Science and Technology in Medicine

Research Project Proforma (School of Medicine)

Research Title:	**The development of novel methods to characterize thrombi as a source of clinically relevant data in acute ischaemic stroke**
Keywords (up to 5)	**Stroke, thrombi, ischamia**
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Type of projects offered (delete as appropriate)	Intercalation (1 year)/Studentship (4-8 weeks)/Both

(1) Outline the broad aims of your research and its medical relevance (150 words):

Approximately 80% of all acute ischaemic strokes are due to intracranial artery occlusion (1) and early restoration of blood flow to the ischaemic brain is pivotal to good recovery and improved outcomes (2). Intravenous thrombolysis (IVT) is the only officially recognised acute stroke treatment worldwide, and is now used in 8% of UK stroke patients (3).

The apparent resistance of some clots to thrombolytic agents supports the idea that clots are heterogeneous in nature. Gathering information on the nature of clots found in stroke patients is important as this could help to identify types of thrombi which may or may not respond to a particular form of treatment such as thrombolytics and/or mechanical extraction. Little has been published on the nature of clots in humans particularly those

pertaining to stroke. Two relatively recent publications have provided information on histological aspects of thrombi in stroke, but these were limited in terms of scope and patient numbers (4, 5).

This study will use a novel multimodal approach to the characterization of thrombi. This will provide us with a better understanding of thrombus structure and component parts and ultimately may lead to the development of non-invasive techniques to analyse thrombi which could allow clinicians to focus treatments on those most likely to respond (6, 7). Therefore, in addition to the important research questions to be addressed in this project, the development of techniques to characterize thrombi is an important preparatory stage of a proposed clinical trial of thrombi removed from acute stroke patients, which will utilize the techniques developed here.

The aims of this study are to analyse and compare porcine thrombi that have been aged or exposed to commonly used thrombolytic agents at varying concentrations and time periods using a multimodal approach. This study is being conducted in three main stages:

1. Further development of apparatus to measure the elasticity of whole clots.

2. Use of existing equipment and techniques to analyse various characteristics of thrombus composition.

3. Comparison and analysis of thrombi exposed to different treatments.

(2) Indicate the skills/techniques the student will learn (100 words)

This project will require the use of a range of techniques including:

Histology and immunohistochemistry

Thrombi will be fixed for histology and histological sections will be stained and then digitally recorded using a microscope and attached camera. Subsequently, thrombi will be classified on the basis of the main components observed such as red (RBC-rich) white (platelet-rich) or mixed.

Microscopy

Light microscopy and laser confocal microscopy will be used on the paraffin sections to identify and analyse the constituent components of the thrombi

Spectroscopy

Confocal Raman spectroscopy can provide data on amino acid residues within sections and these are linked to a database that can identify specific proteins including probable concentrations.

Mechanical assays

For clots already formed we will need to continue development of apparatus that will allow quantitative measures of important physical properties. These will be based on one or more of designs already researched that make use of relatively simple apparatus that can be built in-house.

Pulsatile flow system and cerebral circulation model

Pre-treated thrombi will be exposed to pulsatile flow for varying times to determine if this has any effect on their characteristics compared to controls. Various thrombectomy devices can be used with the system.

References

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2. Rha JH, Saver JL. The Impact of Recanalization on Ischemic Stroke Outcome: A Meta-Analysis. Stroke. 2007;38(3):967-73.
3. Thomassen L, Bakke SJ. Endovascular reperfusion therapy in acute ischaemic stroke. Acta Neurologica Scandinavica. 2007;115:22-9.
4. Marder VJ, Chute DJ, Starkman S, Abolian AM, Kidwell C, Liebeskind D, et al. Analysis of thrombi retrieved from cerebral arteries of patients with acute ischemic stroke. Stroke. 2006;37(8):2086-93. Epub 2006/06/24.
5. Niesten JM, van der Schaaf IC, van Dam L, Vink A, Vos JA, Schonewille WJ, et al. Histopathologic composition of cerebral thrombi of acute stroke patients is correlated with stroke subtype and thrombus attenuation. PLoS One. 2014;9(2):e88882. Epub 2014/02/14.
6. Liebeskind DS, Sanossian N, Yong WH, Starkman S, Tsang MP, Moya AL, et al. CT and MRI Early Vessel Signs Reflect Clot Composition in Acute Stroke. Stroke. 2011;42(5):1237-43.
7. Minnerup J, Kleinschnitz C. Visualization of Clot Composition in Ischemic Stroke: Do We Get What We See? Stroke. 2011;42(5):1193-4.