation for MRD-positive patients, de-

escalation approaches when MRD is absent, clinical data supporting MRD-guided therapeutic strategies, and the prognostic and predictive significance of MRD. Additionally, emerging insights into MRD-driven personalized treatment highlight its growing role in future precision oncology (Teixeira et al., 2023).

3.1. Therapy Intensification Based on MRD Positivity; In cases where MRD remains detectable after therapy, escalating treatment intensity has been associated with markedly improved clinical outcomes. Evidence from several clinical trials indicates that MRD-positive patients generally experience a higher likelihood of relapse and reduced overall survival compared to their MRD-negative counterparts (Munshi et al., 2020). In multiple myeloma, for instance, studies have shown that individuals with persistent MRD respond favorably to more aggressive therapeutic approaches—such as incorporating novel drug combinations or increasing treatment dosage—to eliminate residual malignant cells and prevent disease recurrence (Paiva et al., 2020). This strategy is based on the premise that intensifying therapy can target the residual tumor burden that standard treatment alone may not fully eradicate.

Furthermore, accumulated data suggest that the degree of MRD clearance is closely linked to long-term clinical outcomes, reinforcing the rationale for adopting treatment escalation as a means to improve survival prospects in MRD-positive patients (Lahuerta et al., 2017). Ongoing MRD surveillance empowers clinicians to adjust therapy promptly when residual disease re-emerges, ensuring that treatment intensity is tailored to each patient's evolving disease status (Lahuerta et al., 2017).

3.2. De-escalation Strategies in MRD-Negative Cases; Conversely, individuals who achieve MRD negativity may be suitable candidates for treatment de-escalation, allowing for a reduction in therapy intensity while maintaining disease control. This approach offers the potential to minimize treatment-related toxicity and improve quality of life, particularly beneficial for elderly patients or those vulnerable to adverse effects, aligning with the growing clinical adoption of the “less is more” philosophy (Ikeda et al., 2025). Evidence from clinical studies in chronic lymphocytic leukemia (CLL) indicates that MRD-negative patients can safely decrease treatment intensity without compromising remission durability (Fürstenau, De Silva, Eichhorst, & Hallek, 2019).

More recently, investigations into early-stage breast cancer and head and neck malignancies have explored the feasibility of scaled-down treatment regimens for MRD-negative individuals, transitioning them to less intensive therapeutic strategies (Rosenberg & Vokes, 2021). In such settings, rigorous MRD monitoring is essential, ensuring rapid intervention should molecular signs of recurrence emerge while simultaneously avoiding unnecessary exposure to aggressive therapy.

3.3.Clinical Trial Evidence for MRD-Guided Therapy Decisions; A growing number of clinical trials are currently evaluating how MRD measurement can guide treatment planning in real-world practice. One notable example is the DYNAMIC II study, which investigated whether circulating tumor DNA (ctDNA) could be used to tailor adjuvant chemotherapy in stage II colorectal cancer. Early findings suggest that MRD-negative patients may safely undergo treatment de-escalation, reinforcing the value of MRD as a clinical marker for therapy adjustment (Slater et al., 2023). Comparable progress has been observed in multiple myeloma (MM), where MRD monitoring throughout different treatment stages has demonstrated strong prognostic relevance. Trials incorporating MRD results into treatment modification decisions have reported encouraging improvements in disease-free survival (Burgos et al., 2020). These outcomes illustrate the growing movement toward precision-based care, in which therapeutic strategies are personalized according to the molecular burden of residual disease rather than uniform treatment protocols.

3.4.MRD as a Prognostic and Predictive Marker; MRD evaluation has emerged as a strong indicator of both prognosis and treatment response in numerous cancer types. Detectable MRD is frequently associated with a higher likelihood of relapse, whereas clearance of MRD is typically linked to markedly improved survival outcomes over time (Munshi et al., 2020). Meta-analyses and pooled evidence from multiple clinical studies show that MRD negativity correlates with superior progression-free survival (PFS) across different disease stages, including both newly diagnosed and relapsed multiple myeloma cohorts (Paiva et al., 2020). Beyond its prognostic relevance, MRD also serves as a predictive tool that can guide therapeutic decisions aimed at preventing recurrence. Clinicians can use MRD status to assess residual tumor burden more precisely, allowing for tailored treatment strategies and more refined patient risk stratification (Lahuerta et al., 2017).

3.5. Future Perspectives in MRD-Driven Personalized Treatment; Future directions strongly suggest an expanding role for MRD in therapeutic decision-making. Ongoing research emphasizes the need for increasingly sensitive and standardized detection platforms, including next-generation sequencing-based assays and advanced flow cytometry, aiming to deliver more accurate and timely evaluations of residual disease to better guide individualized care (Herrera & Armand, 2017).

There is also growing interest in leveraging MRD as a tool to inform innovative therapeutic strategies, particularly in the realm of immunotherapies and targeted treatments. As insights into tumor biology deepen, integrating MRD monitoring with emerging modalities is expected to further optimize treatment planning and improve clinical precision (Tiong & Loo, 2023). In parallel, adaptive clinical trial designs incorporating MRD feedback are gaining

momentum, enabling real-time therapeutic adjustments and more favorable long-term outcomes (Abidoye et al., 2025).

Overall, transitioning MRD assessment from primarily research-based practice to routine clinical implementation promises to advance personalized oncology, improving survival while minimizing treatment burden through refined, patient-specific therapeutic approaches.

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CHAPTER 50

Evolution of Cancer Immunotherapy and Its Current Clinical Applications

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1. Introduction

In recent decades, cancer immunotherapy has undergone an extraordinary transformation, reshaping the traditional approach to cancer management. While conventional treatments—surgery, chemotherapy, and radiotherapy—have long served as the cornerstone of oncology, advancements in tumor immunology have unveiled the pivotal role of the immune system in tumor development and eradication. This scientific progress has positioned immunotherapy at the forefront of modern cancer therapeutics, offering novel and powerful strategies to combat malignancies. Among these, immune checkpoint inhibitors (ICIs) and adoptive cell-based approaches, particularly CAR-T cell therapy, have emerged as groundbreaking modalities capable of generating durable clinical responses in selected patient populations.

1.1. Evolution of cancer immunotherapy and current clinical applications

Over the past twenty years, cancer immunotherapy has progressed at an exceptional pace, giving rise to cutting-edge therapeutic strategies that markedly improve clinical outcomes. This rapid advancement has been fueled by deeper insights into the tumor microenvironment, immune checkpoint regulation, and the genetic manipulation of immune effector cells.

Tumor Microenvironment and Immune Checkpoints; The tumor microenvironment (TME) has emerged as a central determinant of tumor progression and therapeutic responsiveness, drawing increasing attention in recent immuno-oncology research. Composed of diverse immune and stromal cell populations, extracellular matrix structures, and a range of cytokines and chemokines, the TME dynamically shapes cancer evolution and influences treatment outcomes. Consequently, targeting TME-associated immune regulatory pathways—particularly immune checkpoints—has become a fundamental approach in modern cancer therapy. Immune checkpoint inhibitors (ICIs) directed against PD-1 and CTLA-4 have demonstrated significant clinical benefit, with agents such as pembrolizumab and nivolumab approved for multiple cancers, including advanced hepatocellular carcinoma and triple-negative breast cancer (Bejarano, Jordão, & Joyce, 2021; Donne & Lujambio, 2023). In TNBC specifically, the FDA approval of atezolizumab combined with nab-paclitaxel for PD-L1-positive cases exemplified the therapeutic potential of checkpoint blockade in historically refractory tumors (Geurts & Kok, 2023; Luo et al., 2022). Current research efforts increasingly focus on strategies to enhance ICI response, particularly through combination regimens incorporating additional checkpoint inhibitors, cytotoxic therapies, or emerging immunomodulatory agents aimed at converting immunologically “cold” tumors into treatment-responsive “hot” phenotypes (Mishra, Ali, Dutta, Banday, & Malonia, 2022).

Cell and Gene-Based Therapies; Cell- and gene-based therapeutic strategies continue to advance rapidly, establishing themselves as a vital component of next-generation cancer treatment. Chimeric antigen receptor T-cell (CAR-T) therapy has particularly transformed outcomes in hematologic cancers, offering durable responses where conventional treatments often fail. Recent studies report encouraging clinical efficacy of CAR-T constructs targeting diverse tumor-associated antigens, especially in multiple myeloma and other B-cell malignancies (Pierro et al., 2022; Ren et al., 2022). Concurrently, significant efforts are directed toward improving treatment safety, with dual-target CAR designs and innovative engineering approaches aimed at reducing severe toxicities such as cytokine release syndrome and immune-mediated neurotoxicity (Garfall et al., 2015; Ren et al., 2022). In addition, tumor-infiltrating lymphocyte (TIL) therapy is gaining recognition as a promising option for solid tumors. Evidence from recent clinical trials demonstrates that TIL-based interventions can induce potent antitumor immune responses, supporting their incorporation into routine therapeutic protocols in selected malignancies (Kumar, Watkins, & Vilgelm, 2021; S. Wang et al., 2021).

Bispecific Antibodies and Novel Therapeutics; Bispecific antibodies (BsAbs) have emerged as a significant addition to the expanding landscape of cancer immunotherapy. Designed to simultaneously recognize two distinct antigens, these engineered molecules enhance immune cell–tumor cell interaction and promote more efficient antitumor activity. Recent evidence underscores their therapeutic promise in multiple myeloma and various other malignancies, particularly due to their ability to redirect T-cell cytotoxicity toward tumor targets (Zhivodernikov, Markina, Kirichenko, Popov, & Markin, 2023).

1.2.Success stories and remaining therapeutic barriers

Although major progress has been achieved, cancer immunotherapy still faces considerable challenges. The remarkable success observed in hematologic malignancies has not been mirrored to the same extent in solid tumors. This limitation is largely attributed to the highly immunosuppressive nature of the tumor microenvironment (TME) and the structural barriers that restrict immune cell trafficking and infiltration (Adachi & Tamada, 2022). Tumors with minimal T-cell infiltration—commonly referred to as “cold tumors”—are particularly difficult to treat, as they tend to respond poorly to immune checkpoint inhibitors, resulting in suboptimal clinical outcomes (Ouladan & Orouji, 2025).

Addressing these obstacles requires a deeper understanding of the biological processes that maintain tumors in a cold, immune-excluded state, along with the development of strategies that convert them into highly inflamed, immune-responsive “hot” tumors. Promising approaches include combination therapies pairing ICIs with complementary modalities such as radiotherapy, which can enhance antigen visibility and increase T-cell infiltration into the tumor

microenvironment (Corrò et al., 2022). Furthermore, advanced biomaterial platforms and innovative drug-delivery systems are being actively investigated to enhance the potency and delivery efficiency of immunotherapeutic agents (Bonaventura et al., 2019; Riley, June, Langer, & Mitchell, 2019).

1.3.Importance of transforming “cold” tumors into “hot” immunogenic tumors

Converting immunologically "cold" tumors into "hot" tumors is a pivotal goal in advancing the efficacy of cancer immunotherapy. Cold tumors are typically deficient in immune-stimulating signals, which limits the initiation of an effective antitumor immune response (Ouladan & Orouji, 2025). This immune resistance can arise from multiple factors, including an abundance of immunosuppressive cell populations within the tumor microenvironment and impaired mechanisms of antigen processing and presentation.

Recent research efforts are increasingly directed toward identifying strategies capable of reprogramming these immune-desert tumor types. Approaches under investigation include the application of oncolytic viruses, therapeutic cancer vaccines, and rational combinations of immunotherapies with chemotherapeutic or molecularly targeted agents (Kim, Kim, & Kim, 2021). By enhancing antigen visibility and promoting immune system activation, these interventions may trigger stronger antitumor responses and potentially improve clinical outcomes, even in cancers historically resistant to conventional therapy (Z. Liu et al., 2021).

Additionally, emerging evidence highlights the influence of the gut microbiome on the efficacy of immune-based treatments, introducing a further dimension to cold-to-hot tumor conversion strategies. Modulating gut microbial diversity and composition may enhance systemic immune tone and create a more favorable environment for immunotherapeutic effectiveness (Z. Liu et al., 2021).

2. Strategies to Convert “Cold” Tumors into “Hot” Tumors

Converting immunologically "cold" tumors—defined by minimal immune cell infiltration—into highly responsive "hot" tumors continues to be a major hurdle in cancer treatment. In response, the past several years have seen the emergence of multiple therapeutic approaches aimed at increasing their immunogenic potential and improving immune-mediated tumor eradication.

2.1. Inducing Immunogenic Cell Death

2.1.1. Radiotherapy + immunotherapy synergy; Radiotherapy has traditionally been valued for its capacity to directly induce cytotoxicity in tumor cells; however, increasing awareness of its immune-modulating properties has led to a paradigm shift in its therapeutic application, particularly as a potentiator of immunotherapy. Radiation promotes immunogenic cell death (ICD), triggering the release of damage-associated molecular patterns (DAMPs)—

including calreticulin (CRT), high mobility group box 1 (HMGB1), and ATP—that facilitate antigen-presenting cell (APC) activation and drive subsequent T cell-mediated antitumor immunity (Krysko et al., 2012; Zhu et al., 2021). These molecules facilitate the activation of antigen-presenting cells (APCs) and promote subsequent anti-tumor T-cell responses.

Duan et al. reported that radiotherapy can function as an in situ tumor vaccine by increasing antigen availability and heightening inflammatory signaling during radiation-induced cell death (Demaria, Golden, & Formenti, 2015). This process enhances dendritic cell stimulation and supports efficient cross-presentation of tumor antigens to CD8⁺ cytotoxic T cells, ultimately generating stronger systemic immune responses (Demaria et al., 2021). Furthermore, advanced radiotherapy modalities such as hypofractionated regimens have demonstrated the ability to augment immune activation, further strengthening radiation's role as a synergistic component of immunotherapy (Demaria et al., 2021).

The integration of radiotherapy with immune checkpoint inhibitors has also gained significant momentum. Accumulating evidence indicates that combining radiation with checkpoint blockade can substantially boost antitumor efficacy by harnessing complementary immune mechanisms (Kong et al., 2021; Ollivier, Labbé, Fradin, Potiron, & Supiot, 2021). This combined strategy frequently results in increased T cell infiltration within the tumor microenvironment (TME), amplifying immune-mediated tumor destruction (Yan et al., 2022).

2.1.2. Chemotherapy-induced immune activation: Chemotherapy similarly contributes to immune activation by inducing immunogenic cell death (ICD). The cytotoxic action of many chemotherapeutic agents triggers the release of DAMPs in a manner comparable to radiotherapy, thereby enhancing antitumor immune responses. Evidence suggests that chemotherapy can generate and expose tumor-associated antigens during cell death, which subsequently engage the adaptive immune system and promote targeted immune recognition (Ruan, Leibowitz, Zhang, & Yu, 2020).

It is equally important to address the immunosuppressive characteristics of the tumor microenvironment commonly observed in solid malignancies. Optimal therapeutic benefit requires the strategic use of both chemotherapy and radiotherapy to overcome tumor-driven immune suppression. Immunotherapeutic modalities—including monoclonal antibodies and oncolytic viral treatments—have demonstrated strong synergistic potential when combined with conventional therapies, effectively reversing immunosuppression and improving tumor antigen visibility (Fang et al., 2025; Z. Li et al., 2022).

2.2. Next-Generation Immune Checkpoint Inhibitor Combinations

The advent of immune checkpoint inhibitors (ICIs) has markedly transformed cancer treatment, particularly through the clinical application of monoclonal

antibodies targeting PD-1/PD-L1 and CTLA-4 pathways. These therapies enhance antitumor immunity by preventing inhibitory signaling that suppresses T-cell activation. Nonetheless, a substantial proportion of patients experience inadequate or transient responses, highlighting the need for innovative combination strategies to improve therapeutic efficacy. The synergistic use of PD-1/PD-L1 and CTLA-4 inhibitors has shown considerable promise, and growing attention is now directed toward additional checkpoint pathways such as LAG-3, TIM-3, and TIGIT, which are emerging as important targets for next-generation immunotherapy approaches.

2.2.1. PD-1/PD-L1 + CTLA-4 combination therapies; Combining PD-1/PD-L1 inhibitors with CTLA-4 blockade has emerged as a powerful strategy to amplify antitumor immunity through complementary mechanisms. The therapeutic benefit of dual inhibition arises from their distinct sites and modes of action: CTLA-4 primarily regulates early T-cell activation within secondary lymphoid tissues, whereas PD-1 suppresses T-cell function in the tumor microenvironment, promoting exhaustion and limited effector activity (Qin et al., 2019). Clinical evidence shows that pairing anti-PD-1 therapeutics such as nivolumab or pembrolizumab with the anti-CTLA-4 antibody ipilimumab improves response rates and overall survival across several malignancies, particularly melanoma and non-small cell lung cancer (NSCLC) (Formenti et al., 2018; Kong et al., 2021). This dual blockade is especially effective in tumors with immunogenic characteristics, where CTLA-4 inhibition enhances T-cell priming and proliferation, and PD-1/PD-L1 inhibition restores the cytotoxic capacity of T cells within the tumor milieu. Studies indicate that this synergistic approach results in greater infiltration of functional effector T cells into the tumor, thereby increasing the probability of tumor regression (Patel & Minn, 2018).

Nevertheless, the combination is often associated with a higher risk of immune-related toxicities compared to monotherapies (Formenti et al., 2018). Therefore, optimizing clinical benefit requires careful management to balance robust antitumor activity with the potential for adverse immune reactions.

2.2.2. Emerging targets: LAG-3, TIM-3, TIGIT, and others; Beyond PD-1 and CTLA-4, several additional immune checkpoints have gained considerable attention as therapeutic targets in modern cancer immunotherapy. Notable examples include LAG-3 (Lymphocyte Activation Gene 3), TIM-3 (T-cell Immunoglobulin and Mucin Domain 3), and TIGIT (T-cell immunoglobulin and ITIM domain), all of which transmit inhibitory signals that suppress T-cell responses and contribute to immune evasion by tumors (Anderson, Joller, & Kuchroo, 2016).

LAG-3; LAG-3 operates through mechanisms parallel to PD-1 and CTLA-4, exerting a key regulatory effect on T-cell activation and effector function.

Evidence from clinical research indicates that blockade of LAG-3 enhances antitumor immunity, particularly when administered in combination with PD-1 inhibitors (Andrews, Marciscano, Drake, & Vignali, 2017). Its elevated expression on exhausted T cells within the tumor microenvironment suggests that targeting LAG-3 may restore the function of T cells rendered inactive by chronic tumor stimulation (Kong et al., 2016).

TIM-3; TIM-3 is likewise associated with T-cell exhaustion and immune suppression in the tumor microenvironment. Because of its involvement in inhibitory signaling pathways, TIM-3 has emerged as an appealing therapeutic target aimed at reversing T-cell dysfunction (Xie et al., 2023). Combination approaches involving TIM-3 blockade alongside PD-1/PD-L1 inhibition have shown promise, outperforming monotherapies in restoring T-cell activity (X. Li et al., 2019; Rodler et al., 2025).

TIGIT; TIGIT functions as another inhibitory receptor that restrains T-cell activation and is frequently co-expressed with PD-1 on tumor-infiltrating lymphocytes (TILs) across multiple tumor types. Recent studies reveal that simultaneous inhibition of TIGIT and PD-1 enhances lymphocyte proliferation and cytokine production (Josefsson et al., 2019). Targeting both checkpoints may offer a means to overcome immune resistance mechanisms limiting the effectiveness of current therapies (F. Li, Chen, Pang, Yang, & Jing, 2021).

As knowledge of immune regulatory pathways expands, it becomes increasingly clear that integrating novel checkpoint inhibitors with existing immunotherapeutic regimens may significantly strengthen clinical responses. Continued clinical investigation is crucial to determine optimal combinations, evaluate long-term treatment benefits, and carefully monitor the risk of immune-related toxicities associated with multi-target inhibition (Z. Li et al., 2024; Rodler et al., 2025).

2.3. Cytokine-Based Therapeutic Approaches

Cytokine-based immunotherapies have become pivotal components of modern cancer treatment, utilizing immune signaling molecules to strengthen antitumor activity. Key cytokines—including interleukin-2 (IL-2), interleukin-7 (IL-7), and interleukin-15 (IL-15)—have been molecularly engineered to enhance clinical efficacy while limiting treatment-related toxicity. Recent advances focus on modified cytokine variants and innovative engineering approaches designed to improve therapeutic performance and reduce adverse effects in patients receiving these agents.

2.3.1. IL-2, IL-7, IL-15 engineered variants; IL-2 has long served as a foundational molecule in cancer immunotherapy for its capacity to drive T-cell expansion and boost antitumor immune responses. Nevertheless, systemic IL-2 administration is frequently accompanied by substantial toxicity, including

capillary leak syndrome and cardiovascular adverse effects (Morris, Neelapu, Giavridis, & Sadelain, 2022). To overcome these challenges, next-generation IL-2 formulations—such as pegylated IL-2 and IL-2–Fc fusion proteins—have been engineered. These modified variants prolong IL-2 circulation time and enhance delivery to immune effector cells, thereby improving therapeutic outcomes while limiting systemic toxicity (Vazquez-Lombardi et al., 2017). Notably, IL-2–Fc fusion constructs have been reported to selectively reduce regulatory T cells (Tregs) while sparing cytotoxic T lymphocytes (CTLs), resulting in more effective antitumor immune activation (Vazquez-Lombardi et al., 2017).

IL-7 and IL-15 are key cytokines involved in T-cell maintenance, survival, and memory development, positioning them as strong candidates for therapeutic engineering in cancer immunotherapy. Novel IL-7 derivatives—such as hybrid IL-7 constructs designed to enhance T-cell expansion—have demonstrated encouraging results, particularly when combined with immune checkpoint blockade (Briukhovetska et al., 2021). Likewise, optimized IL-15 variants have been generated with greater biological activity, supporting the proliferation and persistence of natural killer (NK) cells and unconventional T-cell subsets. These modified IL-15 formulations are currently under investigation in preclinical settings and early-phase clinical studies for their capacity to improve outcomes in solid tumors as well as hematological cancers (Berraondo et al., 2019).

2.3.2. Cytokine engineering to reduce toxicity; As cytokine-based therapies continue to expand in clinical application, managing treatment-related adverse events has become a central challenge. One of the most significant toxicities, cytokine release syndrome (CRS), results from excessive immune activation and can lead to severe systemic complications (Sharma & Allison, 2015). To minimize these risks, engineering approaches aimed at refining cytokine pharmacokinetics and improving target specificity are being actively pursued, offering strategies to enhance therapeutic benefit while reducing toxicity.

Advanced Delivery Systems and Targeted Approaches; Emerging delivery technologies—including nanoparticle-encapsulated cytokines and antibody-cytokine fusion constructs—enable localized release within the tumor microenvironment while limiting systemic exposure (R. Wang et al., 2025). This targeted delivery is further optimized by designing cytokines with pH-responsive activity, allowing selective activation in the acidic milieu of tumor tissue and thereby protecting healthy cells (Gaggero et al., 2022). Such engineered systems not only boost antitumor efficacy but also maintain improved safety profiles compared to conventional cytokine administration (Nabergoj, Mlinarič-Raščan, & Jakopin, 2019).

Dual-Function and Smart Cytokine Platforms; Next-generation "smart" cytokines, designed to exhibit dual functionality—such as enhanced tumor-specific binding while maintaining immunostimulatory effects—offer an

innovative route to reducing toxic responses. Recent advances in cytokine engineering seek to fine-tune immune activation, improving antitumor efficiency without triggering excessive T-cell stimulation or CRS (Rotta et al., 2024).

2.4. Oncolytic Virus Therapy

Oncolytic virotherapy represents a novel modality in cancer treatment, utilizing viruses that preferentially infect and lyse malignant cells while preserving healthy tissue. Beyond direct tumor destruction, oncolytic viruses reshape the tumor microenvironment (TME) and have been further enhanced through genetic engineering to improve antigen exposure and immune recognition.

2.4.1.Modification of the tumor microenvironment; The TME is a central determinant of tumor growth and immune escape. Oncolytic viruses possess the capacity to alter this microenvironment by modulating cellular composition and promoting pro-inflammatory immune activity. Viral infection and subsequent tumor cell lysis stimulate responses in key immune populations, including macrophages, dendritic cells, and natural killer (NK) cells (Hinshaw & Shevde, 2019).

Through the induction of immunogenic cell death, oncolytic viruses trigger the release of tumor-associated antigens (TAAs) together with inflammatory danger signals, thereby recruiting immune cells to the tumor site (Xavier, 2018). This process generates an immune-active environment conducive to cytotoxic T lymphocyte (CTL) priming. Research demonstrates that virotherapy enhances dendritic cell infiltration and facilitates improved antigen presentation, counteracting the immunosuppressive characteristics of the TME (Zhou et al., 2020).

Genetic alterations further increase viral immunogenicity. Engineered viruses capable of producing cytokines or chemokines can intensify immune cell recruitment and activation (Xavier, 2018). Notably, interactions between oncolytic viruses and tumor-associated macrophages (TAMs) have been shown to promote M1-like polarization, a phenotype linked to better antitumor immunity (Yi et al., 2022).

2.4.2.Genetically engineered viruses for enhanced antigen presentation; Advances in genetic modification have enabled the design of viruses with enhanced immunostimulatory properties, particularly by increasing antigen presentation and T-cell priming. Engineered viral platforms can be programmed to express TAAs or costimulatory signals, thereby boosting T-cell activation and enhancing immune detection of tumor cells (C. Li et al., 2024). A well-documented example is the engineering of herpes simplex virus (HSV) strains lacking the ICP34.5 gene, which improves antigen presentation pathways and enhances CTL recognition of infected tumor cells (B. L. Liu et al., 2003).

Additionally, oncolytic adenoviruses and other viral vectors have been utilized to deliver immune-modulating transgenes directly into the tumor site, amplifying localized immune activity (X. Wang, Zhong, & Zhao, 2021). Recent strategies also focus on viruses engineered to express multiple TAAs simultaneously or used in combination with checkpoint inhibitors to broaden immune activation (Volovat et al., 2024). Such combinations help limit tumor escape due to heterogeneity and may yield more durable responses and better clinical outcomes.

2.5. Activation of Innate Immunity: STING & TLR Agonists

Harnessing the innate immune system has become an increasingly attractive avenue in cancer immunotherapy, with particular focus on cyclic dinucleotide (CDN) receptor STING (Stimulator of Interferon Genes) and Toll-like receptor (TLR) agonists. These molecular systems play central roles in coordinating antitumor immune responses and are being actively investigated for their potential to enhance or complement cancer vaccine platforms. Growing research interest surrounds the underlying mechanisms and signaling pathways associated with STING and TLR activation, along with ongoing clinical efforts evaluating their use alone or in combination with other therapies.

2.5.1. Mechanisms of action and signaling pathways; The STING signaling cascade is initiated when cytosolic DNA—originating either from tumor cells or invading pathogens—is detected within the cell. Recognition of this DNA triggers cyclic GMP-AMP synthase (cGAS) to synthesize cyclic GMP-AMP (cGAMP), which then binds to and activates the STING receptor. STING engagement facilitates the recruitment of TANK-binding kinase 1 (TBK1), leading to phosphorylation and activation of key transcription factors, including interferon regulatory factor 3 (IRF3) and nuclear factor kappa B (NF- κ B) (Corrales et al., 2015). Activation of these pathways drives the secretion of type I interferons (IFNs) and pro-inflammatory cytokines, ultimately promoting strong adaptive immune responses (Fu et al., 2015).

Beyond interferon induction, a central outcome of STING activation is the maturation and functional enhancement of dendritic cells (DCs). Once activated, DCs efficiently prime CD8⁺ T cells, amplifying tumor-specific cytotoxic immunity (Fuentes et al., 2011). Additionally, endothelial cells have demonstrated increased type I IFN production following STING engagement, highlighting the pathway's role not only in immune activation but also in reshaping the tumor microenvironment (O. Demaria et al., 2015).

Toll-like receptors (TLRs) constitute a major subset of pattern recognition receptors (PRRs) capable of detecting pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) released during cell death. Engagement of TLRs triggers intracellular signaling events that culminate in the release of type I IFNs and various pro-inflammatory mediators, thus enhancing both innate and adaptive immunity (Luchner, Reinke, & Milicic,

2021). Each TLR responds to specific ligands—TLR9 recognizes unmethylated CpG DNA, while TLR7 and TLR8 sense single-stranded RNA. TLR signaling proceeds through adaptor proteins such as MyD88 or TRIF, which activate downstream pathways including NF- κ B and MAPK cascades (Vermaelen, 2019). The resulting immune activation promotes dendritic cell maturation, stimulates Th1 polarization, and strengthens antitumor immune responses more broadly.

2.5.2. Clinical trials and combination therapy potential; Both STING and TLR agonists have progressed into clinical evaluation, reflecting growing interest in their use as monotherapies and, more notably, as components of combination treatment regimens. The STING agonist MIW815 (ADU-S100), for example, has been assessed alongside PD-1 checkpoint inhibitors and demonstrated enhanced antitumor activity in patients with solid tumors and lymphomas (Meric-Bernstam et al., 2023). This therapeutic synergy leverages STING-mediated T-cell priming while simultaneously counteracting tumor-driven immunosuppression.

TLR agonists are likewise advancing through clinical investigation. In ovarian cancer, TLR9 agonists administered with targeted therapies have yielded encouraging preliminary outcomes (Temizoz et al., 2015). Incorporating TLR agonists into vaccine formulations can heighten antigen immunogenicity and strengthen downstream T-cell responses. Early data indicate that combining STING and TLR agonists produces complementary activation of innate immune pathways, generating more potent antitumor responses than either approach alone (Kocabas et al., 2020). Ongoing research is focused on identifying optimal conditions for integrating STING and TLR agonists as vaccine adjuvants, with simultaneous pathway activation proposed to help overcome limitations associated with current immunotherapies (Ni et al., 2020). Additionally, novel delivery strategies—including nanoparticle-based formulations—are being designed to enhance agonist stability, bioavailability, and efficacy while reducing toxicity, reinforcing their promise within next-generation cancer treatment strategies (Manna, Maiti, Shen, Du, & Esser-Kahn, 2020).

3. Immunotherapy Resistance and Strategies

Immunotherapy has transformed cancer management by mobilizing the immune system to eliminate malignant cells. Despite its success, therapy resistance remains a major limitation, reducing clinical benefit in a substantial subset of patients. A deeper understanding of resistance pathways is essential for developing more effective therapeutic approaches. Four major mechanisms underlie immunotherapy resistance: T-cell exhaustion, an immunosuppressive tumor microenvironment, activation of alternative immune checkpoints, and antigen escape/immune evasion.

3.1. T-Cell Exhaustion

T-cell exhaustion arises from prolonged antigen exposure, commonly observed in chronic infections and cancer. Exhausted T cells gradually lose effector functions and exhibit increased expression of inhibitory receptors such as PD-1, CTLA-4, Tim-3, and LAG-3, which suppress activation and proliferation (Corrales et al., 2015). This dysfunctional state leads to reduced cytokine secretion and limited expansion capacity, ultimately impairing tumor control. Upregulation of these inhibitory pathways in tumor-infiltrating lymphocytes (TILs) further accelerates functional decline (Amouzegar, Chelvanambi, Filderman, Storkus, & Luke, 2021). Immune checkpoint inhibitors can partially restore T-cell activity, yet the coexistence of multiple inhibitory signals often necessitates combination approaches for meaningful response (Patel & Minn, 2018). Recent efforts have focused on pairing checkpoint blockade with metabolic modulators to reverse dysfunction, demonstrating improved outcomes in preclinical tumor models (Boustani et al., 2021).

3.2. Immunosuppressive Tumor Microenvironment

The tumor microenvironment (TME) is a major determinant of treatment outcome. It is frequently enriched with immunosuppressive cell populations—including regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs)—which secrete inhibitory cytokines such as IL-10 and TGF- β (Newman, 2024). This environment suppresses effector T-cell activity while simultaneously promoting tumor survival and dissemination.

Strategies to reprogram the TME include the use of oncolytic viruses and STING agonists, which enhance immune activation and promote a pro-inflammatory milieu (Corrales et al., 2015). Combination approaches using intratumoral STING agonists have shown the ability to convert immunologically “cold” tumors into “hot” ones, increasing treatment sensitivity (Lawler, Speranza, Cho, & Chiocca, 2017).

3.3. Alternative Immune Checkpoints

Conventional checkpoint blockade targeting PD-1 and CTLA-4 has demonstrated clinical success, yet non-responders remain common. Resistance often results from compensatory upregulation of alternative inhibitory receptors—including LAG-3, TIM-3, and TIGIT—that suppress T-cell activation (Zhang et al., 2025). These molecules represent emergent resistance pathways capable of bypassing current therapies.

Dual and multi-checkpoint inhibition approaches are being tested to counteract this escape mechanism. Early clinical trial data suggest that concurrent blockade of PD-1 and LAG-3 enhances antitumor activity in melanoma and non-small cell lung cancer (Lawler et al., 2017). Next-generation agents targeting

these pathways are expected to broaden therapeutic benefit across resistant patient groups.

3.4. Antigen Escape and Immune Evasion

Antigen escape is another challenge in cancer immunotherapy, occurring when tumor cells modify or downregulate antigen expression to avoid immune detection (Ghaffari et al., 2018). Mechanisms include mutations in tumor-specific antigens, epigenetic modifications, and reduced MHC expression, which together impair T-cell recognition and enable immune evasion.

Therapeutic strategies to overcome antigen escape often involve combination modalities. Oncolytic viruses can promote immunogenic tumor cell death and enhance antigen presentation, thereby boosting immune recognition (Peters & Rabkin, 2015). STING agonists similarly amplify antitumor immunity by improving antigen visibility and promoting robust T-cell activation (Boustani et al., 2021). Approaches integrating multiple antigen-presenting and T-cell-activating pathways are essential for preventing relapse driven by immune escape.

4. Advances in Cell-Based Immunotherapies

Cell-based immunotherapies represent a rapidly advancing class of cancer treatments that leverage the patient's own immune cells to recognize and destroy tumor cells. Current progress in the field centers on improving the performance of Chimeric Antigen Receptor T-cell (CAR-T) therapy, developing CAR-NK (Natural Killer) cell strategies, optimizing tumor-infiltrating lymphocyte (TIL) therapy, and identifying new antigen targets alongside personalized therapeutic designs

4.1. CAR-T Therapy Innovations

CAR-T therapy has significantly reshaped the treatment of hematologic cancers, most notably B-cell leukemias and lymphomas. Recent advancements aim to enhance therapeutic potency while minimizing toxicity. Initial CAR-T products were designed to recognize a single antigen—frequently CD19—but the challenge of antigen escape has driven the development of dual- or multi-target constructs. These next-generation tandem CAR-T systems, capable of engaging multiple tumor antigens at once, have demonstrated encouraging potential in reducing relapse associated with antigen loss (Gómez-Melero et al., 2025).

Genetic engineering approaches have further improved CAR-T cell persistence and functionality within the tumor microenvironment. Incorporating co-stimulatory domains such as 4-1BB or CD28 has been shown to enhance proliferation and post-infusion survival (Gaimari et al., 2024). Moreover, emerging “armored” CAR-T cells, engineered to secrete cytokines like IL-12 or express additional immune-modulating molecules, are being explored to boost

antitumor activity and overcome immunosuppression in solid tumors (W. Li et al., 2024).

4.2. CAR-NK Cell Approaches

CAR-NK cells have emerged as a valuable complement to conventional CAR-T therapy, capitalizing on the innate immune system's natural ability to recognize and eliminate malignant cells. In contrast to CAR-T cells, CAR-NK cells exhibit intrinsic tumor-killing activity without the need for prior antigen sensitization, enabling rapid therapeutic response following infusion (Noorafrooz, Gale, & Dashtkoohi, 2025). Additionally, CAR-NK therapies tend to show lower risks of severe adverse events such as cytokine release syndrome, owing to their unique biological properties and activation mechanisms (Noorafrooz et al., 2025).

Recent advances aim to further boost CAR-NK cell efficacy through genetic engineering strategies that enhance their persistence, expansion, and cytotoxic function. One approach involves IL-15 supplementation during ex vivo expansion, which has been shown to improve CAR-NK cell survival post-infusion (Noorafrooz et al., 2025). Ongoing research is also exploring "off-the-shelf" universal CAR-NK platforms, which could simplify production, increase patient accessibility, and reduce overall treatment cost (Noorafrooz et al., 2025).

4.3. TIL Therapy Optimization

Tumor-infiltrating lymphocytes (TILs) have attracted increasing interest as they naturally recognize malignant cells within the tumor microenvironment and can elicit strong anti-tumor immune responses. Contemporary efforts to optimize TIL-based therapies focus on refining ex vivo expansion methods to generate larger populations of highly activated, cytotoxic T cells (Blank et al., 2019).

Clinical efficacy has been improved through approaches such as pre-infusion lymphodepletion and post-infusion administration of high-dose IL-2 (Lu et al., 2024). Additionally, emerging research highlights the role of metabolic reprogramming in enhancing TIL durability and function within tumors, supporting their persistence and therapeutic potency (Y. Liu et al., 2023). Overall, strategies that boost both the quantity and functional fitness of TILs hold promise for advancing clinical outcomes across a range of malignancies.

4.4. Novel Targets and Personalized Designs

The field of cancer immunotherapy is increasingly shifting toward patient-specific treatment designs, particularly through the development of neoantigen-directed approaches. These therapies exploit mutation-derived neoantigens unique to each tumor, enabling a tailored immune response that promotes precise T-cell targeting and tumor destruction while minimizing off-target toxicity (Deniger et al., 2018).

Recent progress has also expanded the spectrum of immune checkpoint targets beyond PD-1/PD-L1 and CTLA-4. Novel inhibitory receptors such as LAG-3, TIM-3, and TIGIT are under active investigation, with emerging therapeutics showing potential for synergistic benefit when combined with existing checkpoint inhibitors, CAR-T, or neoantigen-specific TIL therapies (Stern & Stern, 2021). These next-generation checkpoint strategies offer opportunities to overcome immune resistance and strengthen anti-tumor responses.

Overall, rapid advancements in cellular immunotherapies—including CAR-T, CAR-NK, enhanced TIL platforms, and personalized neoantigen-based approaches—underscore a promising trajectory for next-generation cancer treatment.

5. Clinical Challenges

Despite significant advances in cancer immunotherapy, several clinical challenges must still be overcome to maximize treatment success and enhance patient outcomes. Key issues include toxicity management, manufacturing and accessibility barriers, and the need for improved regulatory and standardization processes.

5.1. Toxicity Management

Immunotherapy, particularly with immune checkpoint inhibitors (ICIs), is known to produce distinct toxicity patterns referred to as immune-related adverse events (irAEs). These reactions can involve multiple organs such as the skin, liver, gastrointestinal tract, and endocrine system (Brahmer et al., 2018). Effective identification and management of these toxicities is vital to maintain treatment continuity while reducing patient discomfort and preventing serious complications.

Professional organizations, including the American Society of Clinical Oncology (ASCO), have published detailed guidelines outlining approaches for irAE management. These include routine monitoring, timely intervention, and corticosteroid administration when toxicities become clinically significant (Brahmer et al., 2018). In severe cases (grade ≥ 3), treatment discontinuation and initiation of high-dose corticosteroids are commonly recommended (Dolladille et al., 2020). Continued research is needed to further optimize toxicity management frameworks, ensuring patient safety while maintaining the therapeutic benefits of immunotherapy.

5.2. Manufacturing and Accessibility

The production of cell-based and antibody-driven immunotherapies brings notable complexities, especially regarding scalability, uniformity, and financial burden. For example, CAR-T treatments rely on the genetic modification of each patient's own T cells, making the process labor-intensive, time-consuming, and

expensive (Riley et al., 2019). The personalized nature of these therapies often results in manufacturing delays and contributes to higher treatment costs for both healthcare systems and patients.

To enhance accessibility, research is increasingly focused on more efficient production platforms, including off-the-shelf immunotherapies generated from universal donor cells (Riley et al., 2019). Such developments have the potential to streamline workflows, lower manufacturing expenses, and expand the availability of these therapies to a wider patient population.

5.3. Regulatory and Standardization Issues

The rapid evolution of immunotherapeutic approaches requires strong regulatory systems to guarantee their safety and clinical benefit. Traditional drug approval processes are being tested by the complexity and individualized nature of many immunotherapies, prompting the need for more adaptive regulatory pathways (Kennedy & Salama, 2020). Regulatory authorities must therefore balance thorough evaluation with the urgency of delivering new treatments to patients who lack effective options.

Establishing standardized clinical trial protocols and consistent outcome metrics is vital for accurately assessing emerging immunotherapies (Couey et al., 2019). Greater uniformity in defining irAEs, determining response criteria, and measuring patient quality of life would improve comparability across studies and support evidence-based decision making (Sibaud, 2018). Close collaboration between regulatory bodies and researchers will be key to advancing therapeutic innovation while upholding high standards of safety and efficacy.

6. Future Perspectives and Conclusion

The emergence of cancer immunotherapy has revolutionized oncology, providing new treatment pathways where traditional approaches often fall short. Looking forward, key areas of development include AI-supported therapeutic decision-making, personalized immune-cell-based interventions, and methods aimed at converting immune-resistant “cold” tumors into responsive “hot” ones. These forward-thinking concepts highlight the direction of future research and their possible impact on patient care.

6.1. AI-Driven Therapy Decision Support

Artificial intelligence (AI) is expected to play a transformative role in cancer immunotherapy by improving clinical decision-making. With the growing availability of genomic data, imaging results, and treatment records, AI systems are capable of processing complex datasets to uncover patterns that predict how patients may respond to immunotherapy (Bulashevskaya et al., 2024).

AI-based decision support tools are already being applied to guide personalized treatment selection by accounting for tumor features, immune

profiles, and patient-specific variables. Machine learning approaches, for instance, can estimate response probabilities to CAR-T therapy by analyzing neoantigen expression and immune receptor signatures (Bhinder, Gilvary, Madhukar, & Elemento, 2021). This predictive power supports quicker, more precise clinical choices and can improve patient stratification for clinical trials, ultimately accelerating therapy development.

Furthermore, natural language processing is increasingly used to extract valuable information from unstructured clinical reports, helping clinicians access insights that might otherwise be overlooked. As AI technologies evolve, their adoption in clinical oncology is expected to streamline treatment workflows and contribute to improved therapeutic outcomes for cancer patients.

6.2. Personalized Immune-Cell Therapies

Personalized immune-cell therapies—especially those involving CAR-T and CAR-NK platforms—continue to be a major area of exploration. Current advances focus on adapting these treatments to match the unique antigen landscapes of individual tumors, improving therapeutic precision and success (Newick, O'Brien, Moon, & Albelda, 2017). Neoantigen-based strategies, including personalized cancer vaccines and engineered T cells that specifically target tumor mutations, highlight the promise of individualized design.

Recent innovations include CAR constructs capable of recognizing multiple antigens simultaneously. These “tandem” or “multi-target” CARs may help prevent antigen escape and enhance tumor recognition (Newick et al., 2017).

6.3. Converting “Cold” Tumors to “Hot” Ones

One of the major barriers in cancer immunotherapy is the difference between “hot” tumors—those rich in immune cell infiltration—and “cold” tumors, which lack immune presence and frequently show poor responsiveness to treatment (Louis et al., 2011). Current research is increasingly focused on methods to reprogram cold tumors into hot ones by modifying the tumor microenvironment to boost immune visibility and activation.

Multiple therapeutic approaches are being explored to achieve this shift. STING agonists and other immune-stimulating agents are being evaluated for their ability to enhance tumor immunogenicity, while oncolytic viruses offer another avenue by inducing immunogenic cell death and improving antigen presentation, thereby enabling stronger anti-tumor responses (Louis et al., 2011).

When combined with checkpoint inhibitors or other immunotherapies, such strategies may enhance treatment efficacy in solid tumors that currently respond poorly. Additionally, the development of biomarkers for immune activation could help identify which tumors are most likely to benefit from reprogramming interventions (Louis et al., 2011).

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