

**Study Title: Understanding the impact of Intraventricular Haemorrhage on Brain**

**Development in the Premature Neonate**

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**Short title or acronym: Sampling of the Lateral Ventricle (SOLVe)**

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**Sponsor**

**Great Ormond Street Hospital for Children NHS Foundation Trust**

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**Signatures**

The Chief Investigator, Principal Investigators and Sponsor have discussed this protocol. All have agreed to perform the investigation as written and to abide by this protocol except in case of medical emergency or where departures from it are mutually agreed in writing.

**Chief Investigator**

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Signature

Date:

**Participating Sites and Local Principal Investigators (PI)**

# Amendment History

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| --- | --- | --- | --- | --- |
| **Amendment No.** | **Protocol Version No.** | **Date issued** | **Author(s) of changes** | **Details of Changes made** |
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1. **ABBREVIATIONS**

|  |  |
| --- | --- |
| CI | Chief Investigator |
| CRF | Case Report Form |
| CSF | Cerebrospinal Fluid |
| EGF | Epidermal Growth Factor |
| FGF | Fibroblast Growth Factor |
| FACS | Fluorescence Activated Cell Sorting |
| GCP | Good Clinical Practice |
| GOSH | Great Ormond Street Hospital |
| GP | General Practitioner |
| ICF | Informed Consent Form |
| ISF | Investigator Site File |
| ICH | International Conference of Harmonisation |
| MACS | Magnetic Activated Cell Sorting |
| NHS | National Health Service |
| NSPC | Neural Stem Progenitor Cell |
| NRES | National Research Ethics Service |
| PI | Principal Investigator |
| PIL | Participant/ Patient Information Leaflet |
| R&D | NHS Trust R&D Department |
| REC | Research Ethics Committee |
| RNA | Ribonucleic Acid |
| SAE | Serious Adverse Event |
| SDV | Source Data Verification |
| SOP | Standard Operating Procedure |
| SVZ | Subventricular Zone |
| TMF | Trial Master File |
| UCL | University College London |

# Study Synopsis

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| --- | --- | --- |
| Title |  | Understanding the impact of Intraventricular Haemorrhage on the wall of the lateral ventricle and subventricular zone. |
| Sponsor name |  | Great Ormond Street Hospital |
| Primary objective |  | The primary aim of this research project is to develop an organotypic model of the human ventricular wall to dynamically examine the impact of haemorrhage on the ependyma and SVZ. |
| Secondary objective (s) |  | **Phase 1** - establishment of techniques for *Scanning Electron Microscopy* to look specifically at the ciliated ependymal cells and *Immunohistochemistry* of the ciliated ependyma and NSPC within the SVZ.  **Phase 2** - optimisation of *dynamic imaging* of the ventricular wall - High speed camera acquisition of the ventricular wall and fluorescence labelled viral infection of the neural stem progenitor cells within the SVZ  **Phase 3** - quantification of the impact of haemorrhage on the wall of the lateral ventricle using techniques optimised through phase 1 & 2 of the study  **Phase 4** – application of technique to explore other aspects of ependymal cell function and impact on the NSPC within the SVZ |
| Study Design |  | Organotypic slice preparation from samples of the ventricular wall taken during operative Neurosurgical cases. |
| Study Endpoints |  | The aim is to establish a robust and reproducible organotypic model, which will facilitate detailed analysis of the impact of IVH on the wall of the lateral ventricle. As such the endpoint for this feasibility and optimisation study will be the collection of 20 cases (Phase 1: n=5; Phase 2: n=5; Phase 3: n=10).  Samples will be stored in research laboratories within Great Ormond Street Hospital or the Institute of child health and used for research for up to 15 years, after which time any remaining samples will be destroyed.  When the pathway for taking and analysing samples has been optimised we envisage application of this technique for other experiments and investigations |
| Sample Size |  | 20 patients will be enrolled to establish the organotypic model. |
| Summary of eligibility criteria |  | The prinicpal inclusion criteria is referral for surgical intervention for the treatment of medically intractable epilepsy. |
| Intervention |  | At the time of neurosurgical intervention for the treatment of medically intractable epilepsy, samples of the ventricular wall will be taken for analysis. A 10ml sample of CSF and 2ml sample of blood will also be taken. |
| Procedures: Screening & enrolment |  | Existing referral pathways will be used. All patients undergoing ‘disconnective’ surgery for the treatment of medically intractable epilepsy will be eligible for enrolment in this study |
| Baseline |  | Existing referral pathways will be used. All patients undergoing disconnective surgery for the treatment of medically intractable epilepsy will be eligible for enrolment in this study. |
| Treatment period |  | Enrolment in the study will result in a sample of the ventricular wall being taken for analysis, the treatment of the patient will not in any other way deviate from standard practice. |
| End of Study |  | The feasibility and optimisation phase of the study will be completed when specimens from 20 patients have been analysed. Following this we envisage the continued sampling of the ventricular wall on an as required basis. |

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# Introduction – Background and Rationale

## Global aims of this research project

The global aim of this research proposal is to work towards developing therapies aimed at reducing the incidence of post haemorrhagic hydrocephalus and improving neurodevelopmental outcome following neonatal intraventricular haemorrhage. To achieve this we propose to develop an organotypic or ‘live-slice’ model of the wall of the human ventricular zone. We intend to develop this model to study ‘normal’ function in the human ventricular zone and further to this to determine how haemorrhage and other insults impact on normal function.

Organotypic slice preparation is a well-established technique in mice models(1) and is increasingly seen as potentially replicable in human tissue(2 3). Essentially a sample of the ventricular wall, taken in theatre can be maintained in artificial culture medium, by keeping the sample ‘alive’ in this way the impact of different factors on the wall of the lateral ventricle and subventricular zone can be studied in a dynamic or ‘real-time’ fashion. A robust and reproducible organotypic model of the lateral ventricle wall has a number of potential research applications for example the reaction of the wall of the lateral ventricle to haemorrhage, inflammation and infection could all potentially be studied.

## Neonatal Intraventricular Haemorrhage

Despite advances in neonatal care, premature birth remains common with up to 15 million babies born preterm (<37 weeks gestation) every year worldwide. Indeed, over the last two decades, studies indicate that rather than decreasing, the incidence of premature birth has actually increased in almost all countries with reliable data (4 5). The reason for the observed increase is incompletely understood but is thought to be related to delayed primigravida and the increased use of fertility treatments in the developed world (6 7).

The increased incidence of prematurity, in combination with the improving survival of smaller neonates, has led to a significant increase in the absolute number of infants (8 9) with neurodisability after premature birth (10 11).

The high metabolic demand of the developing brain, in association with fragile and underdeveloped cerebral vasculature and parenchyma, combine to make the premature neonate prone to haemorrhage within the germinal matrix (GMH) (12 13); its incidence is inversely proportional to the degree of brain maturation (14).

## The wall of the lateral ventricle

The wall of the lateral ventricle has long been recognised as a highly specialised region of the brain, which plays a critical role in brain development. For example, its potential role was first described by the Swiss Neurologist Wilhelm His more than 100 years ago who commented on the presence of multiple mitotic cells in the periventricular region of the developing brain (15).

The ventricular (VZ) and subventricular zones (SVZ) are the primary source of neurogenesis and gliagenesis during embryogenesis and increasingly the role of the ependyma/VZ/SVZ in the postnatal period and indeed throughout life, as the potential source of neural stem progenitor cells and lineage restricted cells is being recognised(2 3 16).

## The role of CSF and the ependyma in the evolution of hydrocephalus

Animal models have shown that damage to the ependyma impacts on the periventricular organs particularly around the cerebral aqueduct, it also affects cilial function disturbing the normal flow of CSF, both of which are postulated to play a causative role in the development of ventriculomegaly and in extreme cases hydrocephalus(17). Manipulation of genes important in ependymal function have been shown to cause hydrocephalus both through an impact on the cerebral aqueduct and also hydrocephalus without an impact on the cerebral aqueduct(18).

Denudation (loss of surface layers) of the ependyma is associated with the development of communicating hydrocephalus(19) for example, ependymal cell loss secondary to viral infection has been shown to cause ependymal cell shedding which is thought to play a role in the development of hydrocephalus following foetal infection(17).

The integrity of the ependymal cell layer is maintained through both homophilic binding between cells and binding to the basement membrane. We hypothesise that IVH impacts directly on the ependymal cells affecting the integrity of these junctions and thus increasing cell shedding into the CSF. Further to this we suggest that IVH results in changes in RNA expression within the ependymal cells, increasing their propensity to shed into the CSF and activating a deprogramming cascade resulting in a change of cell type from ependymal to radial glial cell (20).

## The role of CSF and the ependyma in modulating the behaviour of the Neural Stem Progenitor cells (NSPC)

In addition to the development of ventriculomegaly, it has been shown in mice that the cilia play a role in guiding migration of postnatally developed cells out of the SVZ and further to this that areas of ependymal denudation are associated with changes in the underlying cells of the ventricular and subventricular zones(19).

In the final trimester the ventricular and subventricular zones play an integral role in brain development with behaviour of the NSPC tightly regulated in a temporal and spatial fashion to ensure the coordinated development of the brain parenchyma(21). As such we hypothesise that ependymal damage secondary to IVH also impacts on the NSPC within the SVZ potentially causing aberrant cortical development and impacting on the evolution of neurodisability.

It has been shown in mice that the NSPC within the SVZ are exquisitely sensitive to microenvironmental cues. For example FGF & EGF infusions have been shown to increase proliferation, other soluble factors within the CSF including: Insulin like growth factor 2 - IGF2; Bone morphogenetic proteins (BMPs); Wnts; Sonic Hedgehog &Retinoic acid, have also been shown to influence NSPC behaviour in the VZ/SVZ (22). Using this organotypic model we can further explore the impact of each of these compounds.

Similarly the integrity of the intercellular junctions between ependymal cells and the NSPC has been shown to play a critical role in the control of neurogenesis, for example the protein ankyrin plays an important role is stabilizing the ependymal cell junctions and loss of this protein (through FoxJ1 knockout) causes a reduction in neurogenesis (23). Ependymal cells also secrete Noggin, which has been shown in vitro and in vivo to promote progenitor proliferation and neuroblast formation.

As such we postulate that changes in the proximal / apical domain(24) caused by IVH (changes in the CSF physiology) will impact on cortical development due to changes in the CSF microenvironment and disruption to ependymal integrity, and further to this may lead to disordered brain development contributing to the Neurodevelopmental disability seen following GMH /IVH. Understanding this process may give us an opportunity to introduce new treatment options.

## Specific aims of this research proposal

By understanding the molecular pathways that are impacted by IVH and the cascades through which damage occurs we can begin to develop therapies aimed at intervening in this deleterious process. For example it is envisaged that ependymal cell repair either through cell transplantation into the wall of the lateral ventricle or through modulation of the SVZ to produce ependymal cells may be developed to improve outcome and this study will provide the ideal platform to develop the expertise needed to develop this technology.

### The need for an organotypic model of the wall of the human lateral ventricle

Organotypic models provide a unique insight into how whole body systems react to changes in their microenvironment. The technique been used extensively in mouse models for example Ohata et al 2014(25) & Mirzadeh et al 2008(1) and increasingly this technique is now being used in humans for example Paredes et al 2016(3) & Sanai et al 2011(2). The CSF is increasingly recognised as a biologically active and integral regulator of function within the neural stem cell niche and the organotypic model provides an ideal platform to investigate how haemorrhage impacts on the ependyma and subventricular zone.

The validity of the organotypic model is further enhanced by using autologous blood and CSF taken at the time of surgery.

### Sampling the ventricular wall in cases of disconnective epilepsy surgery

Surgical management of intractable epilepsy either through hemispherotomy, temporal / parietal / occipital disconnection or temporal lobectomy with or without amygdalohippocampectomy is regularly undertaken within the Neurosurgical department at Great Ormond Street. As part of the standard operative procedure of these surgical interventions, the wall of the lateral ventricle is exposed to facilitate disconnection of the white matter tracts.

Standard operative technique involves the use of bipolar diathermy and suction or CUSA (Cavitron Ultrasonic Surgical Aspirator) to cut a trench through the wall of the lateral ventricle. What we propose is that prior to cutting the disconnection trench we will take a sample of the ventricular wall for analysis i.e. rather than discarding this tissue, as is standard practice, we will preserve it for analysis. Sampling of the ventricular wall in this way will pose no extra threat to the patient and will take no more than 5 minutes to accomplish.

Depending on the operation undertaken (as shown above) and the degree of access that the operation facilitates to the ventricular wall, we propose to take up to three samples of the ependyma and SVZ from different regions of the wall of the lateral ventricle, for example from the temporal horn, around the trigone and from the frontal horn. The anatomical location from which the sample is taken will be recorded using a screen capture from the stealth guidance system used in theatre and also by taking a picture of the operative site with the operative microscope.

### Analysis of samples taken from the wall of the lateral ventricle

Phase 1 – (n=5)

The aim of phase 1 will be to establish the baseline investigations, develop the techniques for acquisition of tissue, and to optimise the conditions for robust and reproducible analysis of the tissue. Whole mount en-face preparations as well as cross sectional analysis will be used in conjunction with immunohistochemistry and scanning electron microscopy (discussed in more detail in section 16).

Phase 2 – (n=5)

In phase 2 dynamic imaging of the samples will be undertaken in collaboration with the Alvarez-Buylla lab at UCSF, time lapse confocal imaging and ultra high-speed camera acquisition of the cilia will be established to actively monitor behaviour within the ependyma and SVZ.

Phase 3 – (n=10)

In phase 3 the techniques optimised through phase 1 & 2 will be used to determine the impact of autologous blood on the structure and function of the ependyma and SVZ.

### Why this research should be undertaken at GOSH

Neonatal intraventricular haemorrhage is a critically important disease process, which causes significant morbidity for the individual and their family and incurs a massive socioeconomic cost. As a leading cause of neurodisability and the commonest cause of ventricular peritoneal shunting in the developed world, neonatal post haemorrhagic hydrocephalus represents an important neurosurgical disorder and as a world authority in Paediatric Neurosurgery, Great Ormond Street Hospital should be at forefront of research to combat it’s deleterious impact.

We are in a privileged position at GOSH as a key provider of Epilepsy services in the U.K. The surgery undertaken in this specific patient group, coupled with our interest and expertise in the management of neonatal IVH put us in a unique position to develop this research.

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# Objective and purpose

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| --- | --- |
| **Objectives** | **Outcome Measures/Endpoints** |
| **Primary Objective** | The primary aim of this research project is to develop an organotypic model of the human ventricular wall to dynamically examine the impact of haemorrhage on the ependyma and SVZ. |
| **Secondary Objectives** | **Phase 1** - establishment of techniques for *Scanning Electron Microscopy* to look specifically at the ciliated ependymal cells and *Immunohistochemistry* of the ciliated ependyma and NSPC within the SVZ.  **Phase 2** - optimisation of *dynamic imaging* of the ventricular wall - High speed camera acquisition of the ventricular wall and fluorescence labelled viral infection of the neural stem progenitor cells within the SVZ  **Phase 3** - quantification of the impact of haemorrhage on the wall of the lateral ventricle using techniques optimised through phase 1 & 2 of the study  **Phase 4** – application of technique to explore other aspects of ependymal cell function and impact on the NSPC within the SVZ |

# Study Design

## Description of study design

This is a qualitative study addressing a basic science question: namely, how haemorrhage impacts on the ependyma and neural stem progenitor cell function within the subventricular zone. Sample size is based on the number of samples required to optimise methods of analysis. On going acquisition of samples is envisaged.

This is a single centre study as such all analysis and recruitment will be undertaken at GOSH.

# Population

All patients undergoing disconnective surgery for the treatment of medically intractable epilepsy will be eligible for enrolment in this study. In the first instance we envisage that 20 patients will be required to optimise tissue analysis, following this period of optimisation of techniques further sample acquisition and analysis is planned on an as required basis.

## Inclusion Criteria

All patients undergoing disconnective surgery for the treatment of medically intractable epilepsy will be eligible for enrolment in this study. Acquiring a sample of the ventricular wall does not significantly change the standard operative procedure therefore there are no specific contraindications to enrolment in the study.

## Exclusion Criteria

No specific exclusion criteria are envisaged.

# Study Procedures

## Recruitment

Existing referral pathways via the Epilepsy multidisciplinary team will be used. Information about the organotypic study will be given to the family in the Epilepsy clinic when a decision has been made for surgical intervention, or via post if this decision has been made outside the clinic setting. Further to this, when the patient is admitted to the neurosurgcial ward for intervention, the family of the patient will be asked whether they are prepared to partake in the study and if agreeable then consent for this study will be taken at the same time as consent for surgery.

## Informed Consent

Generic information about the study will be provided to the parents of the patients in the Epilepsy out patient clinic with the option to discuss the study with members of the clinical team if required, this will provide adequate time for the parents to consider whether they wish to be involved in the study.

If parents are willing to enrol in the study then a suitably qualified member of the neurosurgical team will take consent at the time of primary surgical intervention.

## Screening and Eligibility Assessment

All patients undergoing disconnective surgery for the treatment of medically intractable epilepsy will be eligible for enrolment in the organotypic study.

## Baseline Assessments

The acquisition of samples of the ventricular wall for analysis will not impact on standard treatment algorithms and no additional assessments to those routinely performed will be undertaken.

## Subsequent Visits

Following intraoperative sampling of the ventricle wall, no further visits or assessments in addition to those routinely undertaken will be conducted. In every respect other than repurposing the tissue samples taken from the ventricular wall intraoperatively, the treatment of the study participants will not differ from standard practice.

## Study Duration

We predict that between 18 and 24 months will be needed to optimise the organotypic model and analyse the impact of haemorrhage on the ependyma and neural stem cells within the SVZ. On average one patient will undergo disconnective surgery per week. Whilst conditions are being optimised for analysis we envisage analysing one sample every two to three weeks to allow adequate time for analysis and optimisation prior to examining further tissue. We envisage that recruiting 20 patients into the feasibility and optimisation phase of this study will take between 18 and 24 months.

## Discontinuation/Withdrawal of Participants from Study

Parents and patients will have the right to withdraw from the study at any time. All samples collected intra-operatively will be removed from any further analysis and destroyed, all data and analysis collected up to that point will be used for overall analysis.

Withdrawal of the subject will not require any further procedures or observations to be undertaken.

## Definition of End of Study

This is a feasibility and optimisation study, we predict that a sufficiently robust and reproducible method will require analysis of 20 samples. As such the end of the feasibility study will be the recruitment of 20 patients, following this we envisage that the organotypic model will be developed further for specific applications and research questions.

# Intervention

Samples of the ventricular wall are taken for analysis. Sampling of the ventricular wall in this manner in no deviates from standard surgical practice, essentially rather than using suction to cleave the ventricle the lining will simply be removed and taken for analysis.

In addition to the tissue sampling from the wall of the lateral ventricle a sample of CSF (approx. 10mls) and blood (approx. 2mls) is taken at the time of operation.

# Randomisation, Blinding and Code-breaking

There is no indication for randomisation or blinding in this study protocol.

## 11.2 Subject Withdrawal Criteria

If a subject withdraws from either study then all intraoperative samples collected will be destroyed. All data collected up to that point will be used for the analysis and modification of experimental protocols.

# Assessment of Safety

### **Serious adverse event**

The deviation in surgical technique required to sample the wall of the lateral ventricle (rather than remove it using diathermy and suction) does not significantly deviate from standard surgical practice as such there is no increased risk incurred by the patient.

## Reporting Procedures for Serious Adverse Events

Should any participant in the study suffer an unexpected serious adverse event this will be reported to R&D by the Chief Investigator within 24 hours of the event.

# Statistics

## Statistical methods to be employed (plan of analysis)

This is an optimisation and feasibility study; based on previous experience with protocol optimisation we envisage that 20 samples will be adequately powered to fully optimise the organotypic model.

# Data Management

## Source Documents

At the time of enrolment into the study all participants will be assigned a study number and this will be the only identifiable information that will accompany the sample. For the purposes of cross-reference, details of the study participant relating to the assigned study number will be stored on secure drivers on the hospital system and only accessible to members of the research team. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/code and not by name.

## Direct Access to source data / documents

Only members of the study research team and authorised representatives from the sponsor will have direct access to the source data and study documentation. All source data and study documentation will also be available to external auditors if and when required, and inspectors in the event of regulatory inspection. Access to the final data set will remain with the chief investigator

## Data Recording and Record Keeping

All data will be recorded solely on GOSH computers within the hospital and stored on secure NHS servers in compliance with all data management protocols and stored for approximately ten years.

### **Archiving**

Archiving will be authorised by the Sponsor following submission of the end of study report.

Essential documents will be retained for a **minimum** of 5 years after completion of the study. These documents will be retained for longer if required by the applicable regulatory requirements.

# Patient Confidentiality & Data Protection

Patient identifiable data, including initials, date of birth and NHS number will be required for the registration process. The study staff will ensure that the participants’ anonymity is maintained. The participants will be identified only by initials and a participants ID number on the CRF and any electronic database. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

Data will be stored in a secure manner and in accordance with the Data Protection Act 1998.

# Sample Collection, Storage, Transfer and Analysis

In selected epilepsy cases (as discussed above) in which the hemisphere is undergoing disconnection and the wall of the lateral ventricle is accessible, samples will be taken for analysis. The size of samples will depend upon the access to the ventricular wall but we envisage taking approximately 1 cubic centimetre samples.

Samples will be taken from different regions of the wall of the lateral ventricle, for example the temporal horn, trigone and frontal horn of the lateral ventricle, depending on the type of surgery undertaken and accessibility to the ventricular wall. All samples will be stored and labelled separately and the position from which the sample was taken will be recorded using both a screen shot from the stealth guidance system and a screen shot from the operative microscope.

**PHASE 1:** *Structural and functional imaging of the ependyma and SVZ*

Intraoperative samples will be immediately fixed in 4% Paraformaldehyde (4% PFA) in theatre and cryopreserved in 30% sucrose for at least 24 hours.

Following fixation and cryopreservation samples will be analysed as both whole mounts(1) and cross sectional samples. To determine optimal conditions for analysis, samples will be embedded in both OCT and Paraffin and sectioned using the cryostat or vibrotome respectively.

Structural analysis looking at the anatomy of the ependyma and SVZ and functional analysis looking at proliferative activity and cell population analysis within the SVZ will be undertaken. For example, layer thickness and cellularity will be determined using H&E and Nissl staining, structural anatomy of the SVZ will be further analysed using immunohistochemistry (peroxidase and fluorescence as appropriate) for example using GFAP, Vimentin and βIII Tubulin, anatomy of the ependyma will similarly be assessed using CD99 and Limax Flavus Agglutinin as described by Domínguez-Pinos et al(19). Proliferation within the SVZ will be quantified with ki67 and PH3, and cellular identity will assessed with CD133, SOX2 & MASH1 (stains for neural stem progenitor cells) NeuN, Calretinin, Calbindin NCAM (Neuronal) & Olig2 (oligodendrocytes). Microglial response will be quantified with Iba1.

Samples will also be analysed using scanning electron microscopy to determine the anatomy of the ventricular surface.

**PHASE 2:** *Dynamic imaging of the wall of the lateral ventricle*

Intraoperative samples are immediately placed into appropriately labelled tubes containing cooled artificial CSF:

|  |  |
| --- | --- |
| Formulation of culture medium used for organotypic preparations | Final Concentration |
| BME – Basal Medium Eagle | 66% |
| Hanks | 25% |
| FBS – fetal bovine serum (non heat activated) | 5% |
| 50% Glucose | 1.32% |
| Penicillin Streptomycin Glutamine | 1% |
| N2 supplement | 1% |

*Table showing the relative concentrations of the different constituents of the artificial CSF used for preparation of the organotypic slice preparations.*

Tissue samples are held in a hypoxic environment, within a time-lapse confocal microscope. Using a fluorescently labeled virus, dividing cells in the SVZ can be labeled thus allowing analysis of the population of cells actively dividing with the SVZ. By combining this approach with changes in the microenvironment, the impact of blood in the CSF can be quantified. Using the time-lapse imaging allows these changes to be followed dynamically to give us an understanding of the time frame through which damage to the SVZ occurs. Similarly, using an ultrahigh speed camera in combination with CD44 fluorescent labeling of the cilia the beat frequency and behaviour of the cilia to changes in the microenvironment can be studied in detail(25). Quantification will be undertaken using the optical fractionater probe of Stereoinvestigator.

Following dynamic imaging as discussed above all samples will be further analysed using the techniques optimised in phase 1, to further determine the impact of haemorrhage on the ependyma and neural stem progenitor cells within the SVZ. Tissue, which is surplus to this initial analysis, will be stored at -80 in freezer space allocated by Professor Tom Jacques. Samples will be retained for the duration of the project and further project-based applications will be made to continue the project.

Samples will be stored in research laboratories within Great Ormond Street Hospital or the Institute of child health and used for research for up to 15 years, after which time any remaining samples will be destroyed.

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Cover for negligent harm will be provided by the Great Ormond Street Hospital for Children NHS Foundation Trust through the Clinical Negligent Scheme for Trusts (CNST).

# Publications Policy

We intend to publish the data in high impact factor peer reviewed scientific journals and present the results of the analysis at relevant conferences.