MODULATION OF INFLAMMATION IN INTRACEREBRAL HAEMORRHAGE

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A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Life Sciences
May 2014
ACKNOWLEDGEMENTS

Thank you to my supervisors Professor Stuart Allan, Professor Andrew King and Dr. Adrian Parry-Jones for their very patient help and guidance. Thank you to Sharon Hulme for the same. Thank you also to Professor Dame Nancy Rothwell, Professor Pippa Tyrrell and the Heurosurgical Research Fund at Hope Hospital for providing part of the funding towards my studies. Above all, thankyou to my parents Meena and Shafique, Linda and Rick, and to my wife Amy for all their love and support.
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LIST OF ABBREVIATIONS AND ACRONYMS

1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-1-isoquinoline

$[^{11}C]$PK11195  carboxamide

$[^{18}F]$DPA-714  tracer

ABC/2  Formula for haematoma volume estimation

ADC  apparent diffusion coefficient

AF  Atrial Fibrillation

AHA  American Heart Association

APTT  Activated partial thromboplastin time

ASA  American Stroke Association

ATACH  Antihypertensive Treatment of Acute Cerebral Haemorrhage

AVM  Arteriovenous Malformation

CAA  Cerebral Amyloid Angiopathy

CAMARADES  Collaborative Approach to Meta-analysis and Review of Animal Data in Experimental Studies

CHA(2)DS(2) -VASc  Clinical prediction score

Clinical prediction score (Congestive heart failure, Hypertension, Age, Diabetes, Stroke/TIA/thromboembolism)

CNS  Central Nervous System
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>CNS</td>
<td>Canadian Neurological Scale</td>
</tr>
<tr>
<td>CONSORT</td>
<td>CONsolidated Standards Of Reporting Trials</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>CT</td>
<td>Computerised Tomography</td>
</tr>
<tr>
<td>DCE</td>
<td>Dynamic Contrast Enhancement</td>
</tr>
<tr>
<td>DFX</td>
<td>Deferoxamine</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>END</td>
<td>Early neurological deterioration</td>
</tr>
<tr>
<td>EUSI</td>
<td>European Stroke Initiative</td>
</tr>
<tr>
<td>FAST</td>
<td>rFVIIa for Acute Haemorrhagic Stroke Treatment</td>
</tr>
<tr>
<td>FFP</td>
<td>Fresh Frozen Plasma</td>
</tr>
<tr>
<td>GCS</td>
<td>Glasgow Coma Scale</td>
</tr>
<tr>
<td>GMNC</td>
<td>Greater Manchester Neurosciences Centre</td>
</tr>
<tr>
<td>GOS</td>
<td>Glasgow Outcome Scale</td>
</tr>
<tr>
<td>GRE MRI</td>
<td>Gradient Echo MRI</td>
</tr>
<tr>
<td>HTN</td>
<td>Hypertension</td>
</tr>
<tr>
<td>ICH</td>
<td>Intracerebral Haemorrhage</td>
</tr>
<tr>
<td>ICP</td>
<td>Intracranial pressure</td>
</tr>
<tr>
<td>IL-1</td>
<td>Interleukin-1</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>IL-1Ra</td>
<td>Interleukin-1 Receptor Antagonist</td>
</tr>
<tr>
<td>IL-6</td>
<td>interleukin 6</td>
</tr>
<tr>
<td>INR</td>
<td>International Normalised Ratio</td>
</tr>
<tr>
<td>INTERACT</td>
<td>Intensive Blood Pressure Reduction in Acute Cerebral Haemorrhage</td>
</tr>
<tr>
<td>IVH</td>
<td>Intraventricular Haemorrhage</td>
</tr>
<tr>
<td>LREC</td>
<td>Local Research Ethics Committee</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean Arterial Pressure</td>
</tr>
<tr>
<td>MIF</td>
<td>microglial inhibitory factor</td>
</tr>
<tr>
<td>MISTIE</td>
<td>Minimally Invasive Surgery plus t-PA for ICH Evacuation</td>
</tr>
<tr>
<td>MRA</td>
<td>Magnetic Resonance Angiography</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MRIS</td>
<td>Medical Research Information Service</td>
</tr>
<tr>
<td>NADPH Oxidase</td>
<td>nicotinamide adenine dinucleotide phosphate-oxidase</td>
</tr>
<tr>
<td>NF κ-β</td>
<td>nuclear factor kappa-light-chain-enhancer of activated B cells</td>
</tr>
<tr>
<td>NHS</td>
<td>National Health Service</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institute of Health</td>
</tr>
<tr>
<td>NIHSS</td>
<td>National Institute of Health Stroke Scale</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>OAT-ICH</td>
<td>oral anticoagulant therapy-associated ICH</td>
</tr>
<tr>
<td>PCC</td>
<td>Prothrombin complex concentrates</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>PI</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>PMNL</td>
<td>Polymorphonuclear Leukocytes</td>
</tr>
<tr>
<td>PT</td>
<td>Prothrombin Time</td>
</tr>
<tr>
<td>rFVIIa</td>
<td>recombinant Factor VIIa</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>rt-PA</td>
<td>recombinant Tissue Plasminogen Activator</td>
</tr>
<tr>
<td>SAH</td>
<td>Subarachnoid Haemorrhage</td>
</tr>
<tr>
<td>SINAP</td>
<td>Stroke Improvement National Audit Program</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>SPARCL</td>
<td>Stroke Prevention by Aggressive reduction of Cholesterol Levels</td>
</tr>
<tr>
<td>SRH</td>
<td>Salford Royal Hospital</td>
</tr>
<tr>
<td>STAIR</td>
<td><a href="#">Stroke Treatment Academic Industry Roundtable</a></td>
</tr>
<tr>
<td>STICH</td>
<td>Surgical Trial in Intracerebral Haemorrhage</td>
</tr>
<tr>
<td>SWI</td>
<td>Susceptibility-weighted imaging</td>
</tr>
<tr>
<td>TGF-β</td>
<td>transforming growth factor beta</td>
</tr>
<tr>
<td>TNFα</td>
<td>Tumour Necrosis Factor alpha</td>
</tr>
<tr>
<td>WCC</td>
<td>White Cell Count</td>
</tr>
<tr>
<td>WMIC</td>
<td>Wolfson Molecular Imaging Centre</td>
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</tbody>
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ABSTRACT

Kamran Ateeque Abid - University of Manchester

Modulation of Inflammation in Intracerebral Haemorrhage

Intracerebral Haemorrhage (ICH) exhibits the worst mortality and morbidity of any stroke subtype. There are no efficacious treatments for this condition and little improvement in patient outcome has been noted despite advancements in medical science over the previous three decades. Furthermore, current available data is increasingly obsolete as the population suffering the disease burden rapidly ages and develops co-morbidities. It is thought that future therapies for this condition may be able to target neuroinflammatory response triggered by the formation of brain haemorrhage however there is little published evidence that has examined this aspect of ICH pathophysiology.

This study therefore examines the current prognosis of a large cohort of patients with ICH to determine the key factors which result in mortality. We find that patients treated at a specialist centre have a surprising and significantly improved survival advantage. Since clinical practice in the United Kingdom is widely influential, the second part of the study focuses on whether the optimal cases are currently being transferred to these centres. The next part of the study then uses MRI/PET brain imaging for the first time in patients with ICH to establish an important link between the processes of neuroinflammation and Blood-Brain-Barrier breakdown. Finally, the concluding part of the thesis presents functional and radiological data from a rat model of ICH in which the inflammatory cascade has been modulated by the use of an antagonist against IL-1. The thesis thus presents a novel and important contribution in our present understanding of the preclinical and clinical disease process.
DECLARATION

This dissertation is the result of my own work and includes nothing, which is the outcome of work done in collaboration except where specifically indicated in the text. No portion of the work has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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Dr. Kamran Ateeque Abid BSc (Hons) MB ChB MPhil (Cantab) MRCS

01st of January 2015
1 Introduction
Spontaneous Intracerebral Haemorrhage (ICH) is defined as the non-traumatic, abrupt onset of an altered level of consciousness or focal neurological deficit associated with a focal collection of blood within the brain on neuroimaging or at autopsy, which is not due to trauma or haemorrhagic conversion of a cerebral infarction and which may be associated with severe headache[1].

Hospital admissions for ICH have increased by 18% over the previous ten years [2] and the scale of the problem is expected to increase further over the coming decades with an increasingly elderly population, many of whom exhibit poor blood pressure control, susceptibility to risk factors such as Cerebral Amyloid Angiopathy (CAA) and greater use of anticoagulants, antiplatelet agents and thrombolytics [3]. Recent evidence suggests that primary prevention is improving, however case fatality and long-term mortality remain unchanged [4].

Depending on bleed site and severity, ICH patients may have symptoms ranging from mild headache to severe paralysis, though typically this form of stroke results in severe neurological injury. There are currently no effective medical or surgical treatments for ICH and death or major disability occur more often than either Subarachnoid Haemorrhage (SAH) or cerebral infarction [5].

There has been increasing interest in the role that inflammatory processes may play in underpinning neurological injury [6, 7], including ICH, which may provide new therapeutic targets.
1.1 Stroke

Overall, ICH represents 10-15% of all strokes that occur in the US, Europe and Australia and 20-30% of all strokes that occur in Asian countries[8]. In England alone, combined stroke costs the National Health Service (NHS) over £3 billion a year, is one of the top three causes of death and is the single largest cause of adult disability [9]. In the majority (80%) of cases of combined stroke, the cause is due to a critical interruption of the blood supply to the brain from a thrombo-embolic event resulting in ischaemia or infarction [10].

The aim of acute treatment in ischaemic stroke is to re-establish perfusion through recanalisation in order to limit the amount of ischaemic tissue produced. If this is done in a time-critical manner, changes in the penumbral area surrounding the ischaemic core may be reversed and subsequently limit the neurological deficit suffered by the patient. Treatment with tissue plasminogen activator (rt-PA) is now a common treatment for ischaemic stroke, though use of this thrombolytic therapy may in itself cause an unintended ICH. Newer therapies directed against interleukin-1 (IL-1) in both ischaemic stroke and SAH, a key component of the inflammatory cascade, have shown promise in both preclinical and clinical studies [11, 12].

Less commonly, tissue infarction in stroke may also arise following spontaneous vessel rupture resulting in ICH formation, however no efficacious treatments currently exist for this condition.
1.2 ICH

ICH represents a substantial public and economic health problem worldwide [13, 14]. The reported incidence of ICH varies depending on definition (depending on whether haemorrhage due to vascular malformations, anticoagulants, thrombolytic agents or illicit drugs is included) and methodological differences in case ascertainment. Incidence is reported to be 10-20 per 100,000 amongst Caucasians [15, 16] and more common still amongst black, Japanese, Latin Americans, and Chinese populations [17-21].

1.2.1 Outcomes after ICH

The demographic profile of ICH is constantly evolving [371] however amongst all forms of stroke ICH is associated with the highest mortality and morbidity [22-24]. Risk is slightly greater in men [25, 26] and varies with the bleed site amongst different populations [27]. Mortality after ICH at 3 months was reported as 34% in one review of 586 patients with ICH from 30 centres [3] though other studies have shown mortality can routinely approach figures as high as 50% at 30 days [28-30]. Subsequent mortality was 59% at one year post-ICH, 82% at 10 years and more than 90% at 16 years, corroborated by other studies including a recent meta-analysis [31] [23, 24]. Amongst survivors, between 80-90% are left with disability, the majority of which are major neurological deficits allowing only 20% of ICH survivors to regain functional independence [22, 32-35].
1.2.2 Risk factors for ICH

**Non-modifiable risk factors**

Historically, classification of ICH into either primary or secondary groups has been advocated. Primary ICH, accounting for 70-80% of cases, develops in the absence of any underlying vascular malformation or coagulopathy and is generally caused by hypertensive arteriosclerosis in deep brain sites [36]. In lobar brain regions, however, ICH may be unrelated to hypertensive vasculopathy, arising instead from CAA (Cerebral Amyloid Angiopathy) [37]. These cases are characterised pathologically by deposition of amyloid protein in the media and adventitial layers of cortical, subcortical and leptomeningeal vessels [38-41] which renders them prone to rupture following minor trauma or following sudden changes in blood pressure [42].

Secondary ICH may be due to structural lesions such as underlying tumours, arteriovenous malformations (AVMs), cavernous malformations, dural arteriovenous fistulae, capillary telangiectasias and berry aneurysms [43, 44]. Microvascular abnormalities such as leukoaraiosis and asymptomatic cerebral microbleeds have been correlated with ICH during anticoagulation, though data regarding these conditions remains too scarce to construct robust prognostic models or treatment guidelines [45]. Secondary ICH also encompasses disease processes responsible for acute hypertensive changes such as eclampsia in pregnancy or use of sympathomimetic drugs [46].

There may be significant overlap between primary and secondary groups (such as an alcohol-induced coagulopathy exacerbating the effects of an amyloid bleed) and the grading has thus served little purpose in evaluating the clinical effects of the haematoma. Furthermore, the “Boston criteria” used to validate the diagnosis of CAA in elderly patients with lobar ICH have only been validated on a cohort of 13 patients which may render their use clinically inaccurate.
[47, 48]. For both these reasons, the distinction between primary and secondary causes is regarded as increasingly outdated.

A meta-analysis reviewing five ICH cohort studies [26] has established patient age as the most important risk factor for development of ICH. In the elderly, CAA is estimated to account for more than 20% of all ICH in those aged over 70 [38, 49].

In sporadic cases of hypertensive and CAA-associated ICH, an estimate of 47% of risk is attributable to non-modifiable risk factors [50].
Modifiable risk factors

The most important modifiable risk factor for ICH has been demonstrated to be hypertension [51], present in 50-70% of patients developing ICH [26]. Hypertension is predominantly associated with bleeds that occur in deep cerebral and brainstem structures [27, 52] and there is much weaker association with lobar haemorrhage[50]. This finding is consistent with the absence of an effect of hypertension on risk of recurrent lobar ICH in a cohort of lobar ICH survivors [53].

The presence of hypertension confers a higher risk of ICH in younger patients (aged 45 or less) in comparison to the elderly (in whom CAA is more common) [54, 55]. There is evidence from large population-based studies that incidence may also be lower in some populations with improved access to medical care and blood pressure control [19, 21, 56]. Qureshi and colleagues [17] also reported an association of lower levels of education with higher incidence of ICH, which they attributed to possible lower awareness of primary health care value in this group, however there are no corroborating studies to support this observation.

Modifiable lifestyle factors such as alcohol consumption have also been reported to increase the risk of spontaneous ICH [57, 58] with both binge-pattern and chronic abuse [59, 60]. A role for the effect of cigarette smoking as a risk factor for ICH has been suggested [61-63] and some case-control studies have also identified diabetes as a risk factor. A systematic review described an overall significant risk ratio of 1.31 (95% CI, 1.09-1.58) and 1.30 (95% CI, 1.02-1.67) for both of these factors however other data have been conflicting [26, 64]. Recent studies have suggested that current smokers may increase their risk in a dose-dependent manner [61, 62, 65].

The role of cholesterol as a risk factor for ICH remains unclear. Case-control studies have reported an association between increased ICH risk and low cholesterol levels [26], perhaps through reduced platelet aggregation, vascular strength or nutritional deficiencies [66].
study is currently evaluating the use of statin therapies to improve ICH outcome, likely due to their anti-inflammatory actions rather than the absolute lowering of cholesterol levels [57]. On the other hand, the 2006 Stroke Prevention by Aggressive reduction of Cholesterol Levels (SPARCL) trial reported a statistically significant increase in mortality from haemorrhagic stroke amongst the group treated with Atorvastatin [67] [68].

In comparison to an absolute risk of 0.15% ICH risk in patients not taking antiplatelet agents, an increased risk of 0.2-0.3% was found in elderly patients prescribed this medication. Furthermore, patient age, drug dosage and the use of multiple antiplatelet agents were found to be the most potent risk factors in this group [69-71].

Use of Warfarin, most commonly employed for the prevention of ischaemic stroke in patients with atrial fibrillation (AF) [72-75], quadrupled its distribution on a per capita basis during the 1990s [76]. Concomitantly, the incidence of oral anticoagulant therapy-associated ICH (OAT-ICH) also approximately quadrupled [77, 78]. Most trials involving warfarin for the treatment of AF or MI report the risk of OAT-ICH between 0.3% - 1.0% per patient year [79, 80] and a relative risk of 7-10 in comparison to non-anticoagulated patients [81-83]. In anticoagulated patients in whom ICH arises, the first episode usually occurs shortly after the commencement of anticoagulant administration (median 14 months [84]). Both clinical trial and community surveillance data show that OAT-ICH risk is increased in patients aged over 75, those with hypertension (in particular if the systolic blood pressure is above 160 mmHg), a history of cerebrovascular disease, higher intensities of anticoagulation and concomitant Aspirin use [79, 80, 82, 85]. The CHADS2 and CHA(2)DS(2)-VASc clinical prediction scores have been developed to help selectively target patients who may benefit from anticoagulation for non-rheumatoid AF [86].

Thrombolysis, regarded as the most potent anticoagulatory acute treatment, is most commonly administered to patients undergoing myocardial infarctions, and is complicated by an ICH rate of 0.4-1.5% across the various regimens employed [87-89]. The risk is magnified by age, female

Introduction
sex, hypertension, black ethnicity or in those with a low body weight, history of prior stroke or excessive anticoagulation [87-89]. With the increasing use of routine early coronary angioplasty the incidence of ICH in this group has decreased [90]. Thrombolysis for ischaemic stroke is also now an established therapy and in this group the 3-month mortality in patients developing subsequent ICH remains between 5.3% and 9.3% [91]. Use of rt-PA, a history of congestive heart failure, extent of parenchymal hypoattenuation on baseline CT, increasing age and protocol violations are associated with increased risk of post-thrombolysis ICH [92, 93].
1.3 Clinical characteristics of ICH injury

Clinically, ICH patients present with a sudden onset of a focal neurological deficit which may be associated with signs of increasing intracranial pressure such as headache, nausea, vomiting or decreased conscious level [94]. Further delineation of the clinical syndrome and associated deficits are highly dependent on bleeding site with infratentorial and intraventricular bleeding resulting in worsened prognosis [95].

Early neurological deterioration (END), most likely to occur within 48 hours of ictus, occurs in between 20-40% of ICH patients and is associated with a subsequent poor prognosis [96, 97]. Objective assessment of deterioration can be defined with clinical scales such as the National Institute of Health Stroke Scale (NIHSS) or the Canadian Neurological Scale (CNS) [98].

The progression of observed neurological deterioration has previously been postulated to be due to the development of cerebral oedema around a static clot [99]. Baseline oedema volume has been shown in a prospective observational cohort of 142 patients to be the strongest predictor of outcome [100] however a separate study using multivariate analysis to examine oedema and haematoma volume did not corroborate those results [101].

An important sentinel prospective study demonstrated the importance of ongoing bleeding and haematoma expansion alone in early neurological deterioration [102]. Amongst these patients, all presenting within 3 hours of ictus, over a quarter had a significant (defined as growth of more than 33%) haematoma expansion within an hour of the baseline CT and an additional 12% of patients exhibited further haematoma expansion at 20 hours. Subsequent large prospective studies have conclusively confirmed the deleterious effects of dynamic haematoma expansion [103-105].
The presence of hyperthermia, high bleed volume and measures of an acute inflammatory response, including neutrophil count, are associated with END [96, 106]. In addition, the volume of the haematoma and the presence of Intraventricular Haemorrhage (IVH) are powerful predictors of outcome [107, 108]. Immediate seizures in ICH patients have a frequency of less than 10% for all bleeding sites combined [109, 110]. However, lobar ICH (especially within the frontal lobe) is associated with increased risk of seizures within 30 days of ICH onset [111-115]. An acute hypertensive response found immediately after ICH is characterised by its high prevalence, self limiting nature and prognostic significance [116]. Although this hypertensive episode typically resolves spontaneously over the next 24 hours [117], high arterial pressure in ICH patients has been shown to be associated with high early mortality rates [29, 118].

Therapeutic aims following imaging concentrate on the tripartite approach of managing the physiological effect of the blood load deposited, the prevention of further haematoma expansion and finally the consideration of clot removal. Trials directed against a single factor have been physiologically successful but have delivered little clinical benefit [104, 119]. A salient observation is that in-hospital mortality is lower in patients admitted to an intensive-care neurology unit [120] and also in those admitted to urban teaching hospitals [121] which provides evidence that aggressive, well-organised multimodal therapy may make a significant difference to patient outcome.
1.4 Diagnostic Imaging

Computerised tomography (CT) scanning is widely used in the diagnosis of ICH as it is cheap, fast, widely available and has a sensitivity and specificity approaching 100% for the detection of acute blood [122]. Bleed volume, which is an important predictor of mortality and morbidity [28], can be estimated with the ABC/2 method [123] or planimetric methods in both CT and Magnetic Resonance (MR) imaging. Both of these methods are more widely validated in CT scanning in comparison to MR although in clinical practice neither method is commonly employed as estimates of bleed volume are rarely recorded, or indeed sought. CT Angiography (CTA) is a valuable adjunct to detect underlying vascular abnormalities [124]. The ‘Spot sign’ describes small enhancing foci on axial CTA images which may be used to help predict haematoma expansion [125]. Disadvantages of CT –based diagnosis may include radiation exposure, logistical problems in moving the patient, contrast reaction where used and even rebleeding through a transient increase in contrast-laden arterial pressure [126].

MRI is less widely available in ICH, more expensive, has longer scan times and is contraindicated in patients with pacemakers or metallic prostheses. However MRI has superior sensitivity for detection of early cerebral ischaemia and higher field strength scans have improved the ability to diagnose acute ICH to levels comparable with CT and is therefore a viable tool for the investigation of acute and chronic ICH[127]. In particular, MR may be used to date ICH bleeds since blood breakdown products produce reliably predictable paramagnetic residues over time. Historically, MRI was thought to be of inferior sensitivity for detection of acute ICH in comparison to CT due to the complex interactions between these breakdown products and acute blood. Modern scanners, however, incorporate novel MR sequences such as susceptibility-weighted imaging (SWI) that allow the accurate differentiation between blood in the intravascular and extravascular spaces without significant image degradation. Furthermore,
small vascular lesions which may have been missed on conventional CT imaging may be
detected by MR [128-130]. Small and recurrent haemorrhages may also be detected with the
technique of Gradient Echo MRI (GRE MRI) [131, 132]. This sequence is particularly useful for
the detection of hyperacute (<6hr) blood which is sensitive to static magnetic field
inhomogeneity [133-135]. Investigation of ICH patients with MR scanning has thus been
recommended in those patients with lobar haemorrhage or who are younger than 45 with no
history of hypertension (the “Hong Kong” criteria)[136]. Further examination of cerebral
vasculature can be augmented with MR Angiography (MRA) which has high sensitivity and
specificity in screening for underlying disorders in ICH [137].
1.5 Management and Critical Care

**Medical management**

Initial medical stabilization must begin with appropriate resuscitative measures before a neurological assessment including appropriate imaging is initiated to grade the severity of injury. Patients with ICH injury have previously been demonstrated to have a decreased likelihood of admission to specialist acute stroke units and to receive a poorer standard of care in comparison to patients with ischaemic stroke [138-140]. Research data has not, in general, been widely adopted in the clinical setting [46, 138, 141-143]. Treatment decisions have generally encompassed the tripartite approach of managing intracranial hypertension, modulation of systemic blood pressure and achieving haemostasis.

**Clinical grading scales**

Overall prognosis is significantly worsened by low GCS at presentation, age, bleed location, volume of bleed at presentation and the presence of intraventricular extension amongst other factors [95]. Clinical grading scales have become increasingly important for standardising assessment, communication, and management amongst clinicians. Initial multivariate models addressing ICH outcome concentrated on 30-day mortality and identified patient age, GCS, ICH size and IVH (Intra-ventricular Haemorrhage) as the key determinants of short-term outcome [95].

Subsequently, over 25 further scoring systems have been created in order to elucidate a scale that is both simple, generally applicable and predictive of mortality and functional outcome [144]. Although many have been developed with clinically and statistically sound methodologies it remains unclear which of the systems has the most validity post-ICH. A recent paper has
demonstrated the importance of the inflammatory marker, C-Reactive Protein (CRP), in significantly improving prognostic accuracy in ICH patients – particularly those deemed low/medium risk with other scoring systems [145].

Intracranial hypertension

Mass effect from the haematoma, oedematous tissue and obstructive hydrocephalus resulting in intracranial hypertension are the major causes of death in the first few days following ICH [128, 146]. Monitoring ICP may be of benefit [22, 128, 147] in controlling cerebral perfusion pressure between 50 and 70 mmHg in order to improve outcome [147]. A randomised control trial has not shown benefit in neurological improvement functional outcomes or mortality following osmotherapy from regular use of intravenous mannitol boluses [148]. For refractory elevation in ICP, pharmacologically induced coma or decompressive hemicraniectomy may be employed [149-151].

Both ASA Stroke Council [152] and European Stroke Initiative (EUSI) [153] guidelines recognise the paucity of definitive trial evidence but recommend the use of ICP monitoring and the selective use of mannitol, hypertonic saline and short term hyperventilation to maintain cerebral perfusion pressure (CPP).

Blood pressure management

The treatment of hypertension, the commonest cause of spontaneous ICH and acutely associated with haematoma enlargement and poor outcome [149], is of considerable importance however the therapeutic goal remains controversial [154, 155]. In a recent analysis of 45,330 patients presenting with ICH [156], 75% had a systolic blood pressure of more than 140 mmHg and 20% had systolic blood pressure of more than 180 mmHg at presentation.
The previous caveat of disturbing the perihaematomal penumbral zone, postulated to be particularly vulnerable to ischaemic injury, appears not to be supported by CT perfusion, MRI and Positron Emission Tomography (PET) studies [157, 158]. This is corroborated by recent data which suggests that an aggressive reduction of systolic blood pressure may show overall therapeutic benefit [159].

The Antihypertensive Treatment of Acute Cerebral Haemorrhage (ATACH) [160] and the Intensive Blood Pressure Reduction in Acute Cerebral Haemorrhage (INTERACT) phase II trial [103] reported that the reduction of systolic blood pressure to less than 140 mmHg decreased the rate of haematoma enlargement without increasing adverse events. No difference in death or disability at three months was seen in either trial although analyses were limited by sample sizes between approximately 1,300 and 2,800 patients followed up for 90 days. Subgroup analysis from INTERACT furthermore indicated that patients recruited within 3 hours and those with an initial systolic blood pressure of 181 or higher appeared to have the greatest benefit from treatment. Recruitment for the Phase III INTERACT2 trial is currently underway. This approach to blood pressure reduction has been reflected in the most recent American Stroke Association (ASA) guidelines [161] however the precise target blood pressure, duration of therapy and effect on clinical outcome all remain undetermined. Individuals with a pre-existing history of hypertension are at greater risk of critical hypoperfusion at mean arterial pressure (MAP) levels well tolerated by normotensive individuals [162].
Haemostasis

Coagulopathy, usually warfarin related, is also associated with haematoma expansion and resulting poor prognosis [163]. The earlier the first scan is obtained, the more likely subsequent bleeding will be detected on a follow-up scan [164, 165] which suggests that effective haemostatic therapy is time critical [166, 167].

Historically, the use of Vitamin K and Fresh Frozen Plasma (FFP) have been recommended to reverse OAT coagulopathies, however the use of prothrombin complex concentrates (PCCs) have lately emerged as potentially improved therapies. These have the advantage of rapid reconstitution and administration using smaller volume infusions with a better safety profile [168-170]. Nonrandomised retrospective reviews and a small case-control study have shown more rapid correction of INR with fewer adverse events when PCC is used in conjunction with Vitamin K in comparison to FFP however no difference in clinical outcome was noted [171-173].

There was also little evidence for improved outcome in patients with acute ICH injury receiving the haemostatic agent recombinant Factor VIIa (rFVIIa) [104] despite data from an earlier phase II trial [105] that noted increase in haematoma volumes of only 11-16% in treatment groups in comparison to 29% in placebo groups with corresponding 90 day mortality of 18% compared to 29%. Subgroup analysis in the phase III rFVIIa for Acute Haemorrhagic Stroke Treatment (FAST) trial suggested a potential benefit for younger patients with a haematoma volume below 60 mLs and treated within 2.5 hours of onset [174] although an increased frequency of thromboembolic events was also noted (7% vs 2%). The disappointing results obtained for single-modality therapies in this multifactorial disease were emphasised.

Dabigatran is a direct thrombin inhibitor which has recently been approved for the prevention of ischaemic stroke in patients with atrial fibrillation [175]. Knowledge on how to acutely
reverse the effects of this novel agent is limited and complicated by the fact that there is currently no true antidote for the drug [176].
1.6 Surgical management

Although evacuation of haematoma as a treatment in ICH has been available for many years, there remains equipoise about which patients are most likely to derive benefit, if any. Surgical evacuation of the haematoma may theoretically prevent further expansion, decrease mass effect and remove the toxigenic stimulus of the inflammatory haem-thrombin complex however, although there have been encouraging trends noted in mortality rates, there is no firm conclusion that can be drawn from the RCTs and hundreds of observational studies that have addressed the role for surgery in ICH. All RCTs to date have only addressed supratentorial haemorrhage and have acknowledged the significant incidence of severe disability that may result even where mortality rates have improved [177, 178].

The landmark Surgical Trial in Intracerebral Haemorrhage (STICH) trial [119, 179] is the largest completed randomised control trial comparing early surgical treatment (with a median operative time of 20 hours post-ICH) to medical treatment. 1033 patients were randomly assigned to treatment arms across multiple centres in the study. Results at 6 months indicated that no significant benefit was demonstrated with early surgery compared to initial conservative management when numbers achieving either good recovery or moderate disability were categorised with the prognosis-based 8 point Glasgow Outcome Scale (GOS) (24% VS 26% respectively). Investigators from the same group recently reported results from the STICH II trial which examined patients undergoing early surgical intervention for removal of moderately-sized superficial lobar haematomas without intraventricular spread [179]. In this trial there was a trend towards a slight survival advantage for ICH patients however no significant difference was noted between treatment and control groups. Nevertheless no difference in death or disability was noted between groups and the results provided evidence that surgical intervention could prove efficacious for this condition.
ASA Stroke Council [152] and EUSI [153] guidelines do not currently recommend early routine evacuation of supratentorial haemorrhage and acknowledge the increased risk of recurrent bleeding [180] following craniotomy. Both guidelines acknowledge that the patient group targeted by STICH II who are deteriorating neurologically and have operative removal with minimally invasive methods have the most evidence for beneficial effect and that operative removal may also be considered in deep haemorrhage in the presence of mass effect. A recent Cochrane review [181] has suggested a more positive outcome from surgical intervention than demonstrated by the STICH investigators.

In those patients with cerebellar ICH (i.e. posterior fossa, infratentorial haemorrhages which comprise 6-10% of total cases [182-185]), haematoma evacuation can be life saving and is a class 1 recommendation in patients with rapid neurological deterioration or in those patients suffering brainstem compromise with or without hydrocephalus secondary to ventricular obstruction [152, 153].

In all sites, surgical research is currently focused on developing less invasive stereotactic and endoscopic evacuation in combination with the use of thrombolytic drugs [186]. These are anticipated to limit neuronal damage and the risk of recurrent bleeding associated with open craniotomy. A randomised trial [187] has shown the stereotactic evacuation of putaminal haematoma was associated with lower mortality and better recovery to functional independence in patients with mildly reduced consciousness. Currently, the ongoing Minimally Invasive Surgery plus t-PA for ICH Evacuation (MISTIE) trials [188] are designed to find the optimal dose of t-PA capable of removing 80% of haemorrhage volume by using stereotactic aspiration followed by catheter irrigation of thrombolytics. Results from the phase II trials have shown that the technique can remove brain clots in a rapid and safe manner and the third phase of the trial is currently underway to recruit 500 patients from one hundred centres.

A further trial examining the use of low dose recombinant tissue-type plasminogen activator delivered via extra-ventricular drains in patients with ICH and intraventricular extension found
an acceptable safety profile for use of this technique [189] and a phase III trial is currently underway.
1.7 Summary of clinical scenario

ICH represents the most severe form of stroke worldwide and is characterised by the worst incidence, worst morbidity and worst mortality of all stroke subtypes. Current understanding of the disease process has yielded relatively little clinical benefit from the therapeutic treatment options available although there have been encouraging trends noted in recent clinical trials and meta-analyses. The disease process is evolving and there is little consensus regarding treatment modalities although results from further phase III surgical trials are awaited.
1.8 Developing improved animal models of ICH

Much of our understanding of the pathophysiology of ICH has come from animal models of the disease, thereby identifying numerous possible therapeutic targets [190]. Across all animal models of disease however, only a third of highly cited studies translated at the level of human randomized trials and a only tenth of the interventions were subsequently approved for use in patients [191] indicating that little clinical benefit has been accrued. Subsequent improvement in mortality rates despite the wealth of animal data in ICH has therefore not been demonstrated [2]

The clear disparity between results obtained from animal models and clinical trials may be due to methodological problems with trial design, failure to publish negative or neutral results or the extrapolation of overtly favourable conclusions from publication bias [192, 193]. Most studies examining ICH have used young, healthy animals in contrast to the typical range of patients encountered with ICH injury. On a more fundamental level, the methods used to create ICH models are essentially invasive, triggering heightened inflammatory responses which are then modulated in experimental intervention to improve outcome. The trend therefore results in anti-inflammatory treatments showing genuine promise however it remains to be determined, for this reason, whether the research findings can be translated into clinical benefit.

The pharmaceutical industry is also limited by patent law and intellectual property rights which encourage a monotherapy-based approach [194], all of which have so far failed in ICH patients. Like the treatment of sepsis, development of treatments for ICH injury may have to use a multitude of research data to construct complex, multimodal treatments rather than aiming for a ‘magic bullet’ approach to
treatment. The scale of the problem in first constructing, then testing such a treatment in a time-critical manner is therefore significant.

The impact of study quality has also been more rigorously pursued in clinical trials in comparison to animal studies; a number of recommendations to improve methodological standards in the latter have been suggested based on the clinical CONSolidated Standards Of Reporting Trials (CONSORT). Both the recommendations and guidelines for conduct and reporting of animal studies in acute ischaemic stroke (the STAIR criteria), the ARRIVE guidelines and the CAMARADES checklist [195-198] have been developed in order to achieve this aim.

Both the National Institutes of Health priority report [199] and the American Heart Association clinical guidelines [152] emphasise the importance of developing clinically relevant models of ICH that will target new therapeutic approaches.
**General animal models of ICH injury**

A number of primate, feline, canine, porcine, lapine and murine models have been developed [200] to study the pathogenesis of ICH including hypertensive dog and rat models and a murine amyloid angiopathy model. Although the latter are arguably the most clinically relevant in nature, they provide inherently higher degrees of variability in haemorrhage size and extent of injury.

The rodent (i.e. rat or mouse) collagenase-induced and autologous blood - injection models of ICH have been established as, popular, reproducible and comparatively inexpensive methods to study pathogenesis and secondary injury [200-202].

**Comparison of rodent models of ICH**

As with ischaemia, no model perfectly reflects the complexity and heterogeneity of human ICH injury. Both the widely used collagenase and autologous blood injection rodent models [203] of ICH recreate the fundamental insult to the brain. Outcome is primarily determined by size and location of the haematoma [204] which is congruent with human studies [2] and suggests reasonable validity for the model. Methodological differences may nevertheless affect study outcome by altering the pathobiology of the induced lesion including inflammatory response, axonal sprouting and subsequent recovery [205].
Collagenase model

ICH injury is induced by direct injection of bacterial collagenase into the animal brain [206] (see Figure 1.1). Blood leaks into the surrounding tissues following the disruption of blood vessel basal laminae by the enzymatic action of the collagenase.

It is now accepted that clinical injury progresses over days following the original insult, both locally and distally to the injury site [207] [208]. Depending on definition, early haematoma expansion occurs in up to 32% of ICH patients [209] either from continual bleeding or episodes of re-bleeding [165]. The collagenase model, when matched for initial lesion size, results in final haematoma volume double that of the autologous injection model with a corresponding increase in perihaematomal cell loss, corpus callosum damage, cortical thinning and distal injury [207]. Since haematoma expansion is intricately linked to haemostasic and blood pressure control, any studies of interventions involving either of these factors may therefore be better examined with the collagenase model in which a progressive microvasculature breakdown is noted.

In addition, further imaging at 1-6 weeks post injury reveals continuing tissue loss in the collagenase model that was not demonstrated in the blood injection model. Although in vitro assays using equivalent collagenase doses do not appear to cause neuronal death or inflammation [210, 211], higher doses of collagenase are directly neurotoxic. The heightened inflammatory collagenase response demonstrated in vivo leads to greater mass effect, oedema, neurotoxicity [208, 212], BBB extravasation [190] and ischaemic injury due to the differentially greater damage to the vasculature [204, 213] and renders the model less suitable for the characterisation of the inflammatory response in ICH.
Autologous blood injection model

In this model, autologous blood is infused directly into brain parenchyma \[214\]. Rather than the spherical lesion shape obtained in the collagenase model, blood travels along the path of least resistance - in particular white matter tracts - resulting in a narrower, slit-like umbrella-shaped lesion pooling within striatal and corpus callosum areas \[207\] which is similar to the pattern seen in human brains. A double-injection modification designed to prevent blood tracking along the needle injection path or causing ventricular rupture has been described \[215\] however the effect on brain tissue of allowing clot formation at two distinct time points is unknown. There is also little data on the effect of recreating the ICH injury with varying needle calibres and with blood driven at equivalent pressures which may better reflect the natural injury pathogenesis.
Figure 1.1: Collagenase vs blood injection models of ICH injury. These sequential diagrams show the differing lesional outcomes using the two respective methods six weeks after induced ICH injury in the rodent brain. Cross sections are marked in centimetres from injection point. Figure from MaClellan et al [207].
1.9 Pathophysiology of ICH

1.9.1 General pathological response

ICH was originally classified according to the region of the brain in which it occurred; thalamic, basal ganglia, and brainstem bleeds are regarded as ‘deep’ whereas bleeds at the junction of the cortical grey matter and subcortical white matter are classified as ‘lobar’. Deep bleeds account for the majority of ICH with lobar bleeds between 25-35% depending on the population studied [216].

Chronic hypertension leads to segmental constriction of vessels through a pathological series of changes described as lipohyalinosis [217, 218]. In this process, atherosclerosis occurring at the branch points of larger (100-500µm) arteries is combined with arteriosclerosis of the smaller (<100 µm) perforating vessels. The atherosclerotic process, regarded as the intersection between inflammation and lipid metabolism [219], is characterised by the proliferation of subintimal fibroblasts combined with proliferation of subintimal fibroblast proliferation accompanied by the deposition of lipid filled macrophages [220]. Arteriosclerosis involves the replacement of smooth muscle cells in the tunica media with collagen [220] resulting in the development of noncompliant narrowed vessels prone to sudden closure (producing lacunar infarct) or rupture (ICH). Most bleeding occurs at or near the bifurcation of small penetrating arteries arising directly from the large basal cerebral vessels where pressure effects may be focused [221] and which therefore lack the protection normally afforded to those with a gradually declining calibre [220]. The mechanism of haemorrhage is thought to be due to rupture of these fragile vessels at multiple sites, some associated with layers of platelet and fibrin aggregates though this has been difficult to prove pathologically [222]. These lesions are characterised by breakage of elastic lamina, atrophy and fragmentation of smooth muscle, dissections and granular or vesicular cellular degeneration [221, 222]. Fibrinoid necrosis of the subendothelium with subsequent focal dilatations – termed microaneurysms [223] – may also
lead to rupture in a small number of patients [221, 224] however there is little evidence to support their presence in the general pathogenesis of ICH [221, 225].

Haemorrhage is thought to dissect along the white tissue planes of the brain and the initial injury is worsened by the direct mechanical force of the expanding haematoma [2, 190]. However the extent of the secondary damage attributable solely to this effect has failed to be replicated in the laboratory and has probably been overestimated historically [226]. The resulting combination of mechanical disruption of white matter tracts allied to oedema formation and localized pressure changes have been demonstrated to rapidly overwhelm local haemodynamic compensatory mechanisms in animal models of the disease [227, 228]. A number of clinical studies have in addition also demonstrated the importance of pressure effects following ICH injury with worse outcomes following disruption of cerebral autoregulation [229-232]. This important pathophysiological mechanism results in a profound increase in intracranial pressure and a corresponding decrease in cerebral perfusion pressure. Neurotransmitter release, calcium influx and mitochondrial dysfunction are all further exacerbated by physical disruption and stretching of surrounding cells leading resulting in increased oedema formation and cellular necrosis [233].

Further neurological deterioration after this point is attributed to the development of apoptosis, further necrosis and presence of inflammatory cells in the surrounding tissue [234, 235]. Thereafter, the haematomal mass itself begins to degrade in a predictable manner and releases further toxic breakdown products such as iron and thrombin resulting in the activation of downstream pro-inflammatory mediators which result in increased blood-brain barrier (BBB) permeability, initiation of apoptosis, recruitment of further inflammatory cells and the development of further oedema and ultimately neuronal death [2, 190, 236-238].

Pathological changes in patients with CAA are characterised by deposition of amyloid B peptide and degenerative changes including chronic inflammatory infiltrates, microaneurysm
formation, concentric splitting and fibrinoid necrosis [239]. White matter abnormalities (e.g., leukoaraoisis) are also associated with an increased risk of both sporadic and familial ICH suggesting a shared vascular pathogenesis [240, 241] although the pathophysiology remains unclear.

Perihaematomal oedema appears to follow a three-step process, increasing in volume by approximately 75% in the first day after ICH [242], peaking around 5-6 days [243] and lasting up to 14 days [244].

In animal models, the initial oedema is caused by clot retraction and an increase in hydrostatic pressure. The second stage (at 24-48 hours in the animal model) results from thrombin generated oedema; it is thus interesting to note that generation of oedema in post-thrombolytic cerebral haemorrhage is much decreased in comparison to standard ICH [245]. The third stage of oedema is the result of a generalised inflammatory process driven by haem released from lysed cells - this stage may be expedited in the animal model with the infusion of lysed cells rather than intact RBCs into the brain [246]. Following thrombin formation in the haematoma mass, peak oedema correlates with this lysis of red blood cells and both haemoglobin and resultant degradation products have been implicated in direct and indirect neuronal toxicity [190, 235].

The haematoma–oedema complex initially causes injury by mechanical disruption of the neurons and glia [35] followed by mechanical deformation causing neurotransmitter release, mitochondrial dysfunction and membrane depolarisation [247]. A temporary metabolic suppression (or hibernation) phase may result, progressing to cellular swelling and necrosis depending on the severity of mitochondrial dysfunction. The secondary cascade of injury initiated byproducts of coagulation and haematoma breakdown, in particular thrombin, activate microglia by 4 hours post injury [190, 248-250]. Activated microglia release products
encouraging breakdown of the blood-brain barrier (BBB), formation of vasogenic oedema and apoptosis in neurons and glia [251-256].

Haemostasis is initiated by the local activation of haemostatic pathways and mechanical tamponade [257, 258] however the presence of high ICP and low cerebral perfusion pressure (CPP) increases the risk of global ischaemia. A variable reperfusion phase lasts from 2-14 days after which a normalization phase develops with the re-establishment of normal cerebral blood flow (CBF). In contrast to ischemic stroke, there is only a modest and transient reduction in global cerebral blood flow after ICH [237, 259, 260] however perihaematomal cerebral blood flow has been shown to independently predict inpatient functional outcome (though not mortality) [261].

The role of ischaemia in the area surrounding a cerebral haemorrhage, as described by numerous CBF studies remains unclear [262-264]. DNA markers of cell damage and apoptosis have been noted in the perihaematomal area as well as the presence of glutamate and other excitotoxins [106, 247] although a direct association with the presence of ischemia remains unclear. In fact, recent PET data has shown normal relative oxygen extraction ratios in this region suggesting that the decreased perfusion is due to reduced cerebral metabolism [157, 158, 265, 266]. MRI and proton magnetic spectroscopy studies in patients have confirmed increases in apparent diffusion coefficient (ADC) values in eight out of nine ICH patients studied and increased lactate levels in only two out of the five haematomas for which there was data. Taken together, these results indicate the absence of cerebral ischaemia in the perihaematomal zone [267].

Although there is limited evidence for a purely ischaemic penumbral zone in the perihaematomal region, there is strong evidence for the existence of an inflammatory penumbral zone [211, 268] [269, 270] which may alter local tissue mechanics and contribute towards localized haematoma expansion which has previously been shown to worsen outcome
[258, 271]. This has previously been identified as a promising therapeutic target for ICH management [270] and will form the basis for the treatments proposed in this thesis.
In rat models of ICH, the vast majority of cell damage occurs within the lesion site and in the peri-ICH region in contact with normal tissue resulting in significant striatal atrophy [208]. This diminution in striatal size appears to be directly correlated to the volume of ICH as determined by MRI scan two hours post-injury. This is in clinical agreement with the observation that initial ICH size in patients relates directly to functional outcome [272, 273]. This “Black Hole” model of cell death results in the production of various trophic factors and other pro-survival molecules that eventually form a gliotic scar and non-functional cavity within the brain matter. Diagram taken from [2]
Inflammation in the brain

Inflammation is the response of living tissue to injury. A complex array of cellular and molecular mediators interact to help limit proliferation of invading pathogens and repair tissue [274]. This mechanism has been postulated to be beneficial in responding to small intracerebral bleeds however the more robust generalized and local inflammatory response generated by a large ICH may be deleterious to outcome [233].

Modulation of inflammation has become a key target for therapeutic intervention in a wide range of diseases. The historical and inaccurate view of the brain as an immune privileged site, unaffected by systemic inflammatory and immune responses, has been significantly revised although it is apparent that the brain inflammatory response differs greatly from that observed in the rest of the body [7]. The potent inflammatory response following ischemic stroke has been well characterised [275-280] and has generated multiple targets for novel therapies [280] however the data in haemorrhagic models and patients is limited [190, 253, 281, 282]. Nevertheless, deterioration and poor functional recovery in patients correlate with the central nervous system (CNS) inflammatory response following ICH [96]. Inflammation is likely to assume a more important pathogenic role in ICH compared to ischaemic stroke since no immunogenically or biochemically reactive blood is deposited in the extracellular space in the latter. Two microarray studies examining changes in gene expression 24 hours after ICH injury noted a substantially different response from that seen after ischaemia, hypoxia, hypoglycaemia or seizures with up to 50% of uniquely up-regulated genes in ICH [283, 284]. Disruption of the cellular or signaling response in the inflammatory cascade improves markers of disease progression and outcome in ICH models [211, 285]. A recent meta-analysis of all non-surgical interventions in animal models of ICH [286] identified administration of anti-inflammatory drugs such as Clioquinol and Desferoxamine as one of only two interventions which were able
to significantly reduce brain water content, improve neurobehavioural scores and reduce haematoma volume.

Thus, attention has turned to the characterisation of the inflammatory response after ICH and its role within the pathophysiological process, which may provide the basis for the first effective therapeutic targets for this condition [253, 277].

### 1.9.2 Neuroinflammatory response to ICH

Much of the insight into the inflammatory response seen to occur shortly after ICH injury and peaking several days later [287-290] stems from experimental animal models of ICH. Clinical studies are generally limited to blood and cerebrospinal fluid (CSF) sampling after ictus and there is scant histopathological data from human post-mortem studies [281]. The inflammatory process generally originates in and is mediated by damaged brain tissue however a substantial peripheral response also appears to exacerbate tissue injury [288]. In the ischaemic model of stroke, bone marrow has been demonstrated to produce increased numbers of granulocytes and monocytes which both mediate and contribute to progression of the disease process [291-294]. Multiple inflammatory mediators in peripheral lymphoid organs and the circulation help drive the inflammatory response to brain injury [292, 295-298]. Once ICH occurs, the inflammatory response can be divided into cellular and molecular components as depicted in Figure 1.3 below.
Figure 1.3 The major cellular and molecular components of the neuro-inflammatory response to intracerebral haemorrhage.

These selected components of the inflammatory cascade which follow ICH injury have been identified as potential targets for modulation of injury. The molecular messengers may be derived from a number of different cellular sources and interact in a complex manner. IL-1β: interleukin-1 beta, IL-6: interleukin 6, TNF-α: Tumour Necrosis Factor alph, NADPH Oxidase: nicotinamide adenine dinucleotide phosphate-oxidase, SOD: Superoxide dismutase, NF k-β: nuclear factor kappa-light-chain-enhancer of activated B cells, NO: Nitric Oxide, O₂: Oxygen

Diagram from [253]
Cellular components

The major inflammatory cells that accumulate within the brain and become activated after ICH are (blood derived) leukocytes, macrophages and (resident) microglia. These cells are the major CNS sources of cytokines, chemokines, and other immunomolecules [253, 280, 281, 299] (Figure 1.3).

A number of studies provide evidence of haematoma infiltration of leukocytes which, in the rat, appear to begin within 4 hours [281, 300], peak between days 2-3 and almost disappear by day 7 [288, 290, 301]. Neutrophils are positive for TNFα [281, 300] and have been shown to directly damage brain tissue by both generating reactive oxygen species (ROS) and secreting pro-inflammatory proteases [281, 302]. They undergo apoptotic cell death within 2 days of entering the haematoma [281] and further stimulate macrophages to release pro-inflammatory mediators during this process [281, 303].

Clinical studies provide further evidence for increased leukocyte numbers post-ICH injury both in CSF [281, 304] and peripherally, correlating with haematoma size and intraventricular extension [305-307]. Furthermore, high leukocyte count is an independent predictor for early neurological deterioration in primary ICH [96, 308]. Although activation of brain microglia and release of inflammatory mediators occur within minutes or hours, leukocyte recruitment is characteristically delayed in comparison to peripheral organs. Infiltration of peripheral leukocytes to the brain is associated with a concentration gradient of chemokines acting across areas of decreased BBB integrity [309]. Neutrophil and macrophage presence has been demonstrated in human pathological studies to infiltrate at 5-12 hours in perihematomal bordering vessels before polymorphonuclear leukocytes (PMNLs) infiltrate haematoma mass during days 2-4 post-ICH. Thereafter, numbers decrease within 72 hours post-injury [310, 311].
An increased neutrophil count in ICH patients has previously been found to be an independent risk factor for early neurological deterioration in ICH patients [309].

Limited data exists for the beneficial effects of targeting leukocytes after ICH in comparison to those documented in experimental ischaemic models [281, 312-315]. However, whole body irradiation in rodent ICH with subsequent global depletion of circulating leukocytes and platelets has been found to confer protection against subsequent ischaemia and oedema formation [281, 285].

Microglia are the resident macrophages of the CNS and account for 5-20% of the total glial population [316]. They have been shown to have a number of interactions with astrocytes [317-319], a group of cells critical for CNS function [320-322] and outcome of stroke injury [323]. Microglia exhibit migratory and proliferative responses and phagocytic behaviour. The haematomal mass is slow to clear in the initial few days after injury but mostly resolves by day 7 in mouse models. Microglial activation during this period is noted to be prominent in the first day in the peri-ICH region before peaking at day 7 and then continuing to finally become quiescent after three weeks. Similarly, in the autologous-blood injection rat model, microglial activation appears at 1-4 hours post-insult in the peri-haematomal region, peaks between day 3-7 and persists for a month [288, 290, 324].

In addition to their primary role of clearing the haematoma mass, microglia act as the keystone of the inflammatory response in the brain and their activation can be identified morphologically [325]. Microglia express and release a variety of cytokines [326-328], reactive oxygen species (ROS) [251, 317, 329] and nitric oxide [330, 331] (Figure 1.3). Using the collagenase-induced ICH model, Wang and coworkers [251] infused mice centrally with macrophage / microglial inhibitory factor (MIF), tuftsin fragment 1-3 Thr-Lys-Pro 2 days before or 2 hours after injury. Results revealed reduced haematoma size and oedema, decreased neuronal degeneration and
Improved neurological function. Further experiments with tetracycline derivatives, which are known to ameliorate microglial activation [332], given an hour after collagenase-induced ICH in the rat and continued over a week, subsequently resulted in improved morphological appearance of neurons and better functional recovery [332]. Downregulation of the pro-inflammatory TNFα produced by microglia after ICH damage in rats has also been found to reduce haematoma volume and improve subsequent neurobehavioural scores [300, 333]. Furthermore, enhanced levels of brain injury are found in aged rats in which greater degrees of microglial activation are typically noted [334].

The central importance of activated microglia to ICH-induced brain injury in animal models has therefore been widely demonstrated although human data remains limited [199, 233, 243, 244]. Future clinical trials involving microglial modulation will have to address the temporal nature of both their deleterious and neuroprotective effects on injured brain tissue [335-337]. Use of positron emission tomography (PET) scanning with appropriate tracers may be particularly useful in this regard [338-340].

1.9.2.1 Molecular Components

The primary source of cytokines within the brain are activated microglia / macrophages [299] and the white cells [280] that cross the post-injury permeable BBB [341]. Pro- and anti-inflammatory cytokines with the ability to activate a positive feedback cycle of activation [342] have been shown to be released in experimental models of brain injury [6, 280, 299, 343]. Pro-inflammatory genes that are upregulated after ICH include transcription factors, heat shock proteins, cytokines, chemokines, extracellular proteases and adhesion molecules [283]. Nuclear factor kappa-B (NF κ-B) has been shown to regulate many of these in vitro [299, 344] and this
factor has been found to be induced in blood vessels and infiltrating leucocytes in peri-ICH cerebral cortex in humans [253, 281].

In particular, TNF-α and IL-1β are pro-inflammatory cytokines which have been shown to be elevated in repeated experimental studies of ICH [281]. Microarray data in the rat model [283, 345] shows up-regulation of TNF-α, macrophage inflammatory protein 1a and IL-1β at 24 hours post event [253, 281]. The time course of the response appears to vary between species used [253].

TNF-α has an apparently dual role in which it helps to repair injured brain tissue, an effect that is perhaps dependent on the site of the initial injury [346]. Knockdown studies of TNF-α in the rat [300, 333] result in significant reductions in peri-ICH cell death and neurobehavioural deficits. IL-1β has few identified roles in the maintenance of normal brain function, although both TNFα and IL-1β have been proposed to exacerbate ICH injury [347, 348]. Direct injection into rat brain of either appears to open the BBB leading to vasogenic oedema [349-351]. Within the first day following injury, there appear to be deleteriously synergistic effects of IL-1β and TNFα, which are produced by generally separate subsets of activated microglia and macrophages [342].
1.9.2.2 Interleukin-1

Interleukin-1 was first described as the “endogenous pyrogen” following its discovery and acts as strong driver of inflammatory responses when administered exogenously in animal models [352]. Subsequent analysis revealed that it consists of two ligands, IL-1α and IL-1β, encoded by different genes but with high sequence homology [353]. Both are synthesized as large precursor proteins; IL-1α is biologically active in both forms and remains generally intracellularly, IL-1β is active only after cleavage of its precursor by caspase and is secreted by a pathway which has yet to be defined [354].

IL-1β is an endogenous pyrogen that contributes to an exacerbation of neuronal loss [345, 355]. After ischaemic injury, a biphasic response in IL-1 expression is noted, similar to that found with TNFα, for both transient and permanent vessel occlusion with the first peak at 1 hour post-reperfusion and a second after 6-24 hours [356-358]. The first peak is attributable to resident activated microglia, astrocytes, neurons and endothelial cells [345, 359]. The later expression is due to influx of inflammatory cells into the CNS [358, 360]. Recent evidence indicates that IL-1α expression in microglia appears to be the earliest signal of IL-1 system involvement [361].

As with TNFα, IL-1β is capable of stimulating its own production as well as of other pro-inflammatory mediators, including cytokines and adhesion molecules and can activate microglia and astrocytes. Furthermore, IL-1β is capable of stimulating calcium influx into neurons increasing ischaemic vulnerability to these cells and increasing inflammatory response. Finally, IL-1β induces oedema formation and primes the endothelium for adherence of leukocytes [355, 360, 362]. In a rat model where ICH was induced with the use of a double injection autologous
striatal injection, an 8-40 fold increase in IL-1β was observed at 3 hours and 24 hours post injection [253, 281]. In common with other participants in the inflammatory cascade, IL-1β also has neuroprotective effects which it achieves by stimulation of astrocytes which are able, in turn, to produce various survival factors. However this is a delayed response and most evidence suggest that IL-1 is a key mediator of neuronal injury in the early stages after stroke.

1.9.2.3 Inhibition of IL-1

The third ligand discovered in the IL-1 family was the naturally occurring highly selective competitive IL-1 receptor antagonist (IL-1Ra). This molecule is released from the same cells that express IL-1 [6], blocks all effects of the molecule and has no other known actions to date. In the ischaemic stroke model, the temporal induction of IL-1Ra closely follows that of IL-1β suggesting that, in exerting their effect, the relative balance between the two signaling molecules is important rather than their absolute values [280, 363].

It is known that interleukin-6 (IL-6), can upregulate IL-1Ra. It is therefore interesting to note that IL-6 deficient mice fail to show improved outcome after ischaemic stroke [345, 362, 364, 365]. In the same model, the level of tissue damage was strikingly reduced with the direct administration of IL-1Ra even when the intervention was delayed by up to 3 hours [366-369]. More recent evidence [370] has also shown a beneficial effect of systemically delayed IL-1Ra administration in aged and corpulent rats. Furthermore, long-term (25-day) behavioural outcomes of fine motor control were significantly improved in rats who had IL-1Ra administered at the time of ischaemic injury although the effect of delayed administration was not replicated [371]. A subsequent phase II trial in ischaemic stroke patients has already demonstrated the safety profile of an administered recombinant form of IL-1Ra and improved
clinical outcomes in the experimental group as demonstrated by Barthel Index and Modified Rankin Scale [11, 363], though the study was not powered to report such an effect.

It is to be hoped that the inflammatory overlap seen between haemorrhagic and ischaemic models of brain injury may be able to yield similarly encouraging results. No clinical trials have yet been undertaken to target IL-1 in ICH patients however the autologous ICH rat model exhibits attenuated brain oedema formation and thrombin-induced intracerebral inflammation with adenovirus-mediated overexpression of IL-1Ra [372, 373] and thereby provides evidence that modulation of IL-1 may prove to be beneficial in this disease.
1.9.3 Summary

The elucidation of the inflammatory response to ICH injury holds great promise in understanding the pathobiology of the disease and should be an important target for further research, particularly in the area immediately adjacent to the haematoma. Targeting this tissue has given rise to the concept of a “tissue clock” in ischaemic stroke research [374]. There is strong evidence that this tissue is the site of an inflammatory “penumbral” zone in ICH which may be salvageable with the correct modulation of the inflammatory cascade.

Potential therapeutic targets arising from this thesis may present a similar opportunity to develop therapeutic interventions that both have efficacy and can be administered many hours before any currently available medical or surgical interventions. In particular, IL-1 is likely to be a key driver of the pathological effects of ICH as demonstrated in several animal models. Modulation of its effects with the use of the exogenous use of IL-1Ra could therefore potentially herald the first safe and effective treatment for this condition in human subjects.
1.10 Thesis Aims and Objectives

1.10.1 Chapter Two:
The chapter will aim to determine the important factors impacting on current ICH mortality in British patients. This was achieved by conducting a large retrospective cohort study in a group of ICH patients referred to a leading UK hospital in order to determine the relative importance of previously known prognostic factors regarding ICH mortality.

1.10.2 Chapter Three:
This chapter will aim to establish whether a difference in ICH outcomes exists using current clinical management at different UK centres and, if so, to examine the factors responsible for patient transfer between those centres. A retrospective cohort study of clinical patient characteristics was conducted in order to answer this question and the data was compared with prospectively gathered radiobiological records of the ICH injury to determine which patients were subject to transfer for surgical care at a specialist unit. The site-specific survival data for each patient group was then compared in order to elicit whether a survival advantage existed between sites and treatment modalities.

1.10.3 Chapter Four:
The aim of this chapter was to investigate the presence of a direct neuroinflammatory response in the subacute phase following ICH injury in patients. A proof-of-principle MR/PET pilot study was undertaken to look for the presence of BBB breakdown and microglial activation within
ICH-injured brains. Blood samples were also taken to assess the presence of consequent systemic inflammatory response within these patients.

1.10.4 Chapter Five:
The aim of this chapter was to characterize the functional and radiological response of IL-1 modulation in an animal model of ICH injury. In order to elicit this, the study developed a previously described method of inducing ICH injury in the rat brain and compared groups treated with a competitive antagonist of IL-1 with saline-treated controls. The subsequent inflammatory response in both groups was assessed by using a radiological measurement of oedema formation in the brain following ICH in the three days following injury. Furthermore, a radiological measurement of brain atrophy was determined at long term (28 day) follow-up. Lastly, a comparison of the functional outcomes of both animal groups was established using a range of behavioural tests from immediately after ICH injury to 28 days post-injury.
1.11 References


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2 Accuracy and Clinical Usefulness of Intracerebral Hemorrhage Grading Scores: A direct comparison in a UK population
2.1 Introduction

ICH has been established as a condition that has been changing its clinical profile as the population demographic has aged and developed subsequent co-morbidities and different medication regimes. Much of the available clinical data regarding the important prognostic factors in ICH mortality has therefore become increasingly obsolete. The thesis chapter therefore first determined important prognostic factors in a large cohort of patients recently treated for ICH injury in a number of UK centres.
Accuracy and Clinical Usefulness of Intracerebral Hemorrhage Grading Scores
A Direct Comparison in a UK Population

Adrian R. Parry-Jones, MD, PhD; Kamran A. Abid, MD; Mario Di Napoli, MD; Craig J. Smith, MD; Andy Vail, MSc; Hiren C. Patel, MD, PhD; Andrew T. King, MD; Pippa J. Tyrrell, MD

Background and Purpose—Various grading scores to predict survival after intracerebral hemorrhage (ICH) have been described. We aimed to test the accuracy and clinical usefulness of 3 well-known scores (original ICH score, modified ICH score, and ICH grading scale) in a large unselected cohort of typical ICH patients.

Methods—A total of 1364 ICH cases were referred to our center from January 1, 2008, to October 17, 2010. Clinical details were prospectively recorded, and the first computed tomography brain scan was retrospectively reviewed to determine ICH volume and location and to identify intraventricular hemorrhage. The original ICH, ICH grading scale, and modified ICH score were calculated. Receiver operating characteristic and decision curves for 30-day mortality were generated.

Results—A total of 1175 patients were included in the final analysis. All 3 scores and the Glasgow Coma Scale (GCS) divided cases into groups with highly significant differences in mortality. The area under the receiver operating characteristic curve was very similar for original ICH (0.861), ICH grading scale (0.874), and GCS (0.872), but was less for modified ICH score (0.824). Age was much less predictive (0.565). Combining GCS with age, log ICH volume, and intraventricular hemorrhage to derive a multifactorial risk of death at 30 days significantly increased the area under the receiver operating characteristic curve (0.897). All scores and GCS demonstrated a similar net benefit for threshold probabilities of 10% to 95%. Above 95%, the net benefit of GCS became inferior to the prognostic scores.

Conclusions—Although existing grading scores are highly predictive of 30-day mortality, GCS alone was as predictive in our cohort, but age was not. (Stroke. 2013;44:1840-1845.)

Key Words: decision curve analysis ■ Glasgow Coma Scale ■ intracerebral hemorrhage ■ prognosis ■ prognostic scores

Various prognostic scoring systems have been devised and tested to predict survival after intracerebral hemorrhage (ICH) with the intention of improving prediction of prognosis, but none are used routinely in clinical practice. They differ in the factors included, their complexity, and ease of use. There has been concern that prediction of a poor outcome using these scores may lead to inappropriate withdrawal or limitation of care very early after ICH, and thus, these predictions of poor outcome may become self-fulfilling prophecies.1 Because ICH has a 30-day mortality of ≈40%,2 it is, nonetheless, important to provide patients and their families with a personalized assessment of the likelihood of survival after ICH and to do so with reasonable accuracy. Physicians often make an informal assessment of their patient’s likelihood of survival on the basis of their own personal experience, and this assessment may be inaccurate. Prognostic scores on the basis of large data sets may allow physicians to prognosticate more objectively. Whether aggressive supportive care can improve this predicted longer term outcome is currently unclear.3

Level of consciousness and hematoma volume at admission are the most consistent outcome predictors, and grading scores combining these variables with other independent outcome predictors (including the original ICH [oICH] score,4 the modified ICH score [mICH],5 and the ICH grading scale [ICH-GS])6 show the best predictive values.7 These grading scores have been validated previously using measures of discrimination and calibration,8,9 but it remains unclear whether these scores are useful in clinical practice.

Received January 31, 2013; accepted April 5, 2013.
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The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.113.001009/-/DC1.

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DOI: 10.1161/STROKEAHA.113.001009
The aim of our study was to test existing scores in a large cohort representative of typical ICH patients in hospital-based stroke care in the United Kingdom using measures of discrimination, calibration, and decision curve analysis (DCA).\textsuperscript{10} We also compare these grading scores with commonly used clinical characteristics that physicians may informally rely on to inform their assessment of prognosis.

Methods

Clinical Setting

The neurosurgical department, Salford Royal NHS Foundation Trust, Salford, United Kingdom, serves the 2.6 million population of Greater Manchester and receives referrals from 14 hospitals throughout the region. On the basis of known incidence,\textsuperscript{2} the \( n \approx 450 \) acute ICH cases referred annually would represent \( \approx 75\% \) of incident ICH cases in our population. Details of every referral were prospectively recorded by the duty neurosurgeon in an electronic database, including the date and time of the referral, demographic details, clinical findings (including the Glasgow Coma Scale [GCS] score at time of referral), investigation findings, and the agreed management plan. Each case was reviewed within 24 hours by the senior neurosurgeon and neuroradiologist on call. The exact time of symptom onset was not recorded for all cases in the database. For analysis of survival, the date and time of the first computed tomography (CT) brain scan was used as an alternative to onset and is very likely to have been performed within 24 hours of symptom onset in the vast majority of cases. Time from symptom onset to first CT brain scan was recorded for 924 (67.7\%) cases, and the median time was 6 hours (interquartile range, 2–18 hours).

National Health Service (NHS) Research Ethics Committee approval was obtained for our study. We identified 1364 patients referred between January 1, 2008, and October 17, 2010, whose diagnosis had been recorded as ICH, and a study investigator and neurosurgeon (K.A.) reviewed each case for study inclusion. Cases were included in the analysis if the first CT brain scan after onset could be obtained for review. If there was a clear history of a major head injury before presentation, patients were assumed to have sustained a traumatic ICH and were excluded. Cases in which the diagnosis was of hemorrhage into other intracranial compartments without ICH, no hemorrhage at all, or hemorrhagic transformation of an infant were excluded.

The first CT brain imaging study after onset of symptoms was retrospectively reviewed by a study investigator (K.A.) who recorded ICH location as either deep or lobar and supratentorial or infratentorial. Deep ICH was defined as involving deep brain structures, including the basal ganglia, thalamus, vermis, and brain stem. Lobar ICH was defined as involving the cerebral or cerebellar lobes without involvement of deep structures. Where blood was evident in both compartments, the compartment containing the majority of the blood was recorded. Hematoma volume was calculated using the ABC/2 method, as previously described,\textsuperscript{11} and intraventricular hemorrhage was recorded. The oICH score and ICH-GS were derived for each patient as described in their original publications.\textsuperscript{4,6} The mICH score was calculated for all cases in the data set, although it was devised for use in basal ganglia ICH only. Although the mICH score takes hydrocephalus into account, we excluded this because we felt that the presence or absence of this CT finding is subjective and has been shown to have poor interobserver agreement in this setting.\textsuperscript{12} The survival status and date of death of nonsurvivors were obtained on October 21, 2011, via the Medical Research Information Service, Southport, United Kingdom, allowing a minimum follow-up period of 370 days for all cases. Data collection and image analysis were conducted before collection of survival data and were thus blinded to outcome.

Statistical Analysis

Cases with incomplete data were excluded. The \( \chi^2 \) test was used for comparisons of categorical variables, and the \( t \) test or the Wilcoxon rank sum test for continuous variables according to manner of distribution. Kaplan–Meier survival curves were produced for the study population divided by prognostic categories and compared using the log-rank test. To aid clarity, GCS was divided into 7 categories for the Kaplan–Meier curve only. Receiver operating characteristic (ROC) curves were generated for each prognostic measure, and the area under the ROC curve (AuROC) was calculated. Confidence intervals were calculated according to binomial exact formula. To assess calibration, observed 30-day (oICH score, ICH-GS) and 6-month (mICH) mortality and 95\% confidence intervals were derived from the Kaplan–Meier analysis and plotted against predicted mortality as published for each derivation cohort.\textsuperscript{13} A similar analysis was performed to assess the calibration of the GCS against an independent published ICH cohort.\textsuperscript{13}

Logistic regression was performed using GCS, age, intraventricular hemorrhage, and the natural logarithm of ICH volume to derive a predicted risk of death at 30 days for each case and an ROC curve for this was then derived. All data were expressed as median and interquartile range unless otherwise stated.

DCA (see Methods in the online-only Data Supplement) was used to compare the clinical usefulness of the ICH scores and GCS across the full range of threshold mortality risk probabilities.\textsuperscript{14} All statistical analyses were performed in SPSS 16.0 for Windows (SPSS Inc) and the R statistical package (www.r-project.org).

Results

All 1364 cases with a recorded diagnosis of ICH in the neurosurgical referral database for the period of the study were reviewed. After 176 exclusions (Figure 1; Table I in the online-only Data Supplement), 1188 cases were submitted to Medical Research Information Service on October 21, 2011. Thirteen could not be traced, leaving 1175 in the final analysis. Clinical and imaging characteristics at referral are outlined in Table 1. Case fatality rate was 25.4\% (n=298) at 3 days, 41.1\% (n=483) at 30 days, 49.4\% (n=581) at 6 months, and 52.5\% (n=617) at 1 year. The distribution of cases across the range of possible grading scale scores is shown in Table II in the online-only Data Supplement. All 3 prognostic scores and GCS (split into 7 categories) divided cases into groups with highly statistically significant differences in mortality (Figure 2; \( P<0.0001 \) for all, log-rank test).

Figure 1. Flowchart outlining exclusions from the final analysis. CT indicates computed tomography; GCS, Glasgow Coma Scale; ICH, intracerebral hemorrhage; and MRIS, Medical Research Information Service.
Discrimination and Calibration

Discrimination of the prognostic scores for 30-day mortality, as determined by AuROC, showed that the mICH score was inferior to the ICH-GS (P<0.0001) and oICH score (P<0.0001; Table 2). However, when applied to deep supratentorial ICH only, oICH (AuROC, 0.856; 95% confidence interval, 0.822–0.885; P=0.1135) and ICH-GS (AuROC, 0.860; 95% confidence interval, 0.826–0.889; P=0.0977) reduced their discrimination and were similar to mICH.

When performance of the individual components of the scores was tested (age, ICH volume, and GCS), the GCS alone performed as well as the ICH-GS (P=0.9891) and oICH score (P=0.1427). ICH volume was less discriminatory, whereas age was only weakly predictive of death at 30 days. Logistic regression was performed, including GCS, age, log ICH volume, and intraventricular hemorrhage, as predictive factors to derive a multifactorial risk of death at 30 days. The overall performance of this combined risk was better than the GCS and the prognostic scores (P<0.0001, for all comparisons). The 3 components of the GCS were tested individually and were less discriminative than the total score (P<0.0001, for all comparisons). The calibration of the prognostic scores for 30-day mortality (oICH score and ICH-GS) and 6-month mortality (mICH score) between our cohort and the cohorts in which they were derived is shown in Figure 3. Our cohort had similar survival to that predicted from the oICH cohort, higher survival than the ICH-GS cohort, and markedly lower survival than most of the mICH cohort.

Decision Curve Analysis

The relative performance of scores and GCS was similar in DCA (Figure 4) and in the AuROC analysis. The net benefit for all 3 scores and GCS surpasses the strategies of treat all and treat none between threshold probabilities of 10% and 95%. Between threshold probabilities of 20% to 95%, ICH-GS and GCS have a similar net benefit, are slightly superior to the oICH score, and clearly better than the mICH score. Only above a threshold probability of 95% does the net benefit of GCS become inferior to the prognostic scores.

Discussion

We have shown that 3 previously described grading scores for ICH are highly predictive for 30-day mortality when applied to our cohort of patients. In addition to being much larger than the study populations in which these scores were first described,4–6 our study population was drawn from patients in a different country and healthcare system, thus adding support to the external validity of these grading scores as prognostic scores. No substantive difference in the performance of the 3 scores was found except for lower performance of the mICH score when applied to all cases. However, when it was used in the subgroup of patients with basal ganglia ICH, its performance was similar to those of the other scores. It is of note that the GCS alone, which contributes to all 3 scores, was as good at predicting 30-day mortality as the prognostic scores. The GCS has the advantage of being simpler to use, because it does not require analysis of brain imaging and measurement of hematoma volume followed by the calculation of an additional

Table 1. Demographic and Clinical Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
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<th>30-Day Mortality (n=483)</th>
<th>P Value</th>
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<tr>
<td>Men</td>
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<tr>
<td>Women</td>
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<td>Median (IQR)</td>
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<td>34 (7.0)</td>
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<td>50–64</td>
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<td>88 (18.2)</td>
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<td>≥65</td>
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<td>361 (74.8)</td>
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<td></td>
<td></td>
<td>0.8397</td>
</tr>
<tr>
<td>Deep</td>
<td>588 (50.1)</td>
<td>240 (49.7)</td>
<td></td>
</tr>
<tr>
<td>Lobar</td>
<td>587(49.9)</td>
<td>243 (50.3)</td>
<td></td>
</tr>
<tr>
<td>ICH volume, cm³</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>25 (7.4–64.8)</td>
<td>56.8 (23.4–110.1)</td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>641 (54.6)</td>
<td>143 (29.6)</td>
<td></td>
</tr>
<tr>
<td>30–50</td>
<td>180 (13.6)</td>
<td>70 (14.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>&gt;50</td>
<td>374 (31.8)</td>
<td>270 (55.9)</td>
<td></td>
</tr>
<tr>
<td>IVH</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>446 (38.0)</td>
<td>297 (61.5)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>729 (62.0)</td>
<td>186 (38.5)</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
<td>0.8405</td>
</tr>
<tr>
<td>Yes</td>
<td>75 (6.4)</td>
<td>30 (6.2)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1100 (93.6)</td>
<td>453 (93.8)</td>
<td></td>
</tr>
</tbody>
</table>

All data are expressed as median (interquartile range [IQR]) or as a percentage of the total study population (n=1175).

GCS indicates Glasgow Coma Scale; ICH, intracerebral hemorrhage; and IVH, intraventricular hemorrhage.
score. Combining other factors that are included in the score and have previously been shown to predict prognosis did add to the predictive power of GCS, but this can largely be explained by overfitting because the model was tested in the same cohort from which it was derived. Our finding that age alone was a comparatively weak predictor of survival is an important reminder to clinicians not to provide undue weight to this factor when considering likelihood of survival for individual patients.

Thirty-day survival by oICH score was very similar between our cohort and the derivation cohort, suggesting this score is well calibrated to our population. For the ICH-GS, our cohort has a higher 30-day survival relative to the derivation cohort across the range of scores. The derivation cohort for the mICH score included highly selected patients enrolled in a randomized trial of surgery, and this is reflected in the marked differences in outcome relative to our cohort. It is also well recognized that mortality is significantly lower after ICH in Japan, making comparisons between Asian and non-Asian populations difficult.

We used DCA to confirm the clinical usefulness of the scores and GCS. By reducing a curve to a single value, it is possible for AuROC analysis to mask important differences in sensitivity versus specificity across the full range of prognosis. Only at the extreme end of the scale, for patients with <5% survival probability, did the leading models diverge. In our cohort, only the lowest possible GCS score (3) confers a prognosis worse than 5% survival, whereas both the ICH-GS (12, 13) and the oICH (5, 6) contain 2 divisions within this range. Thus, this may favor using the ICH-GS over the GCS, in which distinguishing risks of death >95% is important.

The strengths of our study include our large sample size, the prospective nature of the data collection (except analysis of imaging), and the blinding of image analysis and prognostic scores determination to survival status. ICH grading scores are not routinely used for clinical care at our center and were determined retrospectively for this study, thus preventing them from influencing care decisions and, hence, prognosis. To the best of our knowledge, this is the largest population in which ICH prognostic scores have been tested to date. We were able to ascertain the outcome status of the vast majority of the patients with otherwise complete data (only 13 of 1188 [1%] could not be traced), so very few patients were lost to follow-up. Finally, many previous studies have applied prognostic scores to historical data recorded as part of clinical research studies conducted for other reasons, leading to a sample of patients that may not be representative of typical ICH patients. In our study, prognostic scores were calculated from data collected as part of routine clinical care, and thus, our findings more accurately reflect the performance of these scores across a wide range of ICH patients. GCS was likely to have been measured by nonspecialists in most cases, but it is important to note that those referring patients to the neurosurgical team and providing the GCS were usually the physicians primarily responsible for the patient’s acute care. As our aim was to determine the validity of these scores for routine clinical use, the GCS as determined by the physician responsible for the patient’s acute care is of the most relevance to this.

Our study does have limitations. First, we excluded 107 patients from the analysis because of incomplete data, and 83
of the 107 were excluded because we were unable to access their initial CT brain scans. This may introduce some bias, but this is unlikely to have altered the overall conclusions. Second, our patients were included in this study because they had been referred to our neurosurgical service. Although we believe that ≈ 75% of all ICHs in our population are referred to the neurosurgical service, this is likely to have introduced some selection bias. For example, some older patients may not have been referred as evidenced by the slightly lower mean age of our patients, relative to a UK population-based study\(^1\) (71 versus 76, respectively). This may make the results of our study less applicable to the very old. Third, we do not know the functional outcomes for survivors in our study. Survival and functional outcome are of great importance to patients and their families after ICH, and the ability of these scores to predict good functional outcome needs to be tested in additional large studies. Fourth, we used the GCS at the time of referral to neurosurgery in our analysis because our aim was to assess our ability to predict survival at the point of referral to neurosurgery, when decisions are still being made about patient management. GCS at this time point may have greater prognostic significance than at initial presentation because it would be expected that a minority of patients will have already had early neurological decline. Finally, we were unable to test some newer ICH prognostic scores because some of the factors that inform these scores were not recorded in our data set (eg, National Institutes of Health Stroke Scale Score). However, in a small cohort study, there was little additional benefit to be gained by using these newer scores,\(^2\) relative to the scores we were able to test in our study.

**Table 2. Discrimination: Area Under the Receiver Operating Curve (AuROC) and 95% Confidence Interval for All 3 Scores Tested and for Each Factor Used to Derive the Scores**

<table>
<thead>
<tr>
<th>Factor/Score</th>
<th>AuROC</th>
<th>95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>oICH score</td>
<td>0.861</td>
<td>0.840–0.880</td>
</tr>
<tr>
<td>ICH-GS</td>
<td>0.874</td>
<td>0.853–0.892</td>
</tr>
<tr>
<td>mICH score (all patients)</td>
<td>0.824</td>
<td>0.801–0.845</td>
</tr>
<tr>
<td>mICH score (deep, supratentorial only)</td>
<td>0.836</td>
<td>0.801–0.867</td>
</tr>
<tr>
<td>GCS (total score)</td>
<td>0.874</td>
<td>0.853–0.892</td>
</tr>
<tr>
<td>GCS (motor score)</td>
<td>0.917</td>
<td>0.793–0.839</td>
</tr>
<tr>
<td>GCS (verbal score)</td>
<td>0.841</td>
<td>0.611–0.862</td>
</tr>
<tr>
<td>GCS (eyes score)</td>
<td>0.811</td>
<td>0.787–0.834</td>
</tr>
<tr>
<td>Log ICH volume</td>
<td>0.778</td>
<td>0.753–0.801</td>
</tr>
<tr>
<td>Age</td>
<td>0.565</td>
<td>0.537–0.594</td>
</tr>
<tr>
<td>Multifactorial risk</td>
<td>0.897</td>
<td>0.878–0.914</td>
</tr>
</tbody>
</table>

The probability of death by 30 days was also determined for each case using a multifactorial logistic regression model, including age, log (volume), Glasgow Coma Scale (GCS), and the presence of intraventricular hemorrhage as factors. AuROC was also calculated for this multifactorial risk. A total of 42 cases were excluded from the AUC analysis for GCS components because only the total GCS and not the breakdown was available. AuROC indicates area under the receiver operating characteristic curve; CI, confidence interval; ICH-GS, ICH grading scale; mICH, modified ICH; and oICH, original intracerebral hemorrhage.

\(^*\) Binomial exact.

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**Figure 3.** Graphs showing calibration of the prognostic scores against their original derivation cohorts. The original intracerebral hemorrhage (oICH) score (A, solid squares) was derived from an unselected cohort of 161 ICH patients presenting to 2 hospitals within a large urban area of the United States.\(^1\) The ICH grading scale (ICH-GS; A, open diamonds) was derived from 310 unselected ICH patients presenting to a tertiary referral center in Mexico.\(^6\) The modified ICH (mICH) score (B) was derived from a prospective randomized study\(^5\) undertaken at a single neurosurgical center in China with inclusion of 226 ICH patients on the basis of hematoma location (basal ganglia), volume of hematoma (10–100 mL), time to presentation (<24 h), and patient or family consent to participate in the study. Patients were then randomly assigned to undergo endoscopic hematoma evacuation or conservative treatment only. Because 30-day survival was not reported in the original study, survival is shown at 6 months to allow direct comparison. Survival for the subset with deep, supratentorial hemorrhage (BG; n=504) is shown compared with mortality in the derivation cohort with surgery (solid squares) and without surgery (open diamonds). Calibration for Glasgow Coma Scale (GCS) against an independent cohort is shown in C. Bars represent 95% confidence intervals taken from the Kaplan–Meier survival analysis. Confidence intervals were not reported for the derivation cohorts.
Physicians face an uncertain situation in managing patients immediately after ICH, in which they must balance whether to offer aggressive supportive care with the importance of providing dignified end-of-life care to a patient who may be very likely to die in hospital, with or without treatment. Although physicians should be cautious of using this prognostic information to make important management decisions, we feel that accurate prognostic information should not be denied to patients and their families simply because our ability to improve survival by aggressive supportive care is unclear. The GCS represents the simplest way to estimate survival after ICH.

Conclusions

Our data support the use of ICH grading scores as prognostic scores to provide patients and families with an estimate of the likelihood of survival after ICH. Such scores may also provide a useful tool in the design and conduct of acute intervention studies in ICH as a means of stratification. There is little to discriminate between the scores, but given that the GCS alone is simpler than any of the published scores, performs at least as well as all 3 ICH scores in this study, and removes the need to make important management decisions, we feel that accurate prognostic information should not be denied to patients and their families simply because our ability to improve survival by aggressive supportive care is unclear. The GCS represents the simplest way to estimate survival after ICH.

Disclosures

None.

References

SUPPLEMENTAL MATERIAL

Supplementary methods

**Decision Curve analysis:** Grading scores might be used to support decisions to discontinue aggressive supportive care and allow a patient with a very poor prognosis a dignified and peaceful death. However, the probability of death at which clinicians or relatives would be uncertain about continuing supportive care (or ‘threshold probability’, \( P_t \)) will vary and is highly personal. Decision curve analysis (DCA) is a recently described method that attempts to address this problem by assessing the ‘net benefit’ (NB) of using a prognostic model in supporting decision making across a range of threshold probabilities and is a useful extension to standard statistical methods of assessing prognostic models.\(^{10}\) NB is defined as \( \frac{TP - w \times FP}{N} \), where TP is the true-positive count, FP is the false-positive count, N is the total number of the population, and w is a weight equal to the odds of the threshold \( \frac{P_t}{1 - P_t} \), which is essentially the relative harm of a false-positive and a false negative result.\(^{10}\) In our analysis, we were interested in the NB of receiving aggressive supportive treatment on the basis of 30-day mortality. Thus, for our decision curve, the zero reference line represents a strategy of assuming all patients will survive to 30 days and thus treating all individuals, whilst the line labeled ‘treat none’ represents a strategy of assuming all will die by 30 days and treating none with aggressive supportive treatment. DCA consists of plotting NB against threshold probability of 30-day mortality by applying the strategy of providing aggressive supportive case to an individual based on the NB obtained by the different prognostic scores and only if estimated individual probability (\( P_i \)) of 30-day mortality is greater than \( P_t \), in function of the \( P_t \).\(^{10}\) It facilitates the comparisons among alternative
prediction models used to calculate $P_i$. This analysis goes beyond the AuROC analysis in making it explicit where differences in sensitivity or specificity affect the usefulness of the prognostic measure and may facilitate the decision of which of several models to select.
**Supplementary table 1** Demographic and clinical characteristics of included and excluded patients: All data are expressed as median (interquartile range (IQR)) or as a percentage of the total included or excluded population. The 5 cases that were found to be duplicate database entries are not included in the excluded patient data.

GCS, Glasgow Coma Scale.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Included patients (n=1175)</th>
<th>Excluded patients, (n=184)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>619 (52.7)</td>
<td>108 (58.7)</td>
</tr>
<tr>
<td>Female</td>
<td>556 (47.3)</td>
<td>76 (41.3)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>73 (61-82)</td>
<td>67 (50-79)</td>
</tr>
<tr>
<td>&lt;50</td>
<td>121 (10.3)</td>
<td>45 (25.1)</td>
</tr>
<tr>
<td>50-64</td>
<td>237 (20.2)</td>
<td>36 (20.1)</td>
</tr>
<tr>
<td>≥65</td>
<td>817 (69.5)</td>
<td>98 (54.7)</td>
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<td><strong>Medical History</strong></td>
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<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>396 (33.7)</td>
<td>38 (20.7)</td>
</tr>
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<td>No</td>
<td>779 (66.3)</td>
<td>146 (79.3)</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
</tr>
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<td>Yes</td>
<td>116 (9.9)</td>
<td>16 (8.7)</td>
</tr>
<tr>
<td>No</td>
<td>1059 (90.1)</td>
<td>168 (91.3)</td>
</tr>
<tr>
<td>Hypecholestrolaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>60 (5.1)</td>
<td>6 (3.3)</td>
</tr>
<tr>
<td>No</td>
<td>1115 (94.9)</td>
<td>178 (96.7)</td>
</tr>
<tr>
<td><strong>Medications</strong></td>
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<tr>
<td>Anticoagulant</td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>210 (17.9)</td>
<td>18 (9.8)</td>
</tr>
<tr>
<td>No</td>
<td>965 (82.1)</td>
<td>166 (90.2)</td>
</tr>
<tr>
<td>Antiplatelet(s)</td>
<td></td>
<td></td>
</tr>
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<td>Yes</td>
<td>271 (23.1)</td>
<td>30 (16.3)</td>
</tr>
<tr>
<td>No</td>
<td>904 (76.9)</td>
<td>154 (83.7)</td>
</tr>
<tr>
<td><strong>GCS</strong></td>
<td></td>
<td></td>
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<tr>
<td>14 - 15</td>
<td>568 (48.3)</td>
<td>94 (53.4)</td>
</tr>
<tr>
<td>9 - 13</td>
<td>283 (24.1)</td>
<td>34 (19.3)</td>
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<td>6 - 8</td>
<td>110 (9.4)</td>
<td>20 (11.4)</td>
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<tr>
<td>3 - 5</td>
<td>214 (18.2)</td>
<td>28 (15.9)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>8</td>
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</tbody>
</table>
Supplementary table 2 - Prognostic score frequencies: All data are expressed as number and as a percentage of the total study population (n = 1175). oICH, ICH score; ICH-GS, ICH grading scale; mICH, modified ICH score.

<table>
<thead>
<tr>
<th>Score</th>
<th>All patients (n=1175)</th>
<th>30-day mortality, (n=483)</th>
<th>P-value $\chi^2$ for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>oICH score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>226 (19.2)</td>
<td>15 (3.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1</td>
<td>288 (24.5)</td>
<td>47 (9.7)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>252 (21.4)</td>
<td>88 (18.2)</td>
<td></td>
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<td>208 (17.7)</td>
<td>145 (30.2)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>156 (13.3)</td>
<td>143 (29.5)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>42 (3.6)</td>
<td>42 (8.7)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3 (0.3)</td>
<td>3 (0.6)</td>
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<td>ICH-GS</td>
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<td>203 (17.3)</td>
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<td>10</td>
<td>140 (11.9)</td>
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<td>12</td>
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<td>97 (20.0)</td>
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<tr>
<td>13</td>
<td>18 (1.5)</td>
<td>18 (3.7)</td>
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<tr>
<td>mICH score</td>
<td></td>
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<td>&lt;0.0001</td>
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<tr>
<td>0</td>
<td>340 (28.9)</td>
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<td>1</td>
<td>180 (15.3)</td>
<td>52 (10.7)</td>
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<td>2</td>
<td>276 (23.5)</td>
<td>111 (22.9)</td>
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<td>3</td>
<td>202 (17.2)</td>
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<tr>
<td>5</td>
<td>29 (2.5)</td>
<td>29 (6.0)</td>
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</tbody>
</table>
Accuracy and Clinical Usefulness of Intracerebral Hemorrhage Grading Scores: A Direct Comparison in a UK Population
Adrian R. Parry-Jones, Kamran A. Abid, Mario Di Napoli, Craig J. Smith, Andy Vail, Hiren C. Patel, Andrew T. King and Pippa J. Tyrrell

Stroke. 2013;44:1840-1845; originally published online May 16, 2013; doi: 10.1161/STROKEAHA.113.001009

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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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3 Which factors influence decisions to transfer and treat patients with acute intracerebral hemorrhage and which are associated with prognosis? A retrospective cohort study
3.1 Introduction

With the establishment of relevant prognostic factors related to ICH mortality in the previous chapter, it was important to elicit whether significant differences existed between treating centres. The clinical and radiological data from each patient was therefore compared to determine which of these were responsible for subsequent transfer to a specialist surgical unit. Survival data between the different centres and between medical and surgical management were subsequently compared to determine whether a survival advantage existed between the centres that participated in the study.
Which factors influence decisions to transfer and treat patients with acute intracerebral haemorrhage and which are associated with prognosis?
A retrospective cohort study

Kamran A Abid,1,2 Andy Vail,3 Hiren C Patel,1,4 Andrew T King,1,4 Pippa J Tyrrell,4,5 Adrian R Parry-Jones4,5

ABSTRACT

Objectives: To identify factors associated with the decision to transfer and/or operate on patients with intracerebral haemorrhage (ICH) at a UK regional neurosurgical centre and test whether these decisions were associated with patient survival.

Design: Retrospective cohort study.

Setting: 14 acute and specialist hospitals served by the neurosurgical unit at Salford Royal NHS Foundation Trust, Salford, UK.

Participants: All patients referred acutely to neurosurgery from January 2008 to October 2010.

Outcome measures: Primary outcome was survival and secondary outcomes were transfer to the neurosurgical centre and acute neurosurgery.

Results: We obtained clinical data from 1364 consecutive spontaneous patients with ICH and 1175 cases were included in the final analysis. 140 (12%) patients were transferred and 75 (6%) had surgery. In a multifactorial analysis, the decision to transfer was more likely with younger age, women, brainstem and cerebellar location and larger haematomas. Risk of death in the following year was higher with advancing age, lower Glasgow Coma Scale, larger haematomas, brainstem ICH and intraventricular haemorrhage. The transferred patients had a lower risk of death relative to those remaining at the referring centre whether they had surgery (HR 0.46, 95% CI 0.32 to 0.67) or not (HR 0.41, 95% CI 0.22 to 0.73). Acute management decisions were included in the regression model for the 227 patients under either stroke medicine or neurosurgery at the neurosurgical centre and early do-not-resuscitate orders accounted for much of the observed difference, independently associated with an increased risk of death (HR 4.8, 95% CI 2.7 to 8.6).

Conclusions: The clear association between transfer to a specialist centre and survival, independent of established prognostic factors, suggests aggressive supportive care at a specialist centre may improve survival in ICH and warrants further investigation in prospective studies.

INTRODUCTION

Spontaneous intracerebral haemorrhage (ICH) represents a major worldwide health problem1–2 and is responsible for 10–15% of all strokes. ICH is associated with the highest mortality and morbidity of all stroke subtypes and case fatality rate has remained unchanged for the past three decades.1–3 Although a number of large trials testing new therapies for ICH are underway, at present, there is no proven treatment for this condition and care remains largely supportive.

There has been increasing interest in admitting patients directly to specialist centres to improve outcomes in a diverse range of conditions including trauma, head injury and subarachnoid haemorrhage.4–6 A recent study of 205 patients with ICH presenting to emergency departments in non-tertiary hospitals in the USA found a non-significant trend towards reduced mortality with transfer to a tertiary care centre.7 Another US study reported lower mortality for patients with ICH treated in a neurological intensive care unit (ICU) rather than...
in a general ICU, but did not adjust for imaging findings and had missing premorbid health data for over half of the patients included in the study. These findings suggest that outcomes after ICH may be improved by timely and comprehensive supportive care at specialist centres, but this has not been tested in a large population in a healthcare system outside the USA.

In the UK, ICH is initially managed by physicians from diverse specialties including stroke medicine, emergency medicine and elderly care. Neurosurgical and neurocritical care expertise are typically concentrated in regional neurosciences centres such that the majority of patients are not admitted directly to hospitals with these services. Receiving hospitals typically refer most ICH cases to regional neurosurgical centres shortly after diagnosis. Although national guidelines have been published, there remains considerable uncertainty about the surgical management of ICH and practice is likely to vary.

We first examined the factors associated with the decisions to transfer and/or operate on patients with ICH referred acutely to neurosurgery in a large cohort of unselected patients with ICH representative of hospital-based stroke care in the UK. Second, we sought to identify whether the decisions to transfer and/or operate on patients with ICH were associated with survival, independent of known prognostic factors.

METHODS

Participants
We included all patients referred to the Neurosurgical Department at Salford Royal NHS Foundation Trust (SRFT), Salford, UK with acute ICH from 14 regional hospitals between 1 January 2008 and 17 October 2010. Based on the reported incidence of ICH, it is likely that the majority (around 75%) of new ICH cases in the catchment population of around 2.6 million were referred during the study period.

Procedures
Details of every referral made to the neurosurgical service are prospectively recorded by the duty neurosurgeon in an electronic referral database. The date and time of the referral, demographic details, history, examination (including the Glasgow Coma Scale (GCS) score at time of referral), investigation findings, diagnosis and the agreed management plan are recorded. Each case is reviewed by an on-call consultant neurosurgeon and consultant neuroradiologist within 24 h. All ICH cases during our study period were reviewed by a study investigator and neurosurgeon (KAA) for inclusion in the study, blinded to survival status. Cases were excluded if there was clear evidence on the history of a traumatic cause for ICH, if the diagnosis was not ICH, if there were incomplete clinical data (including the GCS) or if the initial CT scan after presentation was not available for review. To provide a measure of premorbid health, the American Society of Anesthesiologists (ASA) grade was determined based on details of comorbidities and premorbid functional status recorded in the neurosurgical database. A single study investigator (KAA) reviewed each patient’s first CT scan and recorded haematoma location (deep, lobar, brainstem and cerebellum) and the presence or absence of hydrocephalus and intraventricular haemorrhage (IVH). Deep ICH was defined as involving deep supratentorial brain structures, including the basal ganglia and thalamus. Lobar ICH was defined as involving the cerebral lobes without involvement of deep structures. Brainstem location was defined as haemorrhage involving the medulla, pons and/or midbrain. Any haemorrhage within the cerebellum was defined as cerebellar. Where blood was evident in more than one compartment, the compartment containing the majority of the haematoma was recorded. Haematoma volume was determined by the ABC/2 method.

Explanatory factors were log-transformed if positively skewed. A binary logistic regression model was used to investigate associations between decisions to transfer and operate on patients referred to the neurosurgical service. Cox regression analysis was performed to investigate factors associated with the risk of death during the 1-year period after referral.

In modelling, a rational selection procedure was applied to avoid overfitting where data were sparse, with factors exerting little influence on the outcome omitted from final models. Organisational factors (centre and consultant) were first screened in unifactorial analyses. If statistically significant they were included as strata when selecting clinical factors and retained only if they remained statistically significant after adjustment for retained clinical factors. For analysis of decision to operate, it was necessary to reduce the number of model parameters: centres were categorised according to their chance of having a transfer accepted; and ASA grades 3 and above were merged. For analysis of patient survival, follow-up time was censored beyond 1 year.

All analyses were performed with Stata (V.12, StataCorp LP, USA).

Role of the funding source
This research received no specific grant from any funding agency in the public, commercial or not-for-
RESULTS

In total, 1364 cases were coded as ICH in the referrals database for the period of our study and 189 were excluded for reasons outlined in figure 1. Of these, 1175 patients with complete data were included in the final analysis. The number of patients referred from each of the 14 hospitals ranged from 38 to 138, with the exception of one specialist oncology centre (n=5) that does not receive acute patients directly. The baseline characteristics of the study population, categorised by whether they were transferred and had surgery, are outlined in table 1.

After adjustment for other variables, the decision to transfer a patient to the neurosurgical centre was associated with higher GCS score, brainstem and cerebellar location, larger haematoma, younger age and female sex (table 2). Higher GCS was predictive of transfer (OR 1.13; 95% CI 1.06 to 1.21). A strong association was found between infratentorial haemorrhage and transfer (OR 4.41; 95% CI 2.31 to 8.40) and with each doubling in ICH volume the odds of transfer also approximately doubled. The odds of transfer more than halved with every 10 year increase in patient age (OR 0.41; 95% CI 0.35 to 0.48). Patients who had an ASA grade 3 (severe systemic disease) or more were less likely to be transferred than those who were previously healthy (ASA grade 1). There were differences in acceptance rates by referring centre. Post hoc rationalisation suggested that patients at designated stroke centres and those with greater travel times to the neurosurgical centre were less likely to be transferred. There was no evidence of differences in decision-making between the 19 consultant neurosurgeons. Hydrocephalus on the initial radiology report was excluded from the final model as there was no evidence of association with the decision to transfer.

Of the 140 patients transferred to neurosurgical care, 75 (54%) had acute surgery. The decision to perform acute neurosurgery for the 140 transferred patients was associated with brainstem ICH location and lower GCS (table 3). Acute surgery was less common with lobar location, larger haematoma, younger age and female sex (table 3). After taking in to account the GCS, location of haematoma and volume of haematoma, there was no evidence that the other variables (referring centre, responsible neurosurgeon, age, IVH, reported hydrocephalus on initial imaging or ASA grade) were associated with the decision to operate and examination of interaction terms in the model confirmed this was specifically for lobar haemorrhages. After taking into account the GCS, location of haematoma and volume of haematoma, there was no evidence of association with the decision to transfer and operate remained strong predictors of survival (table 4, figure 2).

One-year mortality for all patients was 53% and did not significantly differ between patients referred from the Greater Manchester Comprehensive Stroke Centre based at SRFT (54%), all stroke centres combined (52%) or all teaching hospitals combined (53%), but was significantly lower in those transferred to neurosurgical care (34%). In a multifactorial Cox regression analysis for the whole study population, increasing age, increasing haematoma volume, decreasing GCS, brainstem location, IVH and severe pre-existing patient comorbidity (ASA grade ≥3) were found to be strongly associated with the hazard of death (table 4). After adjusting for these clinical prognostic factors, the decisions to transfer and operate remained strong predictors of survival (table 4, figure 2).

To further understand the reasons for improved survival for neurological patients, we compared the acute management of patients who were under the care of the neurosurgical (n=140) or stroke medicine (n=87) teams at SRFT, as both groups were cared for at the same hospital with access to the same support facilities during the same time period. The differences between the groups are not significant, but neurological patients tended to...
have larger haematomas, more IVH and a lower GCS, but younger age and a better premorbid ASA grade (table 5). We applied the Cox regression model to the subset of patients admitted to SRFT (n=227) and estimated effects were broadly similar to the whole population, although with wider CIs reflecting the reduced sample size. This suggested that the SRFT stroke medicine patients were representative of those not transferred to neurosurgery (table 4).

Within the SRFT patients, marked differences between stroke medicine and neurosurgery were identified in the number of patients transferred to higher level care, the use of DNR orders and whether further vascular imaging was performed (table 5). To determine whether these factors accounted for the improved outcomes seen in neurosurgical patients, the Cox regression was rerun from 24 to 48 h to include these factors, excluding patients who had died before each time point. Results are shown for 24 h as the differences were apparent by this time. Hazard of death was higher for those with DNR decisions in place and lower in those who underwent vascular imaging, regardless of the findings (normal, arteriovenous malformation (AVM) or aneurysm). There was no evidence of association with the acute care setting. After adjustment for these

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline characteristics of study population divided by transfer and surgery decisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic</td>
<td>Not transferred (n=1035)</td>
</tr>
<tr>
<td>Age (years), median (IQR)</td>
<td>75 (65 to 84)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>546 (52.8)</td>
</tr>
<tr>
<td>Referring hospital, ID (n)</td>
<td>1 (4), 2 (94), 3 (87), 4 (78), 5 (65), 6 (47), 7 (58), 8 (43), 9 (129), 10 (88), 11 (101), 12 (33), 13 (95), 14 (113)</td>
</tr>
<tr>
<td>Clinical</td>
<td></td>
</tr>
<tr>
<td>Premorbid ASA grade (%)</td>
<td>1 (14%), 2 (54%), 3 (23%), 4/5 (10%)</td>
</tr>
<tr>
<td>GCS, median (IQR)</td>
<td>14 (7 to 15)</td>
</tr>
<tr>
<td>Imaging</td>
<td></td>
</tr>
<tr>
<td>Haematoma volume (mL), median (IQR)</td>
<td>23.6 (6.8 to 63.4)</td>
</tr>
<tr>
<td>Deep, n (%)</td>
<td>444 (43%)</td>
</tr>
<tr>
<td>Lobar, n (%)</td>
<td>481 (46%)</td>
</tr>
<tr>
<td>Brainstem, n (%)</td>
<td>74 (7%)</td>
</tr>
<tr>
<td>Cerebellum, n (%)</td>
<td>36 (3%)</td>
</tr>
<tr>
<td>Hydrocephalus, n (%)</td>
<td>503 (49%)</td>
</tr>
<tr>
<td>Intra/ventricular haemorrhage, n (%)</td>
<td>383 (37%)</td>
</tr>
</tbody>
</table>

Total number of patients in final analysis=1175.
ASA, American Society of Anesthesiologists grade; GCS, Glasgow Coma Scale.

<p>| Table 2 | Predictors of transfer to neurosurgical care |</p>
<table>
<thead>
<tr>
<th>Factor</th>
<th>OR</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per 10 year increase)</td>
<td>0.41</td>
<td>(0.35 to 0.48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>1.92</td>
<td>(1.22 to 3.01)</td>
<td>0.005</td>
</tr>
<tr>
<td>GCS</td>
<td>1.11</td>
<td>(1.05 to 1.18)</td>
<td>0.001</td>
</tr>
<tr>
<td>ASA grade 2 (vs 1)</td>
<td>0.78</td>
<td>(0.46 to 1.34)</td>
<td>0.37</td>
</tr>
<tr>
<td>ASA grade 3 (vs 1)</td>
<td>0.43</td>
<td>(0.21 to 0.90)</td>
<td>0.03</td>
</tr>
<tr>
<td>ASA grade 4/5 (vs 1)</td>
<td>0.16</td>
<td>(0.05 to 0.54)</td>
<td>0.003</td>
</tr>
<tr>
<td>log ICH volume</td>
<td>1.97</td>
<td>(1.58 to 2.45)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lobar (vs deep)</td>
<td>0.72</td>
<td>(0.43 to 1.19)</td>
<td>0.20</td>
</tr>
<tr>
<td>Brainstem (vs deep)</td>
<td>2.42</td>
<td>(1.00 to 5.82)</td>
<td>0.05</td>
</tr>
<tr>
<td>Cerebellum (vs deep)</td>
<td>6.37</td>
<td>(2.65 to 15.29)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

140 (12%) patients were transferred acutely to neurosurgical care and 1035 (88%) remained at their referring hospital for further care. Referring hospital was included in the regression model and was significantly associated with the decision to transfer (p=0.02).

ASA, American Society of Anesthesiologists grade; GCS, Glasgow Coma Scale; ICH, intracerebral haemorrhage.

<p>| Table 3 | Predictors of surgical treatment in transferred patients |</p>
<table>
<thead>
<tr>
<th>Factor</th>
<th>OR</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCS</td>
<td>0.77</td>
<td>(0.68 to 0.87)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>log ICH volume</td>
<td>1.49</td>
<td>(1.002 to 2.21)</td>
<td>0.049</td>
</tr>
<tr>
<td>Lobar ICH (vs deep)</td>
<td>0.34</td>
<td>(0.15 to 0.77)</td>
<td>0.009</td>
</tr>
<tr>
<td>Infratentorial (vs supratentorial)</td>
<td>4.45</td>
<td>(1.40 to 14.12)</td>
<td>0.011</td>
</tr>
<tr>
<td>GCS</td>
<td>0.77</td>
<td>(0.68 to 0.88)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>log ICH volume</td>
<td>1.54</td>
<td>(1.03 to 2.30)</td>
<td>0.04</td>
</tr>
<tr>
<td>Lobar (vs deep)</td>
<td>0.44</td>
<td>(0.19 to 1.03)</td>
<td>0.06</td>
</tr>
<tr>
<td>Brainstem (vs deep)</td>
<td>18.2</td>
<td>(1.72 to 192.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cerebellum (vs deep)</td>
<td>1.03</td>
<td>(0.27 to 4.01)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Of the 140 patients transferred to neurosurgical care, 75 underwent surgery.
GCS, Glasgow Coma Scale; ICH, intracerebral haemorrhage.

associations, the effect of transfer to neurosurgery on survival was lost (table 6). For the 139 (61%) patients without a DNR order, there were only two calls to the cardiac arrest team. The first was due to a respiratory peri-arrest and the second was the result of a seizure without cardiac or respiratory arrest, and both patients survived.

DISCUSSION
In our large UK cohort of patients with ICH, the decision to transfer to our neurosurgical unit was more likely in those with infratentorial ICH, higher GCS, younger age, larger haematomas and female sex. For transferred patients, the hazard of death, adjusted for established ICH prognostic factors, was less than half that of those remaining at their referring hospital.

Our study has a number of strengths. We have studied a large cohort of over a thousand patients with complete survival data likely to be representative of routine clinical practice in similar population centres in the UK. All clinical data were prospectively recorded in the electronic neurosurgical database by a neurosurgeon at the time of referral and each case was reviewed by a senior neurosurgeon and neuroradiologist within 24 h of referral. Therefore, accurate and complete clinical information was available for the great majority of patients with ICH referred during the study period. Data collection and image analysis for this study were conducted by a single investigator, blinded to outcome, which minimises the introduction of bias. Our study also has some limitations; first, we excluded 107 (8%) cases due to missing data although this was unlikely to have greatly affected our overall conclusion. Second, we have retrospectively estimated premorbid ASA grade from the information recorded in the neurosurgical database. Clinical information recorded in the neurosurgical database was fairly detailed for most cases, but it remains possible that the information was incomplete for some, thus underestimating the effect of premorbid health on mortality. Third, we believe that we have captured approximately 75% of the incident ICH cases in our population, but bias may have been introduced by case selection from referring hospitals. Fourth, because the study was limited to a single receiving centre, it is uncertain whether our findings are likely to be relevant to practice in other neurosurgical centres. However, the large sample size of our study and the fact that decisions were made by 1 of 19 consultant neurosurgeons increases the relevance of our conclusions to other centres and surgeons. Finally, functional outcome is of the utmost importance to patients and carers but we did not have these data in our ICH survivors. A previous study has shown worse 3-month cognitive outcomes in transferred versus non-transferred patients with ICH despite similar admission scores. It is not clear whether improved survival with neurosurgical care may be at the expense of an increased proportion of patients left alive but with

<table>
<thead>
<tr>
<th>Factor</th>
<th>Whole population (n=1175)</th>
<th>SRFT patients only (n=227)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age (per 10 year increase)</td>
<td>1.17</td>
<td>(1.10 to 1.25)</td>
</tr>
<tr>
<td>GCS</td>
<td>0.81</td>
<td>(0.79 to 0.83)</td>
</tr>
<tr>
<td>ASA grade=2 (vs 1)</td>
<td>1.16</td>
<td>(0.89 to 1.51)</td>
</tr>
<tr>
<td>ASA grade≥3 (vs 1)</td>
<td>1.63</td>
<td>(1.24 to 2.14)</td>
</tr>
<tr>
<td>log ICH volume</td>
<td>1.30</td>
<td>(1.18 to 1.38)</td>
</tr>
<tr>
<td>Lobar (vs deep)</td>
<td>0.99</td>
<td>(0.82 to 1.18)</td>
</tr>
<tr>
<td>Brainstem (vs deep)</td>
<td>1.82</td>
<td>(1.35 to 2.45)</td>
</tr>
<tr>
<td>Cerebellum (vs deep)</td>
<td>1.46</td>
<td>(0.96 to 2.22)</td>
</tr>
<tr>
<td>IVH</td>
<td>1.54</td>
<td>(1.28 to 1.84)</td>
</tr>
<tr>
<td>Neurosurgical care, no surgery*</td>
<td>0.41</td>
<td>(0.22 to 0.73)</td>
</tr>
<tr>
<td>Neurosurgical care, surgery*</td>
<td>0.46</td>
<td>(0.32 to 0.67)</td>
</tr>
</tbody>
</table>

*Compared with remaining at referring location for further care.
ASA, American Society of Anesthesiologists grade; GCS, Glasgow Coma Scale; ICH, intracerebral haemorrhage; IVH, intraventricular haemorrhage; SRFT, Salford Royal NHS Foundation Trust.

Figure 2 Kaplan-Meier survival curve for different management groups adjusted for case mix. ICH, intracerebral haemorrhage; transferred, no surgery, n=65/1175 (6%); transferred, surgery, n=75/1175 (6%); not transferred, n=1035/1175 (88%).


Table 5  Clinical characteristics, management and survival at Salford Royal Hospital by responsible care team

<table>
<thead>
<tr>
<th>Factor</th>
<th>Surgical (n=140)</th>
<th>Medical (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years), median (IQR)</td>
<td>55 (45 to 65)</td>
<td>73 (61 to 81)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>73 (52%)</td>
<td>53 (61%)</td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premorbid ASA grade (%)</td>
<td>1 (32%)</td>
<td>1 (18%)</td>
</tr>
<tr>
<td>Lobar, n (%)</td>
<td>56 (40%)</td>
<td>27 (31%)</td>
</tr>
<tr>
<td>Brainstem, n (%)</td>
<td>11 (8%)</td>
<td>10 (12%)</td>
</tr>
<tr>
<td>Cerebellum, n (%)</td>
<td>14 (10%)</td>
<td>5 (6%)</td>
</tr>
<tr>
<td>Hydrocephalus, n (%)</td>
<td>72 (51%)</td>
<td>46 (53%)</td>
</tr>
<tr>
<td>IVH, n (%)</td>
<td>63 (45%)</td>
<td>30 (34%)</td>
</tr>
<tr>
<td><strong>Acute care setting, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICU or NHDU within 24 h</td>
<td>90 (64%)</td>
<td>9 (10%)</td>
</tr>
<tr>
<td>ICU or NHDU within 48 h</td>
<td>92 (66%)</td>
<td>11 (13%)</td>
</tr>
<tr>
<td><strong>Vascular imaging, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within 24 h</td>
<td>51 (36%)</td>
<td>6 (7%)</td>
</tr>
<tr>
<td>Within 48 h</td>
<td>56 (40%)</td>
<td>7 (8%)</td>
</tr>
<tr>
<td>AVM identified</td>
<td>13 (9%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Aneurysm identified</td>
<td>20 (14%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Do not attempt resuscitation, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within 24 h</td>
<td>31 (22%)</td>
<td>56 (64%)</td>
</tr>
<tr>
<td>Within 48 h</td>
<td>32 (23%)</td>
<td>56 (64%)</td>
</tr>
<tr>
<td><strong>Surgery, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No surgery</td>
<td>65 (46%)</td>
<td>87 (100%)</td>
</tr>
<tr>
<td>ICP monitor only</td>
<td>2 (1%)</td>
<td>NA</td>
</tr>
<tr>
<td>EVD</td>
<td>23 (16%)</td>
<td>NA</td>
</tr>
<tr>
<td>Haematoma evacuation</td>
<td>50 (36%)</td>
<td>NA</td>
</tr>
<tr>
<td>Dead at 30 days, n (%)</td>
<td>39 (28%)</td>
<td>38 (44%)</td>
</tr>
</tbody>
</table>

ASA, American Society of Anesthesiologists grade; AVM, arteriovenous malformation; EVD, external ventricular drain; GCS, Glasgow Coma Scale; ICH, intracerebral haemorrhage; ICP, intracranial pressure; ICU, Intensive Care Unit; IVH, intraventricular haemorrhage; NHDU, Neurological High Dependency Unit.

severe long-term neurological deficits. Further prospective studies will be required to definitively answer this question.

Previous US studies have provided some evidence to support improved survival after ICH in those treated in specialist neurological ICUs (when compared with non-specialist ICUs) and/or in tertiary centres. However, transfer to ICU/HDU may be a marker of later neurological/general decline with an associated worsening of prognosis. We do not have information relating to neurological/general decline beyond the acute phase, so no clear conclusions can be drawn from this finding. ICHs caused by AVMs are associated with a lower mortality than ICH due to other causes but aneurysmal ICH carries a fairly poor prognosis. Patients with ICH in whom there are reasons to suspect an underlying AVM or aneurysm (imaging characteristics, age and/or history) are often transferred to neurosurgery, primarily for further vascular imaging. If patients with AVMs (but not aneurysms) are over-represented, this may improve overall survival for those transferred to neurosurgical care and may account for the association seen in our study between performing further vascular imaging and improved survival. However, AVMs were found in 9% and aneurysms in 14% of neurosurgical patients and a systematic review of published studies found an overall incidence of 20% for AVMs and 13% for aneurysms. Although most studies have probably overestimated the incidence of vascular malformations due to selection bias, vascular malformations were found less often in our study than in previous published studies. We found no association with the findings of vascular imaging (when performed) and survival in our study. However, as most stroke medicine patients did not have vascular imaging, we cannot completely rule out the introduction of bias in our study from a differing mix of underlying aetiologies between the neurosurgery and stroke medicine patients.

The overall transfer rate to the neurosurgical unit was 12% (140 patients) of ICH referrals. Despite contemporaneous national guidelines recommending initial medical management for infratentorial ICH, infratentorial location was strongly associated with the decision to transfer. The preference for transferring younger patients may partly be related to the increased likelihood of finding an underlying vascular malformation and hence a lower threshold for early transfer and investigation. Advancing age greatly reduced the likelihood of transfer and this seems to be independent of premorbid health. This may represent a reluctance to operate on older patients as there is evidence of worse outcomes from neurosurgery with older age in other conditions. Why female sex was positively associated with the decision to transfer is not clear, though this may relate to the higher incidence of cerebral aneurysms in women. Among transferred patients, the decision to intervene surgically was more likely with larger haematomas and infratentorial location, probably due to the propensity of infratentorial bleeds to cause herniation syndromes and/or obstructive hydrocephalus. Clearly, there are reasons why neurosurgeons may transfer patients with ICH to their care that will carry an associated survival benefit, independent of further care, which will introduce unmeasured confounding. Although younger patients with better premorbid health...
Although DNR orders should not limit other aspects of care, there is evidence for the potential benefits of aggressive supportive care in ICH. The use of early DNR orders has previously been shown to be independently associated with reduced survival after ICH, so DNR orders within 48 h of admission are advised against in the recent US guidelines. The use of early DNR orders has previously been shown to be independently associated with a higher hazard of death. To further understand this finding, we compared patients within our study population who were under either neurosurgery or stroke medicine at the same hospital (SRFT). DNR orders were in place during the acute phase (<48 h) in a far higher proportion of stroke medicine patients and this was strongly and independently associated with a higher hazard of death. The use of early DNR orders has previously been shown to be independently associated with reduced survival after ICH, so DNR orders within 48 h of admission are advised against in the recent US guidelines. Although DNR orders should not limit other aspects of ICH care, they may subsequently lead to a less aggressive approach to care by the wider care team. Alternatively, they may be markers of less measurable but unalterable prognostic factors and our retrospective design could not determine causation. It may be that a more aggressive approach to supportive care after ICH in neurosurgical patients is responsible for improved survival and that the infrequent use of early DNR orders and high rate of vascular imaging are markers of this. In addition to the studies discussed above, further indirect evidence for the potential benefits of aggressive supportive care in ICH is provided by the surprisingly low mortality rates for patients recruited to the Clot Lysis: Evaluating Accelerated Resolution of Intraventricular Hemorrhage (CLEAR) II trial. Patients included in the trial were expected to have a high mortality based on their clinical and imaging characteristics but had around 20% mortality in the active and placebo groups. Further support for this theory is available for head injury, where observational data have supported improved outcomes with direct admission to a neurosurgical centre, and a randomised controlled trial to test this hypothesis is currently underway (ISRCTN68087745).

Our findings support the hypothesis that aggressive supportive care in specialist centres may improve survival after acute ICH and this warrants further investigation. This may have implications for the organisation of care for patients with ICH in the UK and internationally.

Table 6  Management factors within the first 24 h associated with risk of death in first year for SRFT patients

<table>
<thead>
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<th>Adjusted† HR (95% CI)</th>
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*Unadjusted for specific factors within table but adjusted for factors identified in table 4.
†Additionally adjusted for factors within table.
ICU, intensive care unit; NHDU, neurological high dependency unit; SRFT, Salford Royal NHS Foundation Trust.

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Contributors KAA contributed to the design of the study, collected the data, analysed CT scans and wrote the first draft of the manuscript. AV contributed to the design of the study, undertook the statistical analyses, and reviewed and contributed to the final draft of the manuscript. ATK, HCP and PJT contributed to the design of the study and reviewed the final draft of the manuscript. ARP-J contributed to the study design, had overall responsibility for the work, assisted with the data analysis and wrote the final draft of the manuscript and is the guarantor of the study. All authors, external and internal, had full access to all of the data (including statistical reports and tables) in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

Funding This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None.

Ethics approval Ethics approval was granted for this study by NHS Research Ethics Committee North West 10—GM North; Ref 11/H1011/3.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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REFERENCES


Microglial activation and blood brain-barrier breakdown in acute intracerebral haemorrhage: a combined $[^{11}C]$-(R)-PK11195 PET and DCE-MRI study.

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4.1 Introduction

ICH accounts for at least 10% of strokes in the United Kingdom (UK)\(^1\) and has an incidence of 25 new cases per 100,000 per year\(^2\). Around 40% of ICH patients die before one month and over half of survivors remain dependent on others for day-to-day care\(^2\). Compared to ischaemic stroke, ICH leads to a greater loss of quality adjusted life years\(^3\) and whilst the incidence of ischaemic stroke has fallen\(^4\), the incidence and case fatality of ICH has not changed in the last 30 years\(^1,2\). Despite the considerable and persistent health, social and economic burden of ICH, it has received little attention in comparison to ischaemic stroke\(^5\), and has no proven acute therapy, aside from modest benefits from intensive blood pressure lowering\(^6\) and stroke unit care\(^7\).

Regardless of the cause, extravasation of blood into the brain parenchyma leads to rapid physical tissue injury and may lead to mass effect, brain herniation syndromes and early death\(^8\).

For survivors of the acute phase, secondary brain injury contributes to tissue damage over the subsequent hours to days and is driven by a cascade of cellular and molecular events including the toxic effects of blood components (thrombin, haem, iron) and sterile inflammation\(^8\). Partly driven by these processes, cerebral oedema increases rapidly over 3 days with a further slow increase up to 1-2 weeks after onset\(^9,10\). Preclinical studies have demonstrated that local inflammation occurs in response to diverse acute brain injuries (including ICH), exacerbating early damage and playing an important role in later repair and recovery. Within hours of an acute brain injury, activated microglia take on a pro-inflammatory phenotype, releasing cytokines and chemokines that activate astrocytes and endothelial cells and lead to recruitment of neutrophils to the site of injury. This early inflammatory response profoundly exacerbates tissue injury but over subsequent days, factors released by circulating blood monocytes...
recruited to the site of injury limit damage by switching the resident microglia to an anti-inflammatory phenotype. Previous clinical studies in ICH have largely focused on peripheral inflammatory markers and shown associations between fever, elevated white blood cell count, IL-6, C-reactive protein (CRP), fibrinogen, and c-fibronectin on admission and worse short term outcomes (haematoma expansion and neurological decline at 48 h). Elevated CRP, fibrinogen and matrix metalloproteinases (MMPs) on admission are associated with poor functional outcomes and survival at 1-3 months. These clinical studies demonstrate the importance of the systemic inflammatory response but provide no information on the inflammatory response within the brain, where inflammation contributes directly to secondary injury. Animal models have taught us much about the central nervous system (CNS) inflammatory response to ICH, but current ICH models have limited relevance to the clinical disease and research in ischaemic stroke has been blighted by the failure to translate promising preclinical findings to effective therapies. The blood-brain barrier may impede the delivery of some peripherally administered drugs to the CNS. Activated microglia are key cellular coordinators of the inflammatory response and a major source of inflammatory mediators in acute ICH. IL-1 worsens outcomes in ischaemic stroke via both systemic and central actions but we do not know the relative contribution of each in ICH. To limit central inflammation, anti-inflammatory treatments must enter the brain parenchyma. However, acute brain injury is often accompanied by a localised increase in blood-brain barrier permeability. There is limited data on the blood-brain barrier after ICH, with a single study showing perihematomal blood-brain barrier breakdown at 1 week after symptom onset in 25 patients and no data describing the blood-brain barrier during the more acute stage of the disease. At 1 week, blood-brain barrier breakdown was greater with lobar (vs. deep) ICH and had a positive correlation with ICH volume. If similar relationships are seen in the more acute phase, then delivery of anti-inflammatory drugs will be expected to differ depending on haematoma location and size with implications for efficacy. Furthermore,
understanding how well blood-brain barrier breakdown co-localises with inflammation is important in predicting the delivery of drug to its therapeutic target. CNS inflammation can be imaged \textit{in vivo} by using PET to study the uptake of radiolabelled tracers (e.g. $^{[11]}\text{C}$-$(R)$-PK11195) that bind to the translocator protein (TSPO) present in activated microglia. Our pilot study sought to demonstrate the feasibility of performing these techniques in patients during the acute stage of ICH.
4.2 Methods

All patients were recruited from Salford Royal NHS Foundation Trust (Salford, UK) following NHS Research Ethics Committee (REC ref 12/NW/0435) and local approvals. Five ICH patients were recruited between 4-28 days after symptom onset to undergo a $[^{11}C](R)$-PK11195 PET to identify microglial activation and an MRI brain scan (on day of PET +/- 3 days) for coregistration and identification of the haematoma, perihaematoma oedema, acute ischaemia, blood-brain barrier breakdown and co-existent microbleeds. PET scans were undertaken at the Wolfson Molecular Imaging Centre, University of Manchester. MRI scans were undertaken at Salford Royal NHS Foundation Hospital. Venous blood was taken during the initial hospitalisation (where possible) and again immediately prior to the PET scan to measure levels of CRP and IL-6. All patients admitted to the Greater Manchester Comprehensive Stroke Centre (GMCSC) or the Neurosurgical wards at Salford Royal NHS Foundation Trust with a diagnosis of acute ICH were screened for study eligibility criteria. These included age ≥ 18, ability to provide informed consent, a clinical diagnosis of acute, spontaneous intracerebral haemorrhage, supported by CT brain imaging, mobile with the assistance of 1 person at the time of research scans, able to lie flat for at least one hour at the time of research scans, discharged from hospital or current in-patient on a rehabilitation unit/ward at the time of research scans. Patients were considered ineligible for the study if there were any of the following exclusion criteria; a clear history of recent (<7 days prior to onset) head trauma, thought to be the cause of intracerebral haemorrhage; if intracerebral haemorrhage was secondary to venous sinus thrombosis, intracranial tumour or a vascular malformation; contraindication to magnetic resonance imaging; pregnant or breast feeding patients; significant renal impairment (estimated glomerular filtration rate < 30); neurosurgical procedure performed since admission or booked for surgery; treatment with drugs known to or likely to interfere with PK11195 binding.
(including benzodiazepines, steroids, minocycline) that could not be stopped in sufficient time to prevent interference with PET scanning.

### 4.2.1 PET scanning

All PET scanning was performed with a High Resolution Research Tomograph (HRRT; Siemens/CTI Knoxville, TN). The axial and transaxial fields of view of this 3-D dedicated brain scanner were 25cm and 35 cm respectively, giving an intrinsic spatial resolution of approximately 2.5mm. A 7 minute transmission scan using a $^{137}$Cs point source was acquired for subsequent attenuation and scatter correction. Shortly after the start of the emission scan, $^{11}$C-(R)-PK11195 was injected intravenously as a slow bolus over approximately 15 seconds. Emission data were then acquired over 60 minutes in list mode as 18 time-frames (one background frame of approximately 7 minutes prior to injection, followed by 17 frames: 1 x 15s, 1 x 5s, 1 x 10s, 1 x 30s, 4 x 60s, 7 x 300s and 2 x 600s). The position of the subject's head was indicated using laser beams and monitored via a camera throughout the scan and corrected immediately if necessary.

Iterative ordinary Poisson-ordered subset expectation maximisation (OP OSEM) 3-D method was used to reconstruct a quantitative series of dynamic images from the PET emission scan incorporating normalisation and corrections for random coincidences, scattered radiation and attenuation. A supervised clustering algorithm was used to extract grey matter reference tissue kinetics from dynamic brain PET data to generate a reference tissue input function for each PET scan and was compared with a reference tissue input function from cerebellar grey matter. Parametric images were generated using the Simplified Reference Tissue Model (SRTM). For reconstruction, 16 subsets were used and the reconstruction was run for 12 iterations for the mean activity concentration value at the volume-of-interest level to converge. The voxel size of
the reconstructed PET images was 1.22 x 1.22 x 1.22 mm$^3$. After reconstruction, images were regularised with a 3-D Gaussian filter of 4mm full-width at half maximum to reduce image noise at the voxel level prior to calculation of parametric maps with the SRTM.

4.2.2 MR scanning

All MR scans were performed with a Philips 3T Achieva whole body MR scanner with an 8 channel head coil.

The MR imaging protocol included susceptibility weighted imaging (SWI), T1-weighted volume imaging, fluid attenuated inversion recovery (FLAIR) imaging, diffusion weighted imaging, and dynamic contrast enhanced T1 MRI (DCE-MRI).

A dynamic series of 3D spoiled gradient echo images were acquired with the following scan parameters: Field of view 192 mm x 192 mm and matrix size of 128 giving in-plane resolution of 1.5 x 1.5 mm; 32 contiguous axial slices of 4mm thickness; TE = 0.8ms, TR=2.4ms, flip angle 10 degrees and image acquisition time of 7.6 seconds. 80 images were acquired over 10 minutes. On the 8th dynamic, a gadolinium (Gd-DTPA) bolus was administered using a power injector. The volume administered was proportional to the weight of the subject with a dose ratio of 0.1mmol/kg.

Prior to the dynamic scan, a series of additional 3D spoiled gradient echo images were acquired at 3 flip angles (2, 5 and 10 degrees) in order to calculate a pre-contrast T$_1$ map. The acquisition parameters were the same as for the dynamic series except only 8 dynamics were collected, giving an acquisition time of 60 s per flip angle. An average image was calculated for each flip angle by taking a mean over the 8 dynamics. In order to correct for B1 field inhomogeneities, a B1 mapping sequence was also acquired with the same voxel size and coverage as for the
variable flip angle images. This consisted of a pair of 3D spoiled gradient echo images with TR1 = 25 ms and TR2 = 125 ms, flip angle 60 degrees, TE 5 ms, acquisition time 117 seconds.

In addition, a T2-weighted FLAIR image was acquired with the following parameters: TR 10 s, TI 2.75 s, TE 140 ms, in-plane resolution of 0.69 mm, 100 contiguous axial slices of 1.3 mm thickness with an acquisition time of 450 seconds. A 3D T1-weighted image was also collected with scan parameters: TR 8.4 ms, TE 3.9 ms, flip angle 8 degrees. Data was reconstructed with a resolution of 0.94 x 0.94 x 1 mm, acquisition time 311 seconds.

MRI analysis

A pre-contrast T1 map was calculated using the variable flip angle spoiled gradient echo technique, by fitting the variable flip angle images on a voxel-by-voxel basis for T10 and A0 using the equation:

\[
S = A_0 \sin \theta (1 - e^{-\frac{TR}{T10}}) \\
1 - \cos \theta e^{-\frac{TR}{T10}}
\]

[equation 1]

Deviations from specified flip angles, \( \theta \), due to B1 inhomogeneities were taken into account as follows. The ratio of the images in the B1 mapping sequence (r) was used to estimate the true flip angle \( \theta_T \) on a voxel-by-voxel basis, using:

\[
\theta_T = \arccos \left( \frac{r.n - 1}{n - r} \right)
\]

[equation 2]

where \( n = \frac{TR_1}{TR_2} \).
The percentage deviation of the true flip angle from the specified flip angle \( \theta_S \) is given by \( \theta_T/\theta_S \) and \( \theta \) in [equation 1] is multiplied by this factor on a voxelwise basis when calculating \( T_1 \). Prior to multiplication, the \( \theta_T/\theta_S \) image was smoothed using the Matlab function ‘smooth3’ with a ‘box’ convolution kernel of size [5 5 3] voxels in the x,y and z axes respectively.

**DCE analysis**

Quantitative assessment of BBB integrity was generated from DCE-MRI. The dynamic series of 80 images was first corrected for motion using the ‘realignment’ option in SPM8 (Statistical Parametric Mapping, University College London 2009 [http://www.fil.ion.ucl.ac.uk/spm/](http://www.fil.ion.ucl.ac.uk/spm/)), which aligned all DCE images to the first image in the time-series. An ‘Arterial Input Function’ (AIF) was derived from the saggital sinus which was delineated using MRIcro on the final, registered dynamic image. The saggital sinus showed clear signal enhancement on this image and a region could be easily selected using the 3D intensity threshold tool in MRIcro. A voxel-by-voxel fit of the dynamic data for both the volume transfer constant \( (k_{\text{trans}}) \) and blood volume \( (v_p) \) was performed using the uptake model [29]:

\[
C_e(t) = R^{\text{trans}} \int_0^t C_p(t') dt' + v_p C_p(t)
\]

[equation 3]

where \( C_0(t) \) is the tissue concentration of the contrast agent and \( C_p(t) \) is the plasma concentration at time \( t \) after contrast agent injection at \( t=0 \).

The tissue concentration \( C_e(t) \) is calculated from the signal in the dynamic images \( S_e(t) \) according to:

\[
C_e(t) = \frac{R_1(t) - R_{10}}{R_1}
\]

[equation 4]
Where $r_1$ is the longitudinal relaxivity of the contrast agent, which was assumed to be $3.4 \text{ s}^{-1}\text{mM}^{-1}$. $R_{10}$ is the baseline longitudinal relaxation rate, taken from the pre-contrast $T_1$ map using $R_{10} = 1/T_{10}$.

$R_1(t)$ was calculated using equation [5] below, derived from equation [1] considering $S_t$ as the signal in the post-contrast dynamic images and $S_0$ as the mean signal from the 6 pre-contrast dynamics, ignoring the first image due to equilibrium effects.

$$
R_1(t) = \frac{1}{TR} \ln \left[ \frac{1 - B \cos \theta + \frac{S_t(t)}{S_0}(B - 1)}{1 - B \cos \theta + \frac{S_t(t)}{S_0}\cos \theta(B - 1)} \right]
$$

[equation 5]

where $B = \exp(-R_{10}TR)$.

The plasma concentration $C_p(t)$ is also calculated using equations [4] and [5] with $S_t$ the mean signal from the sagittal sinus region, $S_0$ the mean signal from the 6 pre-contrast dynamics within this region and $T_{10} = 1.6s$. $C_p(t)$ was corrected for haematocrit by dividing by $(1 - \text{Hct})$ where Hct of 0.42 was used.

Equation [3] was then fit to $C_t(t)$ and $C_p(t)$ using constrained least squares minimisation (lsqcurvefit in Matlab) on a voxel-wise basis for 3 parameters: $k^{\text{trans}}$, $v_p$ and $T_0$, where $T_0$ the offset time between $C_t(t)$ and $C_p(t)$. $k^{\text{trans}}$ was constrained to be between 0 and 0.1 min$^{-1}$, $v_p$ between 0 and 1 (i.e. zero blood content to 100%) and $T_0$ between 0 and 15 s (i.e. assuming the sagittal sinus signal always lagged the tissue signal but by a maximum of 2 time points, or 15 s).

In order to avoid local minima, minimisation was performed twice, using the fitted parameters of the first minimisation as starting parameters for the second minimisation.
Registration of MR Images

SPM8 was used to co-register the $k_{\text{trans}}$ and $v_p$ maps to the high resolution 3D T$_1$-weighted image. The first dynamic of the DCE series was used to compute the registration parameters which were then applied to the other images. The FLAIR image was also separately co-registered to the 3D T$_1$-weighted image.
4.3 Results

4.3.1 Pilot patients selected were generally representative of the disease burden

The five patients successfully enrolled in this pilot study were recruited from both sexes, across a wide age range and exhibited a number of different causes of ICH injury in varied locations throughout the brain (see Table 4.1).

The data from the scans ranged from as early as five days post-injury to as late as sixteen days. ICH volumes in the subjects were found to be small and systemic inflammatory markers were within normal limits.

4.3.2 Increased tracer binding appears to correlate with areas of BBB breakdown around the haematoma site

Kinetic DCE mapping indicates that BBB breakdown is pronounced at the periphery of the haematoma despite the lower values of blood flow in comparison to the centre (see Figure 4.2).

All patients exhibited some degree of BBB breakdown at the haematomal site as evidenced by direct contrast enhancement. In four of the patients recruited, increased tracer binding, indicating microglial activation, was also seen both within and around the haematoma (see Figure 4.1). There also appear to be areas of microglial activation distant to the site of injury in the majority of the patients examined.
4.3.3 Microglial activation was decreased in one patient

Patient 1 exhibited an apparent decrease in peri-haematomal binding of tracer despite disruption of the BBB (see Figures 4.3 and 4.4). This lady underwent a subsequent excision of the lesion which was suggestive of an underlying cavernoma.
Modulation of Inflammation in Intracerebral Haemorrhage
Dr. Kamran Ateeque Abid – May 2014

<table>
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Microglial activation and blood brain-barrier breakdown in acute intracerebral haemorrhage: a combined [11C](R)-PK11195 PET and DCE-MRI study.
Figure 4.1: Patient scan summary

Representative Ktrans (top row) and PK1195 (bottom row) mapping for each patient. Post injury scan day is given next to patient identifier.
Figure 4.2 Kinetic DCE mapping.

(A) Ktrans parameter map. Blood plasma flow per unit volume of tissue is plotted at the periphery (top row) and outside (bottom row) of the haematoma and compared with core values (B) The graph shows concentration of contrast agent detected plotted against time in the haematomal core (blue line) and periphery (red line) against control readings from the contralateral hemisphere (green line). Ktrans values are 0.00347 min⁻¹ vp=2.0%, 0.00353 min⁻¹ vp=0.37% and 0.00078 min⁻¹ vp=1.0% respectively.
Figure 4.3: Representative imaging from Patient “2” showing an ICH lesion in the right occipital lobe 10 days post injury. (A) Parametric map of binding potential from $[^{11}\text{C}]$-(R)-PK11195 labelled PET scan in axial (top row), coronal (middle row) and sagittal (bottom row) views. Red indicates increased binding. The images are uncorrected for the centre of the lesion. (B) Co-localised fused image (C) T1 weighted Inversion recovery sensitivity encoding (IRSENSE) MRI image.
4.4 Discussion

The study provided a novel and important contribution to the understanding of the inflammatory process following ICH injury in the human brain.

There is little existing data examining the timecourse of BBB disruption following human ICH. Using DCE MRI we demonstrated the presence of BBB breakdown and quantified its severity in the subacute phase following haematoma formation. This has important implications for the mechanism by which neuronal damage occurs and may determine the efficacy of drug delivery to the site of injury.

It is hoped that results from our study may also better inform preclinical models of ICH injury in order to more closely mirror the inflammatory response seen in the clinical condition. It was noted in our study that BBB disruption was more apparent in peripheral areas of the haematoma as opposed to the core areas previously reported in animal models [30]. Small areas of contrast enhancement were also noted distant from the haematoma area. It is unclear whether this an artefactual finding or whether the BBB undergoes disruption at these sites. Animal studies have previously shown that BBB disruption is a dynamic process [31] and future experiments may benefit from serial measurements in patients to assess how both haematoma and distant sites change with time and neurological function.

Examination of the microglial system provides a useful objective for elucidating the underlying pathophysiology of ICH injury [32] however there is limited published evidence examining microglial activation in animal models of ICH and, to date, no previous studies examining human subjects.
Despite the range of aetiology resulting in ICH in our study, increased [$^{11}$C-($R$)-PK11195 tracer uptake, indicating microglial activation, was noted in the majority of the patients recruited. We have shown that areas of microglial activation appear to correlate well with regions of BBB breakdown at the periphery of the lesion as well as more distant neuroanatomical sites. This important finding highlights the fundamental nature of the inflammatory response in the subacute phase of the disease and may provide a valuable target for future therapies directed against this condition. Further, detailed work is currently underway as part of a larger study to more precisely map the coregistered areas of BBB breakdown with areas of microglial activation; unfortunately the data was not available to include at the time this thesis was completed.

A separate mask with detection algorithm was required in our study to measure tracer uptake in the haematomaal core. Further improvements to future experimental design may include the development of more sensitive and specific techniques to quantify [$^{11}$C-($R$)-PK11195 detection.

Interestingly, previous work examining [$^{11}$C-($R$)-PK11195 uptake in patients with gliomas [33] has noted that tracer kinetics are differentially affected by glioma grade. It is hypothesised that part of the mechanism by which gliomas spread is by suppression of the normal inflammatory response that follows other types of brain injury [34]. One unexpected finding from our data was that, in the case of Patient "1", decreased [$^{11}$C-($R$)-PK11195 uptake was noted both in and around the haematoma. This lady later underwent excision of the underlying lesion which had caused the bleed and histological analysis strongly indicated the presence of an underlying cavernoma. There is only one previous study which has examined the presence of this lesion with PET scanning in four patients [35] and this found decreased accumulation of 11C-glucose tracer. Our study therefore adds a small but important contribution to the limited evidence that cavernoma formation may be dependent on a disruption of the BBB which is distinct from most other forms of brain injury [36].
Peripheral inflammatory marker levels were not found to be affected by the extent of microglial activation and further quantitative analysis of the imaging data awaits completion however more patients will have to be recruited in order to generate results which will be applicable to the wider population. A further limitation of our study was that the ICH volumes examined were generally small however the increased neurological deficit observed with larger bleed volumes precluded the ability of the patients to consent to, and tolerate, the transport and scanning requirements of the study protocol. Future experiments may have to take this into account since haematomal size appears to have a direct correlation with the volume of BBB breakdown [29].

Despite the small number of patients recruited for this prospective cohort pilot study, a diverse range of ICH injury was noted in a number of neuroanatomical locations and from patients of different ages and sexes with differing co-morbidities. By demonstrating the relationship between BBB breakdown and microglial activation we have therefore provided strong evidence that further experiments to explore this topic with these techniques are feasible, necessary and desirable.
4.5 References


5 FUNCTIONAL AND RADIOLOGICAL OUTCOMES FOLLOWING MODULATION OF IL-1 FUNCTION IN A RAT MODEL OF INTRACEREBRAL HAEMORRHAGE.
Modulation of Inflammation in Intracerebral Haemorrhage
Dr. Kamran Ateeque Abid – May 2014

Functional and Radiological outcomes following modulation of IL-1 function in a rat model of intracerebral haemorrhage.

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5.1 Introduction

Spontaneous ICH is common and displays the worst mortality and morbidity of all subtypes of stroke [3-5]. Existing treatments for the condition have resulted in negligible benefits for patients during the previous two decades, an effect which is partly due to the mischaracterisation of the underlying pathophysiology [7-9,11]. In recent years, there has been increased interest in examining the role of the inflammatory process which is known to exacerbate ICH injury in both human and animal models of the disease [7]. In particular, IL-1 has been identified as an early and central component of the cytokine cascade and subsequent modulation of IL-1 function has resulted in improved outcomes following ischaemic stroke injury [1].

Animal models of ICH have been established since the 1960s and have provided a robust, reproducible and reliable means by which the pathophysiological basis of the injury and response to novel therapeutic agents can be assessed [6]. Using a recombinant version of the naturally occurring receptor antagonist (IL-1Ra), which has previously proven safe in humans [1], this study will aim to characterise the functional and radiological results of modulating IL-1 in a rat model of ICH. This is important since any potentially therapeutic compound will have to demonstrate that it can restore or maintain function after ICH as well as addressing the underlying mechanism of injury.
5.2 Methods

Twenty two healthy male Wistar rats between 320g and 590g in weight (Charles River Laboratories, UK) were used in this study in accordance with the Animals (Scientific Procedures) Act (1986), the Directives of the European Union on animal ethics and welfare and also the University of Manchester protocols on the Use and Care of Animals. A previous study was used to estimate the effect size of oedema attenuation, as a surrogate marker of inflammatory response, at 72 hours post-ICH injury to power the study at 80% (see Appendix). Rats were allowed to acclimatise to their holding cages for a week before they were assigned randomized study numbers. A random number generator (http://www.randomizer.org/) was used to generate a list of numbers which was then used to determine the order in which the operations were carried out. A second investigator used a similar method to prepare anonymised samples of IL-1Ra and vehicle for injection thereby blinding the initial investigator during operative, testing and data measurement phases.

All experiments were carried out in paired morning and afternoon operating sessions. The animals were anaesthetised with isoflurane (3.5-5 L/min), Nitrogen (1.2 L/min) and Oxygen (0.6L/min) and core temperature was maintained at 37°C with the use of a feedback controlled heating pad utilising a rectal thermometer. The rats were immobilised in a stereotactic frame and secured with ear bars before they were shaved and prepped with alcoholic iodine solution. A midline longitudinal incision was made through skin before periosteum was stripped away from the midline and the overlying soft tissue retracted. A burr hole to the level of the dura was drilled 1 mm anterior and 3mm right-lateral to the Bregma. A 27-gauge needle was used to withdraw blood from the tail artery once the overlying skin had been cleaned with alcoholic chlorhexidine solution. A fresh needle was then attached to the
syringe and superglued into place. The assembly was attached to an infusion pump and the needle was inserted to a depth of 5.5mm inferior to the dural surface with the bevel turned laterally away from the midline. The blood was then injected at a rate of 10 microlitres per minute for a total of ten minutes. After a pause of a further ten minutes, the needle was then carefully withdrawn from the tissue and the burrhole was sealed with surgical wax. The overlying soft tissue was closed with interrupted, non-absorbable monofilament sutures.

A small patch in the right flank of the animal was then prepared in a similar manner before an anonymised 100mg/Kg dose of either IL-1Ra or vehicle was injected subcutaneously. The animal was transported within four hours to the 3T MR scanner to undergo a scan to check haematoma placement. The animal was then recovered using standard techniques and returned to the holding area after a period of observation. The same animal was then re-anaesthetised in order to undergo further corresponding injections of IL-1Ra or vehicle every 12 hours for the first three days in order to maintain a steady state serum concentration [2]. Animals underwent functional testing at baseline, 1 day, 3 days, 7 days, 14 days and 28 days post-operatively and had two further MR scans at 72 hours and 28 days post-operatively before they were sacrificed (Figure 5.1).

![Figure 5.1: Experiment timeline](image-url)

**Functional and Radiological outcomes following modulation of IL-1 function in a rat model of intracerebral haemorrhage.**
5.2.1 IL-1Ra

The IL-1Ra was produced by Biovitrum (Stockholm, Sweden) in prefilled syringes (Anakinra ® 100mg/0.67ml) and stored at 4°C in light-protected conditions at all times. Prior to administration, the drug was transferred to anonymised eppendorf tubes alongside saline vehicle samples under sterile conditions as part of the randomisation and blinding process.
5.2.2 MR scanning

Rats were anaesthetised according to the procedure given above and allowed to breathe spontaneously whilst they were being scanned. Rectal temperature (RS 51 K-type thermometer, RS Components Ltd., Northants, UK) and respiration rate (MR10 respiration monitor Graseby Medical Ltd., Hertfordshire, UK) were monitored during the procedure. Imaging was carried out using a 7-T horizontal-bore magnet (Magnex Scientific Ltd., Abingdon, UK) connected to a SMIS computer console (Surey Medical Imaging Systems Limited, Guildford, UK) with a transmit/receive birdcage volume coil. RARE-T1 (scan parameters: 23 contiguous axial slices of 1 mm thickness; TE = 9 ms, TR=1300 ms, flip angle 10 degrees and image acquisition time of 7.6 seconds), FLASH T2* (TE=15, TR = 504), TURBO RARE T2 HIRES_FLASH (TE=33, TR=2741) and RARE (TE=10, TR=3000) Inversion recovery images were obtained for each animal with total scan times approximating 44 minutes.

In order to calculate lesion volume, oedema formation and brain atrophy, the image with the largest lesion was chosen as the reference image in the series. Image-J software (imagej.nih.gov, Version 1.6.0-20) was used to trace around the border of the coronal sections on T1 scans in order to calculate the total brain area in the slice using the "area" software tool. The section was further subdivided into the Right ("Ipsi" to lesion) and Left ("Contra" to lesion) areas. The two 1 mm slices both immediately anterior and posterior to the reference image were also measured in order to provide five coronal sections each 1 mm thick to perform brain volume calculations for each animal which were centred on the midpoint of each lesion.

Using the same co-ordinates for the reference image, a similar technique was employed on the T2 scans for each animal in order to calculate lesion and oedema volumes. This series of scans has previously been shown to have a higher lesion sensitivity than the T1 images and all abnormally hyper/hypodense areas around the needle track were included in the final
measurement. Calibration for all scans was done with the internal scale on the image and standardised between scans.
Figure 5.2: Examples of post operative scans (A) MRI T1 coronal section 4 hours post surgery showing needle track in right (ipsilateral) hemisphere (B) Example of area calculation on the preceding scan. Dotted line represents border of area calculated by software (C) MRI T2 coronal section 72 hours post surgery. Hyperintense signal represents haematoma and oedema formation. (D) MRI T2 coronal section 28 days post surgery
Functional testing

*Forelimb placement test* – Rats were held by their torsos allowing their forelimbs to hang free. Ipsilateral vibrissae were then stimulated against the corner edge of a countertop. Intact animals quickly placed their paws on the countertop when vibrissae were stimulated. Animals with unilateral lesions exhibit impaired forelimb placing on the contralateral side [12]. Twelve trials were conducted per animal with stressed or tense rats discounted from recorded attempts.

*Beam walking test* – Rats were placed in the centre of a solid beam suspended approximately three feet in the air and functionally graded crossing the beam in either direction (see Appendix). All rats underwent a training run on a wide beam to acclimatise themselves before being tested on a 3 cm wide beam with first a flat then a hemispheric cross-section and re-graded. Each animal was given three attempts to cross the beam and the final attempt was used for analysis.

*Adhesive removal test* – A standardised piece of adhesive tape large enough to cover three pads on the rat’s front paws and wrap around the wrist was applied and the time taken to remove it was recorded. Rats first underwent pre-operative baseline training with three attempts timed for removal of each piece of tape. At scheduled times post-operatively, rats were given three contiguous attempts to remove the tape and the time was again recorded. The time taken to remove the adhesive tape on the third attempt was compared with the third baseline reading and the difference was calculated.
5.2.3 Exclusion criteria:

Animals were excluded from study analysis if they:

a) Died before the postoperative endpoint either through anaesthetic overdose or termination due to welfare issues

b) Exhibited incomplete blood delivery to the brain (for example due to blood capture within the apparatus). The initial postoperative scan was used to assess both the volume of blood delivered and its correct anatomical location before continuation in the study, as agreed by the three main investigators.

c) Exhibited excess and widespread hemispheric damage from the operation

5.2.4 Data Analysis

Behavioural data from the forelimb placement and beam walking tests were statistically compared with the Mann-Whitney U test. In the adhesive removal test only the 3rd of 3 attempts at each time point was considered to account for training effects. Each post-ICH time point was then normalized by subtracting baseline time and an Area Under the Curve (AUC) was subsequently calculated for each animal. An independent sample t-test was then performed on this data. The same test was used to compare groups measuring changes in brain atrophy and oedema. All statistical tests were calculated using Graphpad (Version 5.0, Graphpad Software Inc.) or SPSS (Version 20.0, ibm.com) software.
5.3 Results

5.3.1 Exclusions
Of the twenty two rats that were entered into the study, two rats died or were terminated for health reasons before completion in the IL-1Ra treated group. An additional rat in both the vehicle and treated group was excluded due to poor haematoma placement. An additional rat in the IL-1Ra group was excluded due to widespread and unrepresentative hemispheric damage following the operation. Due to a fire in the MR scanner, a further nine rats had incomplete imaging data for analysis although their behavioural data was unaffected.

5.3.2 Oedema and atrophy
No significant difference was noted in attenuation of radiological oedema or atrophy between IL-1Ra and vehicle groups.

Baseline lesion sizes exhibited heterogeneity in both IL-1RA and vehicle treated groups (see Figure 5.3). The coefficient of variation was calculated as 8.8% for vehicular groups (n=11) and 9.7% for IL-1Ra treated groups (n=7).

In vehicular groups hemispheric size ipsilateral to the ICH was noted to be smaller than than the contralateral hemisphere. The reverse was noted to be true in IL-1Ra treated animals although the difference did not achieve significance. The finding of increased hemispheric volume 72 hours post operatively was noted in the IL-1Ra treated animals after correction had been made for lesional size (see Fig 4xB and 4xC). No difference was observed in oedema accumulation 72 hours post operatively in comparison to measurements taken at 4 hours in either vehicle or IL-1Ra treated groups.
When brain atrophy following ICH injury was plotted, most animals in both vehicular and IL-1Ra treated groups exhibited decreased brain hemispheric volume in comparison to the contralateral side (see Figures 5.4A and 5.4B). No significant difference was demonstrated between the two groups when a correction for lesional size was made. Furthermore, no significant difference was demonstrated with IL-1Ra treated animals in comparison to the vehicular group when 4hr brain volume was used as a reference point for long-term change.
Figure 5.3 Resultant oedema from ICH animals subject to IL-1Ra and vehicle administration at 72 hours.
Table 5.3D: Oedema calculations from the different brain regions; mean and SD figures are given as ratios or absolute values in mm$^3$

Fig 5.3: (A) Baseline lesional area of both groups including animals excluded from the final analysis shows heterogeneity of lesional area.

(B) Ratio of calculated oedema in ipsilateral vs contralateral hemispheres including correction for lesion size. (C) Oedema volume differentials; this difference was reflected in the plot of oedema volume difference in each group and no significant difference was found between treated and vehicular animals when initial lesion size was accounted for.

Table 5.3d shows oedema calculations as delineated by ipsilateral and contralateral hemispheric and lesional area measurements taken at 72 hours post-operation (unless otherwise stated). The final column shows $p$ values from the independent sample t-test used to compare treated and vehicular groups. Ipsilateral hemispheric volumes were found to be significantly higher in the IL-1Ra treated groups in comparison to contralateral hemisphere.
controls even after a correction for lesional size had been made. Overall, no significant attenuation in oedema was noted in either group 72 hours after the operation.
**Fig 5.4. Brain atrophy calculated 28 days post-operatively.** (A) Atrophy ratios comparing Ipsilateral and contralateral hemispheres before and after correction for lesional size. (B) Comparison of atrophy volumes. No significant difference was demonstrated with IL-1Ra treated animals in comparison to the vehicular group.

The table shows four different atrophy parameters in which treatment and control groups are compared with independent t-tests and the significance level is given in the final column.
5.3.3 Functional data

There was a trend towards improved functional performance on both the narrow and round beam walking test in rats treated with IL-1Ra (see Figures 5.5 & 5.6) however this was not significant at any of the timepoints assessed. No significant difference was detected between groups whether their absolute performance levels or change from baseline were tested.

A similar difference was observed in the forelimb placement test (see Figure 5.7), which also showed a non-significant trend for improvement in functional recovery which decreased as a function of time following the operation.

No significant difference was observed between groups in the adhesive removal test (see Figure 5.8).
**Fig 5.5. Performance of rats on the narrow beam as graded by Feeney score** (Mean +SEM).

The columns represent change from baseline testing (feeney score 7 in all cases). No significant differences are seen between groups compared with the Mann Whitney U Test (p<0.05, n = 11 veh, n=7 IL-1Ra ). The table shows p values from the test.

<table>
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<th>Day</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>14</th>
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<td>p value</td>
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<td>0.280</td>
<td>0.22</td>
<td>0.228</td>
<td>0.533</td>
</tr>
</tbody>
</table>
Fig 5.6. Performance of rats on the round beam as graded by Feeney score (Mean + SEM).
The columns represent change from baseline testing (feeney score 7 in all cases). No significant differences are seen between groups compared with the Mann Whitney U Test ($p<0.05$, n=11 vehicle, n=7 IL-1Ra). The table shows $p$ values from the test.
**Figure 5.7. Left Forelimb Placement Test.** The number of successful trials from 12 attempts at placing the left forelimb on a surface when the contralateral vibrissae were stimulated is plotted. The columns represent change from baseline testing (12 successful forelimb placements in all cases). The significance values from a Mann Whitney test is given in the table below the graph (p<0.05 n=11 vehicle, n=7 IL=1Ra).
Fig 5.8. Adhesive removal test result table and graphs

Control (black line) VS IL-1RA (red line) animal results are plotted. Combined time for removal of adhesive tape calculated after removal of baseline (preoperative) time for each animal. Graph A shows results for Left paw, Graph B shows results for the Right paw. The Area Under the Curve (AUC) was calculated for each animal and compared with an independent sample t-test (see table). No significant difference was found between groups for adhesive removal from either paw (p<0.05).
5.4 DISCUSSION

This study demonstrates that no significant difference was found in radiological markers of inflammation or functional outcome in rats with induced ICH injury following modulation of IL-1 function.

Although previous studies have shown favourable outcomes of IL-1 modulation in rat models of ischaemic stroke [13], this is the first known instance in which this mechanism has been tested using this model. Although published methods [14] were piloted and developed in earlier work, it should be noted that there was marked heterogeneity in blood delivery to the caudate area. Coefficient of variation in lesional sizes were between 8-10% in our study in comparison to a coefficient of variation of 1-2% in brain water content using a similar model of ICH induction [15].

This variation is likely to be due to a combination of four limitations of the study. Firstly, because of the novel nature of the experiment, a calculation to power the study had been performed using data from another group examining the effect of deferoxamine on ICH outcome. Coupled with the number of exclusions from the present study, the group treated with IL-1Ra was sensitive to one animal acting as a data outlier.

Secondly, methodological limitations of the operation resulted in clotting within the delivery apparatus resulting in inconsistent pressures and volume of blood delivery to the brain. Addition of anticoagulant agents has previously been shown to impact upon the subsequent inflammatory response following ICH injury [16] and could therefore not be employed in our study. It is furthermore possible that some of the animals may have been subject to either hypoxic or ischaemic injury since there was no real-time monitoring of blood pressure or oxygenation levels.

Furthermore, the method of blood delivery using this system has been shown to deliver umbrella-shaped lesions within the brain depending on which anatomical structures are subject
to the pressure of the driven blood. Analysis of the two segments immediately anterior and posterior to the reference image may therefore not capture all of the resultant haematoma in comparison to whole brain analysis.

Lastly, due to the novel nature of the experiment, it has not been determined how long IL-1Ra may have to be administered post-injury and at what dosage to prevent long-term atrophy and functional impairment. Future studies may incorporate continuous IL-1Ra infusion until termination of the animal has occurred.

Despite the lack of any significant difference noted in functional outcomes in the study, there were some encouraging trends noted in early timepoints following IL-1Ra treatment for ICH injury. This is an important finding since it may provide the basis for potential therapeutic interventions in addition to elucidating the underlying mechanism of the disease process. In addition, much of the published literature concerning animal models of ICH injury examines the acute nature of the injury and its possible prevention rather than the potential for functional recovery once the injury has occurred.

Pilot work conducted on a similar number of animals previously established that the functional tests were sensitive for detecting haematoma versus saline injected animals however greater numbers and more sensitive tests may be required to demonstrate the benefit of IL-1Ra administration.

The beam-walking test is a reliable measure of hindlimb functioning and provides a focused locomotor assessment that detects placement dysfunction of the hindlimbs after unilateral brain damage. Three different beam sizes were used to detect deficiencies in hindlimb function and all of these were sufficiently sensitive enough to detect behavioural deficits within the first twenty four hours following ICH injury. A strength of the study was that a number of different beams with different sensitivities for injury were employed. Further improvements to the design could incorporate ladder walking and rotating beam tests to increase sensitivity further.
The forelimb placement test, although simple to design, has previously proven amongst the most sensitive measures of brain dysfunction following ICH injury and is particularly helpful in elucidating the extent of oedema formation which has been used as a surrogate marker for pathological inflammatory response. The results from this sensorimotor test in our study were promising, showing the greatest difference between vehicle and drug-treated animals in the initial period following the operation. Experience gained in handling the rats is likely to further improve the outcome of this test since other researchers have noted the importance of consistently handling each animal to achieve optimum results.

The adhesive removal test proved less agreeable in terms of detecting a significantly improved result. Individual differences in the temperament and behaviour of the animals inevitably introduced some bias in the time to contact and time to removal of the tape. In addition, the rats were noted to be relatively phasic in terms of attempted tape removal and would wander around their cages for variable periods if they were unable to remove the tape after a certain number of attempts rather than freeing themselves in a consistent and linear manner. There was also variability introduced into the recording of the timed results when tape would become dislodged and ride up the rodent’s arm for example. In addition, individual animals had greatly altered aptitude for learning following ICH injury. In order to improve this test, more comprehensive baseline testing of the rats could be employed to ensure a consistent learning effect between test subjects.

Despite the absence of a significant result in either arm of the study, the novel data generated serves as an important basis for the design of further experiments to investigate the relationship between IL-1 generated inflammatory response and ICH injury. The data gained from this study will enable the construction of an adequately powered repeat experiment although future work should also include further development of the injury model to decrease the amount of clotting observed within the apparatus – for example the use of intra-arterial...
cannulation – and the extension of test groups to include ages and co-morbid rats with differing administration regimes of IL-1Ra.
5.5 References


6 SUMMARY
This thesis has detailed new and compelling evidence that recharacterisation of ICH injury as an inflammatory condition may lead to significant improvements in patient outcome.

As set out in the introductory chapter, many obstacles remain in the search for progress however the penumbral zone of tissue immediately adjacent to the haematomaal mass has been shown to represent a promising target for future therapies for this condition.

In general, relatively little progress in this regard has been made over the previous few decades. Despite rapid advances in medical science and encouraging trends from meta-analyses and ongoing surgical trials, no new treatments have definitively been identified which have significantly and reliably improved clinical outcomes or reduced mortality. ICH is therefore a common condition which remains the most devastating of stroke subtypes.

Accurate prognostic scoring is essential to identify which patients may benefit from treatment interventions. The aim of the first experimental chapter was therefore to determine which factors currently impact the most on ICH mortality.

This was achieved by performing a retrospective cohort analysis of ICH injury over 1300 patients recently referred to a leading UK hospital. By combining discrimination, calibration and decision curve analysis of the clinical condition in conjunction with the radiological appearance of the haematoma, a simplified decision rule was generated which had equal prognostic power in comparison to existing and more complicated scoring systems.

Of interest it was noted that age, which commonly informs clinical decision-making, is actually a relatively poor prognostic indicator of mortality. Due to the analysis of large numbers of unselected patients as well as the widespread nature of stroke injury, it is likely that our findings are generally applicable in clinical practice.
The next experimental chapter sought to determine whether there were differences in patient outcome when they were treated at different centres with existing care and, if so, which factors determined transfer to those centres.

A retrospective cohort study was again employed to show that transfer to a special surgical unit was correlated with significantly improved outcome even when no surgery subsequently took place. This important finding on a large number of patients suggested that early and aggressive goal-directed therapy could significantly improve outcome from this condition. It is interesting to note that similar recent findings have been noted in septic patients who have since been reclassified as suffering from a systemic inflammatory response syndrome (SIRS)[1].

This provides indirect evidence that targeting the results of a generalised disruption to normal physiology, such as that encountered in an inflammatory response to disease, may lead to improvement in the condition. Although much literature exists regarding the clinical management of ICH, the evidence base relating to this aspect of the condition remains sparse.

This study again highlighted that increasing patient age was negatively correlated with a decision to transfer, a finding that has widespread implications for an ageing population who may otherwise benefit from specialist care. Further prospective studies to determine the extent of improved survival in case-matched individuals would be of great benefit.

Although specialist units tend to employ higher levels of evidence-based care, little progress overall has been made in translating basic science results from the laboratory bench to the bedside. The focus of the fourth chapter was therefore to investigate the direct presence of neuroinflammatory response in the subacute phase following ICH injury.

A pilot study was thus undertaken to evaluate the extent of microglial activation in patients with early haematoma formation.

Our findings have shown for the first time that neuroinflammatory response correlates peripherally and distally with areas of BBB breakdown at the edge of the haematoma. This study provides a novel and important link between neuro-inflammation and disease.
pathophysiology. Since there are important correlates between drug delivery and areas of BBB breakdown, these findings have also provided data which will play an important part in the development of future treatments at this early time point in the disease process. Our protocol has proven that further investigation with these techniques is feasible on a diverse range of patients with differing underlying causes of ICH. In addition the data generated may better inform preclinical models of the disease.

The final experimental chapter utilised a rat model of ICH to test whether modulation of the inflammatory process with a treatment, previously been shown to be safe in humans, would lead to improved outcome. Despite development of the existing model of ICH injury, methodological limitations precluded a definitive answer on whether IL-1Ra will provide the basis of an efficacious treatment for this condition. Nevertheless, there were encouraging trends towards improved functional outcomes in the early time course of the disease and further improvements to the model have subsequently been made as the basis for ongoing experiments.

Much further work is required to characterise the inflammatory response following ICH injury and how this may be altered to improve patient outcome. Part of the reason that so few findings within the lab have translated to clinical benefit have been because the disease is studied by a diverse range of clinicians and scientists with little common ground between them.

Recent data from large surgical series have shown some trends towards improving mortality for this condition however one of the particular strengths of the translational nature of this thesis is that new evidence has been provided at both a preclinical and clinical level using a diverse range of operational, animal-based, radiological and population-based techniques which strongly suggest that improved outcomes may be achievable by targeting the inflammatory nature of this condition.
6.1 References

7 APPENDIX
**Power calculation**

We opted for a two tailed power calculation. Nakamura and colleagues [1] previously used oedema attenuation with deferoxamine (DFX) at 3 days following induced ICH injury as a surrogate marker for inflammatory response.

With DFX administration, they found that brain water content was 80.5 ± 0.5% in comparison with 81.5 ± 0.8% in vehicle-treated controls (P<0.01). Using these figures, a power size calculator ([www.stat.ubc.ca/~rollin/stats/ssize/n2.html](http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html)) was used to calculate that 9 rats in each group would be required to observe a difference powered at 80%. This calculation was checked manually and a 20% contingency rate was employed based on the results of an earlier pilot study. Eleven rats in total were therefore selected for each group.
**Beam walking functional scoring system**

The Feeney scoring system was used to measure beam-walking performance in accordance with earlier studies [Feeney 1982]. The rat was graded as “0” if the rat fell off the beam within 10 seconds), “1” (rat remained on the beam for more than 10 seconds but could not place the affected limb on the beam, “2” (rat was unable to cross but could place the affected limb on the beam and maintain balance), “3” (rat traversed beam while dragging the affected limb), “4” (rat crossed the beam and placed the affected limb on the beam at least once), “5” (rat crossed with more than 50% foot slips with the affected limb), “6” (rat crossed with fewer than 50% foot slips with affected limb), or “7” (rat crossed with 2 or fewer foot slips).
