Bacterial Contamination Is Involved in the Etiology of Soft-Tissue Filler, Late-Onset, Inflammatory Adverse Events

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Background: The treatment algorithm in late-onset inflammatory adverse events with soft-tissue fillers depends primarily on the assumed causative factor: immunologic or bacterial.
Methods: The authors included 29 patients, 13 of whom experienced late-onset inflammatory adverse events to fillers (inflammatory group) and 16 who did not (reference group). Biopsies were acquired from both groups with an 18-G needle. Before taking the biopsy, the authors acquired skin swabs for 25 of the 29 patients. The IS-pro method—a new and very sensitive method to detect microbiota—was used. This is a novel broad-range polymerase chain reaction technique based on length and sequence variations of the 16S to 23S ribosomal interspacer region. IS-pro can detect bacteria at low abundances and identify them up to species level. To exclude contamination from skin microbiota, the authors compared the microbiota found on skin swabs with that found in the corresponding biopsies.
Results: A high level of Gram-positive bacteria was found in biopsies of soft-tissue fillers, predominantly in patients from the inflammation group. This suggests that these bacteria were introduced during the primary filler injection treatment. The composition of the microbiota on the skin differed markedly from that in the filler, indicating that contamination during the sampling process did not influence results.
Conclusions: Bacteria adherent to soft-tissue fillers or bacteremia probably play a causative role in adverse events. Contamination of samples in the biopsies with skin microbiota was excluded. *(Plast. Reconstr. Surg.* 151: 971, 2023.)
CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, III.

PATIENT SAFETY

3 THERAPEUTIC

Soft-tissue fillers are widely used in dermatology, plastic surgery, and aesthetic medicine to reduce the signs of skin aging.[1] The most recent survey of the American Society for Dermatologic Surgery on dermatologic procedures in 2017 reported an increase of 21% in the number of soft-tissue filler injections.[2] With more patients undergoing injections with soft-tissue fillers, adverse events have also increased.[3]

Several studies have suggested that inflammatory adverse events may be caused by biofilms (Fig. 1) resulting from bacterial contamination during the initial treatment.[4–7] This has been reported with other foreign materials[8,9] such as breast implants,[10] pacemakers,[11] and prosthetic joints.[12]

In this article, we address the following research questions: Is there a correlation between adverse events and the presence of

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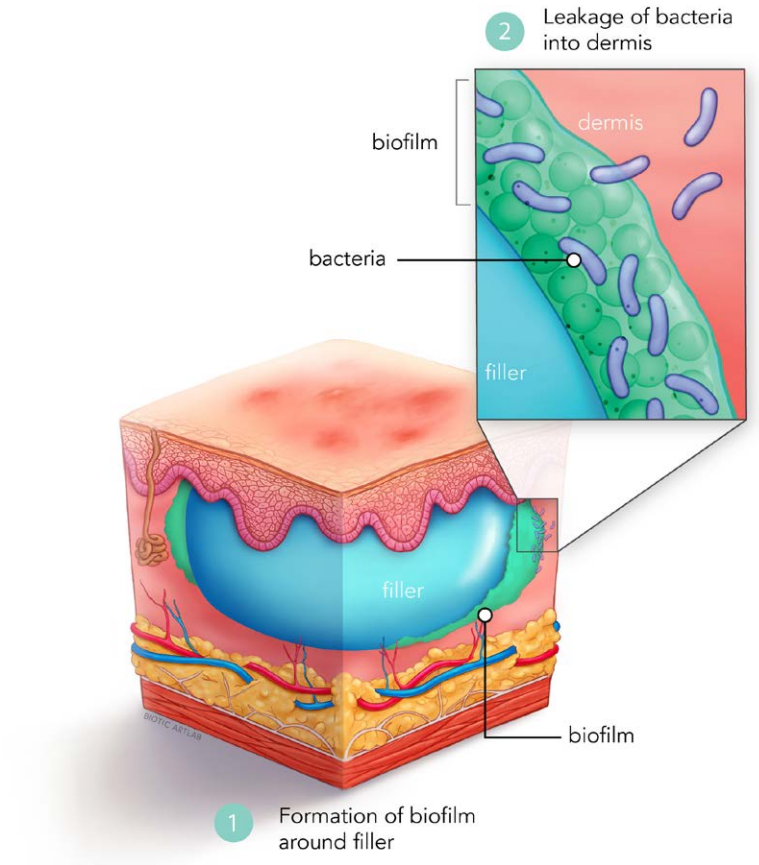

Fig. 1. Biofilm (a heterogeneous structure comprising bacteria embedded within a strong extracellular matrix of secreted polysaccharides) surrounding the soft-tissue filler.

bacteria surrounding the injected filler? If so, could bacterial contamination during the initial injection be the underlying cause of these adverse events?

To analyze the microbiota, we used a new and very sensitive method: the intergenic spacer profiling (IS-pro) assay. This is a novel, broad-range polymerase chain reaction (PCR) technique based on sequence variations and length of the 16S to 23S ribosomal interspacer region.[13] IS-pro can identify bacteria up to species level and detect bacteria at low abundance (fewer than five colony-forming units).

PATIENTS AND METHODS

Samples were collected from patients between 2016 and 2018 at the dermatology department of Erasmus University Medical Center, the Netherlands. The local medical ethics committee approved this study (approval no. MEC-2016-660 NL). Patient characteristics were collected by questionnaire assessment. All participants provided written informed consent.

We included patients who were willing to undergo a biopsy of the filled tissue at a specialized outpatient clinic for soft-tissue filler adverse events. Two groups were defined: an inflammation group with an adverse event and a reference group without such an event. An inflammatory adverse event was defined as the appearance of two or more of the following clinical symptoms or signs of inflammation 3 months or later after initial filler injection: skin induration, edema, nodules with or without tenderness, with or without fistulation or discharge of pus or filler material. The reference group consisted of patients treated with soft-tissue fillers at least 3 months before inclusion who did not report any of these inflammatory signs. Patients with isolated soft lumps caused by migration of the filler substance, but without any of the abovementioned inflammatory signs, were also included in the reference group. Both the inflammation group and reference group

completed a questionnaire assessment including items on ethnicity, autoimmune diseases, smoking status, allergies, and location of the injection. Because there is no national database of soft-tissue fillers injected, no consideration can be made of possible alternation of dilution of the filler, how patients were prepared before the procedure, which antiseptic was used, whether a topical anesthetic was placed, or whether it was placed before or after prepping.

Of the total sample of 29 patients, 25 had two skin swabs taken on the adverse event side, followed by disinfection with chlorhexidine-alcohol and soft-tissue biopsies through fine-needle aspiration. Afterward, the specimens were collected in sterile specimen containers followed by immediate snap freezing in liquid nitrogen and stored at −20°C until further analysis.

Laboratory Methods

IS-Pro Assay

Isolated DNA was amplified with the IS-pro assay (InBiome, Amsterdam, the Netherlands) according to the manufacturer's protocol. IS-pro differentiates bacterial species by the length of the 16S to 23S rDNA interspace region, with taxonomic classification by phylum-specific fluorescently labeled PCR primers that have been extensively evaluated for coverage of the phyla included in the assay. Two multiplex PCR reactions are performed. The first contains two fluorescently labeled forward primers and three unlabeled reverse primers. The first forward primer is specific for the phyla *Firmicutes*, *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia* (FAFV) and the second primer is specific for the phylum *Bacteroidetes*. The second PCR reaction contains one forward primer specific for the phylum *Proteobacteria* and seven reverse primers, together covering the phylum *Proteobacteria*. Amplification was done on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). After PCR, 5 μL of PCR product was mixed with 20 μL of IS-Pro eMix (IS Diagnostics). DNA fragment analysis was performed on an ABI Prism 3500 Genetic Analyzer (Applied Biosystems).

Data Analysis

Data were analyzed with the IS-pro proprietary software suite (InBiome). Automated species calling of IS-pro peaks was done with the dedicated IS-pro software suite (InBiome), in which peaks are linked to a database containing IS-profile information of more than 500 microbial species. Peaks of less than 145 relative fluorescence units (RFU) and *Proteobacteria* peaks less than 500 were regarded as background noise and were discarded from further analysis. Peaks known to be human contamination and peaks detected in the negative controls that were considered to be contamination were discarded from further analysis.

Statistical Analysis

Bacterial loads were measured by the intensity of their associated fluorescent signal in the CE machine as RFU; summed intensities were used to calculate total bacterial loads. The median load (summed intensity) for each of the three phyla was compared between the inflammation group and reference group. For effect size, the nonparametric Hodges-Lehmann estimate of median difference was used. To assess the statistical significance of differences between the groups, the Mann-Whitney *U* test was used.

To estimate the increased risk of adverse events following soft-tissue filler injections in relation to the intensity of the three phyla, a logistic regression analysis was performed, modeling the increased odds of inflammation for each additional 1000 RFU.

For all analyses, the significance level was set to 0.05. Analyses were conducted with IBM SPSS Statistics for Apple software, version 25.0 (IBM Corp., Armonk, NY).

RESULTS

A total of 29 patients took part in this study. This sample was divided into an inflammation group of 13 patients who experienced late-onset inflammation and a reference group of 16 patients who did not experience inflammation. The two groups were first compared on patient characteristics (age, sex, ethnicity, smoking status, autoimmune diseases, allergy, cold sore, filler type, injection location, and filler in situ). Analyses (Table 1) showed that all differences were small and none was statistically significant.

To determine whether bacterial infection plays a role in adverse events after injection of soft-tissue fillers, the presence of bacteria and their loads were compared between the inflammation group and reference group. To further analyze the role of different bacterial phyla, results were stratified into the groups *Bacteroidetes*, FAFV, and *Proteobacteria*.

Figure 2 presents a series of box plots that illustrate the distribution of *Bacteroidetes*, FAFV, and *Proteobacteria* bacterial loads (in 1000 RFU). The median load was markedly higher for the FAFV

Table 1. Descriptive Statistics for Inflammation and Reference Group

Characteristics	Inflammation	Reference	χ^2	*P*
Sex				
Female	11	15	0.66	0.422
Male	2	1		
Age, yrs, mean (SD)	59.7 (10.7)	63.4 (6.4)	t = 1.15	0.261
Race				
Nonwhite	1	1	0.02	0.879
White	12	15		
Smoking				
Yes	3	1	1.71	0.191
No	10	15		
Autoimmune disease				
No	13	14	1.75	0.186
Yes	0	2		
Allergy				
Drugs	3	2	1.52	0.468
Atopy	4	3		
No	6	11		
Cold sore				
Yes	5	6	0.00	0.958
No	8	10		
Filler type				
Nonpermanent	3	0	4.12	0.042
Permanent	10	16		
Injection location				
Periorbital	0	2	6.67	0.155
Lips	1	2		
Cheeks	4	1		
Zygoma	5	3		
Nasolabial folds	3	8		
Time inside the body, months, mean (SD)	12.3 (3.5)	13.4 (4.0)	t = 0.81	0.426

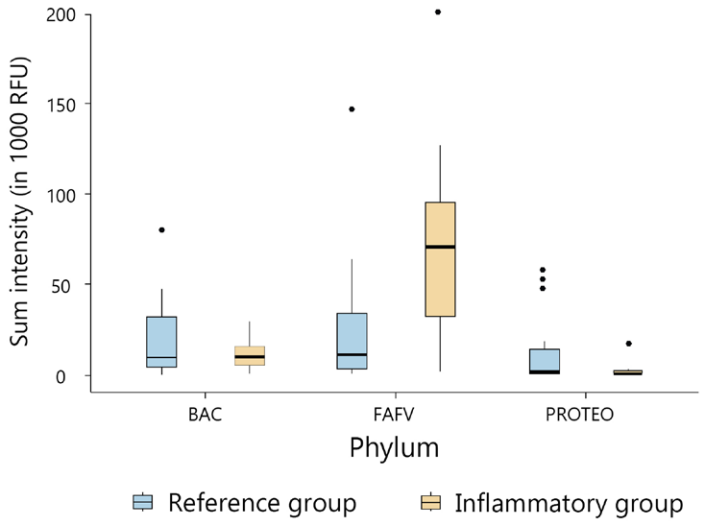

Fig. 2. Sum intensity [in 1000 relative fluorescent units (*RFU*)] for three bacterial phyla in patients with no inflammation (reference group) or inflammation (inflammation group). Only the FAFV phylum showed significantly higher RFU intensity.

phylum [32.9; interquartile range (IQR), 5.6 to 70.6] than for the other two phyla (*Bacteroidetes*: 9.4; IQR, 4.5 to 16.8; *Proteobacteria*: 0.0; IQR, 0.0 to 1.9). Bacterial load was lowest for the *Proteobacteria*. A Friedman test for related samples showed that the differences between the phyla were statistically significant ($\chi^2 = 27.21$, $df = 2$, $P < 0.001$).

Figure 2 further shows that the relatively high load of the FAFV phylum occurred mainly in the inflammation group. To compare the inflammation group and reference group on each of the three phyla, Hodges-Lehmann median differences were calculated. The median loads (with IQR) for the inflammation and reference groups, respectively, were 9.4 (5.3 to 15.1) and 9.7 (3.5 to 31.5) for *Bacteroidetes*, 70.5 (31.3 to 95.6) and 10.6 (2.5 to 33.4) for FAFV, and 0.0 (0.0 to 1.5) and 0.8 (0.0 to 13.4) for *Proteobacteria*. The Hodges-Lehmann median difference was considerably larger for the FAFV phylum (54.0; 95% CI, 11.6 to 82.0) than for the *Bacteroidetes* (−1.8; 95% CI, −17.3 to 5.4) and *Proteobacteria* (−0.0; 95% CI −10.8 to 0.0) phyla. Only for the FAFV phylum was the difference statistically significant (Mann-Whitney *U*, 50.0; $P = 0.018$).

A relatively high level of bacterial contamination was found in the biopsies from filled soft tissue of patients experiencing adverse events, mainly involving the FAFV phylum (which includes most Gram-positive bacteria). This may indicate that high levels of bacterial contamination are predictive for adverse events. To estimate the increased risk of adverse events in relation to the intensity of the three phyla, a logistic regression analysis was conducted with sum intensity of *Bacteroidetes*, FAFV, and *Proteobacteria* (in 1000 RFU) as predictor variables and inflammation status as the binary dependent variable. The analysis showed that, overall, the intensity of the phyla was significantly associated with the odds of adverse events (Hosmer-Lemeshow goodness-of-fit test $\chi^2 = 6.22$, $P = 0.623$, Nagelkerke $R^2 = 0.65$, overall model test $\chi^2 = 19.33$, $P < 0.001$). However, of all three phyla, only FAFV appeared a risk factor (OR, 1.06; 95% CI, 1.01 to 1.11; $\chi^2 = 13.66$; $P < 0.001$).

Bacterial Contamination

The relatively high sum intensity for the FAFV phylum in the inflammation group suggested that bacterial contamination during the initial treatment could have caused the high bacterial load that leads to adverse events. To assess this possibility, we collected a total of 40 soft-tissue filler biopsies and 26 double skin swabs from the total group of 29 patients. For eight of the patients, 15 additional biopsies were taken from the sites of multiple injected soft-tissue fillers. In those cases, the IS profile measurements of multiple biopsies were aggregated on the patient level by taking the arithmetic mean. For three patients, it was not possible to obtain skin swabs. These three patients were removed from the analysis.

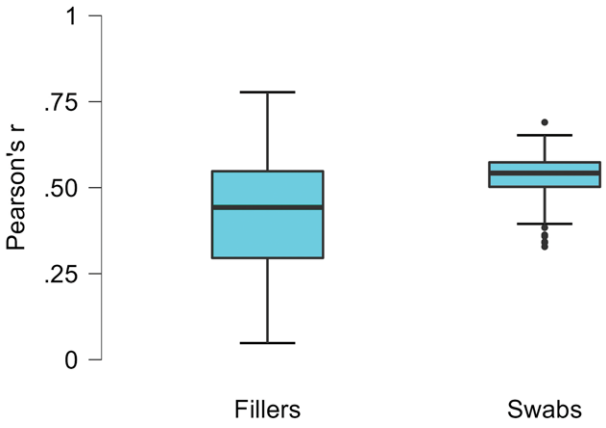

Fig. 3. Pearson correlation coefficients between patients. Fillers: boxplot showing all correlation coefficients comparing bacterial loads in the soft-tissue filler biopsy from each patient with every other patient; swabs: boxplot showing all correlation coefficients comparing bacterial loads on the skin swabs from each patient with every other patient.

For all 26 patients included in the analyses, two skin swabs were collected. Similarities of these 26 pairs of IS profiles were calculated with the Pearson correlation coefficient. As expected, similarities between the two skin swabs were generally high, with an average correlation coefficient of 0.85. Therefore, to avoid redundancy in the results, analyses of all between-patients similarities involving skin swabs were conducted using only the first skin swab.

Between-Patients Similarity

The two boxplots in Figure 3 show the distributions of the correlation coefficients for each type of between-patients similarity for soft-tissue filler biopsies and skin swabs. As can be seen, the distributions of correlation coefficients for the two types of similarity not only have clearly different locations, with relatively low similarity between skin swab and soft-tissue filler biopsy IS profiles, but also different degrees of variation. The largest variation in similarity is found among soft-tissue filler biopsy IS profiles, and the lowest among skin swab IS profiles.

The markedly low similarity of skin swab and soft-tissue filler biopsy IS profiles (6.5% on

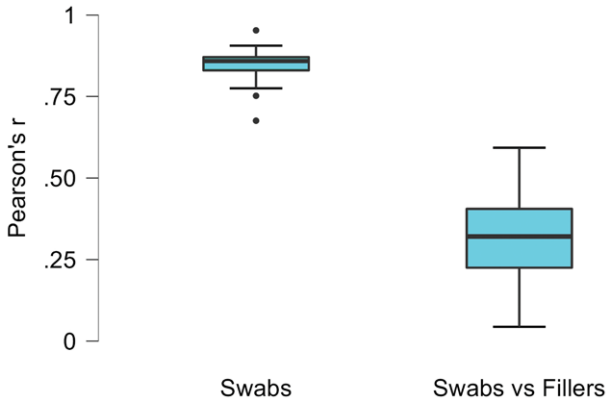

Fig. 4. Pearson correlation coefficients within the same patient. Swabs: boxplots showing all correlation coefficients comparing bacterial loads on the skin swabs of the right and left side of the face in every patient; swabs versus filler: boxplots showing all correlation coefficients comparing bacterial loads on the skin swab from each patient and bacterial load in the soft-tissue filler biopsy from the same patient.

average) indicates that soft-tissue filler biopsy IS profiles are even more distinctive from skin swab IS profiles than they are among other soft-tissue filler biopsy IS profiles (14.8% similarity on average).

Within-Patient Similarity

In 26 patients, IS profiles were determined for two skin swabs and one soft-tissue filler biopsy. This enabled us to determine the within-patient similarity between two skin swabs as well as between skin swab and soft-tissue filler biopsy profiles.

The two boxplots in Figure 4 show the distributions of the correlation coefficients for the two types of within-patient similarities. One is in regard to skin swabs of the affected side of the face and the corresponding area on the other side ("swabs"). The other is in regard to skin swabs compared with soft-tissue filler biopsies within the same patient ("swabs versus filler"). It is apparent in the figure that the distributions of correlation coefficients for these two types of similarities have different locations. There is high similarity in skin swabs from both sides of the face, but relatively low similarity between skin swab and soft-tissue filler biopsy. Both also differ markedly in terms of variation.

A Wilcoxon signed-rank test for related samples showed that the difference between the two median correlation coefficients is statistically significant ($Z = 4.37$, $P < 0.001$). Because the IS profiles representing the type of bacteria in the filler biopsy differ so substantially, this indicates that contamination of biopsies with skin bacteria during the sampling process is very unlikely.

Comparing Figures 3 and 4, it appears that the within-patient and between-patients similarities of skin swab and soft-tissue filler biopsy IS profiles are of the same order (9.0% and 6.5%, on average). This suggests that a soft-tissue filler biopsy IS profile from a patient is just about as different from other patients' skin swab IS profiles as it is from the patient's own skin swab IS profile.

DISCUSSION

This study shows a strong correlation between bacterial load and late-onset inflammatory adverse events in a group of soft-tissue filler recipients. This correlation applied to the FAFV phyla only and not to the *Proteobacteria* or *Bacteroides* phyla. The FAFV phyla contains the group of Gram-positive bacteria including common skin-associated bacteria such as *Staphylococcus*, *Streptococcus*, and *Cutibacterium* (Fig. 5).

Although no microscopy was performed, many of the samples taken macroscopically showed filamentous opacities, which is suggestive of biofilm formation. When analyzed on species level, each patient generally showed dominance of a single species (eg, *Streptococcus pyogenes* or *Staphylococcus epidermidis*). For the entire patient group, an abundance of different bacteria from the FAFV phyla was found, but with only one dominant species per patient.

This may be indicative of long-term formation of a microbial biofilm, in which over time some bacteria benefit more from the environment than others (ie, circumstances may be more favorable for some bacteria than for others), which can lead to selective outgrowth of a single species (or a very limited number of species). Although the presence of bacteria does not necessarily indicate an infection, we found FAFV bacteria to be significantly more abundant in diseased cases than in nondiseased ones, concurring with the first two postulates of Koch. This suggests a causative relationship.

It is possible that the bacteria found in the biopsy originated from contamination on the patient's skin during sampling. Other studies investigating the role of bacteria in adverse events of soft-tissue fillers have raised this issue,[14] but these studies did not compare skin swabs and tissue samples. In our study, this contamination is unlikely; as shown in Figure 2, no correlation between the bacteria phyla surrounding the filler in comparison with the bacteria phyla from

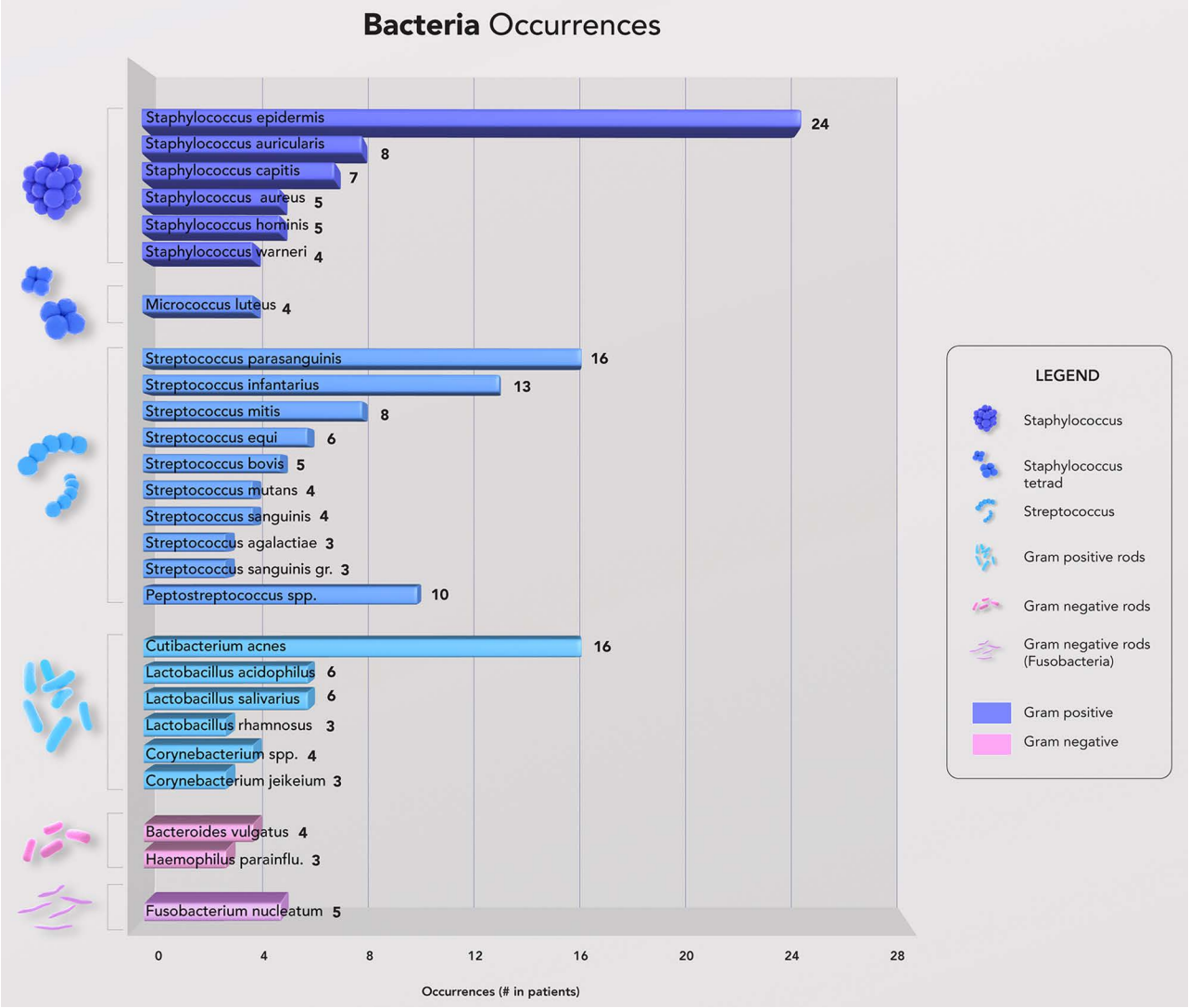

Fig. 5. The occurrences of different bacteria. Research was conducted on a group of 29 patients. A total of 27 types of skin bacteria were found to occur in two or more patients. A total of 42 other types of bacteria occurred in two or fewer patients and were not included in this figure.

the skin swabs was found in individual patients. However, it is possible that bacteria from the skin microbiota were coinjected with the filler material, giving rise to a bacterial biofilm with a very distinct signature as compared with the original skin microbiota caused by selectional pressures within the host environment. Therefore, the use of a disinfection agent (eg, chlorhexidine) for cleaning of the skin before a filler treatment is very important. A second origin of bacteria that contaminate the soft-tissue filler is contamination through bacteremia. Bacteremia is defined as an invasion of the bloodstream by live bacteria.[15]

In late-onset inflammation after filler injections, the type of antibiotic to be chosen is subject to debate. Many studies have been published on this topic, with many antibiotic treatment options being presented,[16–18] which is attributable in part to geographic differences in bacterial resistance. However, our data indicate that antibiotics active against Gram-positive bacteria, such as vancomycin, may be the best choice. Additional support with an antibiotic that penetrates biofilms, such as rifampicin, can be advantageous. Of course, removal of the filler material with biofilm should remain the cornerstone of every therapeutic regimen.

Our findings suggest a causative role for bacteria, probably by biofilm formation, in the development of late-onset inflammatory adverse events to soft-tissue fillers. Bacteria were most likely

introduced during the primary filler injection treatment or, alternatively, by bacterial contamination through bacteremia.

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DISCLOSURE

Dr. A. Budding has proprietary rights to IS-pro platform technology and is a cofounder of a spinoff company developing this technique. The remaining authors have no financial interest to declare in relation to the content of this article.

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