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Does lobar intracerebral haemorrhage differ from non-lobar intracerebral haemorrhage?

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I confirm that I composed this thesis and that it is my own original work. I have not submitted the work for any other degree or professional qualification.

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2. Fonville, A.F.F., Samarasekera, N., Hutchison, A., Perry, D., Roos, Y.B., Al-Shahi Salman, R. 2013 Eligibility for Randomized Trials of treatments specifically for intracerebral haemorrhage Community-based study. *Stroke*, Oct; 44(10):2729-34.

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3. Samarasekera, N. Smith, C., Al-Shahi Salman, R. The association of cerebral amyloid angiopathy with intracerebral haemorrhage: systematic review and meta-analysis of case-control studies.

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   European Stroke Conference, 2013, London

3. Samarasekera, N., Lerpiniere, C., Farrall, A., White, P., Wardlaw, J., Smith, C., Al-Shahi Salman, R. Does spontaneous intracerebral haemorrhage location influence survival? A prospective community-based study.

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Abbreviations

95% CI	95% confidence interval
Aβ	Amyloid-beta
ANOVA	Analysis of Variance
AVM	Arteriovenous malformation
BMB	Brain microbleed
BOMBS	Brain Observer MicroBleed rating Scale
CAA	Cerebral amyloid angiopathy
CCM	Cerebral cavernous malformation
CSF	Cerebrospinal fluid
CT	Computed tomography
DNA	Deoxyribonucleic acid
DVT	Deep vein thromobosis
DWI	Diffusion weighted imaging
ECG	Electrocardiogram
EDTA	ethylene-diamine-tetra-acetic acid
EPVS	Enlarged perivascular spaces
FLAIR	Fluid-attenuated Inversion Recovery
FoV	Field of view
GCS	Glasgow Coma Scale
GP	General practitioner
GRE	Gradient recalled echo
HTI	Haemorrhagic transformation of an infarct
IADSA	Intra-arterial digital subtraction angiography

ICD	International Classification of Diseases
ICH	Intracerebral haemorrhage
INR	International Normalised ratio
IQR	Interquartile Range
ISD	Information Services Division
LINCHPIN	Lothian Study of INtraCerebral Haemorrhage, Pathology, Imaging and Neurological Outcome
MARS	Microbleed Anatomical Rating Scale
MRI	Magnetic resonance imaging
MI	Myocardial infarction
NEX	Number of excitations
NHS	National Health Service
NTC	No template control
OR	Odds Ratio
PCR	Polymerase chain reaction
PE	Pulmonary embolism
SD	Standard Deviation
SNP	Single nucleotide polymorphism
TE	Echo time
TIA	Transient ischaemic attack
TR	Relaxation time
UK	United Kingdom
UV	Ultraviolet
WMH	White matter hyperintensities of presumed vascular origin
WTCRF	Wellcome Trust Clinical Research Facility

Abstract

Spontaneous (non-traumatic) intracerebral haemorrhage accounts for ~10% of all strokes in Western populations. Investigations may identify intracerebral haemorrhage (ICH) as 'secondary' to underlying causes such as tumours or aneurysms, but ~80% of ICHs which have no apparent underlying cause (so-called 'primary' ICH) tend to be attributed to small vessel vasculopathies such as arteriolosclerosis or cerebral amyloid angiopathy (CAA), on the basis of an adult's risk factors and clinical and radiographic features of the ICH.

The commonly accepted hypothesis is that CAA contributes to lobar ICH and arteriolosclerosis causes non-lobar ICH. In the following thesis, I set out to explore whether (a) the baseline demographic, clinical features and apolipoprotein E genotype of adults with lobar and non-lobar ICH differ, (b) the prognosis of adults with lobar and non-lobar ICH differ and (c) the neuroimaging correlates of small vessel disease in adults with lobar and non-lobar ICH differ since this might provide clues to the vasculopathies underlying lobar and non-lobar ICH. I explored (d) the strength of the association between CAA and ICH by systematically reviewing neuropathological case control studies and (e) the radiological and pathological features of lobar ICH to examine the nature of CAA in persons with lobar ICH and whether any computed tomography (CT) features of ICH are associated with CAA-related lobar ICH.

I set up a prospective, community-based inception cohort study of adults with ICH in South East Scotland. Adults with spontaneous primary definite ICH had the opportunity to consent to participate in the Lothian Study of IntraCerebral Haemorrhage, Pathology, Imaging and Neurological Outcome (LINCHPIN), an ethically-approved, prospective community-based research study examining the causes of ICH using apolipoprotein E genotyping, brain MRI and research autopsy in case of death.

Of 128 adults with first-ever spontaneous primary definite ICH diagnosed during 2010-2011, age and pre-morbid hypertension did not differ by ICH location but a history of dementia was more common in adults with lobar ICH. The proportion of adults with one or more non-lobar brain microbleed (BMB) was significantly higher in adults with

non-lobar ICH but I did not find any other differences in the severity or distribution of other neuroimaging correlates of small vessel disease between lobar and non-lobar ICH. The apolipoprotein ε4 allele was more common in participants with lobar ICH in comparison to those with non-lobar ICH but the frequency of the ε2 allele did not differ by ICH location. Adults with lobar ICH were significantly more likely to survive one year after their ICH in comparison to those with non-lobar ICH after adjustment for other known predictors of outcome.

From a systematic review of neuropathological case control studies of CAA and ICH, stratified by ICH location, I found a significant association between CAA and lobar ICH but not with ICH in other locations. I examined the radiological and pathological features of 33 adults with first-ever lobar ICH. The presence of CAA or vasculopathy and the severity of CAA in a lobe affected by ICH was concordant with that of the corresponding contralateral unaffected lobe. Capillary CAA was associated with severe CAA. Subarachnoid extension of the ICH tended to be more frequent in those with CAA-related strictly lobar ICH.

Having explored the incidence, risk factors and prognosis of lobar and non-lobar ICH, in future work I would aim to establish the strength of the association between CAA and ICH in different brain locations in a neuropathological case control study. Future work should examine the radiopathological features of lobar ICH in a larger cohort and the coexistence of other small vessel diseases, in particular arteriolosclerosis in persons with ICH.

Section A Introduction

Chapter 1 Introduction

Chapter 1 Introduction

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1 Introduction

1.1 Intracerebral haemorrhage

1.1.1 *The importance of intracerebral haemorrhage*

Stroke is the third commonest cause of death worldwide, after ischaemic heart disease and all cancers, combined [Strong, Mathers, & Bonita 2007;Lozano et al. 2013]. Intracerebral haemorrhage (ICH) accounts for ~15% stroke in the UK [Lovelock, Molyneux, & Rothwell 2007] and two million [Sudlow & Warlow 1997] of about 15 million strokes globally each year. As the incidence of ICH increases with age [van Asch et al. 2010], and the world's population continues to age, there will be an increasing demand placed on stroke services [Di Carlo A. 2009].

Spontaneous non-traumatic ICH results from the rupture of blood vessels into the brain parenchyma. The ICH is not thought to have been caused by a head injury [Steiner et al. 2011].

ICH should be distinguished from other types of intracranial haemorrhage [Al-Shahi Salman, Labovitz, & Stapf 2009] including subarachnoid haemorrhage, pure intraventricular haemorrhage, subdural haemorrhage and extradural haemorrhage although an ICH may extend into one or more of these intracranial compartments (Figure 1 on page 52). The relevance of this distinction is that the risk factors, causes and management options for each differ [Al-Shahi Salman, Labovitz, & Stapf 2009].

1.1.2 *Incidence*

A systematic review and meta-analysis of population-based studies of ICH found that the overall incidence of ICH was 24.6 per 100 000 person-years (95% confidence interval [CI] 19.7-30.7) [van Asch et al. 2010]. Five population-based studies [Broderick 1993;Inagawa et al. 2003;Labovitz et al. 2005;Zahuranec et al. 2006;Lavados et al. 2010] which quantified the incidence of lobar and non-lobar ICH found an excess of non-lobar in comparison to lobar ICH and one study found a higher incidence of lobar ICH [Tatu et al. 2000].

1.1.3 *Prognosis*

Based on a recent meta-analysis, the one month case fatality following primary ICH is 40% [van Asch et al. 2010]. The pooled one year survival estimate from nine population-based studies is 46% [Poon, Fonville, & Al-Shahi Salman 2013].

Two studies [Nilsson et al. 2002;Sacco et al. 2009], one of which was population-based [Sacco et al. 2009], have reported lower one year survival in those with lobar ICH in comparison to supratentorial deep ICH although this was not reported by two other hospital-based studies [Faught et al. 1989;Franke et al. 1992].

The recurrence rate of all strokes in survivors of primary ICH is 4% per person-year [Bailey et al. 2001a]. In two studies of 696 patients, the recurrence rate of lobar ICH was higher than that of non-lobar ICH [Poon, Fonville, & Al-Shahi Salman 2013]. This may support lobar and non-lobar ICH having different contributory causes; lobar ICH may be more strongly associated with cerebral amyloid angiopathy (CAA) than non-lobar ICH (Chapter 9).

1.1.4 *Classification of ICH*

1.1.4.1 Primary vs. secondary ICH

ICH has been traditionally classified as either 'primary' or 'secondary' [Al-Shahi Salman, Labovitz, & Stapf 2009].

Primary ICH has no detectable underlying secondary cause [Ikram, Wieberdink, & Koudstaal 2012] and is commonly attributed to a presumed small vessel vasculopathy, most commonly arteriolosclerosis or CAA. This decision is usually made on the basis of an adult's risk factors and the clinical and radiological features of the ICH [Cordonnier et al. 2010a].

Secondary ICH is attributable to a variety of structural causes including tumours, arterial aneurysms, intracranial venous thrombosis, arteriovenous malformations (AVMs), systemic diseases such as vasculitis and haemostatic disorders [Warlow et al. 2008;Al-Shahi Salman, Labovitz, & Stapf 2009].

Approximately 80% ICH are 'primary' [Warlow et al. 2008] although in practice, determining whether an ICH is 'primary' or 'secondary' will also be dependent upon the

extent to which a clinician investigates a patient for secondary causes and is therefore subject to detection bias [Cordonnier et al. 2010a].

1.1.4.2 SMASH-U classification

The recent SMASH-U classification subdivides ICH into one of the following causes: Structural, Medication (antithrombotic medications, intravenous thrombolysis), Amyloid angiopathy, Systemic or other disease (except for anticoagulation, hypertension or CAA), Hypertension and Undetermined [Meretoja et al. 2012]. Although it has been validated in a retrospective notes review and shown to have good inter-observer agreement, each category is mutually exclusive which is unlikely to be representative of ICH in clinical practice where one or more causes may overlap.

A single ICH may be the result of several factors [Warlow et al. 2008]; for example: predisposing risk factors such as older age, male sex, hypertension and high alcohol consumption [Ariesen et al. 2003], precipitants such as antithrombotic medications or structural causes. One such example is an elderly patient with a past history of ischaemic heart disease for which he is prescribed aspirin and hypertension who is admitted with an ICH. Both antithrombotic use and hypertension are risk factors [Ariesen et al. 2003;Gorelick & Weisman 2005] which are likely to have contributed to their ICH. Even in patients with a known structural cause such as an aneurysm, other factors such as hypertension contribute to the risk of rupture [Rinkel 2005].

1.1.5 *Lobar vs. non-lobar ICH*

Supratentorial ICH is typically subdivided by location as 'lobar' or 'deep', where deep commonly refers to ICH involving the basal ganglia and/or thalamus [Warlow et al. 2008]. The assumption underlying this is that the risk factors and causes of lobar and deep ICH may differ. Lobar ICH in the elderly is commonly attributed to CAA [Viswanathan & Greenberg 2011] and deep ICH is attributed to hypertension-related arteriolosclerosis [Cordonnier et al. 2010a]. The location of the ICH has also been used to classify cause; that is lobar ICH is labelled as probable 'CAA-related' and non-lobar ICH as probable 'hypertension-related' [Arima et al. 2010], although this dichotomisation is likely to be too simplistic since any single ICH is likely to be caused by several factors.

1.1.5.1 Previous definitions of lobar ICH

Of 41 observational studies which have compared lobar vs. other types of ICH, 20 did not define lobar location [Brott, Thalinger, & Hertzberg 1986;Fieschi et al. 1988;Franke et al. 1992;Berlit & Tornow 1994;Graffagnino et al. 1994;Giroud et al. 1995;Greenberg et al. 1996;Rosenow et al. 1997;Inagawa et al. 2003;Karapanayiotides et al. 2004;Labovitz et al. 2005;Zahuranec et al. 2006;Zia et al. 2006;Seifert et al. 2006;Telman et al. 2010;Kuramatsu et al. 2011;Ruiz-Sandoval et al. 2011;Matsukawa et al. 2012;Chen et al. 2012;Hu, Wang, & Luo 2013] and 21 used various definitions of 'lobar' ICH, including ICH which was: cortical and/or subcortical [Broderick 1993;Anderson et al. 1994;Nilsson et al. 2000;Bilbao et al. 2005;Weimar et al. 2011;Biffi et al. 2011b], or cerebellar [Pezzini et al. 2013], predominantly cortical and/or involving underlying white matter [Woo et al. 2002;Tveiten et al. 2012;Martini et al. 2012], subcortical or in a hemisphere excluding the basal ganglia or thalamus [Lipton et al. 1987;Massaro et al. 1991;Boonyakarnkul et al. 1993] or in any lobe(s) of the brain [Radberg, Olsson, & Radberg 1991;Yaqub et al. 1991;Ruiz-Sandoval, Cantu, & Barinagarrementeria 1999;Tatu et al. 2000;Sacco et al. 2009;Lavados et al. 2010;Jamieson et al. 2012;Arboix & Grive 2012].

1.1.5.2 Previous definitions of non-lobar ICH

There may be more of a consensus regarding how to define non-lobar ICH. Of 28 studies which defined 'non-lobar' ICH, 20 defined 'non-lobar' as involving the basal ganglia or infratentorial regions [Brott, Thalinger, & Hertzberg 1986;Lipton et al. 1987;Massaro et al. 1991;Radberg, Olsson, & Radberg 1991;Yaqub et al. 1991;Boonyakarnkul et al. 1993;Greenberg et al. 1996;Rosenow et al. 1997;Ruiz-Sandoval, Cantu, & Barinagarrementeria 1999;Tatu et al. 2000;Inagawa et al. 2003;Zahuranec et al. 2006;Lavados et al. 2010;Weimar et al. 2011;Ruiz-Sandoval et al. 2011;Biffi et al. 2011b;Matsukawa et al. 2012;Arboix & Grive 2012;Tveiten et al. 2012;Pezzini et al. 2013], seven included deep periventricular white matter [Broderick 1993;Anderson et al. 1994;Nilsson et al. 2000;Woo et al. 2002;Bilbao et al. 2005;Jamieson et al. 2012;Martini et al. 2012] and one included 'subcortical' structures [Sacco et al. 2009].

1.1.5.3 Mixed ICH

'Mixed' ICH, involving both lobar and deep regions of the brain has only been accounted for in six studies [Fieschi et al. 1988;Boonyakarnkul et al. 1993;Anderson et al. 1994;Bilbao et al. 2005;Telman et al. 2010;Biffi et al. 2011b].

1.1.5.4 Definition of lobar and non-lobar ICH

I have defined an ICH as lobar if it involves one or more lobes of the brain (Figure 2 on page 53). The following ICHs are lobar:

- a single ICH involving any of the following areas: frontal, frontotemporal, frontoparietal, parietal, parieto-temporal, parieto-occipital, temporal, temporo-occipital or occipital or
- an ICH which involves both lobar and supratentorial 'deep' regions of the brain or
- multiple ICHs in either solely lobar locations or where at least one ICH involves a lobar location.

I have defined an ICH as non-lobar if it does not extend to a lobar area (Figure 3 on page 54). The following ICHs are non-lobar:

- a single infratentorial ICH (involving the brainstem and/or cerebellum) or
- a single supratentorial deep ICH (involving the basal ganglia, internal capsule, external capsule and/or thalamus) or
- multiple ICHs in solely non-lobar locations (either supratentorial deep or infratentorial).

1.1.5.5 Risk factors for lobar and non-lobar ICH

Studies which have examined the risk factors for lobar and non-lobar ICH have reported conflicting results (Table 5 on page 140). Four hospital based studies reported that female sex was more common in lobar ICH [Massaro et al. 1991;Weimar et al. 2011;Matsukawa et al. 2012;Arboix & Grive 2012] and in seven studies (only one of which was population-based [Labovitz et al. 2005]) those with lobar ICH were older than non-lobar ICH [Graffagnino et al. 1994;Greenberg et al. 1996;Weimar et al. 2011;Kuramatsu et al. 2011;Biffi et al. 2011b;Matsukawa et al. 2012]. Non-lobar ICH has been associated with Hispanic and Mexican-American ethnicity [Labovitz et al. 2005;Zahuranec et al. 2006].

1.1.6 *Diagnosis of ICH*

To date a scoring system to reliably clinically differentiate between ICH and infarction does not exist [Weir et al. 1994]. The only reliable method of detecting ICH in life is early brain imaging [Davenport & Dennis 2000].

1.1.6.1 Computed tomography and magnetic resonance imaging

The widespread availability of computed tomography (CT) in many countries and its rapid acquisition time makes it the first test an individual with a suspected stroke might have following their initial presentation. The CT imaging characteristics of ICH are determined by the extent to which X-rays are attenuated by the haematocrit and haemoglobin content of the ICH [Kidwell & Wintermark 2008]. In the hyperacute and acute phases the ICH is hyperdense in comparison to the brain parenchyma. The density of the haematoma increases in the first seven days with clot retraction and extrusion of serum [Chewning & Murphy 2010]. It then becomes less dense due to lysis of red blood cells and haemoglobin, so that within weeks it is indistinguishable from an infarct [Kidwell & Wintermark 2008].

Early studies of magnetic resonance imaging (MRI) for the diagnosis of ICH suggested that MRI was insensitive for the detection of acute blood, although this may have been related to the weak field strengths of the scanners used and a lack of haem-sensitive MRI sequences [Weingarten et al. 1991]. The strengths of MRI lie in its ability to distinguish ICH from haemorrhagic transformation of an infarct (HTI) using diffusion-weighted imaging (DWI) [Lovelock et al. 2009] and the detection of chronic haemorrhages; since haem-sensitive gradient recalled echo (GRE) T2* images show haemosiderin-related signal drop-out in the majority of people indefinitely [Wardlaw & Statham 2000]. However, the use of MRI continues to be limited by both its availability and the difficulty using it in acutely unwell patients who have metal implants or a pacemaker, or are unable to lie flat for the scan duration [Kidwell & Wintermark 2008] (Chapter 6).

1.1.6.2 Detection of secondary ICH

Detection of a structural cause for an ICH may influence a patient's management and prognosis. There is no standardised imaging approach to detect structural lesions. Clinicians are more likely to investigate a patient with ICH if they are younger, do not

have a past history of hypertension or have ICH in a lobar location [Cordonnier et al. 2010a]. However, the diagnostic utility studies on which these assumptions are based have been small, retrospective and suffered from both selection and work-up biases [Laissy et al. 1991;Zhu, Chan, & Poon 1997;Yeung et al. 2009].

Non-contrast CT may reveal certain clues to a structural cause such as calcification or enlarged vessels along the margin of an ICH, suggestive of an AVM or an empty delta sign within the superior sagittal sinus [Lee 2002], suggesting cerebral venous sinus thrombosis. These features have been combined with age (18-45 years = two points, 46-70 years = one point, ≥ 71 years= zero points), gender (female=one, male=zero) and neither a known history of hypertension or coagulopathy (Yes=one, No=zero) to form the secondary ICH score. A total score of >two has 86% sensitivity and 72% specificity for the detection of vascular lesions [Delgado Almandoz et al. 2010], although the score is yet to be independently prospectively validated.

MRI is helpful in demonstrating tumours and cerebral cavernous malformations (CCMs) [Steiner et al. 2006]. Although intra-arterial digital subtraction arteriography (IADSA) is regarded as the reference standard for the diagnosis of aneurysms and arteriovenous malformations [Chewning & Murphy 2010], its limited availability and small (~0.7%) but appreciable risk of stroke [Cloft, Joseph, & Dion 1999;Willinsky et al. 2003], make it unsuitable for use in every case of ICH.

Non-invasive angiographic techniques (CT angiography, MR angiography) have 90% sensitivity for the detection of aneurysms larger than three millimetres when compared to IADSA in patients with subarachnoid haemorrhage or patients without a known aneurysm but with symptoms that could be attributed to an aneurysm [White, Wardlaw, & Easton 2000]. CT angiography and venography have been shown to have 100% sensitivity (95% CI 92%-100%) and 99% specificity (95% CI 94%-100%) in comparison to IADSA for the diagnosis of intracranial vascular malformations [Wong et al. 2011].

1.2 What causes spontaneous primary ICH?

Hypertension-related arteriolosclerosis and CAA are commonly thought to be the main contributory factors causing spontaneous primary ICH. In the following section I will

outline the early pathological studies of arteriolosclerosis and describe the clinical, pathological and genetic basis of CAA.

1.2.1 *Arteriolosclerosis*

In 1868 Charcot and Bouchard first noted tiny (250-400 micrometre) outpouchings from the walls of cerebral blood vessels at autopsy in individuals who had died from ICH [Charcot & Bouchard 1868]. The outpouchings were preferentially seen in the thalamus and basal ganglia. They termed these 'miliary aneurysms' and postulated that these were the source of bleeding. Nearly a century later, in an autopsy study of 54 cases, Ross Russell replicated these findings, and by comparing cases with and without hypertension (defined as an initial diastolic recording on hospital admission of at least 110mmHg), found that the aneurysms were associated with hypertension [Ross Russell 1963].

The role of these aneurysms remains contentious since it has rarely been possible to link an ICH to the rupture of a particular aneurysm [Caplan 1992]. In 1971, Miller-Fisher published the findings from a case series of three patients with putaminal and pontine ICH in which he had identified multiple sources of bleeding [Fisher 1971]. He also noted degenerative changes of arteriolosclerosis, manifesting as both fibrinoid necrosis and segmental disorganisation with thickening of the walls of deep perforating arterioles. The latter was later called 'lipohyalinosis', because lipid containing macrophages within the wall stained readily for fat. Although other studies [Ooneda et al. 1973] have reported these changes and aneurysms in patients with so called 'hypertensive ICH' studies have been small case series which have not defined hypertension.

1.2.2 *Clinical manifestations of cerebral amyloid angiopathy*

In the 1970s clinicopathological case reports and case series first noted the co-existence of CAA in individuals with ICH [Vinters 1987]. Since then, there have been several case control and cross-sectional studies which have noted an association between CAA and lobar ICH (Chapter 9).

ICH attributable to CAA has been postulated to be multiple [Gilles et al. 1984;Zhan et al. 2004], recurrent [Zhan et al. 2004] and associated with dementia [McCarron et al. 1999b] but studies have been small case series [Gilles et al. 1984;McCarron et al. 1999b],

hospital-based [Zhan et al. 2004] and lacked standardised methods for assessing CAA [Gilles et al. 1984;McCarron et al. 1999b;Zhan et al. 2004].

There are at least three other manifestations of CAA: cognitive impairment, transient focal neurological symptoms and rapidly progressive cognitive and neurological decline.

Two population-based studies have confirmed an association between pathologically proven CAA and dementia [Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) 2001;Pfeifer et al. 2002], which remains after adjustment for age, brain weight, neurofibrillary tangles, neuritic plaques, Lewy bodies and hippocampal atrophy [Matthews et al. 2009]. There is only one study of the nature of cognitive impairment found in CAA which showed that moderate to severe CAA was associated with decreased perceptual speed and episodic memory although not other cognitive domains [Arvanitakis et al. 2011].

There has been an increasing number of case series in recent years of persons with recurrent episodes of transient stereotyped positive or negative neurological symptoms [Okazaki, Reagan, & Campbell 1979;Raposo et al. 2011]. A subsequent MRI brain scan with T2*-weighted imaging may show lobar ICH in up to three quarters of patients, brain microbleeds (BMBs) in a lobar distribution or cortical subarachnoid haemorrhage, suggestive of CAA [Charidimou et al. 2012]. Of note however, is that as yet there have been no larger prospective studies of this phenomenon which have correlated clinical and radiographic with pathological findings.

Rarely, CAA may present as Aβ-related angiitis (also called CAA-related inflammation) with rapid cognitive decline, headache, behavioural changes, seizures and focal neurological deficits [Scolding et al. 2005]. MRI may show extensive white matter hyperintensities on T2-weighted sequences or FLAIR but for a definitive diagnosis brain biopsy or autopsy is required showing CAA within the vessels of the affected area and perivascular or transmural inflammation [Chung et al. 2011].

1.2.3 *Neuroimaging correlates of cerebral amyloid angiopathy*

The neuroimaging correlates of CAA on MRI include BMBs, superficial siderosis and cortical subarachnoid haemorrhage.

BMBs are small hypointense foci on T2*-weighted haem-sensitive MRI sequences and BMBs in a strictly lobar distribution are thought to be a biomarker of CAA (Chapter 6).

Superficial siderosis is defined as superficial, cortical, linear deposits of haemosiderin on T2*-weighted images [Linn et al. 2008] and is distinguished from convexity subarachnoid haemorrhage in which the bleeding extends into the adjacent subarachnoid space without involvement of adjacent parenchyma, interhemispheric fissure, basal cisterns or ventricles [Kumar et al. 2010].

Both superficial siderosis and subarachnoid haemorrhage have been associated with CAA on the basis of case reports [Karabatsou 2007;Linn et al. 2008], hospital-based retrospective case series [Kumar et al. 2010;Charidimou et al. 2012] and cross-sectional studies [Linn et al. 2010] in which the diagnosis of CAA has typically been made using MRI imaging without pathological confirmation of CAA [Kumar et al. 2010;Charidimou et al. 2012]. In one retrospective study of 60 participants which compared ICH attributed to pathologically-confirmed CAA vs. ICH without CAA, superficial siderosis was detected in 61% participants with CAA-related ICH and none of those without CAA [Linn et al. 2010], but the study used a highly selective cohort with no mention of the time interval between the ICH and imaging, despite the imaging appearances of haemorrhage being known to change over time [Kidwell & Wintermark 2008]. More recently, disseminated superficial siderosis (involving more than three sulci) has been associated with an increased risk of symptomatic lobar ICH in those with CAA [Charidimou et al. 2013d] diagnosed according to the Boston criteria [Knudsen et al. 2001].

Larger prospective studies using haem-sensitive MRI sequences performed at a standard time interval after presentation with pathological correlation are needed, especially since superficial siderosis is not unique to CAA and may be seen in brains without pathologically proven CAA [De Reuck et al. 2013].

1.2.4 *Ante-mortem diagnosis of cerebral amyloid angiopathy*

The reference standard for the diagnosis of CAA is pathological confirmation using biopsy or autopsy specimens. Since participants with ICH may not undergo surgery and with a declining number of autopsies in recent years [Kretzschmar 2009], efforts have been made to develop criteria for the ante-mortem diagnosis of CAA which utilise not only pathological findings but other clinical and radiographic features of the ICH. The Boston criteria for the diagnosis of 'CAA-related ICH' categorise first-ever or recurrent lobar ICH as definite, probable or possible CAA-related ICH by using pathological

findings if available, radiographic features such as multiple ICHs or BMBs and clinical features such as the absence of coagulopathy [Knudsen et al. 2001].

1.2.5 *Definition of cerebral amyloid angiopathy*

Although the definition of a disease entity is traditionally described before its clinical and imaging features, I define CAA at this point, since it is by using this definition that the subtypes (Section 1.2.7, page 44), genetic basis (Section 1.2.7, page 44) and pathogenesis (Section 1.2.9, page 46) of CAA are understood.

CAA is an 'umbrella' term which describes a group of diverse disorders [Revesz et al. 2009], unified by the pathological findings of deposition of amyloid fibrils in leptomeningeal and cortical arteries, arterioles, capillaries and rarely veins; sometimes associated with additional spread into the adjacent neuropil [Attems 2005]. 'Amyloid' is a morphological term referring to at least 20 different proteins [Attems 2005] which form insoluble fibrils that unite in a β-pleated sheet structure.

CAA is thought to be distinct from systemic amyloidoses, in part because some amyloidogenic precursor proteins are too large to diffuse across the blood brain barrier [Banks 2009;Sattianayagam, Hawkins, & Gillmore 2009]. It is not associated with senile systemic amyloidosis [Tanskanen et al. 2006]. Although amyloid-beta (Aβ) is found both in the systemic circulation and in the cerebrospinal fluid (CSF) and brain interstitial fluid, most Aβ is derived from neurons (Section 1.2.9.4, page 47) and transgenic mice which produce increased amounts of systemic Aβ do not develop cerebral Aβ deposits, providing further evidence against CAA being derived from systemic Aβ [Kawarabayashi et al. 1996].

The most common form of CAA is due to Aβ protein [Revesz et al. 2009] and I will focus on the subtypes, genetic risk factors and pathology of Aβ CAA below.

1.2.6 *Amyloid precursor protein and Aβ*

Aβ protein is derived from amyloid precursor protein (APP); the gene for which is sited on the long arm of human chromosome 21. The function(s) of APP in the central nervous system are not well described, although evidence from in vitro studies suggests that APP may function as a cell surface receptor [Zheng & Koo 2011] and mediate the adhesion and growth of neurons and non-neuronal cells [Coughlan & Breen 2000].

Aβ protein is routinely produced by many cell types. It is thought that Aβ may regulate synapses; that is, increases in neuronal activity lead to increased production of Aβ which then via a negative feedback loop reduce synaptic function [Pena et al. 2006]. Consistent with this, Aβ has been shown to reduce glutamatergic transmission by reducing the expression of NMDA receptors in neurons in vitro [Snyder et al. 2005].

Unlike the parenchymal Aβ plaques found in Alzheimer's dementia which are predominantly composed of Aβ of 42 amino acid length ($A\beta_{42}$), vascular Aβ largely consists of a shorter more soluble Aβ fragment which is 40 amino acids long ($A\beta_{40}$) and has a higher $A\beta_{40}$:$A\beta_{42}$ ratio than in an Aβ plaque [Roher et al. 1993;Attems, Lintner, & Jellinger 2004].

1.2.7 *Sporadic and Hereditary CAA*

Aβ-CAA may be sporadic or hereditary. Hereditary CAA may result from mutations of the APP gene or mutations of genes which control the processing of APP or Aβ within the brain or chromosomal abnormalities such as trisomy 21 (Down's syndrome) since the extra copy of the APP gene leads to increased APP, $A\beta_{40}$ and $A\beta_{42}$ [McCarron, Nicoll, & Graham 1998].

1.2.7.1 Sporadic CAA

The majority of Aβ-CAA is sporadic. It rarely occurs under the age of 50 years [Masuda et al. 1988] and its prevalence increases each decade and approaches 50% in those aged 90 years and above [Masuda et al. 1988;Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) 2001]. Males and females are equally affected. The prevalence of CAA varies from 21% in a population-based cohort [Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) 2001] to 80% in hospital based cohorts of patients with Alzheimer's dementia [Mandybur 1975;Esiri & Wilcock 1986] and is significantly higher than in controls of a similar age without dementia [Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) 2001]. CAA is also more severe in those with Alzheimer's dementia compared to controls without [Yamada 2002].

1.2.7.2 Hereditary CAA due to Aβ deposition

Missense mutations of the APP gene can occur either within or outside the coding region of the Aβ peptide. Mutations within the coding region of the peptide lead to a phenotype in which CAA is prominent [Revesz et al. 2009], one example of which is Hereditary Cerebral Haemorrhage with Amyloidosis of Dutch type (HCHWA-D) characterised by recurrent lobar ICH and dementia [Bornebroek et al. 1996]. Mutations outside the coding region lead to clinicopathological phenotypes of early onset Alzheimer's dementia [Revesz et al. 2009].

1.2.7.3 Genetic risk factors for sporadic CAA

The most well known genetic risk factor for CAA is apolipoprotein E although other gene polymorphisms implicated in Alzheimer's dementia are also known risk factors for CAA such as presenilin-1 and neprilysin.

The apolipoprotein E gene codes for a protein of the same name, which is expressed in several organs including the liver and the brain. The protein assists in the transport of triglycerides to the liver. In the brain, astrocytes and microglia express apolipoprotein E which functions as a ligand to assist in the endocytosis of lipoproteins [Kim, Basak, & Holtzman 2009].

The epsilon form of the apolipoprotein gene, is contained on the long arm of chromosome 19 [Verghese, Castellano, & Holtzman 2011]. Single base changes in the gene determine the three common alleles ε2, ε3 and ε4 [Zannis et al. 1982] and lead to changes in the function of the resulting protein isoforms. The E4 isoform may accelerate the formation of Aβ plaques by acting as an Aβ binding protein [Wisniewski & Frangione 1992].

The ε2 and ε4 alleles are both associated with sporadic Aβ-CAA [Verghese, Castellano, & Holtzman 2011] with the ε4 allele having a dose-dependent effect on CAA severity [Premkumar et al. 1996].

Neprilysin is a proteolytic enzyme responsible for the catabolism of Aβ in the brain and inhibition of the enzyme leads to increased accumulation of Aβ42 in rat brains [Iwata et al. 2000]. Moreover increased CAA severity has been associated with decreased neprilysin activity in human brain tissue [Miners, Kehoe, & Love 2011]. Mutations of

presenilin-1, a protein involved in the processing of APP, can lead to early-onset familial Alzheimer's dementia [Sherrington et al. 1995]. One small study showed an association between presenilin-1 polymorphisms and CAA severity [Yamada et al. 1997] although this requires further exploration.

1.2.8 *A historical perspective*

The earliest report of CAA is likely to be by Oppenheim [Oppenheim 1909] when he described 'drusige Nekrosen' (translated as gland-like necrosis) in the brain parenchyma adjacent to hyalinised capillary walls in six out of 14 brains of individuals with dementia and the pathological changes of Alzheimer's disease. Scholz published the first study of CAA in 1938 [Scholz 1938]. In 15 of 104 autopsied brains he noted an abnormality of the cerebral blood vessels and immediately adjacent brain parenchyma with the morphological and staining properties of 'drusige Entartung' (literally glandular degeneration) and postulated that this might be amyloid.

Sixteen years later Pantelakis provided the first description of CAA limited to the blood vessels without adjacent parenchymal involvement and coined the term 'congophilic angiopathy' because it appeared apple green when stained with Congo Red and viewed under polarised light [Pantelakis 1954].

1.2.9 *Pathology of cerebral amyloid angiopathy*

1.2.9.1 Morphology

Severe CAA is visible as acellular thickening of blood vessel walls on haematoxylin and eosin stained tissue sections although this appearance is non-specific, occurring with other small vessel diseases such as arteriolosclerosis [Attems 2005]. Stains for Aβ include Thioflavin S or T (which fluoresce under ultraviolet light) and Congo Red. In recent years immunohistochemical stains for Aβ have become more widely used because they offer greater standardisation and increase the specificity of diagnoses as accumulation of abnormal proteins can be detected with morphology and location [Dickson 2005].

CAA deposition is progressive, initially occurring in the tunica media of the blood vessel wall. In the early stages, the vessel wall structure remains intact but as the severity of CAA increases, there is loss of smooth muscle cells as Aβ infiltrates all layers of the

wall. With severe CAA the vessel wall splits akin to an onion skin (so called 'double barrelling') and leakage of Aβ into the neuropil may occur (Figure 4 on page 55) [Attems 2005].

1.2.9.2 Pathological subtypes of CAA

Cross-sectional studies of patients with Alzheimer's dementia and controls suggest that there are two subtypes of CAA: CAA type one, which is characterised by the presence of CAA in capillaries with or without CAA in larger vessels and CAA type two, which is restricted to leptomeningeal and cortical arteries, arterioles and occasionally veins [Thal et al. 2002a]. Although the presence of capillary CAA has been associated with more extensive Aβ plaques deposition in Alzheimer's dementia [Thal et al. 2010], most studies have taken place in the setting of dementia and the significance of it in those with ICH is yet to be determined.

1.2.9.3 Distribution of CAA

Sporadic CAA is thought to preferentially distribute in the occipital lobes followed by either frontal, temporal or parietal lobes but studies have had differing results [Mann et al. 2001], been limited by a lack of blinding of neuropathologists to sampling location [Yamada et al. 1987;Premkumar et al. 1996;Pfeifer et al. 2002;Tian et al. 2003;Tian et al. 2004;Attems, Jellinger, & Lintner 2005], variation in the assessment of CAA severity with no assessment of inter-observer agreement [Yamada et al. 1987;Premkumar et al. 1996;Pfeifer et al. 2002;Tian et al. 2003;Attems, Jellinger, & Lintner 2005] and sampling bias [Tian et al. 2003]. The basal ganglia, thalamus, cerebellum and brainstem are less frequently affected [Attems 2005].

Although Aβ plaques in Alzheimer's dementia appear to be deposited sequentially with the plaques initially seen in the neocortex, followed in order by the allocortex, diencephalon and striatum, brainstem nuclei and cerebellum [Thal et al. 2002b], sequential deposition of CAA is yet to be proven.

1.2.9.4 Pathogenesis of CAA

Several transgenic mouse models exist that carry human gene mutations known to cause hereditary Aβ-CAA. The APPDutch mouse has an APP gene containing the familial Alzheimer's disease mutation E693Q leading to a phenotype with prominent CAA and

rarely any Aβ plaques [Herzig et al. 2004]. The Tg2576 mouse overexpresses a human APP transgene containing the Swedish familial Alzheimer's disease mutation [Hsiao et al. 1996] leading to increased expression of human APP above the levels of endogenous mouse APP and consequent increased production of both parenchymal and vascular Aβ [Shin et al. 2007]. The Tg-SWDI mouse expresses the human Swedish, Dutch and Iowa mutations in the APP gene [Davis et al. 2004], leading to production of Dutch/Iowa mutant Aβ and subsequent development of early onset and progressive capillary CAA [Davis et al. 2004].

The greatest limitation of murine models is that their generalisability to humans is limited. Even with prominent CAA, ICH may not occur in APPDutch mice and does not occur in Tg2576 and Tg-SWDI mice [Herzig, Van Nostrand, & Jucker 2006]. In Tg-SWDI mice, CAA occurs in a different distribution (thalamic and subiculum regions) to that seen in humans [Davis et al. 2004] and CAA occurs in these animals when they are younger than humans who may also have multiple comorbidities.

Despite these caveats, they have provided the following valuable insights into the pathogenesis of sporadic Aβ-CAA.

(i) **Most vascular Aβ is derived from neurons** [Herzig, Van Nostrand, & Jucker 2006]**.** Previous hypotheses regarding the pathogenesis of CAA proposed that Aβ in vessel walls may have derived from vascular smooth muscle cells. However, mice that overexpress human Aβ solely in neurons develop CAA [Calhoun et al. 1999] and CAA can occur in capillaries that do not have smooth muscle cells [Herzig, Van Nostrand, & Jucker 2006].

(ii) **CAA is likely to result from reduced Aβ clearance through perivascular lymphatic drainage pathways in the brain** [Weller et al. 2008]**.** CAA may result from increased production of Aβ or decreased clearance. Although several gene or chromosomal defects (for example: mutations in presenilin-1 and presenilin-2 [Revesz et al. 2009] or Down's syndrome [Naito, Sekijima, & Ikeda 2008]) lead to increased production of Aβ-CAA the majority of cases of CAA and Alzheimer's dementia are sporadic [Nicoll et al. 2004] and therefore not accounted for by these mechanisms. The drainage pathways of interstitial fluid and solutes have been outlined in transgenic mice. If a fluorescent tracer is

injected into the mouse brain it rapidly reaches and delineates the perivascular lymphatic pathways [Carare et al. 2008]. Moreover, drainage of intracerebral injected solutes is impaired in ageing Tg2576 mice with CAA [Hawkes et al. 2011].

(iii) **Increases in the ratio of $A\beta_{40}$: $A\beta_{42}$ lead to increased vascular Aβ (rather than parenchymal Aβ) deposition.** This is shown by humans with HCHWA-D and APPDutch mice who have a very high ratio of $A\beta_{40}$:$A\beta_{42}$ and develop severe CAA [Herzig et al. 2004]. However, $A\beta_{40}$ alone may not be sufficient to develop CAA since mice that produce increased $A\beta_{40}$ without $A\beta_{42}$ do not develop CAA, whereas those which produce both $A\beta_{40}$ and $A\beta_{42}$ produce both CAA and parenchymal Aβ [McGowan et al. 2005].

(iv) **Apolipoprotein ε4 increases the ratio of $A\beta_{40}$:$A\beta_{42}$ and promotes the formation of CAA in Tg2576 mice.** Tg2576 mice expressing the human apolipoprotein E4 isoform have prominent CAA with very little parenchymal Aβ in contrast to the combination of CAA and plaques seen in Tg2576 mice with murine apolipoprotein E [Fryer et al. 2005].

1.2.10 *Why might sporadic CAA be associated with lobar ICH?*

There are several reasons why sporadic CAA may be associated with lobar ICH. Firstly, sporadic CAA is most common in the lobar regions of the brain (Section 1.2.9.3, page 47). Secondly, hereditary amyloidoses such as HCHWA-D cause recurrent lobar ICH [Bornebroek et al. 1996]. Thirdly, a plausible explanation for an association between CAA and lobar ICH might relate to the differing structures of the walls of cortical and deep arteries. The tunica media of the walls of cortical arteries is adjacent to a single layer of leptomeninges which in turn is next to the perivascular glia limitans. There is no expandable perivascular space. In contrast, the deep arteries have a double layer of leptomeninges which functions as an expandable perivascular space (Figure 5 on page 56) [Weller, Boche, & Nicoll 2009].

Interstitial fluid and solutes are thought to drain out of the brain along the basement membranes in the walls of capillaries and arteries in the opposite direction to blood flow [Weller et al. 2009a]. It is postulated that each arterial pulsation produces a wave in the

opposite direction which propels solutes out of the brain. In older people, arteriolosclerosis leads to stiffening of the arterial walls [Nagasawa et al. 1979] which may reduce the elastic recoil of vessels, both reducing cerebral blood flow and compromising the drainage of solutes out of the brain. The lobar regions may be more prone to CAA because they lack a perivascular space and therefore are more vulnerable to deposition of Aβ which cannot be drained.

1.3 Aims of the thesis

ICH continues to be a devastating condition with a high early case fatality, whose incidence is likely to increase in an ageing population. If advances are to be made in the primary prevention and management of ICH, it will be essential to understand more about the risk factors and underlying causes of the condition. This will require large clinico-radio-pathological prospective studies of ICH with comprehensive case ascertainment and careful phenotyping of participants.

In June 2010 I set up a prospective community-based study of ICH in the Lothian region of Scotland with the aim of answering the following questions.

- How should I design a community-based study of ICH? (Chapter 2)
- What is the incidence of ICH in the Lothian region of Scotland and do the risk factors differ for lobar and non-lobar ICH? (Chapter 5)
- What are the frequencies of definite, probable and possible CAA according to the Boston criteria in a community-based cohort of participants with lobar ICH? (Chapter 5)
- Do neuroimaging correlates of CAA such as BMBs differ between lobar and non-lobar ICH? (Chapter 6)
- Which variables determine prognosis following ICH? (Chapter 7)
- Does apolipoprotein E genotype differ between lobar and non-lobar ICH? (Chapter 8)
- Does the presence of an ε2 or ε4 allele influence outcome following an ICH? (Chapter 8)
- What is the strength of the association between CAA and ICH? (Chapter 9)

- Do any imaging features of lobar ICH on CT discriminate between ICH related to CAA and those which are unrelated to CAA? (Chapter 10)
- What proportion of participants consent to a research autopsy limited to the brain and do any demographic or clinical factors differ in those who consent vs. those who do not? (Chapter 11)

Figure 1 Schematic diagram of the brain showing types of intracranial haemorrhage reproduced from [Al-Shahi Salman, Labovitz, & Stapf 2009]

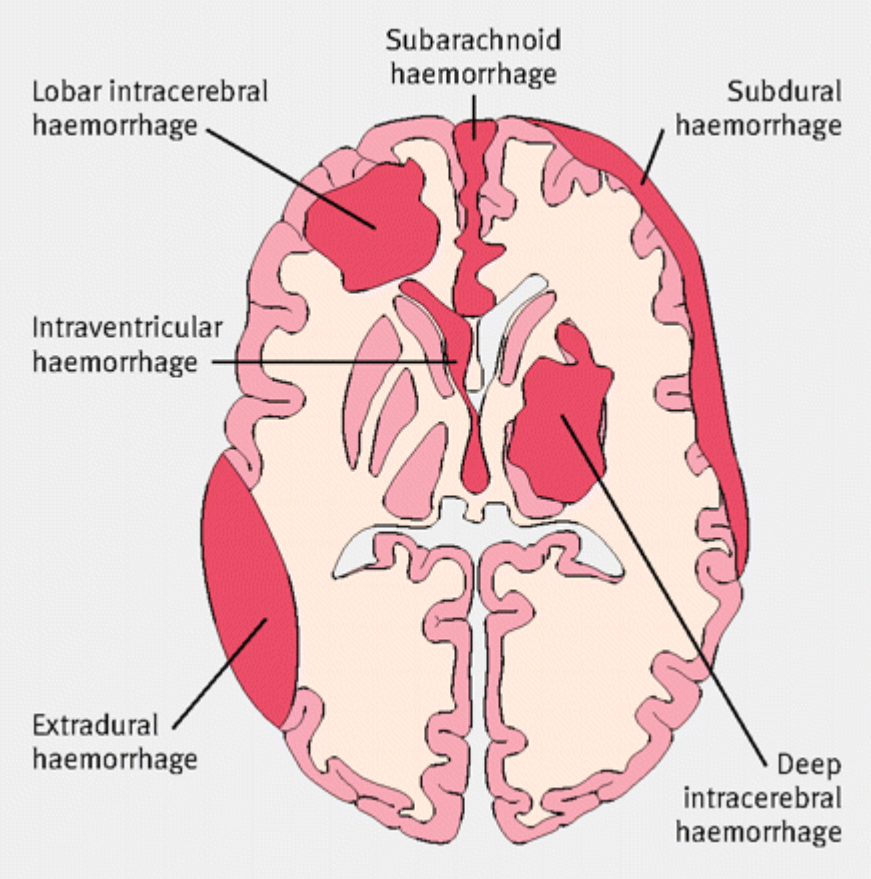


Figure 2 Lobar ICHs

A Axial CT demonstrates a left frontal lobar ICH
B Coronal CT demonstrates a right frontal lobar (periventricular) ICH
C Axial CT demonstrates a right hemisphere lobar ICH with compression but no involvement of the right lentiform nucleus (arrow)
D Sagittal CT demonstrates a left frontoparietal lobar ICH
E Axial CT demonstrates a left hemisphere ICH involving both lobar and deep regions
F Axial CT demonstrates a right hemisphere ICH involving both lobar and deep regions

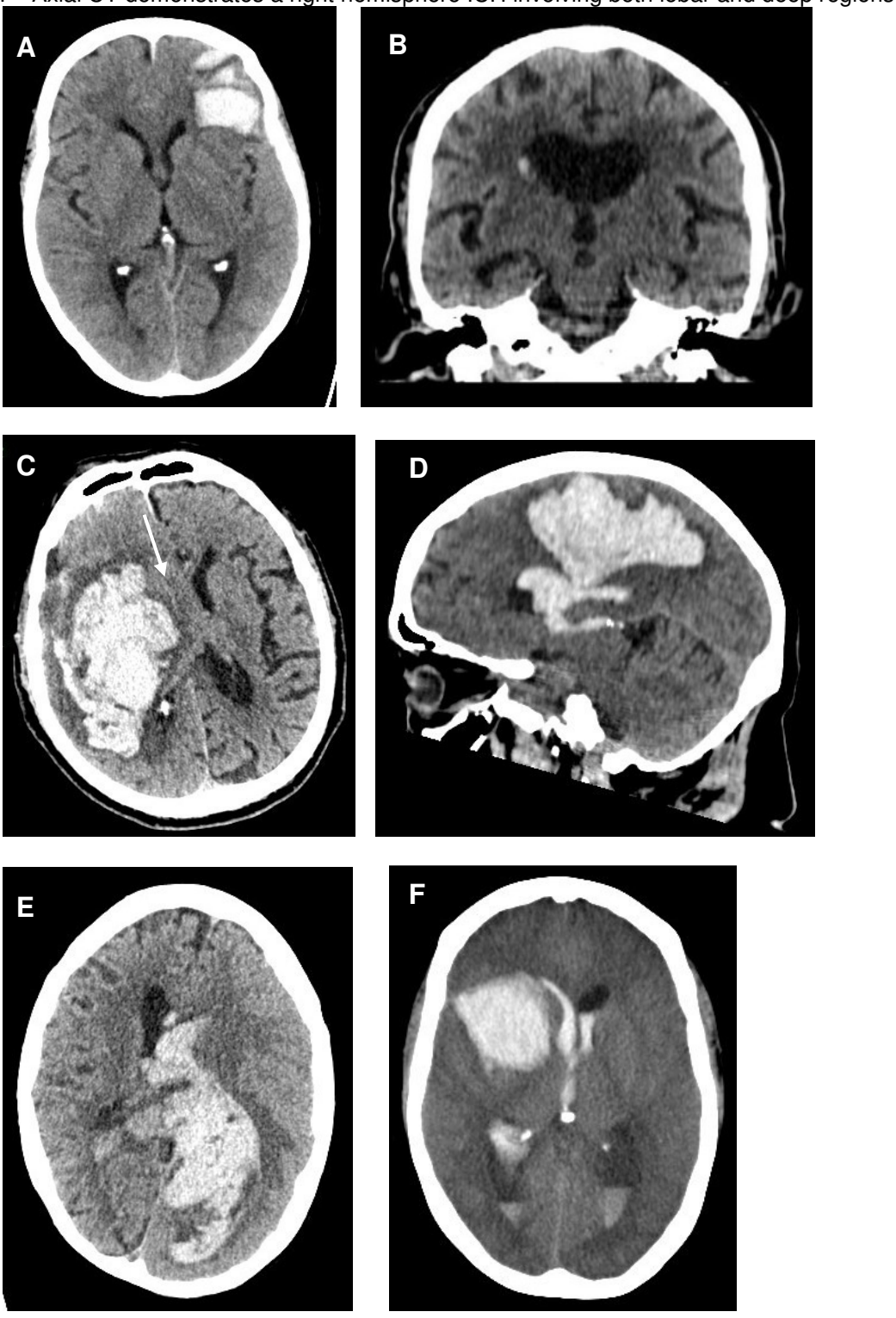


Figure 3 Non-lobar ICHs

A Axial CT demonstrates a left thalamic ICH

B Axial CT demonstrates multiple cerebellar ICHs

C1 and C2 Multiple non-lobar ICHs in the same patient: C1 Axial CT demonstrates a left lentiform nucleus ICH and C2 Axial CT demonstrates an acute pontine ICH

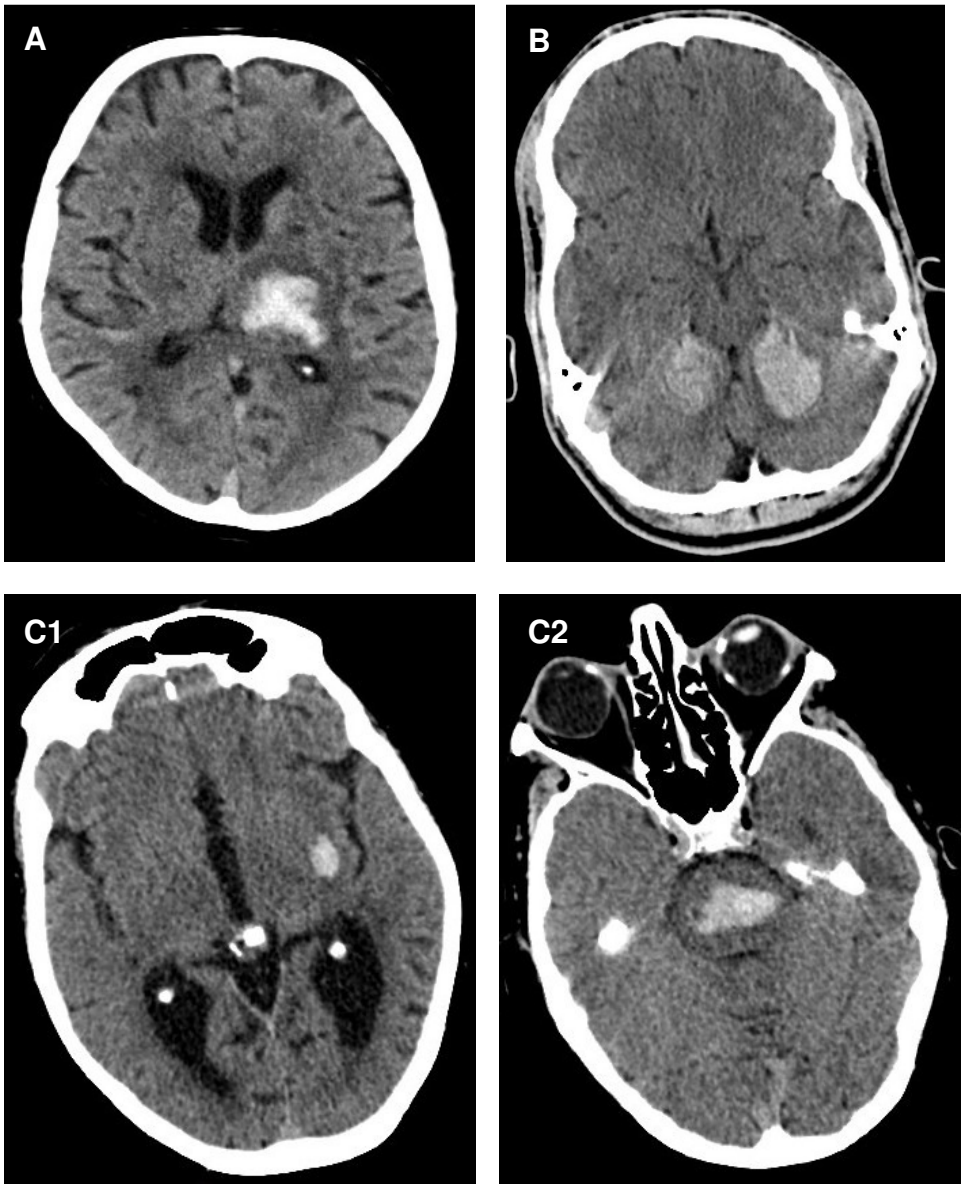


Figure 4 Progression of CAA (reproduced from [Attems 2005])
Normal: normal blood vessel wall
Mild: some Aβ deposition within the tunica media but with preservation of smooth muscle cells in tunica media
Moderate: Abundant Aβ deposition through all layers of blood vessel wall with loss of smooth muscle cells
Severe: Blood vessel wall replaced by Aβ deposition
Severe with double barrelling: Disruption and fragmentation of blood vessel wall
Severe with dyshoric changes: Fragmentation of blood vessel wall leads to leakage of Aβ into the adjacent neuropil

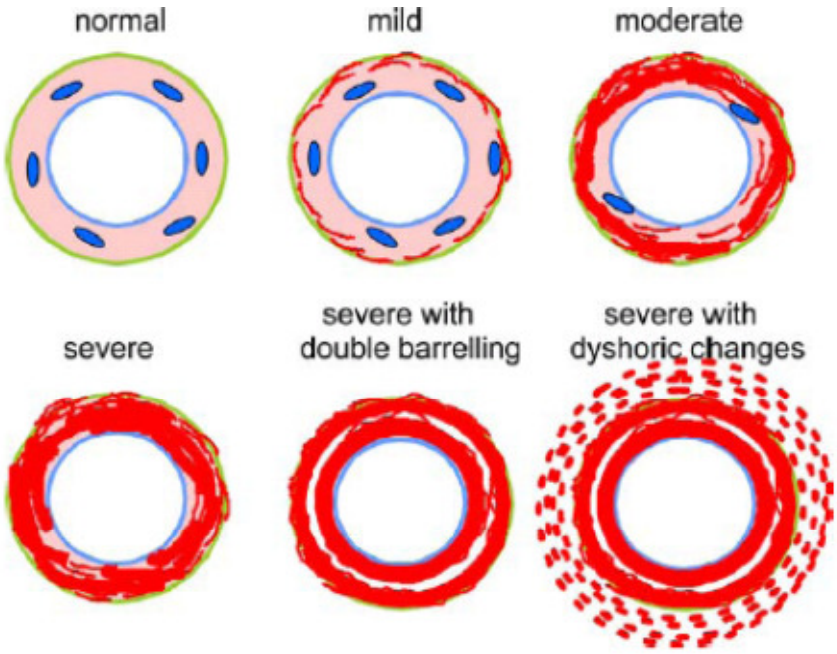
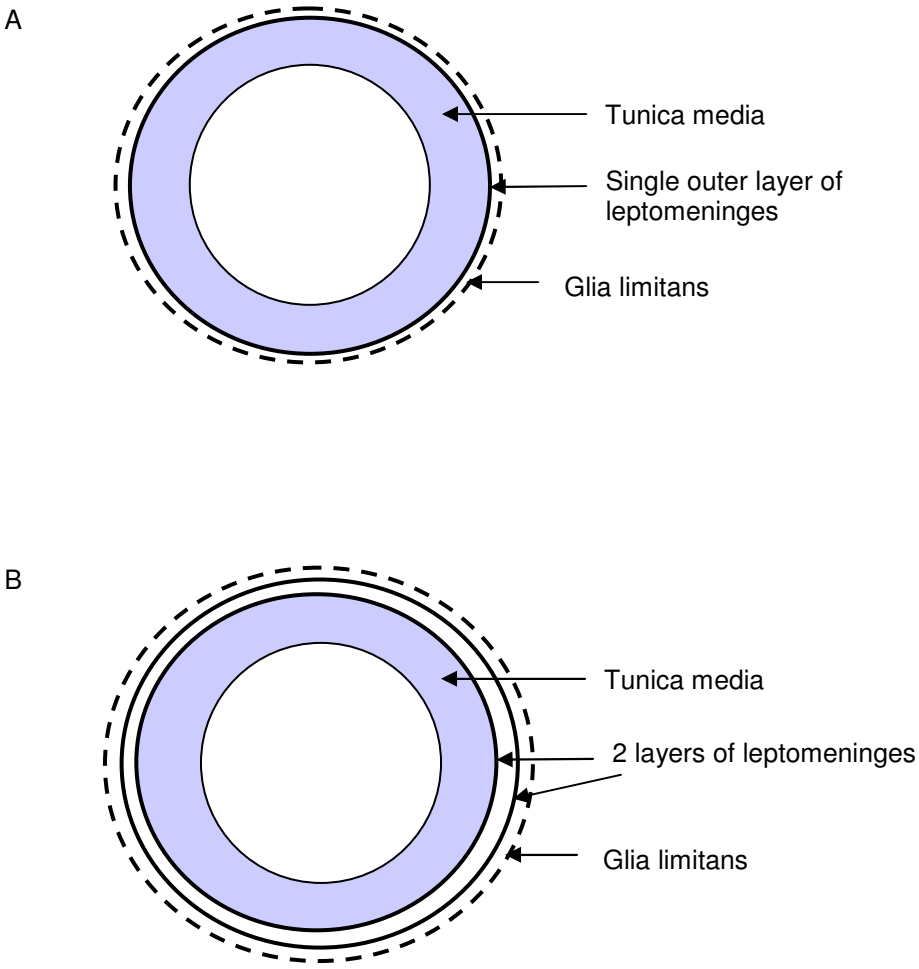


Figure 5 Schematic diagram comparing structure of vessel walls of a cortical artery (A) and basal ganglia artery (B) (adapted from [Weller, Boche, & Nicoll 2009])

A Cortical artery with a single outer layer of leptomeninges and no expandable perivascular space

B Basal ganglia artery with a second layer of leptomeninges. A perivascular space separates the two layers of leptomeninges.



Section B The epidemiology, imaging and genetics of intracerebral haemorrhage in the Lothian region of Scotland-Study design and methods

Chapter 2 Methods of a prospective community-based study of intracerebral haemorrhage

Chapter 3 Methods of a cross-sectional study of MRI brain imaging features in lobar vs. non-lobar intracerebral haemorrhage

Chapter 4 Methods of a cross-sectional study of apolipoprotein E genotype in lobar vs. non-lobar intracerebral haemorrhage

Chapter 2 Methods of a prospective community-based study of intracerebral haemorrhage

Chapter contents

2 Methods of a prospective community-based study of intracerebral haemorrhage

2.1 Introduction

Although there have been a considerable number of incidence studies of spontaneous ICH, prior studies have had various limitations. Studies have lacked a clear reproducible definition of ICH [van Asch et al. 2010], and frequently not distinguished primary from secondary ICH and first-ever from recurrent ICH (Chapter 1). Comprehensive case ascertainment is essential for an incidence study of ICH [Sudlow & Warlow 1997]. ICH is one cause of sudden death which may occur before a person reaches hospital. Even population-based incidence studies of ICH which have reasonable case ascertainment methods often do not screen sudden deaths or deaths that occur soon after reaching hospital emergency departments, leading to potential under-ascertainment of cases [van Asch et al. 2010]. Measures of disease incidence will also be dependent upon defining and enumerating a stable population at risk [Sudlow & Warlow 1997], which ideally should be sufficiently large to obtain a precise estimate.

Cross-sectional studies which have compared the risk factors for lobar and non-lobar ICH have reported inconsistent associations between risk factors such as dementia and hypertension and ICH (Chapter 1). Studies have lacked both clear reproducible definitions of risk factors, based on a participant's pre-ICH health status [Jackson & Sudlow 2006] and clear definitions of ICH location (Chapter 1).

Therefore I set up a prospective, community-based observational cohort study (with nested cross-sectional comparisons) in Spring 2010 to study the incidence of spontaneous primary lobar and non-lobar ICH and its risk factors.

Data collection started on 1st June 2010. This chapter describes the study design, case ascertainment, definition of ICH and its date of onset and methods of data collection at baseline and during follow up. I will illustrate the complexities in diagnosing spontaneous primary ICH using several case histories and describe the strengths and limitations of my methods of case ascertainment and follow up.

2.2 Study design

2.2.1 Inclusion criteria

I recruited patients if they met all of the following criteria.

- First-ever or recurrent spontaneous (non-traumatic) ICH confirmed by brain imaging or pathology (biopsy or post-mortem examination) or sudden deaths where a diagnosis of ICH was suspected but the diagnosis remained unconfirmed.
- Aged 16 years or over at the time of diagnosis.
- Resident in the area served by the National Health Service (NHS) Lothian Health board at the time of the ICH.
- Date of diagnosis 1st June 2010-31st May 2016.

2.2.2 Exclusion criteria

I excluded patients if they met any of the following criteria.

- ICH definitely attributable to trauma.
- Exclusively extra-axial intracranial haemorrhage.
- ICH attributable to HTI.

2.3 Study setting

I recruited participants who were resident in the region served by the NHS Lothian Health board. The Registrar General for Scotland's mid-2010 estimate for the population served by NHS Lothian is 836,711 of whom 695 335 were adults aged 16 and above. The population is stable with net migration of 8786 (population change +1%) from mid 2009 to mid 2010 (www.gro-scotland.gov.uk). The sex ratio (male population divided by the female population) is 0.94.

The region is served by three hospitals with emergency departments or acute medical receiving units in NHS Lothian (Western General Hospital, Edinburgh; Royal Infirmary of Edinburgh, or St John's Hospital at Howden, West Lothian). Each hospital provides acute in-patient stroke services with access to specialist stroke unit care. There are rapid access neurovascular clinics for patients with suspected transient ischaemic attacks and strokes at the Western General Hospital and St John's Hospital.

2.4 Sources of case ascertainment

I used multiple overlapping sources of case ascertainment to identify all potentially eligible adults meeting our inclusion criteria. I prospectively identified the majority of cases using a 'hot pursuit' model, enabling assessment of possible cases of ICH soon after their presentation to hospital. I also used various retrospective sources to supplement my case ascertainment methods.

Sources of case ascertainment

Prospective sources

- Screening of all CT brain scans
- Collaborative network
- Multidisciplinary meetings

Retrospective sources

- Electronic patient records system
- Office of the Procurator Fiscal
- Liaison with stroke audit personnel
- Centralised coding of hospital discharge data

2.4.1 Prospective sources of case ascertainment

2.4.1.1 Screening of all CT brain scans

I reviewed all CT brain scans done in the three hospitals in NHS Lothian. On a Monday morning or following a public holiday, this included all brain scans done over the weekend or holiday period. I reviewed radiology reports for scans when they were available.

2.4.1.2 Collaborative network

I developed a collaborative clinical network comprising consultants, trainee doctors and specialist nurses in the following specialties: stroke (n=17), neurology (n=25), neurosurgery (n=21), (neuro)radiology (n=15), neurorehabilitation (n=4), acute and general medicine (n=24), care of the elderly (n=7), emergency care (n=15), pathology (n=7) and stroke audit personnel (n=3). Collaborators were able to notify us of potential cases in person, use a designated mobile telephone, fax to a designated NHS

office or email to a secure nhs.net email address. Our collaborators received a monthly email reminder of the ongoing study and I placed study posters in their respective departments to encourage notification. I also provided teaching sessions about intracerebral haemorrhage for medical and nursing staff and allied health professionals to increase awareness. I updated our list of collaborators as staffing at the three recruiting hospitals changed.

2.4.1.3 Multidisciplinary meetings

I attended a daily multidisciplinary neuroradiology meeting (comprising neurologists, neurosurgeons and neuroradiologists.) I also attended a weekly multidisciplinary stroke radiology meeting. If I was unavailable, other doctors and stroke fellows notified me of patients.

2.4.2 Retrospective sources of case ascertainment

2.4.2.1 Electronic patient records system

Each hospital attendance to one of the hospitals in NHS Lothian is recorded using an electronic patient records system called TRAK. Every six months I searched the system to identify patients who had either been brought in dead to the emergency department or acute medical receiving unit or who had died in any of these departments. I screened the medical records of any patients listed. I identified two groups of patients:

(i) those who had died suddenly or soon after the onset of symptoms who had a confirmed diagnosis of ICH before death using brain imaging or at post-mortem examination; and

(ii) those who had a history suggestive of stroke but died before they could undergo brain imaging and did not undergo post-mortem examination.

I recorded these cases to ascertain the number of potentially eligible patients with ICH who might have been missed by the lack of further investigation.

2.4.2.2 Office of the Procurator Fiscal

In Scotland, the Crown Office and Procurator Fiscal Service is responsible for the investigation of sudden or suspicious deaths. The Procurator Fiscal Service keeps paper records of all calls concerning sudden deaths made to the office and their subsequent outcomes. The log includes both unexplained in-hospital and all out-of-hospital sudden

deaths referred to the Procurator Fiscal. The Fiscal office may decide that there is sufficient clinical information available to issue a death certificate or if this is not possible, that a fiscal post-mortem examination is required. The Procurator Fiscal office in Edinburgh receives all calls from the Lothian region.

With the necessary approvals from the Procurator Fiscal, in October 2011 I searched their record of deaths from 1st June 2010-31st May 2011 to identify any sudden deaths confirmed as ICH at post-mortem examination and cases where an ICH or other type of stroke was suspected to be the primary cause of death. If I identified a case of ICH which had been diagnosed at post-mortem examination, I recorded the fiscal post-mortem examination number which allowed the post-mortem examination report to be obtained from the Pathology Department based in the Western General Hospital, Edinburgh.

2.4.2.3 Liaison with stroke audit personnel

The Scottish Stroke Care Audit was established in 2002 and audits the care of all stroke patients at hospitals with stroke services in Scotland. The Scottish Stroke Care Audit ascertains cases using a variety of different methods including: screening registers of admissions to the emergency department and stroke units, screening of discharge summaries from stroke units, liaison with multidisciplinary stroke services, review of radiology reports and reviewing handover lists in medical, neurology and care of the elderly wards [Borthwick et al. 2005]. In addition, clinicians seeing patients in neurovascular outpatient clinics complete an audit form which includes the final diagnosis. The audit codes ICH as 'haemorrhagic stroke' and distinguishes it from HTI or ischaemic stroke. The audit does not include patients with subarachnoid haemorrhage.

I liaised with stroke audit personnel weekly to ascertain cases from all three hospitals. Every six months during 1st June 2010-31st May 2011 I liaised with the audit personnel to cross-check their known cases of ICH with cases that I had identified.

2.4.2.4 Centralised coding of hospital discharge data

In NHS Lothian, every episode of hospital care is coded using the tenth revision of the International Classification of Diseases (ICD-10) to categorise the patient's main diagnosis and comorbidities (up to six subsidiary diagnoses). This information is held

by the Information Services Division (ISD). In September 2011, after acquiring the necessary regulatory approvals, ISD supplied a list of hospital discharges during the period 1st June 2010-31st May 2011 in which ICH was listed as any one of the six diagnoses. I used CT brain imaging and the electronic patient records system to check whether any cases ascertained by this search, which I had not already identified prospectively, were eligible.

2.5 Diagnosis of ICH

To increase the reproducibility and internal validity of the study I have based my diagnosis of ICH on imaging or pathological criteria supported by a participant's clinical history.

2.5.1 Clinical diagnosis

I defined spontaneous ICH as 'a symptomatic event (new headache, altered level of consciousness or neurological symptoms), with or without new neurological signs, referable to a focal collection of blood within the brain parenchyma (seen on brain imaging or at post-mortem examination), with signal characteristics on brain imaging or organisation of the haematoma at post-mortem examination consistent with the time of symptom onset, which was not attributable to prior trauma or HTI or an alternative explanation.'

Those with first-ever ICH did not have any previous ICHs but may have had a previous ischaemic stroke. If a case had a previous stroke of undetermined type, I reviewed their history, primary and secondary care records and imaging to determine whether the stroke may have been ischaemic or haemorrhagic. If the type of stroke remained unclear, I included the case as a 'first-ever ICH' but recorded a history of prior stroke of undetermined type in the database.

2.5.2 Imaging diagnosis

Acute ICH is visible on CT as a hyperdense area in the brain parenchyma (although it may also extend into one or more extra-axial compartments), typically in the range of 50-80 Hounsfield units [Dennis et al. 1987]. Interpretation of imaging findings in the context of the clinical history helps to differentiate blood from other causes of hyperdensity on CT, most commonly calcification (Figure 6 on page 77). Although

fresh blood will appear hyperdense, as haemoglobin breaks down, the attenuation of blood declines so that within two weeks a haematoma may appear isodense with brain tissue. Therefore a diagnosis of ICH may be missed if CT imaging is delayed. In those who present late, MRI using haem-sensitive GRE sequences will show previous ICH as a low signal ring indefinitely [Wardlaw & Farrall 2004].

2.5.3 Pathological diagnosis

If a biopsy or post-mortem is done soon after an ICH, ICH is macroscopically visible at post-mortem examination as extravasated blood. It may be large enough to disrupt the architecture of adjacent structures and cause rupture into the ventricles. If an ICH is not fatal, macrophages clear the blood over months leaving a cavity, which may be impossible to distinguish from a prior infarct [Love 2011].

2.5.4 Certainty

A diagnosis of ICH cannot always be established with certainty on imaging. It may be difficult to determine whether a haemorrhage has any parenchymal component or is purely extra-axial or to distinguish ICH from HTI [Lovelock et al. 2009] (Figure 7 on page 78). If there is a history of trauma, it is necessary to differentiate clinically whether the trauma preceded the haemorrhage or vice versa (Figure 8 on page 79).

2.5.4.1 Haemorrhagic transformation of infarction vs. primary ICH

Two types of HTI may occur: petechial haemorrhage without mass effect or parenchymal haemorrhage, manifesting as a blood clot with mass effect [Larrue et al. 1997]. It is the latter which can mimic primary ICH. It has previously been estimated that 7% of primary ICH may be major HTI [Mead et al. 2002].

Certain clinical factors may lead a clinician to consider a diagnosis of HTI ahead of ICH, such as a step-wise clinical deterioration following an initial diagnosis of ischaemic stroke or a delay in the time of onset of symptoms to scan since 9% cases of HTI may occur in the first week following an ischaemic stroke [Paciaroni et al. 2008]. Although prior use of antithrombotic medication is a risk factor for both HTI and ICH, use of these medications in combination with suggestive radiological features should lead to consideration of HTI. There are no validated radiological features of HTI but some features are suggestive including an occluded cerebral artery visible on CT imaging supplying the territory of the ICH or a patchy appearance to the haematoma rather than

the haematoma being uniformly white [Mead et al. 2002]. In addition the area of hypodensity around a haematoma may reach the cortex and be wedge-shaped, indicative of an arterial blockage affecting a vascular territory (Figure 7 on page 78).

2.5.4.2 Traumatic ICH

When a patient presents with ICH it may difficult to disentangle the sequence of events leading to presentation and distinguish ICH resulting from head trauma from spontaneous ICH which led to a fall and subsequent injury (Figure 8 on page 79).

Certain clinical and radiological factors may aid differentiation:

(i) **Clinical history** – A good history from either the patient or a witness is essential to identify whether there were any symptoms of stroke prior to trauma.

(i) **Examination findings** – The patient may have signs of head trauma such as bruising or a scalp haematoma implying significant head trauma.

(ii) **Radiology findings** – Despite an absence of validated signs of traumatic ICH, some features on non-contrast CT imaging would be indicative. There may be multiple foci of blood in different brain areas, contusions and blood may be seen in both parenchymal and extra-axial compartments. Imaging may show signs of 'coup-contrecoup' injury where at the site of head trauma (coup site) a skull fracture or extra-axial blood is seen. Injury on the opposite side of the brain (contrecoup) results from the brain on the opposite side initially being pulled away from the dura but striking the dura once again on recoil. Although haemorrhage can occur at both the coup and contrecoup sites, it is thought to be more common at the contrecoup site [Gean 2004].

However, the examination and radiology findings outlined in (ii) and (iii) may occur with both spontaneous ICH (which results in a fall) and traumatic ICH so the clinical history remains essential to differentiate them. Review of brain imaging is valuable to assess if the distribution of blood is consistent with both spontaneous and traumatic ICH.

2.5.5 Neuroradiology review

To minimise these uncertainties, at a weekly stroke radiology meeting I presented the history and examination findings after which all available diagnostic brain imaging was reviewed by a neuroradiologist with a special interest in stroke imaging. The meeting comprised an expert panel of at least one neuroradiologist in addition to at least one of

each of the following: an experienced consultant stroke physician, a neurologist and a stroke fellow. At most meetings more than one member of each category was present. Where there was uncertainty, the panel reached a consensus decision through discussion, and recommended further imaging if appropriate. If additional information became available subsequently, I presented the case again and revised the diagnosis if necessary. Any participant found to have an incorrect diagnosis of ICH was excluded.

2.5.6 Suspected ICH

Since ICH is one cause of sudden death, those affected may die before reaching hospital or before the diagnosis can be confirmed by imaging. By reviewing cases that died before or soon after reaching hospital, I aimed to identify patients who might have had ICH as well as those who definitely did. A case met the criteria for a suspected ICH if the ICH was unconfirmed by imaging or pathological means and the person met both of the criteria listed below:

(i) **The clinical history was suggestive of a stroke**. Features suggestive of a stroke were either the development of headache, which is common at the onset of ICH [Tatu et al. 2000] or focal neurological symptoms. Both of these may have been of new onset or represented marked worsening of prior symptoms. I also reviewed records for any factors in their medical history which might make an ICH more likely, such as a previous ICH, haematological disorders, use of anticoagulant or antiplatelet medications or alcohol misuse.

(ii) **The examination findings were suggestive of a stroke** – for example, a new focal neurological deficit on examination, signs of raised intracranial pressure (pupillary asymmetry, hypertension with associated bradycardia), or a progressive reduction in conscious level.

2.6 Onset of ICH

When a patient develops symptoms of ICH they typically present to hospital where a diagnosis of ICH may be confirmed using radiological or pathological methods. However, patients may die before a diagnosis can be reached or conversely, if symptoms are mild, their presentation and subsequent investigations may be delayed. I sought to

identify patients at an early and uniform point in the course of their ICH (inception point, [Sackett, Haynes, & Tugwell 1991]) which I have defined below.

2.6.1 Date of ICH

For the date of the ICH, I used the date on which symptoms attributable to the ICH started. I determined this from the clinical history obtained from the patient or the person who witnessed the stroke or found them. This date was the inception point for participants entering the study and prospective follow up began from this point onwards. I did not use the date of admission to hospital or scan date as the inception point, since neither of these dates applied to all participants. If a patient presented with a recurrent ICH, the date of the ICH remained as the date of onset of symptoms attributable to the ICH which had led to their notification although I recorded the date(s) of their prior ICH(s).

2.6.2 Timing & onset of ICH

I recorded the nature of onset of the ICH according to whether the person was awake at the onset of symptoms, awoke from sleep with symptoms or whether the time of onset was uncertain but the person was known to have last been well at a certain time. If the person (or their relative) knew the time when symptoms relating to the ICH began (or were first noted), this was documented. If an exact time was not known but the person was last seen well in the morning the time of onset was recorded as 0800hrs, in the afternoon it was recorded as 1200hrs and in the evening it was recorded as 2200hrs. To obtain a best estimate of the time of onset of symptoms I also compared information from participants and their families with records from the Scottish Ambulance Service and GP notes (where a patient's GP had been contacted prior to the patient reaching hospital).

2.6.3 Incidental ICH

Exceptionally, an ICH may be found incidentally or as part of investigations for other conditions such as cognitive impairment or hyponatraemia (Figure 9 on page 80). In such cases it was often not possible to determine a time or nature of onset. In these cases, the date of the ICH was the date of hospital admission (or the date the person was first seen in an outpatient clinic) which precipitated imaging.

2.6.4 Suspected ICH

If a patient died of a suspected ICH before the diagnosis could be confirmed, the date of the ICH was the date of onset of symptoms which were thought to be attributable to a suspected ICH or if this was unknown, the date the person was last seen well.

2.7 Baseline data collection

I obtained a history from each patient and where this was not possible, I obtained collateral information from relatives or from both primary care records and hospital electronic and paper records. I collected clinical, radiological and pathological information (where applicable) on a proforma; (Appendix).

2.7.1 History

Clinical variables included demographic data (date of birth, address and GP), sources of case notification, past medical history, with particular reference to any past history of ICH, ischaemic stroke, acute coronary syndrome and vascular risk factors, history of the current ICH, current & recent medications (including any exposure to antithrombotic medications or recent intravenous thrombolysis), a family history of ICH or dementia and social history. I assessed a participant's premorbid level of functioning using the modified Rankin Scale [van Swieten et al. 1988] having successfully completed the modified Rankin Scale training programme (http://www.rankinscale.org/links.shtml).

If the participant was dead at presentation, I recorded the date and time of death, the documented cause of death on their death certificate and whether a post-mortem examination was undertaken. I collected post-mortem reports from the Pathology Department, University of Edinburgh to obtain information on a participant's presenting symptoms, salient past medical history and the features of their ICH.

2.7.2 Examination findings

I recorded the following examination findings on admission: systolic and diastolic blood pressure, Glasgow Coma Scale (GCS) and severity of impairment as measured by whether the participant had the ability to talk, was orientated to time (morning or afternoon), place and person, could lift both arms from bed and was able to walk unaided [Counsell et al. 2002].

2.7.3 Investigations

If blood samples had been taken on the day of admission, I recorded their results. I collected the following radiological variables on review of the diagnostic imaging: the location of the ICH, the presence of extension of the ICH from the parenchyma into the ventricles or subarachnoid or subdural compartments.

2.8 Data collection during follow-up

2.8.1 Survivors of ICH

I followed up each survivor of ICH on an annual basis using three methods:

(i) **NHS Lothian's electronic patient records system (TRAK)**: This records if a patient is deceased and any attendances to a hospital within NHS Lothian.

(ii) **Scottish Care Information Store (SciStore)**: This is a database developed by NHS Scotland, accessible by GPs and secondary care doctors in the Lothian health board, which stores clinic letters, discharge summaries, test results (both blood and imaging) and if applicable, a participant's date of death.

(iii) **GP annual questionnaires**: A one page annual GP questionnaire requests confirmation of a participant's address and that they are still alive, their most recent GP electronic summary of comorbidities and current medications and information regarding any new diagnoses of vaso-occlusive events [stroke, transient ischaemic attack (TIA), acute coronary syndrome, deep vein thrombosis (DVT) or pulmonary embolism (PE)], haemorrhagic events (intracerebral haemorrhage or other intracranial haemorrhage, gastrointestinal haemorrhage or other extracranial haemorrhage), dementia or hypertension. The GP is asked to assess the patient's level of disability according to the modified Rankin scale. If I did not receive a response I sent a repeat questionnaire after one month before telephoning the practice if a response was still outstanding.

(iv) I also gave participants the opportunity to participate in a research study on ICH, Lothian Study of INtracerebral Haemorrhage, Pathology, Imaging and Neurological Outcome (LINCHPIN). I followed up those who gave consent to the research study in person six months after their ICH, typically with a phone

call. However, if participants wished they could attend a specialist ICH clinic which was an opportunity for any ongoing issues to be managed. In addition, I provided all participants with our contact details, giving them the opportunity to contact me if they had any questions.

2.8.2 Deaths

When a participant dies or if an ICH is diagnosed at either a fiscal or hospital post-mortem examination, I obtained their entire GP records from Practitioner Services. Practitioner Services is a division of NHS National Services Scotland. It manages the transfer of medical records between GP practices and assists practitioners in maintaining accurate patient registers. After death, a GP practice sends paper copies of a participant's primary care record to Practitioner Services. I also sought copies of their hospital notes, a copy of their death certificate from the General Register Office for Scotland and if applicable, a copy of the post-mortem examination report.

2.9 Data management

The study team designed a relational database for the purposes of data entry, analysis, and effective follow-up (Figure 10 on page 81). The password protected database was built using Microsoft SQL Server 2000 with identifiable information held securely on an NHS server and only anonymised data held on a University of Edinburgh server. I entered data from the data collection sheet and kept these sheets in files within a locked cabinet held in a locked office within the Division of Clinical Neurosciences, Western General Hospital. I restricted access to the database to the core members of the study team with each member being given appropriate rights for their role. Certain range checks were programmed into the database to minimise errors; for example – it was not possible to enter a participant with an age of under 16 years on the date of their index ICH. I linked the database to a spreadsheet containing all residential postcode sectors served by the NHS Lothian Health Board so that any potential participant who was not resident in one of these sectors could not be entered into the database either.

When I had completed our first year of case ascertainment, I checked the entire data set for missing data and inconsistencies. I performed various consistency checks including:

- ensuring a date of death was inserted if the patient was deceased;

- the date of the ICH was on the same day as or preceded the date of death;
- the date of the ICH was on the same day as or preceded the date of admission;
- the imaging features of each ICH visible on diagnostic imaging were recorded if the number of bleeds was recorded as 'multiple';
- the fields indicating whether a patient was on anticoagulant or antiplatelet medications at the time of their ICH were concordant with their medications list;
- their list of comorbidities (coded using the ICD-10 classification) matched the fields indicating if they had a past history of ischaemic stroke, TIA, DVT, PE, atrial fibrillation, diabetes mellitus or cardiovascular disease;
- the verbal component of the GCS on admission was five if the participant was able to talk and orientated; and
- if an International Normalised Ratio (INR) was elevated there was an explanation; typically anticoagulant use or liver disease.

I also performed various range checks including:

- Systolic and diastolic blood pressure on admission; and
- Full blood count, INR and renal function on admission.

I also checked the completeness of all variables included in analyses including the following:

- Demographic variables: age, gender,
- Clinical variables: first-ever vs. recurrent ICH, hypertension, diabetes, dementia, smoking, alcohol use, antithrombotic use, GCS; and
- Investigation variables: INR, ICH location, single vs. multiple ICH, ICH volume, presence of intraventricular extension, presence of subarachnoid extension, presence of subdural extension.

2.10 Evaluation of sources of case ascertainment

I ascertained 166 ICH cases during 1st June 2010-31st May 2011. Figure 11 on page 82 shows that 140 cases out of 166 identified (84%) were ascertained by more than one source. Where a case was identified by one source alone, review of CT brain scans was the most common source of ascertainment. Although identification of every ICH case

by at least two sources of notification would be ideal, >80% overlap between sources is good.

Figure 12 on page 83 shows the degree of overlap between sources of case ascertainment.

The majority of cases were ascertained by review of CT imaging, liaison with our collaborative network and ISD. Although CT brain imaging was the most fruitful source of ascertainment, it was not sufficient alone to detect all cases of ICH. Six cases of ICH were detected by methods other than screening of CT brain scans. Three cases were fatal out-of-hospital deaths where the diagnosis was confirmed at post-mortem examination. In two more cases, patients had had CT imaging performed but the ICH was missed when the scan was reviewed and both these cases were detected by other sources of ascertainment. In a further case, the patient died soon after the development of symptoms and before CT brain imaging could be conducted. The diagnosis was confirmed at post-mortem examination.

2.11 Summary

- This is a community-based, prospective incidence and longitudinal cohort study of spontaneous primary ICH which uses multiple sources of case ascertainment and follow-up.
- The study includes adults aged 16 years or over at the time of their ICH, resident in the region served by the NHS Lothian Health board and diagnosed between 1st June 2010-1st June 2016.
- I confirmed the diagnosis of ICH by review of diagnostic imaging or post-mortem examination reports.
- I ascertained cases of suspected ICH in which the history was suggestive of ICH but the diagnosis of ICH was unconfirmed before or after death.

2.12 Discussion

2.12.1 Case ascertainment

This study meets almost all the criteria for an 'ideal' stroke incidence study [Sudlow & Warlow 1997;Feigin & Carter 2004].

(i) It has a prospective population based design using multiple overlapping sources for both case and outcome ascertainment.

(ii) It uses a standard generalisable definition of ICH and has an inception cohort assembled at an early point during the course of the illness.

(iii) The population is well defined and stable, allowing at least 100 000 person-years of observation.

(iv) I confirm the diagnosis of ICH by imaging or post-mortem examination in ≥80% cases.

(v) I distinguish first-ever in a lifetime ICH from recurrent ICH.

(vi) The denominator for incidence calculations uses reliable current data.

(vii) I present incidence data separately for each sex and age band (Chapter 5).

A recent systematic review of incidence studies of ICH categorised studies which had used multiple case finding methods, including regional hospitals, family doctors and death certificates as excellent [van Asch et al. 2010]. In addition to these methods our study uses surveillance of Procurator Fiscal records and electronic patient records to ascertain those who died of an ICH confirmed at pathological examination before reaching hospital and those who died of a suspected ICH before investigations could confirm a diagnosis. In a disease such as ICH with a high early case fatality, this is essential to begin to identify the unmeasured burden of the disease (the iceberg concept, [Bhopal 2002]) since cases of ICH who die before reaching hospital are likely to represent more severe cases of the disease.

2.12.1.1 Limitations of case ascertainment

However, despite the study fulfilling many of the criteria for an 'ideal' stroke incidence study potentially eligible patients may have been missed if:

(i) MRI was the imaging modality used to diagnose an ICH rather than CT, since I did not routinely screen all MRI brain scans. Although CT remains the most frequently used imaging modality if an acute ICH is suspected [Muir & Santosh

2005], MRI may be used occasionally, particularly if the presentation is atypical or delayed [Muir et al. 2006].

(ii) A resident served by NHS Lothian had an ICH whilst in a different region.

(iii) Neither radiographic nor pathological examination was performed on a person who died suddenly or rapidly in the community after an illness suggestive of stroke.

I mitigated the effects of (i) and (ii) by using overlapping sources of case ascertainment. In particular our extensive collaborative network did occasionally notify me of patients found to have an ICH on MRI or if a patient returned to NHS Lothian having had an ICH elsewhere and accessed in-patient or outpatient stroke services. I considered routine screening of MRI brain scans, but since MRI is used only very occasionally in the diagnosis of acute stroke in the Lothian region with two out of three hospitals having on-site access to MRI within working hours, I suspected that the potential yield would be low.

I mitigated the effects of (iii) by screening the electronic patient records system to identify people who were brought into hospital dead or people who died soon after admission to hospital with a history suspicious of a stroke but before a diagnosis could be confirmed. I did not ascertain all potential cases who had died suddenly in the community without admission to hospital or post-mortem examination to confirm a diagnosis and whose cause of death on their death certificate was listed as a 'stroke.'

In future, the study design could be improved by use of post-mortem imaging techniques to confirm a diagnosis of ICH in those who die rapidly soon after the onset of symptoms and routine screening of all MRI brain scans and screening of all death certificates to ascertain cases where 'stroke' has been listed as one of the primary causes of death.

2.12.1.2 Evaluation of completeness of case ascertainment

Evaluation of the completeness of case ascertainment is challenging. Both indirect statistical methods such as capture-recapture and direct assessment have been proposed. Inherent in capture-recapture methods are assumptions that sources of ascertainment are independent and that all individuals have the same probability of being captured [Tilling 2001]. In our population neither of these assumptions holds true since some sources of ascertainment such as the collaborative network and neuroradiology meetings

are inter-dependent and ICHs which either cause sudden death or less severe symptoms may be missed. Although adjustment can be made for these assumptions using covariates, in our population this would lead to small numbers in certain categories and imprecise estimates.

An example of direct assessment is accessing all primary care records in a defined population to quantify the number of cases that had presented to primary care with symptoms of stroke but had not been notified to the study [Coull et al. 2004]. The size of the population (~700,000 adults in Lothian) is likely to preclude screening of all adults' primary care records but in the future it may be possible to use information held by ISD to produce a sampling frame of all adults in NHS Lothian and select a random age-stratified sample of adults whose records could be screened for previously unidentified cases of ICH.

2.12.2 Methods of follow up

I used several methods of follow up which are designed to ensure that I have a minimum of survival data at one year for each patient, and for the majority, have a record of other key outcomes including disability, reported by the participant's current GP according to the modified Rankin scale. Although the reliability of using a proxy report of the modified Rankin Scale in comparison to patient report has recently been shown to be limited [McArthur et al. 2012], the patient's health status should be well known to their GP and the modified Rankin Scale is the most commonly used functional outcome measure in studies of stroke [Quinn et al. 2009] . Face-to-face interview with ICH survivors would not have been possible and other methods such as extrapolation from records is not valid [Quinn et al. 2008]. Structured telephone interview with participants would be an alternative since it has been shown to have good validity in comparison to face-to-face interview in patients with subarachnoid haemorrhage [Janssen et al. 2010].

Figure 6 Unilateral basal ganglia calcification

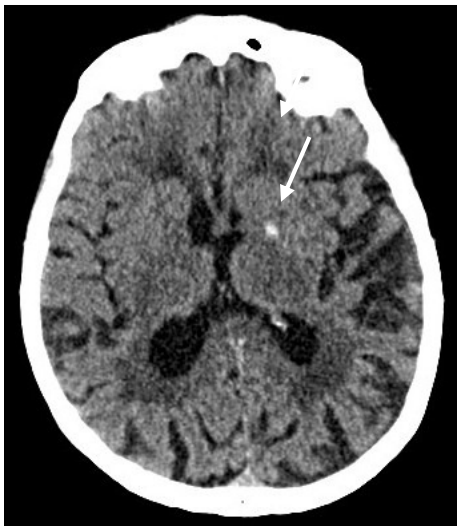


Image A

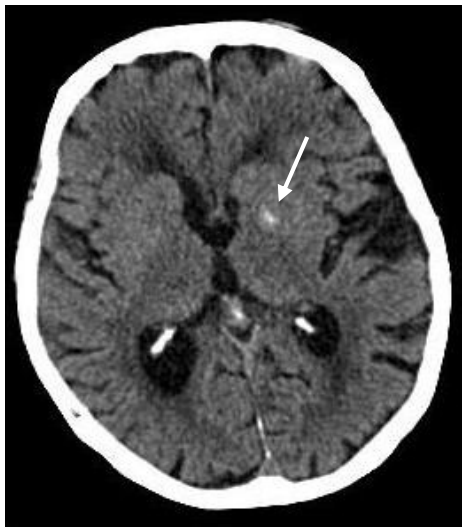
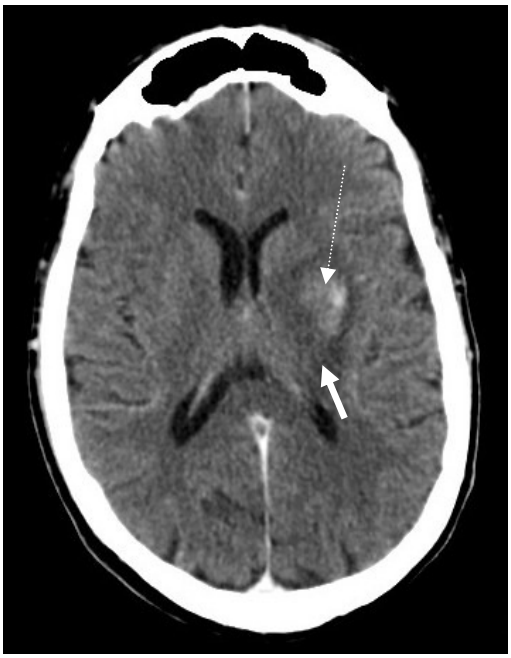


Image B

A 96 year old lady was admitted to hospital with a two day history of urinary frequency. She was noted to be disorientated to time and place which was unusual for her. The medical team requested a CT brain scan to look for an intracranial cause of her confusion (image A). A high density area was noted in left lentiform nucleus (arrow) which was thought most likely to represent a small ICH.

Two months later the lady was readmitted with delirium and she was treated for a suspected lower respiratory tract infection. However when she failed to respond to treatment, she had a repeat CT brain scan (image B). The repeat CT scan shows that the high signal lesion is unchanged in appearance and therefore in retrospect, the lesion was most likely to be calcification.

Figure 7 Haemorrhagic Transformation of an Infarct



A 54 year old gentleman was found at the bottom of his stairs by his neighbour. He was able to recall falling and described weakness of his right arm and leg which occurred before the fall. He was certain that the weakness had actually started three days previously but he had not sought medical help. He had a past medical history of ischaemic heart disease with coronary artery bypass grafting, non-insulin dependent diabetes mellitus and atrial fibrillation for which he was taking warfarin.

On examination he had weakness affecting his right arm and leg with no facial droop, and no higher cortical symptoms. His INR was 7.9.

The axial CT image (above) shows an elliptical area of low attenuation affecting the left basal ganglia (short solid arrow) within which there is some high signal consistent with haemorrhage (long dotted arrow).

I felt that the low attenuation lesion was most likely to be a striato-capsular infarct. The area of haemorrhage within it could be explained by the prior history of warfarin use. The presumed HTI was lower attenuating than what would be expected for a three day old primary ICH.

Figure 8 Traumatic ICH

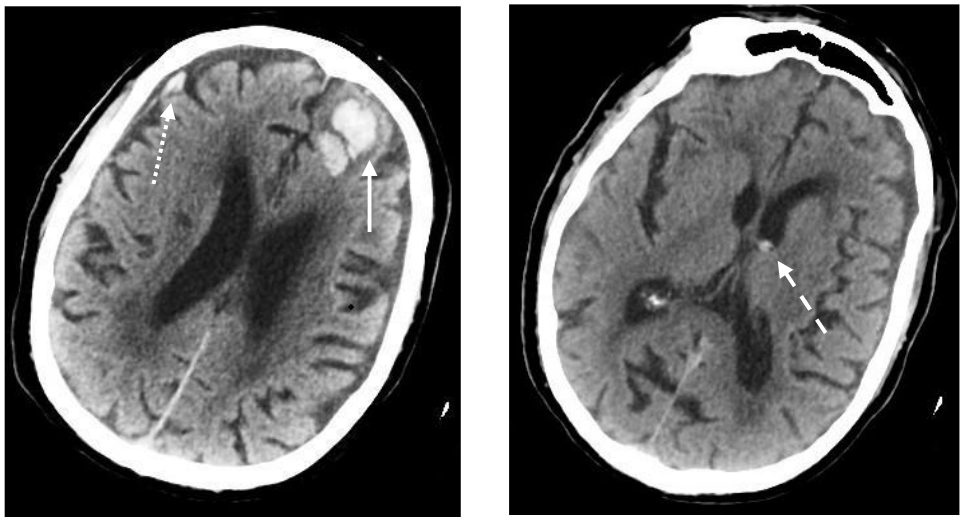
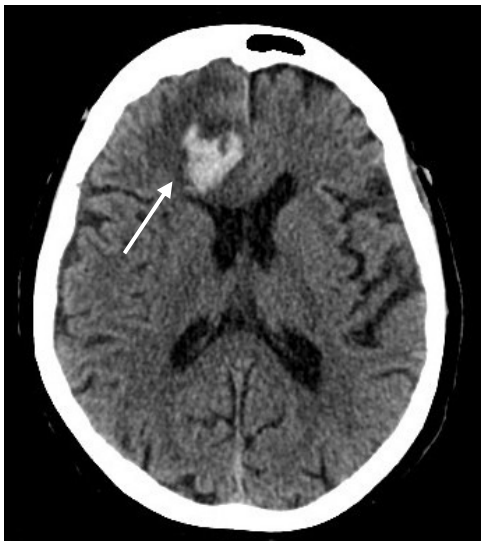


Image A **Image B**

A 90 year old gentleman with a past medical history of Type 2 diabetes mellitus and hypertension was at home with his wife when he stood up and fell. His wife heard a thud when he fell but did not witness the fall. She noted that he had not been complaining of any symptoms earlier in the day. Since the fall he had been unable to communicate. On examination he was agitated and disorientated. A limited neurological examination did not reveal any focal deficit.

He underwent a CT brain scan and two axial slices are shown above. Image A shows a left frontal haematoma (solid arrow) with additional blood in the right subdural space (dotted arrow). Image B shows a small subependymal ICH (dashed arrow). In view of the multiple areas of intracranial haemorrhage in locations typical for traumatic ICH, I felt that the left frontal haematoma may have been traumatic in origin.

Figure 9 Atypical presentation of spontaneous primary ICH



A 93 year old lady was admitted to hospital with a two day history of vomiting and diarrhoea. She had had a recent admission with constipation and had been discharged one week previously. She was alert and fully orientated on examination although a full neurological examination was not performed. Her blood tests were unremarkable except for her sodium level which was 107mmol/litre. The initial diagnosis was of probable intestinal obstruction.

An abdominal x-ray was done which showed dilated small bowel loops although a subsequent CT abdomen did not show evidence of obstruction or perforation. She was managed conservatively and began to improve with her sodium also increasing to126 mmol/litre over the following couple of weeks.

However, on day 16 of her admission she fell on the ward. Over the preceding three days she had been complaining of dizziness, the nature of which was not clear. There was no loss of consciousness or head injury. A subsequent electrocardiogram (ECG) showed trifascicular block. The clinical team also requested a CT brain scan because of ongoing (although improved) hyponatraemia.

The axial CT image (above) shows a right frontal lobar ICH (arrow) with extension into the subdural space (not shown) and surrounding hypodensity which was thought to be at least several days old. The ICH may have been a contributory factor to her both her dizziness and hyponatraemia. Her hyponatraemia improved and she was discharged to a rehabilitation ward.

Figure 10 Entity-relationship diagram for the database

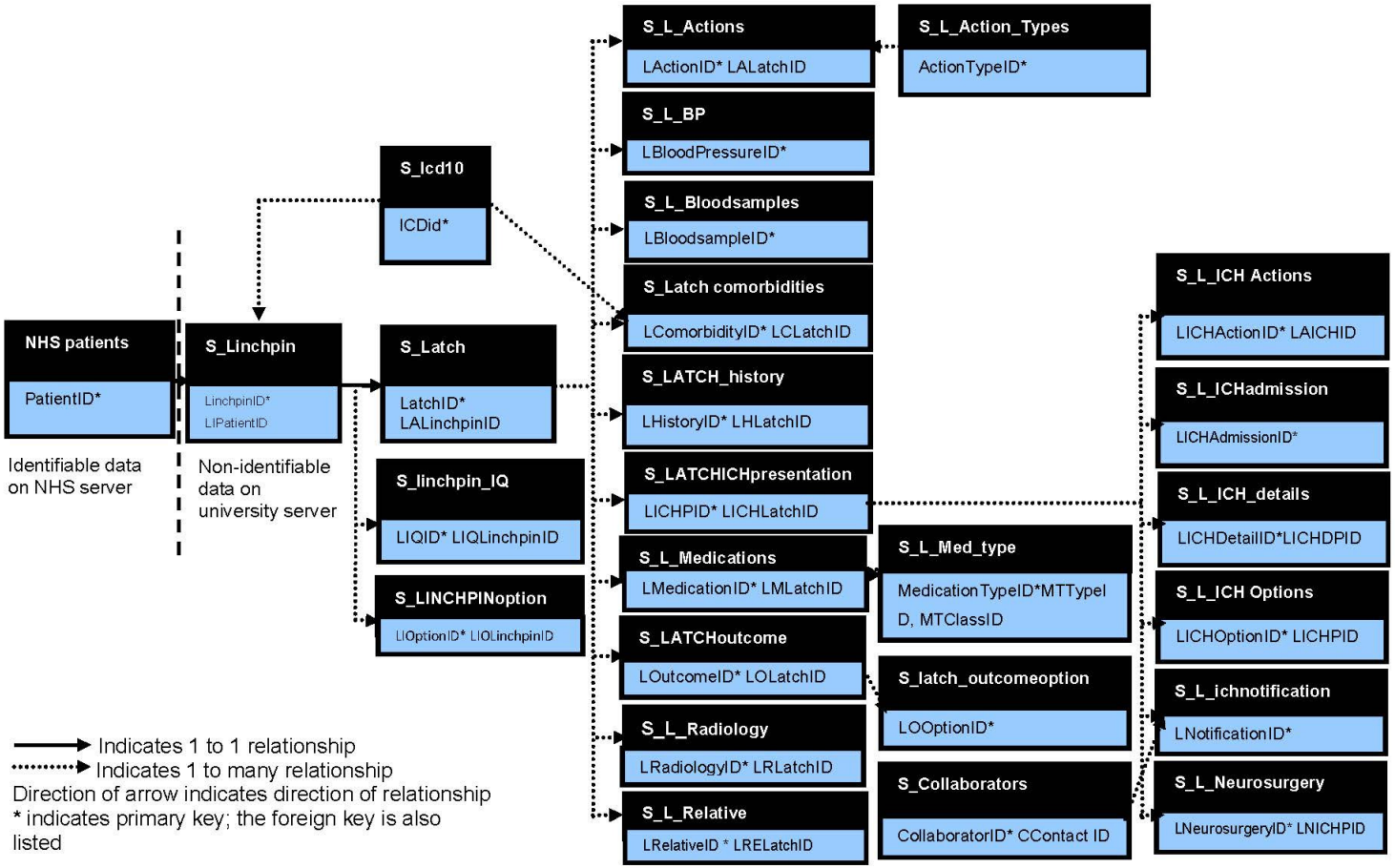


Figure 11 Histogram showing the frequency of cases of ICH with one or more notifications

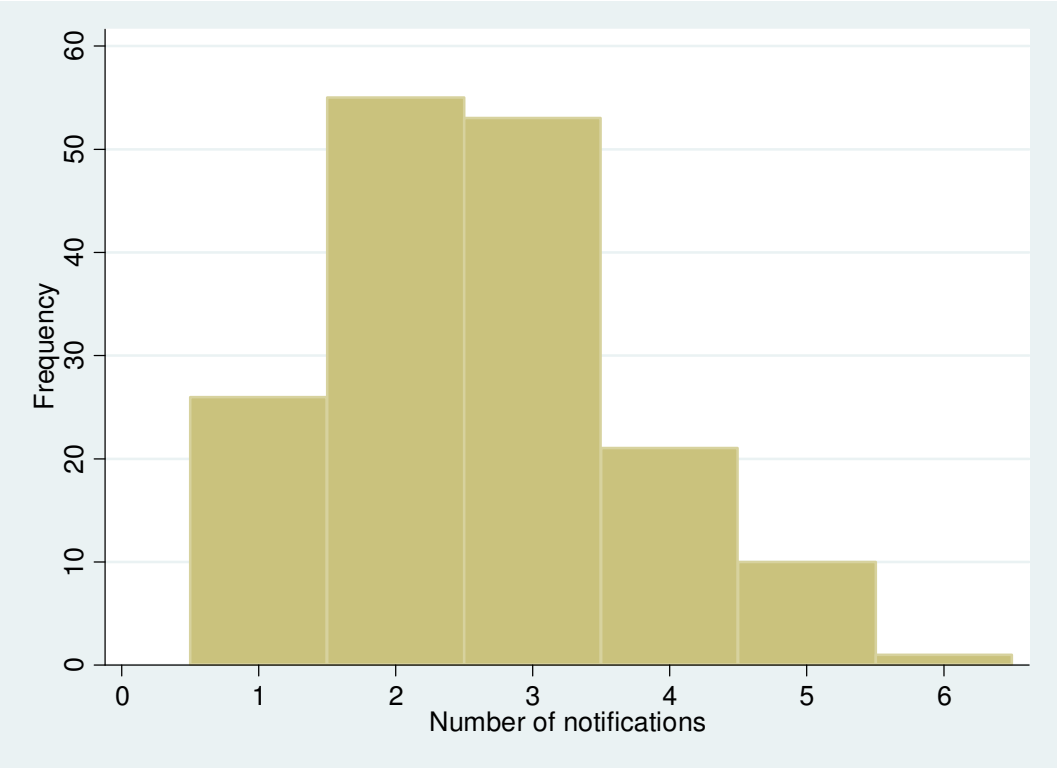
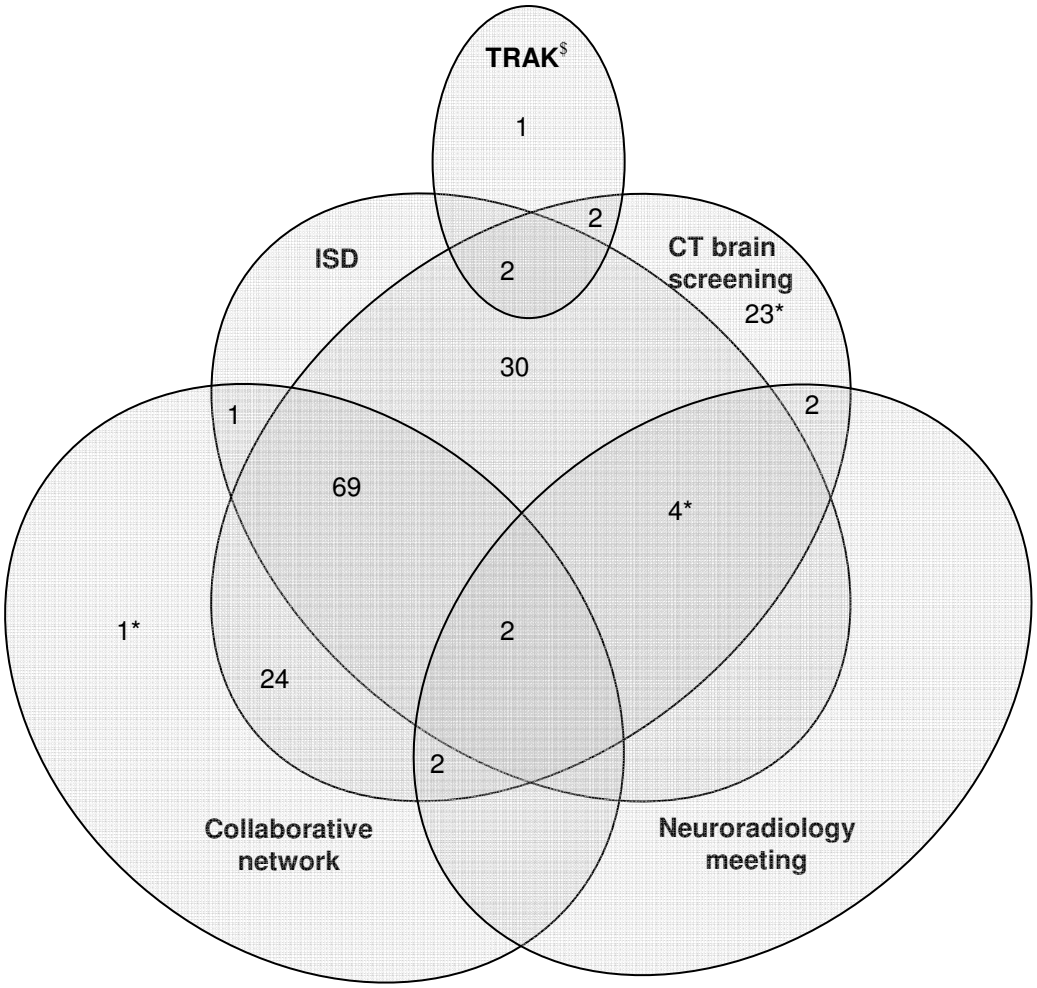


Figure 12 Overlap between sources of case ascertainment (n=166)
[$]TRAK is the electronic patient records system
* indicates one case in each group also notified by the Office of the Procurator Fiscal
Three cases notified by the Office of the Procurator Fiscal alone (these are not shown)



Chapter 3 Methods of a cross-sectional study of MRI brain imaging features in lobar vs. non-lobar intracerebral haemorrhage

Chapter contents

3 Methods of a cross-sectional study of MRI brain imaging features in lobar vs. non-lobar intracerebral haemorrhage

3.1 Introduction

Ideally, studies of markers of small vessel disease should be population-based using participants recruited at a uniform inception point, standard definitions of variables, pre-specified MRI sequence parameters and validated rating scales which differentiate markers of small vessel disease from their mimics and enable calculation of inter-observer agreement [Cordonnier, Wardlaw, & Al-Shahi Salman 2007;Wardlaw et al. 2011]. In the context of ICH, the aim of the study would be to describe the distribution and severity of small vessel disease in patients with ICH with the aim of determining whether the nature of small vessel disease differs according to ICH location. This might provide clues to the underlying contributing causes of lobar and non-lobar ICH.

Neuroimaging correlates of small vessel disease are heterogeneous, including BMBs, white matter hyperintensities of presumed vascular origin (WMH) and enlarged perivascular spaces (EPVS) [Pantoni 2010;Doubal et al. 2010].

BMBs seen as small hypointense foci on haem-sensitive MRI sequences [Cordonnier, Wardlaw, & Al-Shahi Salman 2007], are thought to be an indicator of CAA if in a strictly lobar distribution [Greenberg et al. 2009]. WMH appear as regions of patchy hyperintense signal on T2-weighted and fluid- attenuated inversion recovery (FLAIR) sequences on brain MRI in the deep and periventricular white matter, basal ganglia and brainstem [Wardlaw, Ferguson, & Graham 2004]. ICH has been associated with higher WMH volumes in comparison to other stroke subtypes [Rost et al. 2010]. EPVS are CSF filled cavities that surround small penetrating arterioles and can be visualised on brain MRI. They are commonly seen in patients with ICH [Charidimou et al. 2013c].

In the following chapter, I will describe the development of an MRI rating proforma which was used to rate BMBs, WMH and EPVS on training scans and LINCHPIN study scans and quantify inter-observer and intra-observer agreement for markers of small vessel disease and their mimics.

3.2 Methods

3.2.1 Development of an MRI rating proforma

Since a validated automated method for measuring features of small vessel disease does not exist [Wardlaw et al. 2011], I developed a proforma to assess both scans used for training in the rating of small vessel disease and those done as part of the LINCHPIN study. The aim was to rate both BMBs and other neuroimaging correlates of small vessel disease. The imaging definition for each variable and its method of rating is described in the following section.

3.2.1.1 Acute ischaemic change

I defined acute ischaemic change as a lesion(s) suggestive of a recent cerebral infarct, which was hyperintense on diffusion-weighted imaging, hypointense on the corresponding apparent diffusion coefficient sequence map and normal to hyperintense on T2-weighted and FLAIR sequences. The lesion was typically wedge-shaped or elliptical (if lacunar) and occupied a vascular territory. The side of the ischaemia referred to the side of the cerebrum affected. If the lesion was in the midline, then this was indicated.

3.2.1.2 Acute parenchymal haemorrhage

Acute parenchymal haemorrhage relates to a lesion(s) suggestive of a 'recent' haemorrhage. Since signal changes vary on T1-weighted and T2-weighted MRI sequences as haemoglobin evolves from oxy-haemoglobin (hyperacute stage, hours after the event) to deoxy-haemoglobin (acute stage, one-two days) through to met-haemoglobin (subacute stage, two days to several weeks) [Weissleder et al. 2011], 'recent' haemorrhage referred to a lesion possessing signal changes which indicated that it was either hyperacute, acute or subacute and therefore was likely to have occurred within four weeks of the scan. If more than one acute haemorrhage was visible, I rated the hemisphere and anatomical location of each haemorrhage listing the largest haemorrhage first. I differentiated primary intracerebral haemorrhage from HTI by referring to other MRI sequences; for example by looking for the presence of a wider area of infarction surrounding the haemorrhage using DWI and apparent diffusion coefficient maps [Lovelock et al. 2009].

3.2.1.3 Further characterisation of the haemorrhage

I characterised the location of the ICH as lobar or non-lobar. I used a standard definition of intracerebral haemorrhage location, as described in Chapter 1.

3.2.1.4 Chronic infarcts and haemorrhages

I rated the hemisphere and anatomical location of any chronic infarcts or haemorrhages. Since chronic infarcts may be confused with white matter lesions, I aimed to differentiate these entities using a scale validated in previous cohorts of stroke patients [Wardlaw et al. 1998;Wardlaw et al. 2007].

Chronic infarcts were defined as cortical, lacunar or infratentorial lesions which were hyperintense on T2-weighted and FLAIR sequences and hypointense on T1-weighted sequences. I defined lacunar infarcts as lesions >three millimetres and ≤ 20 millimetres in maximum diameter [Potter et al. 2010] in the basal ganglia, subcortical white matter or brainstem and looked for evidence of cavitation and surrounding gliosis suggestive of chronicity.

Chronic haemorrhages were defined as lesions which were hypointense on T1-weighted and gradient-echo sequences and hyperintense accompanied by a dark rim on T2-weighted sequences [Weissleder et al. 2011].

3.2.1.5 Basal ganglia mineral deposits

Pathological studies reveal that insoluble minerals which accumulate within the basal ganglia comprise different elements including iron due to haemosiderin deposition (most commonly), calcium, zinc and magnesium [Casanova & Araque 2003]. These deposits are visible as typically symmetrical hypointense regions on gradient-echo MRI sequence (Figure 13 on page 99) and may mimic BMBs [Samarasekera, Potter, & Al-Shahi Salman 2011]. Calcium deposits can be differentiated from iron deposits since the former appears hypointense on T1-weighted sequences in comparison to iron which is undetectable. There is no existing validated scale to rate mineral deposits. I used a simple visual scale which graded deposits zero to three, which although unvalidated, has been used in previous studies [Penke et al. 2012].

3.2.1.6 White matter hyperintensities of presumed vascular origin

I defined WMH as parenchymal lesions which were hyperintense on T2-weighted and FLAIR sequences (although not as hyperintense as CSF on T2 weighted sequences) [Fazekas et al. 1987]. WMH were categorised as being periventricular (where they were contiguous with the lateral ventricles) or deep (where they were located in the white matter tracts) [Payne et al. 2002]. Deep hyperintensities became continuous with periventricular hyperintensities in those with a preponderance of white matter lesions. I rated WMH using the Fazekas scale [Fazekas et al. 1987](Figure 14 on page 100). Although there are several different scales available for rating of WMH the Fazekas scale has been widely used in previous studies of stroke, increasing the generalisability of our findings. It also comprises ratings for both deep and periventricular WMH which may be relevant when examining the profiles of small vessel disease which underlie lobar and non-lobar ICH. Moreover, it is easy to apply and differentiates between grades of severity of WMH even at extremes unlike some of the other rating scales used [Wardlaw, Ferguson, & Graham 2004].

3.2.1.7 Atrophy

I defined atrophy as loss of grey and white matter leading to either increased prominence of the ventricles (deep atrophy), or enlargement of CSF spaces and increased prominence of cortical sulci (cortical atrophy) or both. I used a validated template developed from T2-weighted images of 79 subjects aged 65-70 years and 75-80 years who were involved in studies of normal ageing [Farrell et al. 2009]. I used a scale of zero to three, where zero=none, one=mild (5^{th}-25^{th} centile on atrophy template), two=moderate (50^{th}-75^{th} centile) and three=severe (95^{th} centile.)

3.2.1.8 Enlarged perivascular spaces

I defined EPVS, as CSF filled cavities which surround small blood vessels as they traverse from the subarachnoid space into the brain parenchyma [Braffman et al. 1988], which appear hyperintense on T2-weighted sequences and hypointense on T1 and FLAIR sequences. I used a validated scale (http://www.bric.ed.ac.uk/documents/epvs-rating-scale-user-guide.pdf) to rate the number of EPVS in the centrum semiovale, basal ganglia and hippocampal regions in each hemisphere separately and then overall (Figure 15 on page 101).

3.2.1.9 Brain microbleeds

I defined BMBs as punctuate homogeneous foci of less than 10mm in diameter seen in the brain parenchyma which appear hypointense (due to their haemosiderin content) and display 'blooming' properties on gradient-echo sequences [Greenberg et al. 2009]. Raters were aware of potential mimics of BMBs including flow voids in blood vessels, partial volume artefact from bone, intracranial shunt tips and cavernous malformations [Samarasekera, Potter, & Al-Shahi Salman 2011](Figure 16 on page 102).

Two validated scales for rating the number and distribution of BMBs have emerged in recent years: the Brain Observer Microbleed Rating Scale (BOMBS) [Cordonnier et al. 2009] and the Microbleed Anatomical Rating Scale (MARS) [Gregoire et al. 2009].

BOMBS records certain and uncertain BMBs which are either less than five millimetres or five-10mm in size in the following areas: cortex/grey-white matter junction, subcortical white matter, basal ganglia grey matter, internal and external capsule, thalamus, brainstem and cerebellum. MARS similarly rates certain and uncertain BMBs in different anatomical locations but for lobar BMBs, assessors can ascribe their location to a lobe of the brain. It also distinguishes BMBs in the insula and corpus callosum as distinct from other areas and asks raters to provide totals of certain and uncertain BMBs in lobar, deep and infratentorial regions. Differentiation of certain BMBs from those which are uncertain improves inter-rater reliability [Cordonnier et al. 2009].

I modified BOMBS; removing the microbleed size categories (since there was 93% inter-observer agreement on size in the sample used to validate BOMBS) [Cordonnier et al. 2009], incorporating total numbers of BMBs for lobar and non-lobar regions, and listing each lobe of the brain in the anatomical regions. The latter may be relevant when comparing the distribution of BMBs with other neuroimaging correlates of small vessel disease such as WMH. Some authors have also suggested that BMBs in CAA may be more likely to distribute in the parietal and occipital lobes of the brain [Greenberg, Finkelstein, & Schaefer 1996].

3.2.2 Selection of MRI training scans

Prior to rating the LINCHPIN MRI scans, I practised rating markers of small vessel disease using a selection of training scans.

A neuroradiologist selected 15 MRI scans from a collection previously used in the development of the Brain Observer MicroBleed Scale (BOMBS) [Cordonnier et al. 2009]. The scans selected had been the subject of inter-observer disagreement during the development of BOMBS. The scans were from a subset of patients in the Edinburgh Stroke Study; an ethically-approved prospectively-collected hospital based stroke register of consecutive patients with stroke and TIA seen between April 2002 and May 2005 [Jackson et al. 2009].

Following either an ischaemic or haemorrhagic stroke, participants underwent 1.5 Tesla MRI (GE Signal LX EchoSpeed scanner, Milwaukee, WI, USA.) The scan protocol included T1 sagittal sequences and the following axial sequences in all cases: T2-weighted (TR 6300, TE 107, matrix 256 ×256, FoV 24×18 slice thickness 5mm,slice gap 1.5mm, NEX 2 [where TR=relaxation time, TE=echo time, FoV=field of view, NEX = number of excitations]), DWI (TR 9999, TE 98.8, matrix 128×128, FoV 24×24, slice thickness 5mm, slice gap 1mm, NEX 1), FLAIR (TR 9002, TE 147, matrix 256 ×256, FoV 24×24, slice thickness 5mm, slice gap 1.5mm, NEX 1) and GRE (T2*, TR 620, TE 15, flip angle 20, FoV 24×18, matrix, 256×192, slice thickness 5mm, slice gap 1mm, NEX 2.)

3.2.3 Rating of training scans

In July 2012, the training scans were anonymised and viewed independently by both raters blind to all clinical details, the original scan report, previous ratings of BMBs during the development of BOMBS and each other's ratings. For the intra-observer study I rated all scans again six months later. Only the first set of ratings was used to quantify inter-observer agreement.

3.2.4 LINCHPIN MRI scans

Participants recruited into the LINCHPIN study (an ethically-approved interventional study of patients with spontaneous primary ICH), underwent brain MRI.

3.2.4.1 MRI inclusion criteria

Participants were eligible for brain MRI in the LINCHPIN study if they met all of the following inclusion criteria:

- They had a first-ever or recurrent spontaneous primary ICH during 1st June 2010-

31st May 2012.

- They were resident in the Lothian region at the time of their ICH.
- They (or their nearest relative) gave written informed consent to MRI.

3.2.4.2 MRI exclusion criteria

Participants were excluded if they met any of the following exclusion criteria.

- They had a secondary ICH.
- They were unable to tolerate brain MRI because they were too unwell, claustrophobic or unable to lie flat for the scan duration.
- They had a contraindication to MRI; for example – a permanent pacemaker or metal implants elsewhere in their body.
- They were too wide and were therefore unable to fit into the scanner.

3.2.4.3 MRI procedure

To minimise the travelling distance for participants, participants were able to undergo brain MRI at one of two hospitals (Western General Hospital or Royal Infirmary at Edinburgh) serving the Lothian health board. MRI was done using one of two 1.5T MRI scanners (GE Signal LX EchoSpeed scanner, Milwaukee, WI, USA and Philips Gyroscan Intera scanner, Philips Ltd, Best, The Netherlands.) The scan protocol consisted of T1 sagittal sequences and the following axial sequences: T2- weighted, FLAIR and gradient echo for all participants. The sequence parameters are provided in Table 1 on page 98. The total scan time was approximately 25 minutes.

3.2.4.4 LINCHPIN MRI ratings

I rated all brain scans independently, blinded to the participants' names and clinical characteristics using the proforma described above (Appendix). A consultant neuroradiologist with a special interest in stroke, who was also blinded to the participants' names and clinical characteristics, rated BMBs and other imaging parameters to assess inter-observer agreement.

3.2.5 Statistical analysis

I assessed inter-observer agreement for both the training scans (n=15) and LINCHPIN MRI scans (n=50).

I rated binary categorical variables (presence or absence of acute ischaemic change and acute parenchymal haemorrhage) for which I quantified inter-observer agreement using an unweighted κ [Cohen 1960]. In contrast to participants whose scans were used as training scans who may have had either an ischaemic or a haemorrhagic stroke, LINCHPIN participants were all scanned following an ICH and therefore these variables were rated for training scans only.

I used a weighted κ for ranked ordinal variables (basal ganglia mineral deposits, periventricular and deep WMH, deep and cortical atrophy, EPVS.)

For chronic infarcts and BMBs, I dichotomised the data (presence of zero vs. one or more chronic infarcts and presence of zero vs. one or more BMB respectively) and used an unweighted κ to calculate observer agreement for certain BMBs and uncertain and certain BMBs combined in lobar, deep and infratentorial areas. To obtain an overall measure of the agreement regarding the numbers of lesions noted as certain or uncertain BMBs, I calculated the total number of certain and uncertain BMBs noted by each rater for all locations (lobar, infratentorial and deep) and used an intraclass correlation coefficient (two-way random ANOVA) model [Shrout & Fliess 1979].

All analyses were performed in STATA version 11.1, except 95% CI for weighted κ which were calculated in StatsDirect (version 2.7.8).

3.3 Results

3.3.1 MRI training scans

Inter-observer agreement for the presence of acute and chronic lesions ranged from fair to substantial (Figure 17 on page 103). The commonest reason for disagreement was misclassification of chronic ischaemic and haemorrhagic lesions as acute and vice versa. Some chronic ischaemic lesions still appeared hyperintense on diffusion-weighted imaging and I labelled these as acute without reference to the corresponding apparent diffusion coefficient map, which showed that no signal drop out was present.

There was fair-to moderate agreement on ordinal variables including WMH, EPVS and basal ganglia mineralisation, although 95% CI were broad reflecting the relatively small

number of training scans. In almost all cases of disagreement, observers differed by only one grade on the respective rating scales used.

Intra-observer agreement ranged from less than chance to substantial (Figure 17 on page 103). Although intra-observer agreement was less than inter-observer agreement for certain variables, when my second set of ratings was compared with those of the neuroradiologist, inter-observer agreement was invariably greater than the intra-observer study (acute ischaemic change κ 0.17; 95% CI -0.30 to 0.55 acute haemorrhage κ 0.58; 95% CI 0.05 to 0.88; chronic infarcts and haemorrhages κ 0.59; 95% CI 0.06 to 0.85); indicating that inter-observer agreement may have improved at the expense of intra-observer agreement.

Inter-observer agreement for the presence of zero vs. ≥ one BMB ranged from moderate to almost perfect (Figure 18 on page 104). Common causes for disagreement were lesions being too pale to be sure that they were a BMB and interpretation of BMB mimics such as basal ganglia mineralisation, vascular flow voids and partial volume artefacts from the orbit or petrous temporal bone (Figure 16 on page 102). Intra-observer agreement was similar to inter-observer agreement for BMBs in all locations.

The intraclass correlation coefficient across all locations for the overall number of certain BMBs was 0.59 (95% CI 0.14-0.84) and for the total number of BMBs (certain and uncertain) was 0.87 (95% CI 0.64-0.95).

3.3.2 LINCHPIN MRI scans

The neuroradiologist rated all scans for BMBs, atrophy, WMH and chronic infarcts and a subset of 38 scans to assess inter-observer agreement for EPVS and basal ganglia mineralisation.

Inter-observer agreement for ordinal variables (basal ganglia mineralisation, WMH, EPVS, atrophy) ranged from slight to moderate (Figure 19 on page 105). Inter-observer agreement for the presence of zero vs. ≥ one BMB ranged from moderate to substantial (Figure 20 on page 106). The intraclass correlation coefficients across all locations for the overall number of certain BMBs was 0.88 (95% CI 0.79-0.93) and for the total number of BMBs (certain and uncertain) was 0.88 (95% CI 0.80-0.93).

3.4 Summary

- Inter-observer agreement when rating WMH, EPVS and atrophy on both the training scans and LINCHPIN MRI scans predominantly ranged from fair to moderate.
- Inter-observer agreement when rating the presence of zero vs. ≥ one BMB on LINCHPIN MRI scans was similar to the inter-observer agreement achieved for training scans and ranged from moderate to substantial.

3.5 Discussion

3.5.1 Selection of imaging variables

The variables which comprise the MRI rating proforma were selected to assess the presence and/or severity of neuroimaging correlates of small vessel disease in participants with ICH. Variables were either markers of small vessel disease (WMH, EPVS, atrophy, BMBs) or potential mimics of small vessel disease; for example – chronic infarcts which needed to be differentiated from other periventricular and deep WMH or basal ganglia mineralisation which is acknowledged to be a microbleed mimic [Samarasekera, Potter, & Al-Shahi Salman 2011]. Although all the variables are subject to inter-observer variation, I have sought to minimise inter-observer variation by using a standard definition for all variables, validated scales and a systematic rating proforma ensuring that all variables are considered.

3.5.2 Strengths of the study

This study fulfils the Guidelines for Reporting Reliability and Agreement studies [Kottner et al. 2011] in that I have:

(i) Explained how the sample of raters and scans was chosen;

(ii) Specified the method of rating scans, including the MRI rating proforma used and the time interval between ratings in the intra-observer study;

(iii) Specified that scans were rated independently and blinded to clinical information and the scan report (therefore minimising bias);

(iv) Stated the number of raters and scans used and the number of replicate observations conducted;

(v) Described the sample characteristics of participants who underwent MRI in the LINCHPIN study (Chapter 6);

(vi) Reported estimates of both inter-observer and intra-observer agreement.

3.5.3 Inter-observer study

On most parameters, inter-observer agreement did not improve when rating LINCHPIN MRI scans in comparison to training scans. This study was small and exploratory in nature and factors which are likely to explain these results include the small numbers of both training and LINCHPIN MRI scans and restrictions in the use of the κ statistic.

3.5.4 Selection of training scans

Although I used a small subset of scans for training purposes, the scans selected were those which were subject to inter-observer variation when rating BMBs during the development of BOMBS [Cordonnier et al. 2009]. Therefore these scans were likely to be the most complex in terms of distinguishing BMBs from other haemorrhagic lesions and mimics. The scans all contained identical sequences. All sequence parameters were consistent and known, increasing the generalisability of these findings.

To obtain a more precise estimate of inter-observer agreement I would require a larger number of scans which would reduce the width of the 95% CI around the κ estimates. I did not assess whether the use of the proforma improved inter-observer agreement by enabling a structured assessment of both correlates of small vessel disease and their mimics and it would be interesting to do so in a future study.

3.5.5 Use of the κ coefficient

The κ coefficient is the most commonly used statistic to assess agreement between two observers. Unlike measuring the overall percentage of agreement, κ takes into account the agreement between observers which could have occurred 'purely by chance.' κ is a measure of agreement beyond that expected by pure chance, so called 'true' agreement [Cohen 1960].

3.5.5.1 Assumptions of κ

The study design fulfilled the assumptions inherent when using κ – the subjects were independent (so each subject only contributed one paired rating) and the observers were independent. For both nominal and ordinal variables the categories used were mutually exclusive and exhaustive.

The magnitude of κ is influenced by various factors including the prevalence of an attribute [Brennan & Silman 1992] and bias [Feinstein & Cicchetti 1990].

3.5.5.2 Prevalence of an attribute

Prevalence may influence κ when the number of paired ratings in which both raters have rated a variable as being 'present' (positive ratings) differs substantially from the number of paired ratings in which both raters have assessed a variable as being 'absent' (negative ratings). This is shown overleaf for the variables 'acute haemorrhage' and 'acute ischaemic change'.

		Rater 1	
		✓	×
Rater 2	✓	1	1
	×	2	11

Acute haemorrhage

		Rater 1	
		✓	×
Rater 2	✓	7	3
	×	0	5

Acute ischaemic change

For both variables the raters agreed on 12 out of 15 training scans. For the variable 'acute intracerebral haemorrhage' the prevalence of negative ratings is 11 and the prevalence of positive ratings is one leading to the chance agreement also being high, and κ is consequently low (0.29). However, for 'acute ischaemic change' the number of positive and negative ratings is more equally distributed, and κ is higher (0.61). This effect is likely to explain the relatively modest κ's for some variables in which both the observed agreement and expected agreement were high, such as the presence of EPVS and atrophy.

3.5.5.3 Observer bias

Observer bias may also affect κ, for example the tendency of one rater to systematically rate a variable as being present or absent or under or overestimate the severity of an

ordinal variable. In the example above, for the variable 'acute ischaemic change' all disagreements result from rater two assessing ischaemic change as being present. The effect of bias is larger when κ is small [Byrt, Bishop, & Carlin 1993]. I was not able to avoid observer bias in the study design, but this should be taken into account when interpreting the study findings.

3.5.6 Interpretation of weighted and unweighted κ

The following classification of κ is the most widely used [Landis & Koch 1977] : <0 = agreement less than chance, 0-0.2 slight agreement, 0.2-0.4 fair, 0.4-0.6 moderate 0.6-0.8 substantial 0.8-0.1 almost perfect. However, the choice of these categories is recognised as being somewhat arbitrary [Brennan & Silman 1992]. For ordinal variables, the size of κ is influenced by the number of categories and the method of weighting used. As the number of categories per variable increases, there is a higher likelihood of disagreement, resulting in a lower κ, although a linearly weighted κ is less vulnerable to this effect in comparison to quadratic weighting [Brenner & Kliebsch 1996]. I used κ with linear weighting for all ordinal variables. All ordinal variables were rated using four point scales thus making it easier to compare κ across variables. However, it is arguable as to whether unequal weighting might be used to reflect certain key scale divisions for example (no vs. mild WMH).

Table 1 MRI scan parameters used for LINCHPIN scans
*T1WI-T1 weighted imaging, T2WI-T2 weighted imaging

Sequence	TE		TR		Slice thickness		Slice gap		Matrix		Field of view		Flip angle	
	GE	Philips	GE	Philips	GE	Philips	GE	Philips	GE	Philips	GE	Philips	GE	Philips
T1WI*(sagittal)	MIN	15	380	623.0	5	5	1.5	1	384×256	256×205	24	23		
T2WI* (axial)	86.2	100	5000	4841.6	5	5	1	1	384×384	384×242	24	23		
FLAIR (axial)	140	100	9000	6000	5	5	1	1	384×224	256×161	24	23		
T2* GRE (axial)	40	40	300	300	5	5	1.5	1.5	256×160	268×166	24	24	20	20

Figure 13 Basal ganglia mineralisation
A No basal ganglia mineralisation visible; rated as 0
B Subtle linearity bilaterally in Globus pallidi (arrows); rated as 1
C Signal drop out bilaterally in lentiform nuclei (solid arrows); rated as 1 with haemosiderin visible from an old right occipital infarct (dashed arrow)
D Moderate basal ganglia mineralisation (arrows); rated as 2

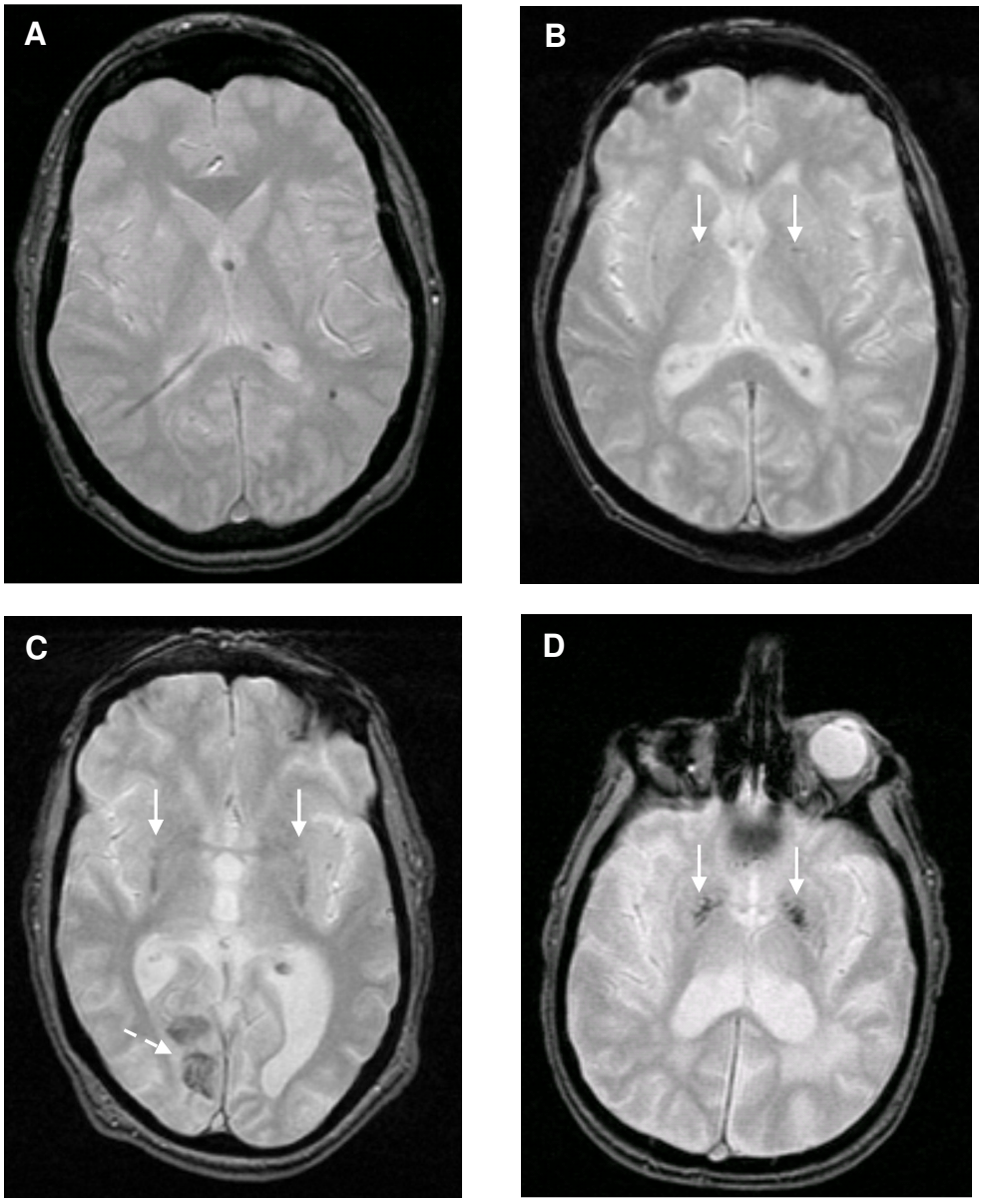


Figure 14 White matter hyperintensities of presumed vascular origin

A Periventricular WMH-1 (Fazekas scale) and right occipital infarct (solid arrow)
B Deep WMH-1 (Fazekas scale)
C Periventricular WMH-2 (Fazekas scale) & right centrum semiovale infarct (circle)
D Deep WMH-2 (Fazekas scale)
E Periventricular WMH-3 (Fazekas scale)
F Deep WMH-3 (Fazekas scale) & two left hemisphere cavitated lacunar infarcts (arrows)

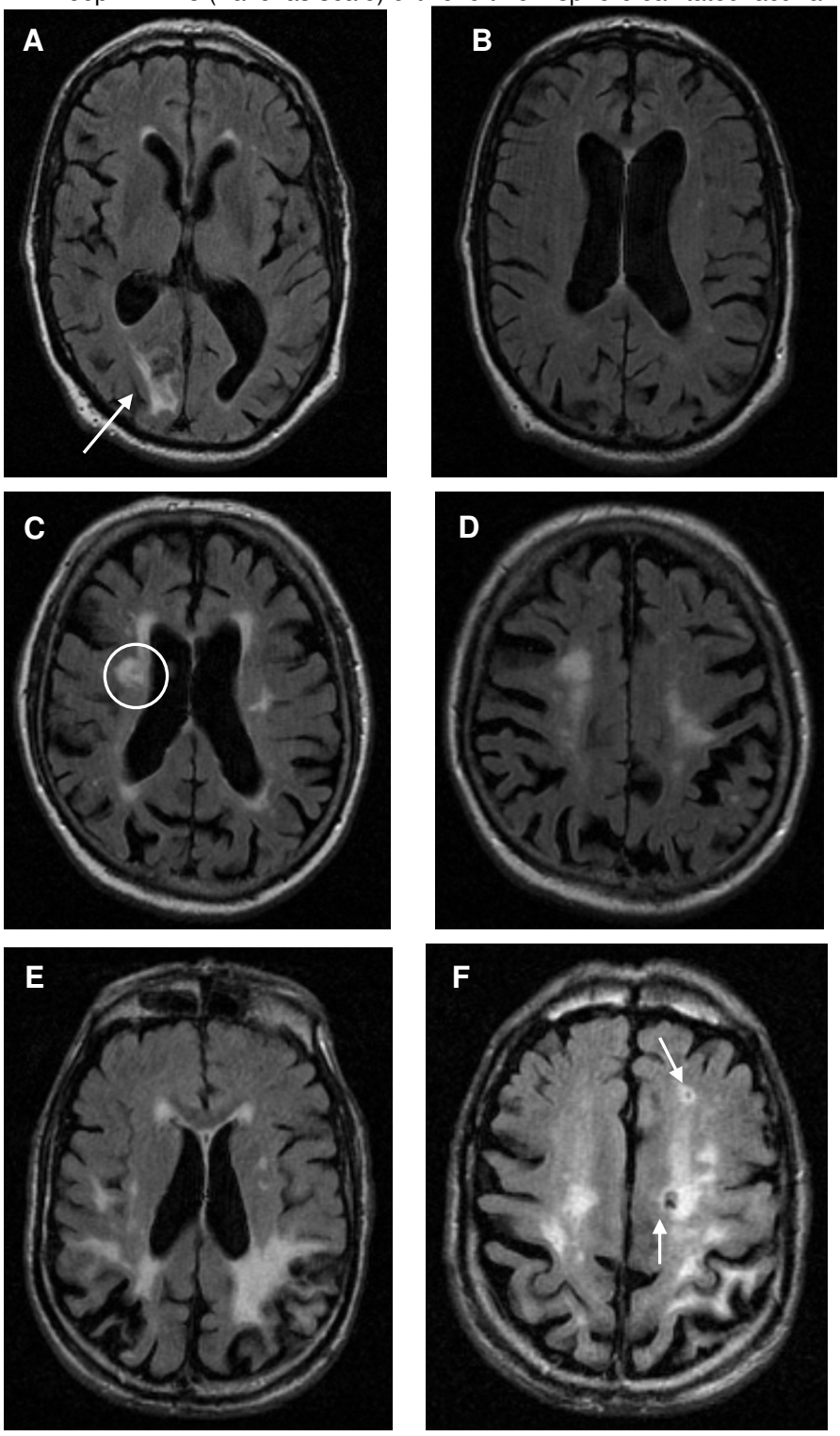
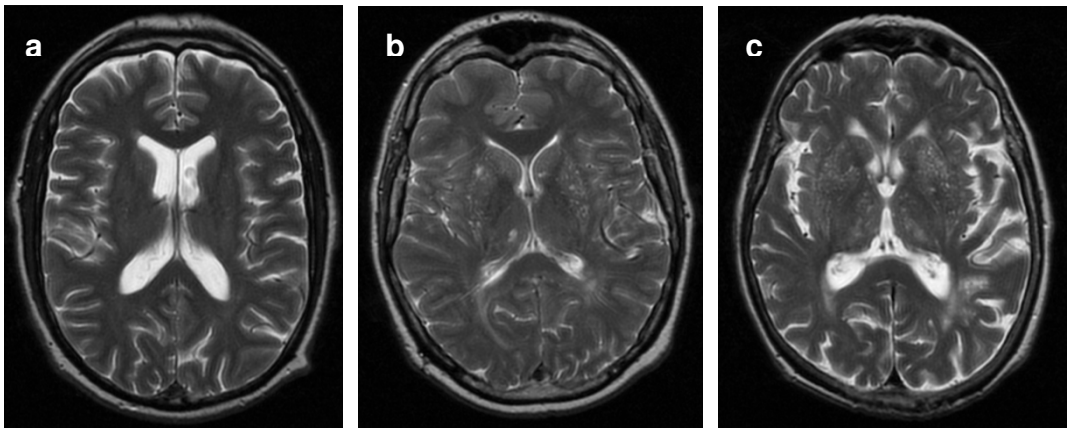


Figure 15 Enlarged perivascular spaces

A Basal ganglia EPVS; a-rated 1, b-rated 2, c-rated 3



B Centrum semiovale EPVS; a-rated 1, b-rated 2 (with 2 old right frontal cortical infarcts,arrowed), c-rated 3

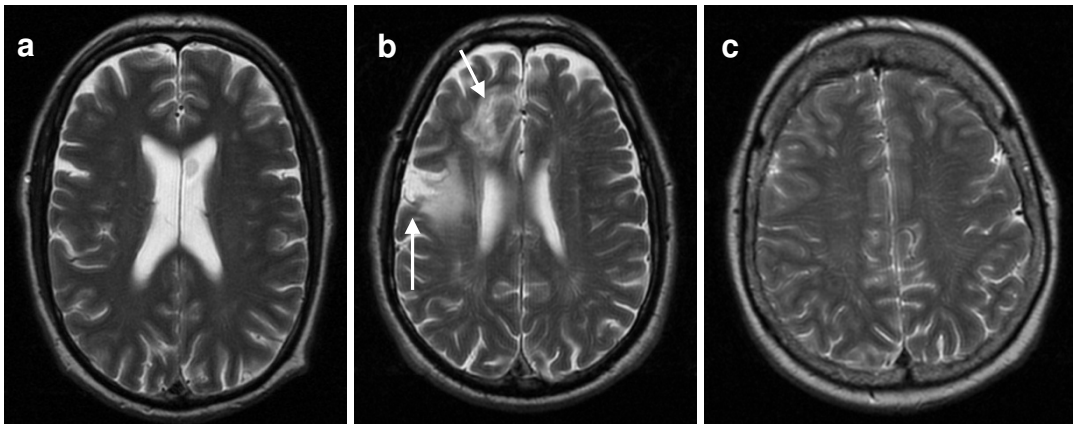


Figure 16 Brain microbleeds

A Left occipital lobe BMB at grey-white matter junction (solid arrow) and two paler BMBs in right internal capsule (dashed arrow)

B Multiple bilateral BMBs in the basal ganglia (solid arrows) and a hypointense focus R frontal lobe secondary to partial volume artefact mimicking a BMB (dashed arrow)

C R parafalcine BMB (solid arrow) and a blood vessel in a cortical sulcus mimicking a BMB (dashed arrow)

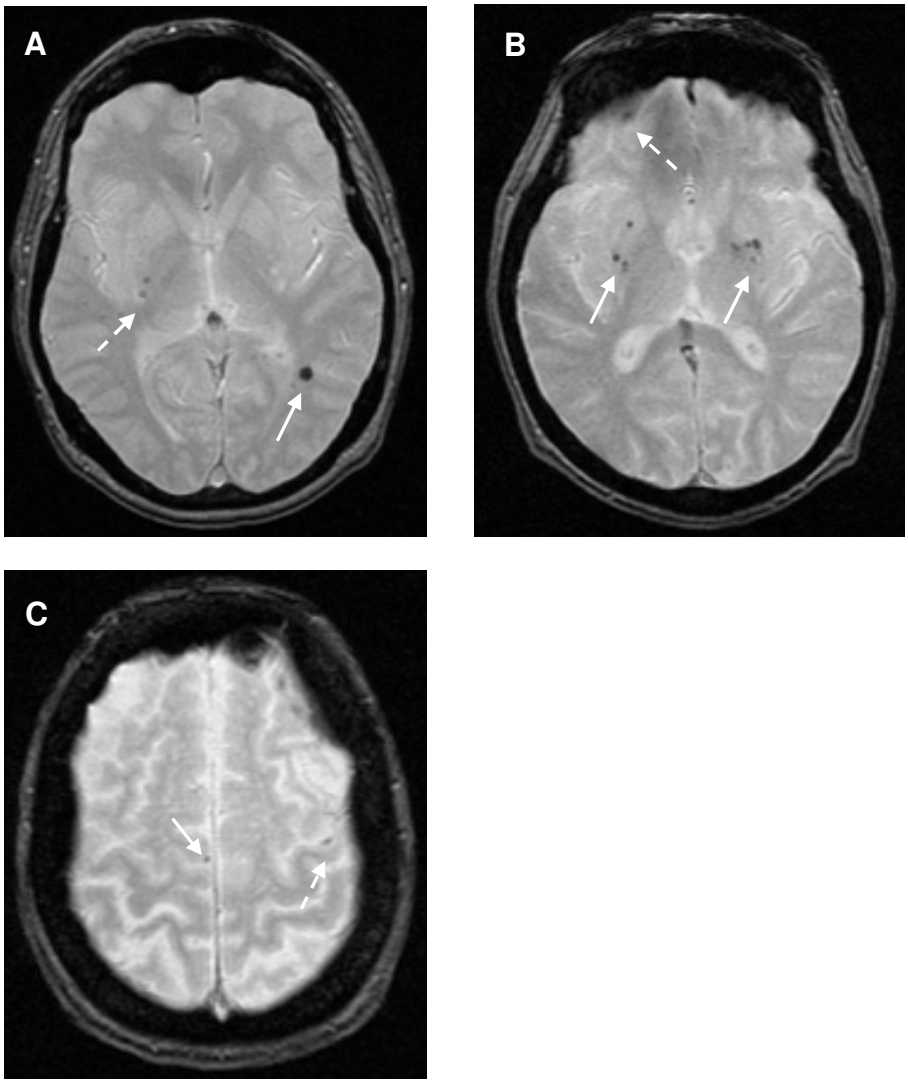


Figure 17 Inter-observer (filled boxes) and intra-observer (open boxes) agreement for 15 MRI training scans, measured by weighted (asterisked) & un-weighted κ, shown as point estimates with 95% confidence intervals

Variable	Inter-observer agreement (kappa)	Intra-observer agreement (kappa)
Acute ischaemic change	0.61 (0.09 to 0.86)	0.00 (-0.58 to 0.27)
Acute haemorrhage	0.29 (-0.1 to 0.74)	-0.19 (0.20 to 0.40)
Chronic infarcts & haemorrhages	0.34 (-0.17 to 0.70)	0.22 (-0.29 to 0.59)
Basal ganglia mineralisation*	0.48 (0.08 to 0.87)	0.15 (-0.12 to 0.42)
White matter hyperintensities		
Periventricular*	0.47 (0.15 to 0.79)	0.49 (0.16 to 0.81)
Deep*	0.32 (0.07 to 0.56)	0.45 (0.09 to 0.82)
Atrophy		
Cortical*	0.32 (-0.01 to 0.65)	0.45 (0.09 to 0.81)
Deep*	0.55 (0.22 to 0.90)	0.50 (0.20 to 0.80)
Enlarged perivascular spaces		
Centrum semiovale*	0.52 (0.17 to 0.88)	0.69 (0.28 to 1.10)
Basal ganglia*	0.54 (0.19 to 0.89)	0.30 (-0.08 to 0.69)
Hippocampus*	0.34 (-0.02 to 0.70)	0.35 (0.00 to 0.70)

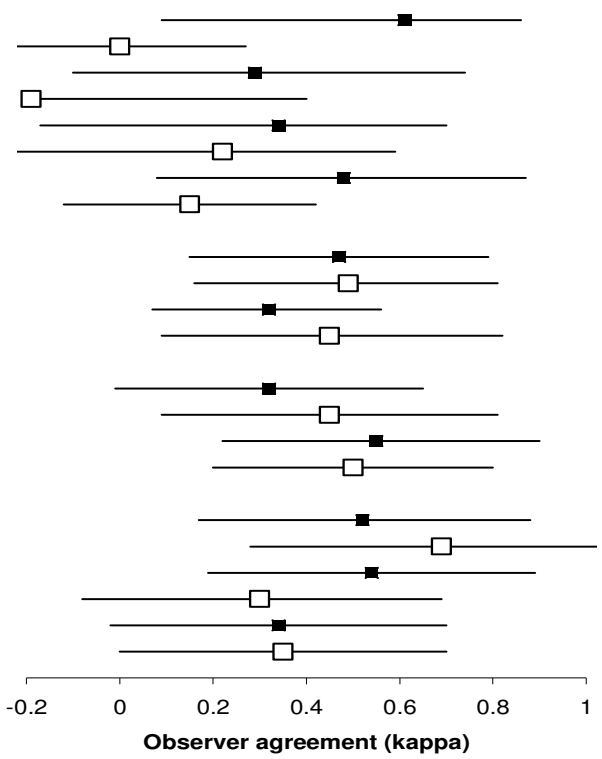


Figure 18 Inter-observer (filled boxes) and intra-observer (open boxes) agreement for 15 MRI training scans, measured by unweighted κ, shown as point estimates with 95% confidence intervals

BMB Location	Inter-observer agreement (0 vs ≥1 BMB)	Intra-observer agreement (0 vs ≥1 BMB)
All locations		
Certain	0.73 (0.22 to 0.92)	0.73 (0.22 to 0.92)
Certain & uncertain	0.84 (0.33 to 0.97)	0.53 (0.02 to 0.83)
Lobar		
Certain	0.86 (0.40 to 0.98)	0.86 (0.40 to 0.98)
Certain & uncertain	0.87 (0.40 to 0.98)	0.73 (0.24 to 0.92)
Deep		
Certain	0.47 (-0.04 to 0.80)	0.38 (-0.13 to 0.72)
Certain & uncertain	0.84 (0.33 to 0.97)	0.71 (0.19 to 0.92)
Infratentorial		
Certain	0.86 (0.37 to 0.97)	0.73 (0.23 to 0.92)
Certain & uncertain	0.72 (0.22 to 0.92)	0.86 (0.40 to 0.98)

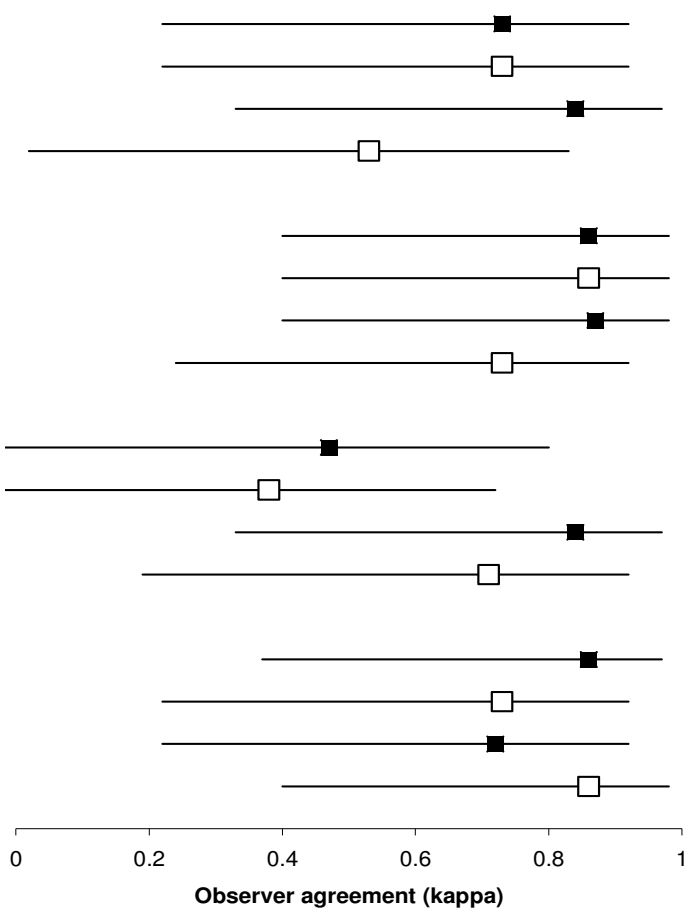


Figure 19 Inter-observer agreement for 50 LINCHPIN MRI scans, measured by weighted (asterisked) & un-weighted κ, shown as point estimates with 95% confidence intervals

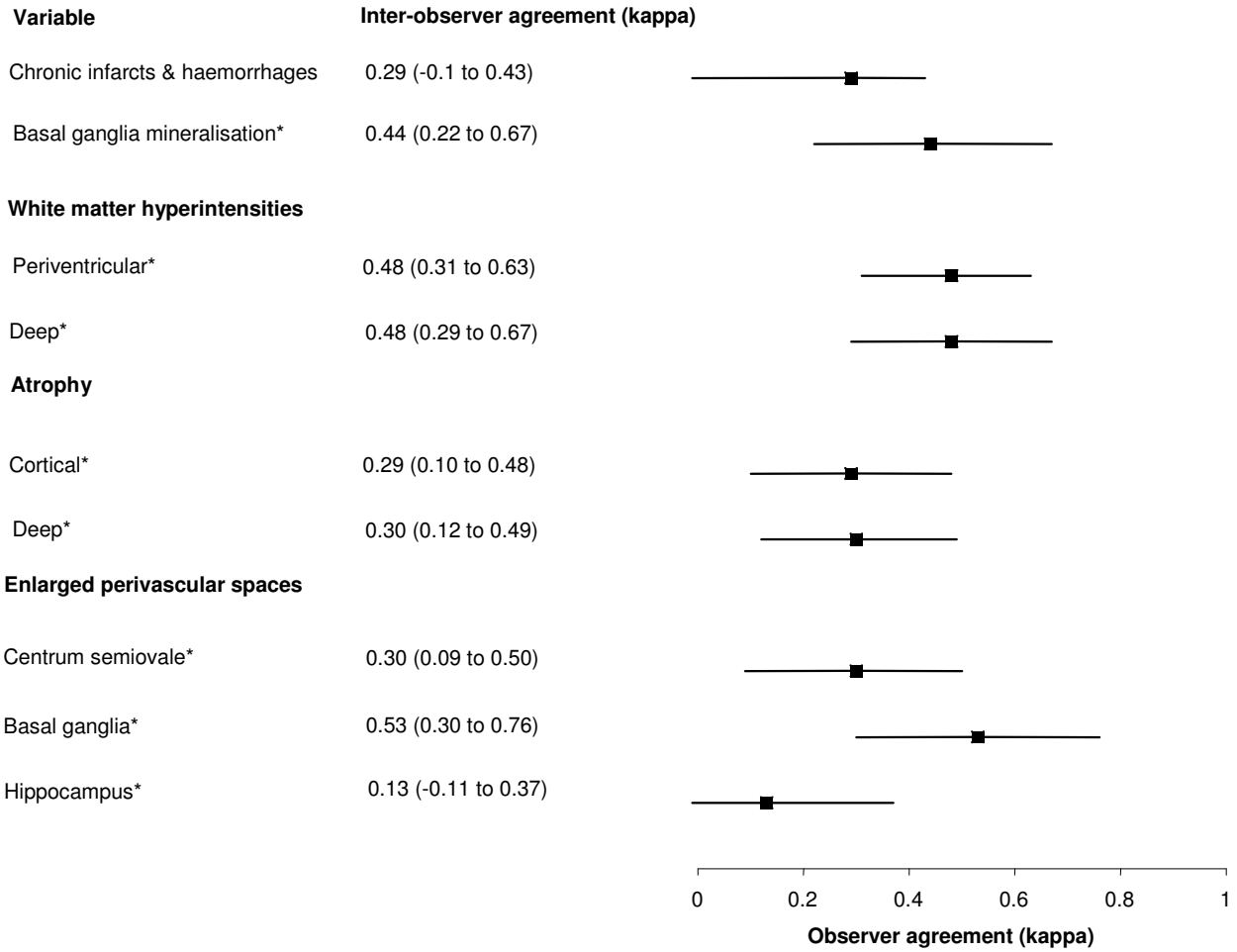
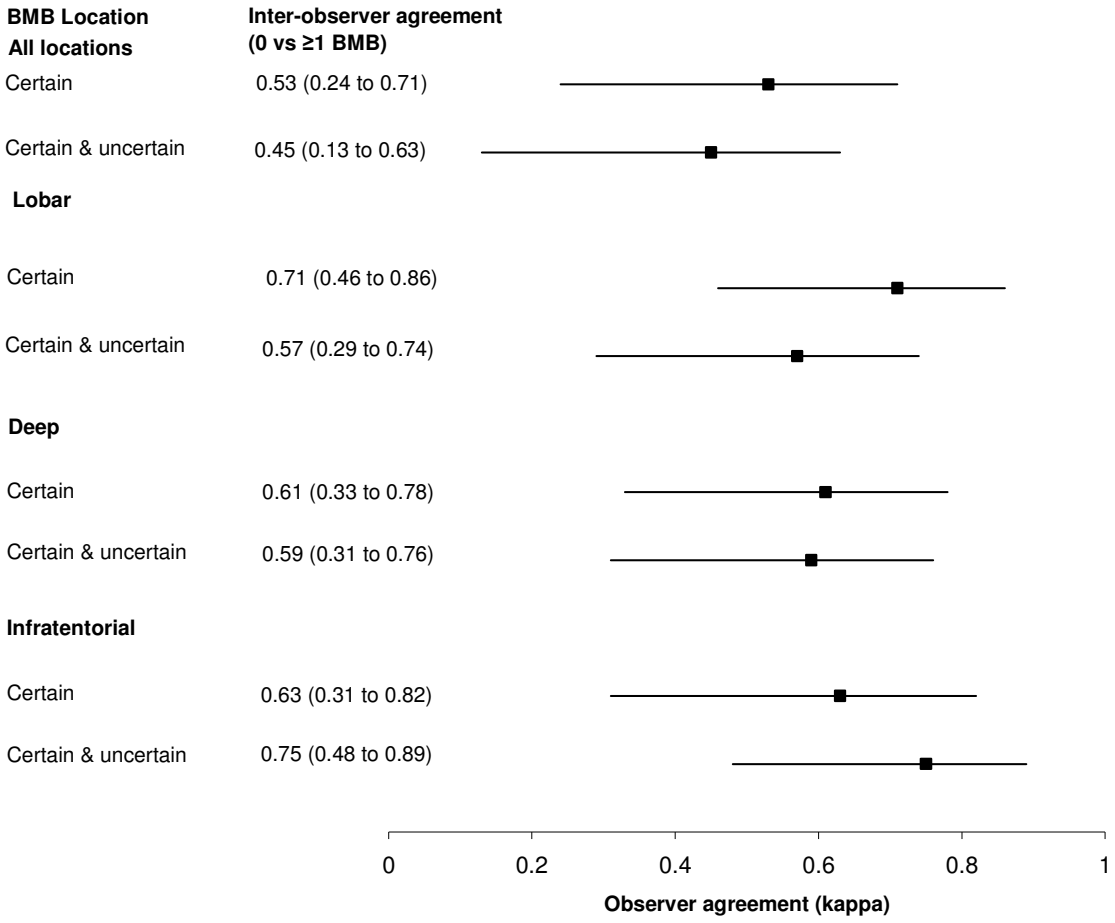


Figure 20 Inter-observer agreement for 50 LINCHPIN MRI scans, measured by unweighted κ, shown as point estimates with 95% confidence intervals



Chapter 4 Methods of a cross-sectional study of apolipoprotein E genotypes in lobar vs. non-lobar intracerebral haemorrhage

Chapter contents

4 Methods of a cross-sectional study of apolipoprotein E genotypes in lobar vs. non-lobar intracerebral haemorrhage

4.1 Introduction

In recent years there have been numerous studies examining the associations between different polymorphisms of the apolipoprotein E gene and stroke phenotypes.

The apolipoprotein E gene codes for a protein of the same name, which is expressed in several organs including the liver and the brain. The protein assists in the transport of triglycerides to the liver. In the brain, astrocytes and microglia express apolipoprotein E which functions as a ligand to assist in the endocytosis of lipoproteins [Kim, Basak, & Holtzman 2009].

The apolipoprotein E gene, is contained on the long arm of chromosome 19 [Verghese, Castellano, & Holtzman 2011]. The three common alleles, ε2, ε3 and ε4 are determined by two single-base changes (single nucleotide polymorphisms, SNPs) in the coding region of the gene at codon positions 112 and 158 [Zannis et al. 1982]. Both SNPs are given RefSNP (rs) numbers: rs429358 at position 112 and rs7412 at position 158. Cytosine bases at both loci equal apo ε4, thymine bases at both loci equal ε2 and a thymine base at position 112 with a cytosine base at position 158 equals ε3. The ε3ε3 genotype which is the common variant of apolipoprotein E, has a cysteine amino acid at position 112 and an arginine at position 158. The ε2 allele has a cysteine at position 158 and the ε4 allele has an arginine at position 112 (Table 2 on page 116).

Although the three common protein isoforms differ by only one or two amino acids, these conformational changes produce important alterations in the function of the protein and there is some evidence that the E4 isoform may accelerate the formation of amyloid plaques by acting as an Aβ binding protein [Wisniewski & Frangione 1992]. The ε4 allele has been associated with an increased risk of ICH in comparison to unaffected controls [Biffi et al. 2010b].

Ideally, a genetic association study of apolipoprotein E and ICH should have: a population-based design with both comparison groups drawn from the same

population, careful phenotyping of participants according to a standard classification and explicit definitions of risk factors [Dichgans & Markus 2005]. Further recommendations include: reporting genotype frequencies (in preference to or, as well as, allele frequencies, since it is the genotype that confers risk of a certain phenotype and allele frequencies can be calculated from genotype frequencies), reporting markers of quality control of genotyping such as the proportion of samples for which a genotype can be obtained (genotype call rate) and reporting whether polymorphisms deviate from Hardy-Weinberg equilibrium, in which case potential reasons should be explored [Little et al. 2002;Salanti et al. 2005;Little et al. 2009].

Therefore in a small preliminary study I sought to determine the apolipoprotein E genotypes of participants with lobar and non-lobar ICH. The participants in this study were recruited from 1st June 2010-31st May 2012.

In the following chapter, I will describe:

- the method of deoxyribonucleic acid (DNA) extraction and genotyping;
- the quality control measures used to maintain the internal validity of the study; and
- the results of the quality control assessment of genotyping.

4.2 Methods

4.2.1 Subjects

I recruited participants into the LINCHPIN study. The full inclusion and exclusion criteria for the study are given in Chapter 2. All participants had a primary ICH during 1st June 2010-31st May 2012 and lived in the Lothian region at the time of their ICH.

Participants or their nearest relative gave written informed consent to a clinical assessment, access to their medical records and two blood samples – one for genotyping of apolipoprotein E and the other to be held in storage for use in future ethically-approved stroke studies.

4.2.2 Data and blood collection

I collected data on baseline demographic, clinical and imaging characteristics as outlined in Chapter 2. As described in Chapter 2, I used multiple overlapping sources of follow-up to determine if a participant had died and if applicable, their date of death. I used

information from questionnaires sent annually to participants GPs to obtain their level of disability one year after their ICH using the modified Rankin scale.

I performed venesection and transferred two ethylene-diamine-tetra-acetic acid (EDTA) tubes containing up to nine millilitres blood to the Wellcome Trust Clinical Research Facility, (WTCRF; Western General Hospital, Edinburgh) for storage and future genotyping. All samples were anonymised, labelled with a unique three digit ID number and the participant's date of birth.

4.2.3 Phenotype definition

I classified participants as having either lobar or non-lobar ICH using the definition outlined in Chapter 1. Non-lobar ICH included participants with both supratentorial ICH involving the basal ganglia, internal capsule and/or thalamus and infratentorial ICH. The neuroradiologist(s) who classified ICH location were blinded to the participant's genotype.

4.2.4 DNA extraction

After venesection, WTCRF extracted DNA from whole blood on the day that they received the sample. To extract the DNA, one blood sample was centrifuged before resuspending the DNA and transferring it to a flip tube which was then stored at -80°C.

4.2.5 Genotyping

4.2.5.1 DNA concentration

We first quantified the concentration of DNA using Quant-iT™ PicoGreen® double stranded DNA reagent; a highly sensitive nucleic acid stain that binds to double stranded DNA producing a strong fluorescent signal. After obtaining the concentration of DNA in each sample, WTCRF standardised each sample to a DNA concentration of 10ng/μl and froze the sample at -40°C.

4.2.5.2 Polymerase chain reaction (PCR)

A single sample of DNA is the starting point for the PCR. PCR enables amplification of a single copy of the target DNA sequence by using primers and free nucleotides to produce multiple DNA copies (Figure 21 on page 117). Two TaqMan assays (Assay-On-Demand, Applied Biosystems) were used to determine the SNPs. The assays

contained sequence specific primers to amplify the region of interest and fluorescent dye-labelled probes which anneal to the sequences and enable discrimination of alleles. The probe for allele X was labelled with VIC® dye and the probe for allele Y was labelled with FAM™ dye. The numbers of the assays for SNPs rs429358 and rs7412 were c3084793 and c904973 respectively.

The laboratory aliquoted 2µl DNA from each sample by robot (Beckman®) into a 384 well plate allowing the DNA to dry completely. The DNA was mixed with the two Taqman genotyping assays (Assay-On-Demand, Applied Biosystems). The well plate contained DNA from 92 blood samples and four no template controls (NTCs). The controls were used for quality control purposes (Section 4.2.5.3, below). Samples were plated in the order in which they arrived in the laboratory.

PCR reactions were conducted according to the manufacturer's protocol, with Taqman genotyping master mix (Applied Biosystems, PN 4731355) and DNAase RNAase free water using the following cycling conditions - 50°C for two minutes, followed by 95°C for ten minutes, followed by 40 cycles of denaturation at 92°C for fifteen seconds and annealing and polymerisation at 60°C for one minute.

The laboratory ran the reaction on a ABI Prism TaqMan 7900HT Sequence Detection System (Applied Biosystems) and genotypes were indicated by Sequence Detection Software version 2.4 (Applied Biosystems). All genotyping calls were double checked by two laboratory researchers and genotyping personnel were blinded to the participant's phenotype. WTCRF performed genotyping of all samples simultaneously.

4.2.5.3 Quality control

The laboratory conducted various quality control measures.

- WTCRF internally validated their genotyping analysis by using no template controls (NTC's) which contained all elements of the reaction except the DNA template. Since the NTC's do not contain the template they should not produce any fluorescent signal when displayed in an allelic discrimination plot (Figure 22 on page 118 and Figure 23 on page 119). This allows detection of contamination occurring either when loading DNA templates onto the well plate or through contaminated reagents.

- WTCRF checked whether the observed and expected genotype frequencies at rs429358 and rs7412 were in Hardy-Weinberg equilibrium [Salanti et al. 2005].
- WTCRF calculated the genotype call rate for each SNP by dividing the number of SNPs receiving a genotype by the number of SNPs on the well plate.
- WTCRF measured the purity of DNA in each sample. One of the commonest measures of DNA purity is assessment of ultraviolet (UV) light absorption [Manchester 1995]. DNA light absorption peaks at 260nm and proteins (and some other contaminants) peak at 280nm. Therefore the ratio of absorbance of UV light at 260nm and 280nm ($A_{260/280}$) can be used to assess DNA purity using a Nanodrop™ ND-8000 spectrophotometer (NanoDrop, USA). A ratio of 1.7-2.0 is generally accepted as 'good quality' DNA [Manchester 1995].

4.2.6 Genotyping definition

I combined the SNPs obtained from each sample to determine the alleles and genotypes for each participant (ε2ε2, ε2ε3, ε3ε3, ε3ε4, ε4ε4, ε2ε4). I compared the proportions of participants with an undetermined genotype in the lobar and non-lobar ICH groups.

4.2.7 Statistical analysis

I used parametric statistics for between group comparisons when the data had a normal distribution and log –transformed variables or used non-parametric statistics when it did not. If an estimated cell count was less than five, I used the Fisher's exact test. I conducted all statistical analyses using Stata version 11.1 (StataCorp LP, Texas, USA). All p values are 2-sided.

4.3 Results

4.3.1 Study participants

92 participants had blood taken for apolipoprotein E genotyping. Two participants were excluded from the study following genotyping because they were found to be living outside of the Lothian region at the time of their ICH (n=1) or their ICH was subsequently found to be HTI (n=1). Of the remaining 90 participants, 48 had lobar ICH. 84 participants had a first-ever ICH. Three participants had recurrent lobar ICH and three had recurrent non-lobar ICH.

4.3.2 Quality control assessment of genotyping

4.3.2.1 DNA quality

The mean A260/A280 ratio was 1.80; standard deviation (SD) 0.07; indicating that DNA samples were of adequate quality.

4.3.2.2 Allelic discrimination plots

Figure 22 on page 118 and Figure 23 on page 119 show the allelic discrimination plots for the two assays for SNPs rs429358 and rs7412 respectively.

Each axis represents the reporter fluorescent signal intensity (Rn) for one of two probes (each relating to an allele). Homozygotes (shown in blue and red) show increased fluorescence on either the horizontal or vertical axis; depending on which probe they were detected by whereas heterozygotes (shown in green) appear in the middle of the plot since they contain copies of both alleles and therefore produce fluorescence with both dyes.

The four NTC's (black crosses, circled) do not produce any fluorescence indicating that there is no evidence of cross contamination between samples. Undetermined alleles (dashed circles) do not cluster within either the homozygote or heterozygote groups and produce an indeterminate fluorescent signal.

4.3.2.3 Genotype call rate

For each SNP, 88 out of 90 DNA samples were called with two alleles undetermined; a call rate of 98%. No control samples were called. All the undetermined alleles occurred in first-ever ICHs (n=84), giving a genotype call rate for each SNP for first-ever ICHs of 98%. The proportion of participants with an undetermined genotype did not differ between those with lobar and non-lobar ICH (lobar: 2/48 (4%) vs. non-lobar 2/42 (5%), χ^2=0.02, p=0.891.)

4.3.2.4 Genotype call frequencies

Table 3 on page 116 shows the genotype frequencies for SNPs rs429358 and rs7412 and the corresponding epsilon genotypes of the 90 participants. Table 4 on page 116 shows the genotype frequencies for SNPs rs429358 and rs7412 and the corresponding epsilon genotypes of the 84 participants with first-ever ICH. Both SNPs conformed to Hardy-Weinberg equilibrium expected proportions (rs429358 p=0.88; rs7412 p=0.32).

4.4 Summary

- The genotyping process for apolipoprotein E fulfilled pre-specified quality control measures.
- The genotype frequencies for SNPs rs429358 and rs7412 conformed to Hardy-Weinberg equilibrium.
- There was no difference in the proportion of participants with an undetermined genotype between the lobar and non-lobar ICH groups.

4.5 Discussion

The findings presented in this chapter should be viewed as a baseline assessment of quality control measures of genotyping of the apolipoprotein E gene.

4.5.1 Quality control assessment of genotyping

The study fulfilled established measures of quality control for genotyping in a genetic association study [Little et al. 2002] which have been included in the STrengthening the REporting of Genetic Association studies (STREGA) statement [Little et al. 2009] including:

(i) successful internal validation of the genotyping process using NTC's;

(ii) blinding of laboratory personnel to participants' phenotypes and the study hypothesis;

(iii) classification of ICH location done blind to participants' genotypes;

(iv) a high genotype call rate;

(v) conformation of genotype frequencies to Hardy-Weinberg equilibrium;

(vi) automated data entry of genotypes called with third party adjudication; and

(vii) presentation of both genotype and allele frequencies.

4.5.1.1 Hardy-Weinberg equilibrium

The Hardy-Weinberg law states that the genotype frequencies and allele frequencies of a population remain constant from one generation to the next [Lunetta 2008]. It predicts how gene frequencies will be inherited assuming that the following assumptions are met:

(i) the population is large, (ii) there is no natural selection, (iii) mutations do not occur, (iv) there is no migration in or out of the population and (v) all members of the population breed randomly and produce on average, the same number of offspring. Deviation from Hardy-Weinberg equilibrium may result from one or more of the assumptions above not being met; for example if there is non random mating or a small non-representative study sample leads to selection bias [Salanti et al. 2005] or if there is genotyping error, especially with non-differential misclassification of genotypes between comparison groups.

Both loci in our study conformed to Hardy-Weinberg equilibrium, indicating that the Hardy-Weinberg assumptions were not rejected and providing some evidence against the existence of selection bias or genotyping errors.

4.5.1.2 Genotype calls

This small genotyping study benefits from all blood samples having been processed by the same laboratory with genotypes assigned simultaneously, therefore avoiding the misclassification bias which may occur when specimens are processed by different laboratories [Plagnol et al. 2007]. Bias may also be introduced by treating undetermined genotypes as 'missing' when the proportion of undetermined genotypes varies between comparison groups, since this may lead to a false association between a genotype and phenotype (Type one error) [Sampson & Zhao 2009]. The proportion of undetermined genotypes may be higher when multiple laboratories are used for genotyping because of variation in the processing of samples [Clayton et al. 2005]. In this study, the proportion of participants with an undetermined genotype was small (4%) and did not differ between comparison groups. However, it is a small study in which all samples were processed by a single laboratory. To obtain meaningful results in genetic-association studies, larger scale studies are necessary, using pooled data from multiple cohorts. These problems might then be overcome by all samples being genotyped by a single centre or using statistical techniques such as the gene counting method in which missing information is imputed based upon estimates of haplotype frequencies [Hawley & Kidd 1995].

Table 2 Variation in gene nucleotides (SNPs) at codon positions 112 and 158 with corresponding amino acids
T= thymine, G=guanine, C=cytosine

Apolipoprotein E isoform	**Gene nucleotide position (corresponding amino acid)**	
	3937 (112)	**4075 (158)**
SNP ID	**rs429358**	**rs7412**
ApoE2	**T**GC (Cysteine)	**T**GC (Cysteine)
ApoE3	**T**GC (Cysteine)	**C**GC (Arginine)
ApoE4	**C**GC (Arginine)	**C**GC (Arginine)

Table 3 Genotype number (with frequencies, %) for SNPs rs429358 and rs7412 in 90 participants [with the corresponding alleles]

	rs429358			
rs7412	**TT**	**TC**	**CC**	**Undetermined**
CC	47 (53) [ε3/ε3]	19 (21) [ε3/ε4]	3 (3) [ε4/ε4]	2 (2)
CT	11 (12) [ε2/ε3]	6 (7) [ε2/ε4]	-	-
TT	0 (0) [ε2/ε2]	-	-	-
Undetermined	1 (1)	1 (1)	-	-

Table 4 Genotype number (with frequencies, %) for SNPs rs429358 and rs7412 in 84 participants with first-ever ICH [with the corresponding alleles]

	rs429358			
rs7412	**TT**	**TC**	**CC**	**Undetermined**
CC	44 (52) [ε3/ε3]	17 (20) [ε3/ε4]	3 (4) [ε4/ε4]	2 (3)
CT	11 (13) [ε2/ε3]	5 (6) [ε2/ε4]	-	-
TT	0 (0) [ε2/ε2]	-	-	-
Undetermined	1 (1)	1 (1)	-	-

Figure 21 Schematic diagram of the Polymerase Chain Reaction (PCR)

A The target DNA sequence

B DNA is denatured at 92°C separating the strands by breaking the hydrogen bonds.

C The temperature is reduced to 60°C enabling primers (shown in red) in the genotyping assays to anneal to the complementary base pair sequences in the target DNA.

D The DNA TaqPolymerase enzyme begins polymerisation, adding nucleotides to the end of each primer (shown in green), eventually forming two copies of the target sequence of DNA. The assay contains many copies of the primer and nucleotides to perform amplification of the DNA.

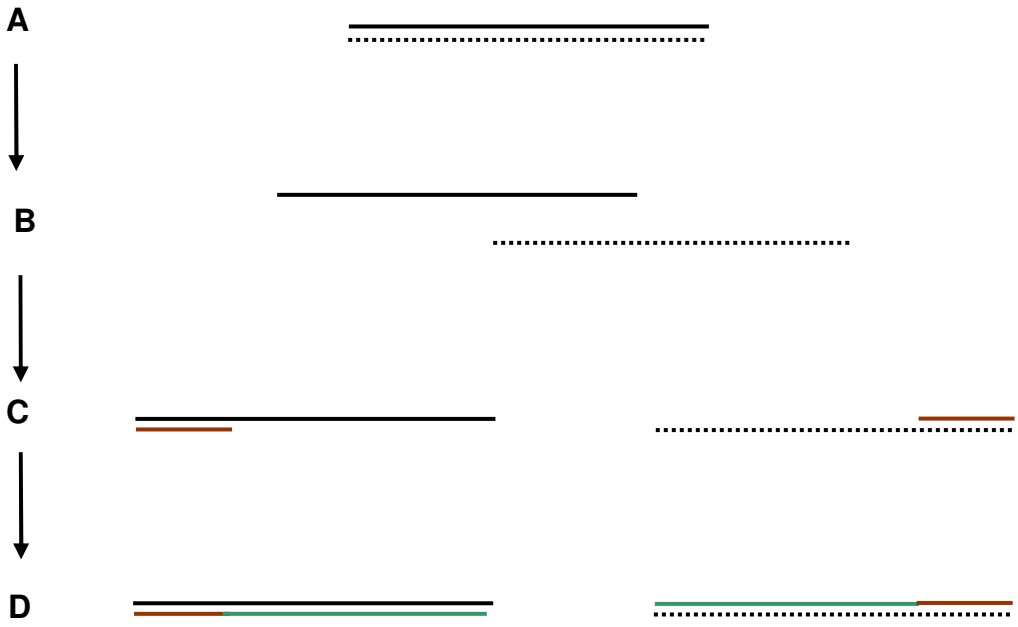


Figure 22 Allelic discrimination plot for assay c3084793 (rs429358) for 92 participants from the LINCHPIN study

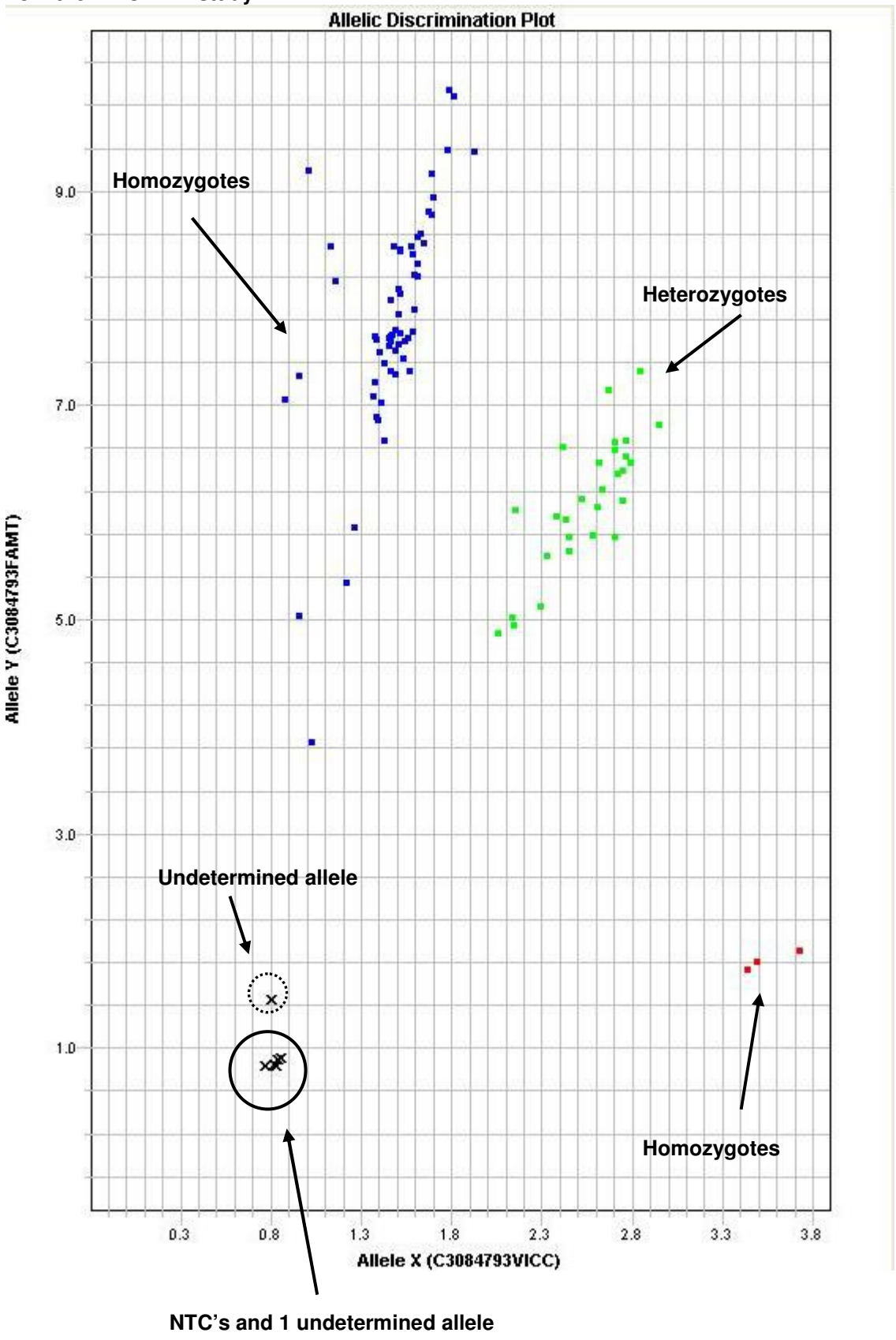
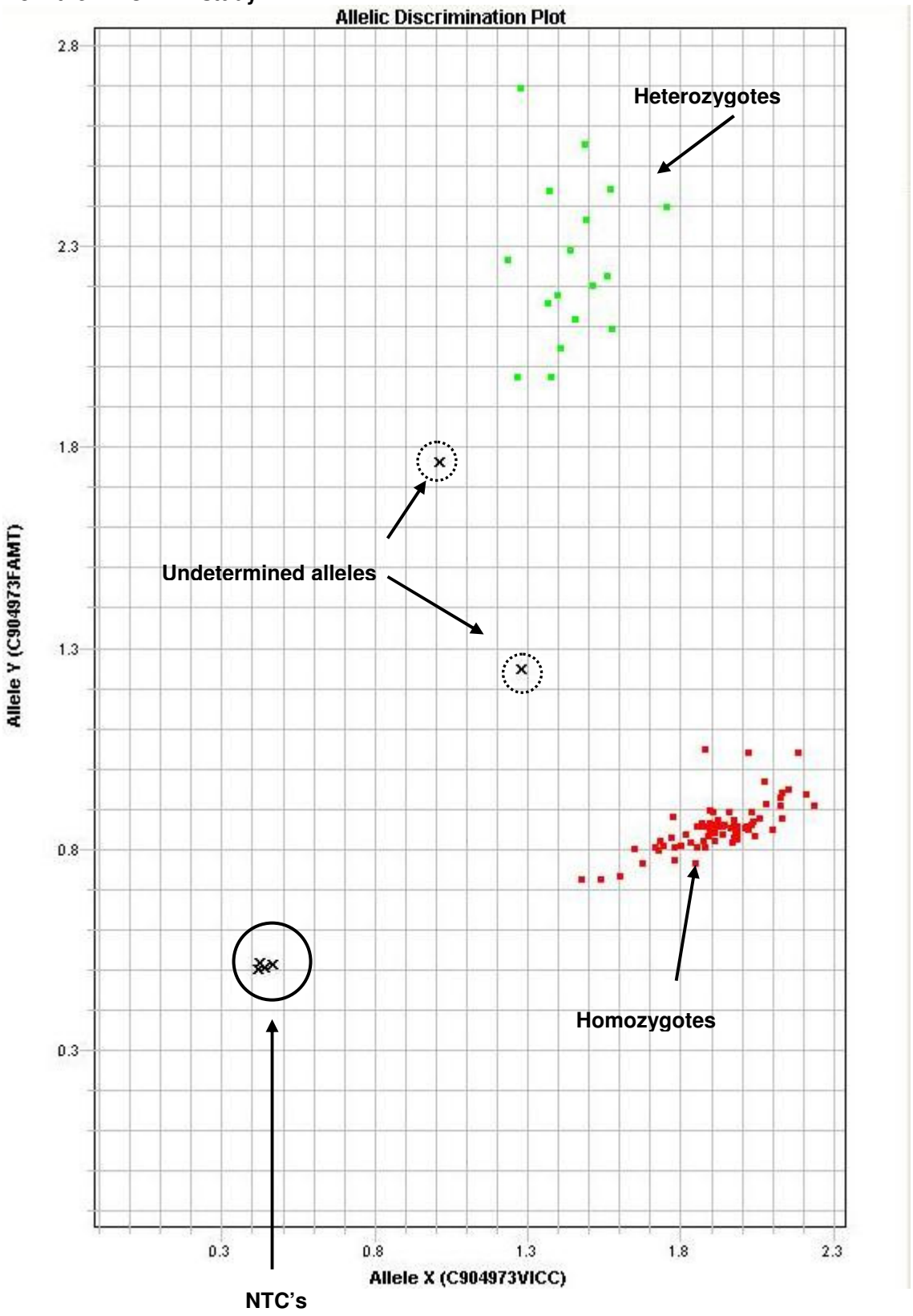


Figure 23 Allelic discrimination plot for assay c904973 (rs7412) for 92 participants from the LINCHPIN study



Section C The epidemiology, imaging and genetics of intracerebral haemorrhage in the Lothian region of Scotland - Results

Chapter 5 Results of a community-based, cross-sectional study of intracerebral haemorrhage-incidence and baseline characteristics

Chapter contents

5 Results of a community-based, cross-sectional study of intracerebral haemorrhage-incidence and baseline characteristics

5.1 Introduction

From my review of studies of ICH including those which have compared lobar and non-lobar ICH (Chapter 1), it was apparent that there is a paucity of larger population-based studies of spontaneous primary ICH, which have used multiple overlapping sources of case ascertainment, explicitly defined ICH location (lobar vs. non-lobar vs. mixed ICH), distinguished primary from secondary ICH and first-ever from recurrent ICH and defined pre-ICH risk factors.

Moreover the findings from previous studies of lobar vs. non-lobar ICH have been inconsistent (Table 5 on page 140). 40 studies involving 4923 individuals with lobar and 7473 with non-lobar ICH have compared the risk factors for these ICH subtypes. Associations between certain risk factors and subtypes of ICH (such as hypertension and non-lobar ICH [Jackson & Sudlow 2006], older age and lobar ICH [Greenberg et al. 1996;Labovitz et al. 2005;Biffi et al. 2011b], and female gender and lobar ICH [Weimar et al. 2011;Matsukawa et al. 2012]) have been more commonly reported in hospital based studies rather than population-based ones but these findings are likely to be biased by the cohorts selected and the definitions of risk factors used. There are no consistent associations between prior smoking, diabetes, ischaemic stroke, ischaemic heart disease, cognitive impairment or dementia and subtypes of ICH. A recent case control study [Martini et al. 2012] which compared cases of ICH vs. controls without ICH suggested that hypertension and hypercholesterolaemia were associated with non-lobar ICH in comparison to controls (matched for age, race and gender) but the cases represented only 23% of all ascertained ICHs and they were significantly younger, less likely to die in the first 30 days after their ICH and more likely to have a past history of hypercholesterolaemia compared to those who did not become cases.

Despite the development of the Boston criteria [Knudsen et al. 2001] and more recent modified Boston criteria [Linn et al. 2010] for the diagnosis of 'CAA-related ICH' in patients with lobar ICH and their increasing application over the last decade, especially

in research settings for classifying those participants without pathological confirmation of CAA with a probable CAA-related ICH, the criteria are yet to be applied to a population based cohort of participants with ICH.

Therefore I sought to describe the incidence of both first-ever and recurrent spontaneous primary and secondary definite ICH in a population based study in South East Scotland and compare the prevalence of certain predefined risk factors in those with lobar vs. non-lobar ICH. In addition, I integrated the MRI brain findings and post-mortem examination results (where applicable) of study participants to describe the frequencies of possible, probable and definite CAA-related ICH according to the modified Boston criteria in a population-based cohort of participants with first-ever lobar ICH. These analyses are based upon participants recruited in the first complete year of the study (1st June 2010-31st May 2011).

5.2 Methods

5.2.1 Incidence

I used the first complete year of the study (1st June 2010-31st May 2011) to calculate the incidence of spontaneous ICH in adults meeting our inclusion and exclusion criteria (Section 2.2, page 60). The crude incidence is the proportion of the adults (aged ≥16 years) on 1st June 2010 who had an ICH during the first year of the study. I used mid-2010 United Kingdom (UK) population estimates from the Office for National Statistics (http://www.ons.gov.uk/ons/taxonomy/index.html?nscl=Population+Estimates) to calculate age standardised incidence estimates using direct standardisation. I distinguished primary from secondary ICH and first-ever from recurrent primary ICH, since the causes of each may differ.

5.2.2 Comparison of lobar vs. non-lobar ICH

I have compared baseline demographic and clinical characteristics in adults with first-ever lobar and non-lobar ICH.

5.2.3 Definition of lobar and non-lobar ICH

5.2.3.1 Lobar ICH

I categorised an ICH as lobar if it involved one or more lobes of the brain. The following ICH(s) were classified as lobar:

- a single ICH involving any of the following areas: frontal, frontotemporal, frontoparietal, parietal, parieto-temporal, parieto-occipital, temporal, temporo-occipital or occipital;
- an ICH which involved both lobar and supratentorial 'deep' (Section 5.2.3.2 below) regions of the brain; or
- multiple ICHs in either solely lobar locations or where at least one ICH involved a lobar location.

5.2.3.2 Non-lobar ICH

I categorised an ICH as non-lobar if it did not extend to a lobar area and was:

- a single infratentorial ICH (located in the brainstem or cerebellum);
- a single supratentorial deep ICH (located in the basal ganglia, internal capsule or thalamus); or
- multiple ICHs in solely non-lobar locations (either supratentorial deep or infratentorial).

5.2.3.3 Definitions of clinical variables

I defined the clinical characteristics of participants as follows.

(i) Hypertension: If this diagnosis had been made prior to their ICH and recorded in GP or hospital notes, or if the participant had been prescribed medications for the treatment of hypertension.

(ii) Diabetes: If this diagnosis had been made prior to their index ICH and recorded in their GP or hospital notes.

(iii) Dementia: If these diagnoses had been made prior to their ICH and recorded in their GP or hospital records. Where a relative or close friend was available I also used the short form of the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) with a cut-off ≥ 64 for dementia [Jorm 1994].

(iv) Smoking: I classified participants as never having smoked, being an ex or a current smoker at the time of their ICH. The definition was based on the history obtained from the participant or their relative and if neither was available, their medical records.

(v) Alcohol: I obtained a history of average alcohol consumption in units per week in the month prior to the ICH from the participant or their relative where available, or their GP or hospital records.

(vi) Antithrombotic use: A participant was classified separately as taking antiplatelet or anticoagulant medications if they had been taking either medication until their ICH.

5.2.3.4 Definitions of other radiological variables

I defined other radiological characteristics of ICHs as follows.

(i) Multiple ICH: If there was more than one acute ICH present on the diagnostic brain imaging obtained at presentation. 'Acute' ICH was determined radiologically by the presence of a hyperdense area in the brain parenchyma (Section 2.5.2, page 64).

(ii) Intraventricular extension: If any blood was present within the ventricles of the brain on review of the imaging by a neuroradiologist with a special interest in stroke.

(iii) Subarachnoid extension: If any blood was present within the subarachnoid compartment of the brain on review of the imaging by a neuroradiologist with a special interest in stroke.

(iv) Subdural extension: If any acute blood was present within the subdural compartment of the brain on review of the imaging by a neuroradiologist without any clinical or radiological evidence of trauma (Section 2.5.4.2, page 66).

(v) ICH volume: Arthur Fonville (Medical Student, University of Amsterdam) measured ICH volume on the first CT brain scan following the participant's presentation with ICH (the diagnostic scan) using Quantomo computerised planimetry software (Cybertrial Inc, Calgary, Canada) [Kosior et al. 2011], which has been shown to have greater reliability for quantifying ICH volume in

comparison to the ABC/2 method [Kothari et al. 1996]. In the ABC/2 method, A is the longest diameter on the axial CT slice which shows the largest volume of ICH, B is the diameter perpendicular to A and C is the depth of the haemorrhage as measured by the number of axial CT slices on which the ICH is visible [Kothari et al. 1996]. Quantomo allows tailoring of individual haematoma shapes whereas ABC/2 approximates haematomas to ellipsoids which may lead to an overestimation of ICH volume.

5.2.4 Statistical analyses

I calculated 95% CI around incidence estimates using the Wilson score interval [Newcombe 1998]. I used parametric statistics for between group comparisons when the data had a normal distribution and log-transformed variables or used non-parametric statistics when it did not. If an estimated cell count was less than five, I used the Fisher's exact test. I conducted all statistical analyses using Stata version 11.1 (StataCorp LP, Texas, USA). All p values are two-sided.

5.2.5 Modified Boston criteria for cerebral amyloid angiopathy

I applied the modified Boston diagnostic criteria for CAA [Knudsen et al. 2001;Linn et al. 2010] to our cohort to determine the frequency of possible, probable and definite CAA-related ICH in a population-based cohort of those with first-ever lobar ICH.

5.2.5.1 Pathological specimens

Since the modified Boston criteria require confirmation of CAA on at least one pathology specimen for a diagnosis of 'definite CAA' or 'probable CAA with supporting pathology' I reviewed the pathology findings for participants with lobar ICH who had undergone post-mortem examination (Chapter 10).

To fulfil the criteria for 'definite CAA', vasculopathy and 'severe' CAA must be present in at least one pathology specimen. To fulfil the criteria for 'probable CAA with supporting pathology' CAA of any severity must be present in at least one pathology specimen without vasculopathy and without an alternative cause of ICH [Knudsen et al. 2001].

Dr Colin Smith (Consultant Neuropathologist, University of Edinburgh) rated CAA according to the Bristol rating scale [Love et al. 2014] which rates the severity of both

parenchymal and meningeal CAA, presence or absence of CAA in the capillaries and extent of vasculopathy in each of the lobes of the brain (Table 6 on page 144). Vasculopathy is defined as the presence of fibrinoid necrosis, thrombosis, haemorrhage or circumferential splitting of the vessel wall. I used parenchymal and meningeal CAA ratings of two ('some circumferential amyloid') and three ('widespread circumferential amyloid') as markers of 'severe' CAA.

5.2.5.2 Brain imaging

The presence of multiple ICHs restricted to lobar, cortical or subcortical regions (including the cerebellum) differentiates probable CAA-related ICH (without pathological confirmation) from possible CAA-related ICH, in which only a single ICH is present [Knudsen et al. 2001].

The definition of 'multiple' ICHs is:

- more than one acute lobar or cerebellar ICH on axial CT or MRI;
- more than one lobar or cerebellar ICH on T2*-weighted sequences (in which case the haemorrhage(s) may be acute or chronic);
- one ICH in addition to at least one BMB on T2*-weighted MRI sequences, both of which are restricted to lobar or cerebellar regions of the brain; or
- recurrent lobar or cerebellar ICH; that is: one acute ICH in addition to evidence of at least one previous ICH on either CT or T2*-weighted MRI sequences, both restricted to lobar or cerebellar regions of the brain.

Since I applied the modified Boston criteria to those with first-ever lobar ICH, I did not categorise any participants with recurrent ICH as having probable CAA-related ICH. A neuroradiologist with a special interest in stroke rated all MRI scans according to a modified version of the Brain Observer MicroBleed rating scale (BOMBS [Cordonnier et al. 2009]; Chapter 3 and Appendix). The scale rates BMBs according to their certainty, and for the purposes of this analysis, only 'certain' BMBs were used.

5.3 Results

5.3.1 ICH cases

Figure 24 on page 147 illustrates the identification of adults with spontaneous ICH from 1st June 2010 to 31st May 2011. Of 216 possible cases 27 were excluded, leaving 166 definite cases of primary and secondary ICH and 23 suspected (but unconfirmed) cases of ICH. Of 166 definite cases, the diagnosis of ICH was confirmed by CT brain imaging in 162 (98%) cases with the remainder diagnosed at post-mortem examination. CT brain imaging was done within two days of ICH onset in 144 (87%) cases. There were 141 cases of spontaneous primary ICH of which 128 (91%) were first-ever.

5.3.2 Secondary ICH

The causes of 25 secondary ICHs are shown in Figure 24 on page 147. Adults with secondary ICH were significantly younger than those with primary ICH (median age (years) 57, interquartile range [IQR] 49-63 vs. 79; IQR 66-83; $p<0.001$). 19 (76%) were lobar.

5.3.3 Suspected ICH

23 adults died from suspected but unconfirmed spontaneous ICH. These adults tended to be older than adults with a confirmed diagnosis of spontaneous primary ICH (median age (years) 86, IQR 79-91 vs.79; IQR 66-83; $p<0.001$). 13 died in the Emergency department and were identified through surveillance of the electronic patient records system and the remainder died in the community before a diagnosis of ICH could be reached and were identified through calls made by GPs to the Office of the Procurator Fiscal. When a participant's final illness had been witnessed, the modes of presentation were rapid onset loss of consciousness (n=11), hemiparesis (n=3), sudden severe headache (n=2) and seizure followed by cardiac arrest (n=1). Four adults were found dead at home and a stroke was suspected because of their past medical histories of stroke. In two cases, 'stroke' had been listed as the main cause of death on their death certificates without any further details being available.

5.3.4 Crude incidence of definite ICH (confirmed by imaging or post-mortem examination)

The crude incidence of first-ever and recurrent spontaneous (primary and secondary) definite ICH in adults in the Lothian health board region of Scotland during 1st June 2010-31st May 2011 was 0.24 per 1000 per year (95% CI 0.21 to 0.27). The incidence of first-ever spontaneous primary definite ICH was 0.18 per 1000 per year (95% CI 0.16 to 0.22). If all suspected ICH cases (Section 5.3.3, page 128) were confirmed as spontaneous primary ICH the incidence would be 0.22 per 1000 per year (95% CI 0.19 to 0.25).

5.3.5 Age standardised incidence of first-ever spontaneous primary definite ICH

The age-standardised incidence of first-ever spontaneous primary definite ICH, directly adjusted to the mid-2010 population estimates for the UK was 0.21 per 1000 per year (95% CI 0.20 to 0.21), which is very similar to the crude incidence of spontaneous primary definite ICH (Section 5.3.4, above).

5.3.6 Age and sex-specific incidence of first-ever spontaneous primary definite ICH

As expected, the incidence of first-ever spontaneous primary definite ICH increased with age (Figure 25 on page 148). Although males had a higher incidence of ICH in the 45-54 years age band there was no overall difference in the incidence of ICH in males vs. females (Table 7 on page 145).

5.3.7 Age specific incidence of lobar and non-lobar first-ever spontaneous primary definite ICH

The incidence of both first-ever lobar and non-lobar ICH increased across all age groups (Table 7 on page 145). The incidence of lobar ICH was higher than non-lobar ICH in the 75-84 years age band but 95% CI were broad and the overall incidences of lobar and non-lobar ICH were similar.

5.3.8 Locations of haemorrhages in adults with first-ever spontaneous primary ICH

Primary first-ever ICH locations were single lobar (n=61), single non-lobar (n=59), single lobar extending to non-lobar regions (n=4), multiple lobar and non-lobar (n=2), multiple lobar (n=1) and multiple non-lobar (n=1). 68 participants (53%) had a lobar

ICH (an ICH which involved at least one lobe of the brain) and 60 participants had non-lobar ICH.

5.3.9 Baseline characteristics of adults with first-ever lobar and non-lobar ICH

The characteristics of adults found to have an ICH involving any lobar region (n=68) are compared with those with ICH in non-lobar regions (n=60) in Table 8 on page 146.

An IQCODE was available for 47 participants (37%). There was no difference in age (has IQCODE: mean 75 years, IQR 66-81 vs. no IQCODE: mean 79, IQR 68-85; p=0.14), gender (males 19/47 (40%) vs. 36/81 (44%); χ^2 =0.20, p=0.66) or ICH location (any lobar 27/47 (57%) vs. 41/81 (51%); χ^2 =0.56, p=0.46) between participants who had an IQCODE and those who did not. A history of dementia was more common in adults with lobar ICH. Pre-morbid hypertension did not vary according to ICH location.

65 out of 128 participants (51%) were using antithrombotic medication prior to their ICH and this proportion did not vary according to ICH location. The incidence of primary first-ever ICH associated with anti-thrombotic medication use was 0.09 per 1000 per year (95% CI 0.07 to 0.12).

Admission GCS scores were significantly higher and ICH volumes were larger in the lobar ICH group. Extension of the ICH into the subdural and subarachnoid compartments was more common in the lobar ICH group. In a post-hoc analysis, extension of ICHs into the subdural and subarachnoid compartments of the brain was associated with larger ICH volumes (subarachnoid extension; present - median ICH volume (ml^3) 44; IQR 24-77; absent -12; IQR 4 -37; p<0.001 and subdural extension; present - median ICH volume (ml^3) 42; IQR 15-78; absent - 20; IQR 5-49; p=0.046).

5.3.10 Application of the modified Boston criteria to adults with first-ever spontaneous primary lobar ICH

Of 68 participants with first-ever lobar ICH, five were aged less than 55 years at the time of their ICH and four had ICH involving both lobar and supratentorial deep regions and therefore could not be considered as having CAA (Figure 26 on page 149). 14 participants out of the remaining 59 had MRI with T2*-weighted sequences available for interpretation of brain BMBs and a further 13 had post-mortem examination specimens.

Six participants with lobar ICH fulfilled the criteria for definite CAA-related ICH; five had probable CAA-related ICH (without supporting pathology) and one had probable CAA-related ICH with supporting pathology. 47 out of 59 eligible participants (80%) fulfilled the criteria for possible CAA-related ICH. No participants had superficial siderosis on MRI.

5.4 Summary

- During 1st June 2010-31st May 2011 the crude incidence of spontaneous definite ICH was 0.24 per 1000 per year (95% CI 0.21 to 0.27) and the incidence of first-ever spontaneous primary definite ICH was 0.18 per 1000 per year (95% CI 0.16 to 0.22) and did not vary by ICH location.
- Lobar ICHs were more likely to have a past history of dementia in comparison to non-lobar ICH, but there were no other differences in pre-ICH characteristics between lobar and non-lobar ICH.
- Lobar ICHs were associated with a lower GCS score on admission.
- Lobar ICHs were larger and associated with extension of the ICH into the subarachnoid and subdural compartments of the brain.
- 12 out of 59 participants with first-ever lobar ICH eligible to be considered under the modified Boston criteria, fulfilled the criteria for definite or probable CAA-related ICH.

5.5 Discussion

5.5.1 Strengths of the study

Strengths of the study include its prospective, population-based design with multiple overlapping sources of case ascertainment, comprehensive data collection and the availability of post-mortem examination findings using an extensive sampling protocol for CAA. Although I did not formally assess the inter-rater reliability of classifying ICH location, all ICHs were classified as lobar or non-lobar by an experienced neuroradiologist with a special interest in stroke, in a multi-disciplinary meeting in which usually more than one neuroradiologist was present, and a consensus decision was

reached. There has been good inter-observer agreement in the assessment of supratentorial ICH location even for larger haematomas [Wermer et al. 2002].

5.5.2 Limitations of the study

It was difficult to apply the modified Boston criteria to this population-based cohort of participants with ICH because I was only able to obtain brain MRI, despite being funded to do so, on 24% of patients with first-ever lobar ICH (Section 5.5.3.9, page 138).

5.5.3 Study findings

5.5.3.1 Incidence of spontaneous primary definite ICH

The incidence of spontaneous primary definite ICH in our study is comparable to the findings of a recent systematic review and meta-analysis in which the incidence of ICH had not changed for several decades [van Asch et al. 2010]. The age-standardised incidence of ICH when adjusted to the population of the UK was very similar to the unadjusted incidence of ICH, indicating that the Lothian health board population is likely to be demographically representative.

In keeping with other studies [van Asch et al. 2010], the incidence of ICH increased with age with the highest incidences in those aged 65 years or older. This is of particular significance given the ageing population of the UK in which the proportion of those aged 65 years or older is set to increase from 17% to 24% between 2010 and 2051 [Office for National Statistics 2012].

5.5.3.2 Incidence of suspected (unconfirmed) ICH

12% of potential ICH cases were suspected but unconfirmed. These patients tended to be older, and were either found dead or deteriorated rapidly soon after symptoms were first noted so that nearly half died before reaching hospital. A diagnosis of stroke is recognised as being harder to confirm in the elderly [Giroud et al. 1991]. Prior comorbidities may dictate that a patient is too unwell for hospital transfer and further investigation. Presentations may be atypical leading to a delay in suspecting a diagnosis of stroke. Although this raises the possibility that I under-ascertained ICH in older age groups, the incidence of ICH in those aged 85 and above is almost identical to that found in other studies [van Asch et al. 2010]. Future studies might seek to determine

the incidence of ICH in those who die suddenly in the community by use of post-mortem imaging techniques [Roberts et al. 2012].

5.5.3.3 Secondary ICH

It is unsurprising that those adults with secondary ICH were younger than adults with primary ICH since clinicians are more likely to investigate younger adults with ICH for underlying causes whereas an ICH in an older person may be attributed to CAA or pre-existing hypertension [Cordonnier et al. 2010a]. The findings are therefore subject to detection bias since the label of 'primary' ICH is dependent on no other secondary cause having been ascertained. Ideally, the impact of detection bias might be evaluated by screening all ICH participants routinely using angiographic imaging +/- MRI. However, since many participants are too unwell this would be impossible. A systematic review of studies investigating spontaneous ICH for a secondary cause, revealed that the prevalence of underlying AVMs and aneurysms in participants who had undergone IADSA was 20% and 13% respectively [Cordonnier et al. 2010a]. Even though the incidence of AVMs and aneurysms in my cohort is lower (3% [95% CI 1-7%] and 6% [95% CI 3-11%]) respectively, the participants formed an unselected cohort, the majority of whom were older than patients included in studies identified by the systematic review [Cordonnier et al. 2010a]. Moreover, in the 58 participants (45%) with first-ever spontaneous primary ICH who underwent further investigation (MRI (n=30), post-mortem examination (n=25), CT angiography (n=2), IADSA (n=1)) I did not find a secondary cause, and therefore it is less likely that I have under-ascertained a considerable number of secondary ICHs.

5.5.3.4 Lobar and non-lobar ICH

If the main causative factor for non-lobar ICH is hypertension and CAA is implicated in lobar ICH, one might expect a decrease in the incidence of non-lobar ICH given the decreases in mean systolic blood pressures in recent years in Western European populations [Danaei et al. 2011].

I did not demonstrate this and possible reasons are:

(i) I had small numbers of ICHs producing broad estimates of incidence and a lack of power to detect such a difference.

(ii) Since both hypertension and CAA are more prevalent with increasing age, they are likely to co-exist with each other and therefore dichotomisation of CAA as the main cause of lobar ICH and hypertension for non-lobar ICH may be too simplistic.

5.5.3.5 Pre-ICH clinical characteristics

The frequency of antithrombotic medication use was comparable to that of another contemporary population-based cohort [Bejot et al. 2013]. A past medical history of dementia was more common in participants with lobar ICH but I did not demonstrate any other significant differences in pre-ICH characteristics between non-lobar and lobar ICHs.

There are several possible explanations for this. The size of the cohort is relatively small and may lack sufficient power. Continued recruitment of participants over the next few years will determine whether the lack of difference in most baseline characteristics in the first year remains in a larger cohort. Although I was unblinded to the location of the participant's ICH when assessing characteristics, any information bias introduced by this would have been more likely to lead to a Type one error rather than mask a significant difference between the groups.

It is plausible that some exposures such as smoking or alcohol consumption were incorrectly reported if participants or their relatives provided an answer which they thought would appear favourable. However, I sought to minimise this by corroborating information with primary care records in which the documentation of exposure predated the occurrence of ICH. Participants were also unaware of the study background and therefore any misclassification of exposure is likely to have been non-differential (equal likelihood of subjects in both lobar and non-lobar ICH having their baseline characteristics misclassified) and therefore biased results towards the null. The extent of misclassification bias could in the future be assessed by comparing the responses obtained for variables such as smoking or alcohol consumption when the participant is interviewed vs. a relative vs. review of their GP records.

5.5.3.6 Dementia

I demonstrated an association between dementia and lobar ICH which is consistent with the findings of three previous hospital-based studies [Cordonnier et al. 2010b;Biffi

et al. 2011b;Jamieson et al. 2012] although two other hospital-based studies failed to find any association [Lipton et al. 1987;Greenberg et al. 1996].

The prevalence of prior dementia of 13% (95% CI 9-20%) in our population-based cohort is very similar to the only previous study which recruited consecutive patients with ICH [Cordonnier et al. 2010b].

I classified prior cognitive impairment and dementia on the basis of medical records and used an IQCODE where available. An IQCODE was available for 37% (n=47) of the cohort. Its use was limited by the substantial number of participants who died either prior to hospital admission (n=3) or soon after admission when it was not appropriate to approach a close relative for completion of a questionnaire (n=37). The IQCODE formed part of the clinical assessment in the LINCHPIN research study and therefore its use was limited to those who gave written informed consent. Some participants either did not consent to participate in the study (n=37), lacked a suitable friend or relative of longstanding (n=2) or did not want a relative to complete a questionnaire regarding their memory (n=2).

It is notable that when the IQCODE was compared to medical records alone for the detection of pre-existing cognitive impairment or dementia (using the IQCODE as the gold-standard), medical records had 30% sensitivity (95% CI 15-52%) and 100% specificity (95% CI 88-100%). Screening of medical records alone did not detect two cases of dementia and 12 cases of cognitive impairment. Therefore it is plausible that I failed to detect some cases of dementia which would have been detected by more widespread use of the IQCODE and therefore the frequency of dementia might be even higher than demonstrated. However, since there was no difference in demographic characteristics or ICH location between participants for whom an IQCODE was obtained and others any misclassification of exposure is likely to have been non-differential.

Since both lobar ICH and senile dementia of Alzheimer's type are associated with CAA [Esiri & Wilcock 1986;Chalmers, Wilcock, & Love 2003] it is plausible that dementia is associated with lobar ICH. However, the majority of those with lobar ICH do not have dementia and therefore other factors such as a past history of hypertension are likely to contribute to lobar ICH. It would be useful in a larger sample to compare the characteristics of demented and undemented participants with lobar ICH to assess

whether the clinical picture of lobar ICH differs, for example in risk factors, radiological features or prognosis. One hospital-based study has shown an association between dementia and mortality from lobar ICH at 90 days (although not at earlier time points) [Jamieson et al. 2012]. However, the authors assessed dementia retrospectively, entered numerous variables into their multivariate analysis and did not account for other indicators of poor prognosis such as intraventricular extension of the ICH.

Future studies should seek to link clinical diagnoses of pre-existing dementia in patients with ICH with their neuropathological correlates, which may guide further research into the roles of small vessel disease such as CAA in causing ICH.

5.5.3.7 Hypertension

I did not demonstrate any association between hypertension and either lobar or non-lobar ICH.

There are several possible explanations. Firstly, a recent systematic review of hypertension in lobar and deep supratentorial ICH showed that although overall, hypertension was more common in deep ICH the association was weaker in population-based studies which used a pre-stroke definition of hypertension and did not include recurrent ICH [Jackson & Sudlow 2006]. Moreover, given that my sample was relatively small, I may have been underpowered to detect any association if one does exist. Since prescription of antihypertensive medications reduces the risk of recurrence of both ICHs attributed to CAA and hypertension [Arima et al. 2010], hypertension is likely to be a risk factor for both lobar and non-lobar ICH, so a much larger sample is likely to be needed to demonstrate an association between hypertension and non-lobar (in comparison to lobar) ICH.

Secondly, there is the possibility that incorrect categorisation of supratentorial ICHs as lobar or non-lobar may have caused misclassification bias. I think this is less likely since one or more neuroradiologists with a special interest in stroke reviewed every diagnostic scan. However, radiologists were unblinded to the clinical history to enable consideration of alternative diagnoses such as HTI and secondary causes of ICH which may have inevitably led to bias.

Thirdly, the proportion of people classified as hypertensive may also differ depending upon whether the definition is based upon medical records, recent blood pressure

measurements or both and whether those with treated hypertension in which the most recent blood pressure measurement is ≤ 140/90 mmHg are excluded [Lovelock, Molyneux, & Rothwell 2007]. I classified a participant has having pre-ICH hypertension if they had a diagnosis of hypertension listed in their medical records or they were taking antihypertensive medications prior to their ICH. One study which compared different definitions of hypertension for using in a model to predict future diabetes found that using a combination of medical records and antihypertensive medications to define hypertension had the highest sensitivity for detection of diabetes [Gulliford, Charlton, & Latinovic 2006], although the impact of using different definitions of hypertension in a population-based cohort with ICH is unknown.

Lastly, I included infratentorial ICH in my definition of non-lobar ICH. A further possibility is that the association between hypertension and infratentorial ICH is weaker than it is for supratentorial deep ICH and by classifying both types as non-lobar, any association is obscured.

5.5.3.8 Imaging variables

I demonstrated that lobar ICHs were larger and more commonly associated with subarachnoid and subdural haemorrhage in comparison to non-lobar ICH. ICHs which extended into these compartments were also larger than those that did not. This is anatomically plausible given the proximity of superficial lobar ICHs to the subarachnoid and subdural compartments of the brain and consistent with previous studies [Patel et al. 2009;Kuramatsu et al. 2011].

I did not record whether any subdural haemorrhage occurred in the contralateral hemisphere to the ICH although one previous study did not observe any contralateral subdural haemorrhages in association with ICH [Patel et al. 2009]. Subdural haemorrhage is commonly associated with trauma but I think it is unlikely that I misclassified traumatic ICHs as spontaneous because of comprehensive review of both the participant's clinical history and their brain imaging (Chapter 2).

Both subarachnoid and subdural haemorrhage (without ICH) have been found in association with CAA in neuropathological case series studies [Vinters 1987] and one study which correlated radiological with pathological findings in seven people with ICH found that ICHs related to CAA extended into the subarachnoid space [Millar,

Wardlaw, & Lammie 1999]. Radiopathological correlation of lobar ICHs (Chapter 10) should clarify whether these radiological features discriminate between lobar ICHs related to CAA and those where no CAA is found.

5.5.3.9 Application of the modified Boston criteria to my cohort

The study shows the difficulty in distinguishing probable or definite CAA-related ICH cases from possible CAA-related ICH cases using the modified Boston criteria in an unselected cohort of participants with lobar ICH, even though 42% of them (n=25) had either MRI or post-mortem examination specimens. 47 (80%) participants out of 59 who were eligible to be categorised under the modified Boston criteria had possible CAA-related ICH. Although many of our participants did not have MRI, 16 participants (27% of those eligible for consideration under the modified Boston criteria) underwent MRI as part of the LINCHPIN research study, none of whom would have had MRI routinely, since CT remains the most frequently used imaging modality for the diagnosis of stroke in the UK [Muir & Santosh 2005].

The Boston criteria were validated in a selected sample of 39 participants at a tertiary referral centre and their sensitivity for detection of CAA was 45% (95% CI 28-62%), (specificity 100%; 95% CI 77-100%) [Knudsen et al. 2001]. The number of neuropathological samples viewed per brain and their locations is unclear, which may influence the likelihood of finding other pathologies in the brain such as small vessel disease and determining the relative contribution of each to the ICH.

The presence of superficial siderosis on T2*-weighted MRI sequences is thought to improve the sensitivity of the criteria for detecting CAA [Linn et al. 2010] but this did not increase the proportion of participants in our cohort with probable CAA-related ICH since only two participants had superficial siderosis; one of whom was less than 55 years of age (and therefore not eligible for consideration under the modified Boston criteria) and another, who had BMBs in a lobar distribution and therefore already had probable CAA-related ICH.

5.5.4 Future directions

The findings of this study indicate the need to increase our understanding of the causes of ICH by using uniform definitions of both risk factors and outcomes (especially ICH

location) and improving the sensitivity of the modified Boston criteria for diagnosing CAA.

Since the association between hypertension and ICH is likely to vary depending on the definition of hypertension used, it would be useful in future studies to compare how the association between hypertension and ICH location varies with exposure definition and whether its effect is modified when adjusted for other risk factors such as age.

It is essential to ensure consistency in assigning ICH locations. There are only two studies to my knowledge which have examined the inter-rater reliability of ICH categorisation as lobar or deep in a total of 93 selected CT brain scans [Wermer et al. 2002;Parameswaran et al. 2003]. Both studies used radiologists with extensive experience in neuroradiology and reported excellent inter-rater reliability as measured by the kappa coefficient (κ 0.78-0.96). In the future, it would be useful to study the inter-rater and intra-rater reliability of categorisation of ICH location on consecutive scans (both blinded and unblinded to clinical details) using clinicians with a variety of radiology experience (such as general radiologists, neurologists and stroke physicians) and explore whether certain factors are associated with lower inter-rater agreement; for example, larger ICHs or involvement of regions such as the insula.

The majority of those with first-ever lobar ICH in my community-based cohort had only possible CAA-related ICH according to the modified Boston criteria which is of limited clinical value in determining ICH cause. Consideration of other features such as a pre-ICH history of dementia or the distribution of WMH on MRI, since an occipital-predominant WMH distribution has been associated with lobar ICH [Zhu et al. 2012], might improve the sensitivity of the criteria. Radio-pathological correlation studies of lobar ICH may lead to ascertaining CT imaging features which distinguish lobar ICH related to CAA from other lobar ICH. Addition of these would increase the external validity of the criteria which are currently limited by their need for MRI which is often unavailable or will not be tolerated by patients with an ICH.

Table 5 Population-based and hospital-based studies comparing risk factors for lobar and non-lobar ICH

Study (year)	N (non-lobar/lobar)	Female sex commoner in lobar ICH	Older age associated with lobar ICH	Race	Hypertension more common in non-lobar ICH	Atrial fibrillation	Diabetes more common in non-lobar ICH	Ischaemic heart disease more common in non-lobar ICH	Prior stroke of any type	Prior ischaemic stroke	Peripheral vascular disease	Hyperlipidaemia	Anticoagulants/coagulopathy more common in lobar ICH	Antiplatelet use	Alcohol use more common in lobar ICH[$]	Current smoking more common in non-lobar ICH	Cognitive impairment/Dementia more common in lobar ICH	↑Body mass index in non-lobar ICH
Population-based studies																		
Boonyakarnkul (1993)	57 (27/30)	○			○													
Broderick (1993)	163 (97/66)				○													
Anderson (1994)	60 (32/28)				○													
Giroud (1995)	117 (64/53)				○		○					○	○		○	○		
Nilsson (2000)	341 (165/176)	○	○		○		○	○	○		○		○	○				
Tatu (2000)	326 (128/198)				○								○		○			
Inagawa (2003)	350 (297/53)				●		○								○	○		○
Karapanyiotides (2004)	330 (163/167)				●		○											
Labovitz (2005)	150 (101/49)	○	●	●[2]	●		○								○	○		
Zahuranec (2006)	260 (173/87)			●~[2]														

Study (year)	N (non-lobar/lobar)	Female sex commoner in lobar ICH	Older age associated with lobar ICH	Race	Hypertension more common in non-lobar ICH	Atrial fibrillation	Diabetes more common in non-lobar ICH	Ischaemic heart disease more common in non-lobar ICH	Prior stroke of any type	Prior ischaemic stroke	Peripheral vascular disease	Hyperlipidaemia	Anticoagulants/coagulopathy more common in lobar ICH	Antiplatelet use	Alcohol use more common in lobar ICH[$]	Current smoking more common in non-lobar ICH	Cognitive impairment/Dementia more common in lobar ICH	↑Body mass index in non-lobar ICH
Zia (2006)	141 (90/51)				○		○					○			○	○		○
Sacco (2009)	415 (205/210)	○	○		●													
Lavados (2010)	58 (43/15)	○	○		○		○					○	○	○	○	○		
Other studies																		
Brott (1986)	140 (67/73)				○													
Lipton (1987)	112 (70/42)	○	○	●[1]	●	○			○				●				○	
Fieschi (1988)	101(60/41)				○													
Massaro (1991)	172 (107/65)	●	○				○	○	○						○			
Radberg (1991)	200 (120/80)				○													
Yaqub (1991)	13 (10/3)	○	○				○	○										
Franke (1992)	157 (97/60)		○		○								○					
Berlit (1994)	321 (67/254)				○													
Graffagnino (1994)	45 (29/16)	○	●	○	○													

Study (year)	N (non-lobar/lobar)	Female sex commoner in lobar ICH	Older age associated with lobar ICH	Race	Hypertension more common in non-lobar ICH	Atrial fibrillation	Diabetes more common in non-lobar ICH	Ischaemic heart disease more common in non-lobar ICH	Prior stroke of any type	Prior ischaemic stroke	Peripheral vascular disease	Hyperlipidaemia	Anticoagulants/coagulopathy more common in lobar ICH	Antiplatelet use	Alcohol use more common in lobar ICH[$]	Current smoking more common in non-lobar ICH	Cognitive impairment/Dementia more common in lobar ICH	↑Body mass index in non-lobar ICH
Greenberg (1996)	63 (18/45)		●		○												○	
Rosenow (1997)	871 (430/441)				●													
Ruiz-Sandoval (1999)	179 (69/110)				●													
Woo (2002)	188 (121/67)				●		●	○	○				○	○	○	○		
Bilbao (2005)	356 (190/166)				●													
Seifert (2006)	189 (128/61)				○		○											
Telman (2010)	464 (282/182)	○~	○~		○~		○~					○~				○~		
Biffi (2011)	384 (196/188)	○	●	○	●	○	○	●		○			○	○			●	●
Ruiz-Sandoval (2011)	540 (342/198)	○	○		●													
Kuramatsu (2011)	174 (96/78)	○	●		○		○			○	○	○		○	○	○		○
Weimar (2011)	780 (509/271)	●	●		●	○	●	○	○		○	○			○	○		
Arboix (2012)	189 (92/97)	●	○		●	○	○	○		○	○	○	○		○	○		●
Chen (2012)	2009 (1491/518)												●					

Study (year)	N (non-lobar/lobar)	Female sex commoner in lobar ICH	Older age associated with lobar ICH	Race	Hypertension more common in non-lobar ICH	Atrial fibrillation	Diabetes more common in non-lobar ICH	Ischaemic heart disease more common in non-lobar ICH	Prior stroke of any type	Prior ischaemic stroke	Peripheral vascular disease	Hyperlipidaemia	Anticoagulants/coagulopathy more common in lobar ICH	Antiplatelet use	Alcohol use more common in lobar ICH[$]	Current smoking more common in non-lobar ICH	Cognitive impairment/Dementia more common in lobar ICH	↑Body mass index in non-lobar ICH
Jamieson (2012)	136 (83/53)	○	○		○		○		○				○	○			●	
Matsukawa (2012)	361 (283/78)	●	●		●			○		○		○	○	○	●	○		○
Martini (2012)*	597 (380/217)		○		●					○			○		○	○		
Tveiten (2012)	110 (61/49)	○	○		●	○	○	○	○							●		
Pezzini (2013)	777 (490/287)																	●

○=no association ●=significant association [1]lobar ICH more common in whites, [2]Hispanic and Mexican American ethnicity more common in non-lobar ICH, [$]- variable definitions of alcohol use, ~ – multivariable analysis, *case control comparison

Table 6 Bristol rating scale for the assessment of CAA

Score	Parenchymal CAA	Meningeal CAA	Capillary CAA	Vasculopathic score
0	Absent	Absent	Absent	Absent
1	Scant deposition	Scant deposition	Present	Occasional vessel
2	Some circumferential amyloid	Some circumferential amyloid		Many vessels
3	Widespread circumferential amyloid	Widespread circumferential amyloid		

Table 7: Crude incidence of first-ever spontaneous primary ICH in the Lothian health board region during period 1st June 2010-31st May 2011 with 95% confidence intervals, by gender and ICH location (lobar vs. non-lobar ICH)

Age band (years)	Incidence (per 100,000 adults/year); (95% CI)		Incidence (per 100, 000 adults/year); (95% CI)	
	Males	Females	Lobar ICH	Non-lobar ICH
16-44	0.6 (0.1-3.2)	1.1 (0.3-4.0)	0.6 (0.2-2.0)	2.8 (0.05-1.6)
45-54	14.3 (7.2-28.1)	5.0 (1.7-14.7)	3.4 (1.3-8.9)	6.0 (2.9-12.4)
55-64	13.2 (6.1-28.9)	14.5 (7.0-29.9)	8.5 (4.3-16.9)	5.3 (2.3-12.5)
65-74	39.9 (22.8-69.7)	34.3 (19.6-59.9)	15.4(8.4-28.3)	21.5 (12.8-36.1)
75-84	97.6 (61.0-156.3)	118.7 (83.2-169.4)	72.6 (51.2-103.1)	37.5 (23.1-60.9)
85+	216.8 (121.1-387.8)	170.3 (109.1-265.8)	80.1 (46.8-137.0)	104.7 (65.4-167.7)
Overall	19.3 (15.1-24.6)	21.2 (17.0-26.5)	9.8 (7.7-12.4)	8.6 (6.7-11.1)

Table 8 Baseline characteristics of 128 adults with any lobar vs. non-lobar first-ever spontaneous primary ICH

	Any lobar (n=68)	Non-lobar ICH (n=60)	p value
Sex (male), (%)	26 (38)	29 (48)	0.25
Age (years); median (IQR)	79 (15)	76 (19)	0.84
History of hypertension*			
Yes (n, %)	40 (59)	41 (68)	0.21
History of diabetes*			
Yes (n, %)	5 (7)	8 (13)	0.25
History of dementia			
Yes (n, %)	14 (21)	3 (5)	0.01
History of smoking*			
Never (n, %)	30 (44)	26 (43)	
Ex-smoker (n, %)	20 (29)	24 (40)	
Current (n, %)	17 (25)	10 (17)	0.40
Alcohol consumption[$]	2 (0-14)	4 (0-14)	0.70
Premorbid medication			
Antiplatelet use* (n, %)	28 (41)	19 (32)	0.24
Anticoagulant use (n, %)	8 (12)	10 (17)	0.43
Admission GCS score**			
median (IQR)	13 (9-14)	14 (10-15)	0.03
ICH volume (ml)^			
median (IQR)	38 (17-74)	11 (4-25)	<0.001
Intraventricular extension^			
Yes (n, %)	33 (51)	31 (53)	0.85
Subarachnoid extension^			
Yes (n, %)	37 (57)	3 (5)	<0.001
Subdural extension^			
Yes (n, %)	10 (15)	2 (3)	0.02

*Data missing in one case
[$]average number of units per week (median; IQR); data missing in 9 cases
** data missing in five cases (three of whom died in the community)
^data missing in four cases for whom the diagnosis was confirmed at autopsy

Figure 24 Flowchart of participants with spontaneous ICH included in the study from 1st June 2010-31st May 2011

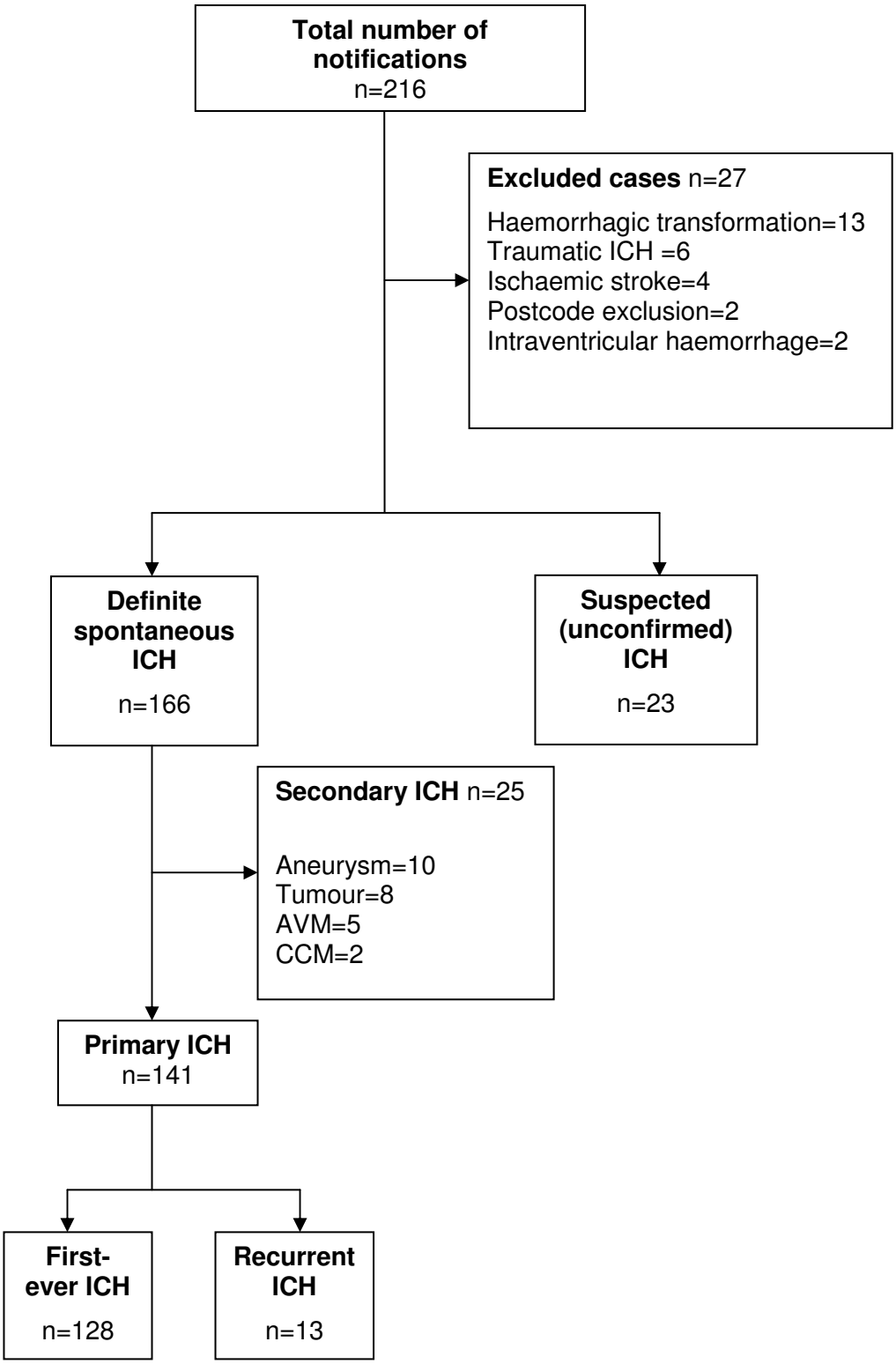


Figure 25 Age specific crude incidence of first-ever spontaneous primary ICH in the Lothian health board region during 1st June 2010-31st May 2011 with 95% confidence intervals

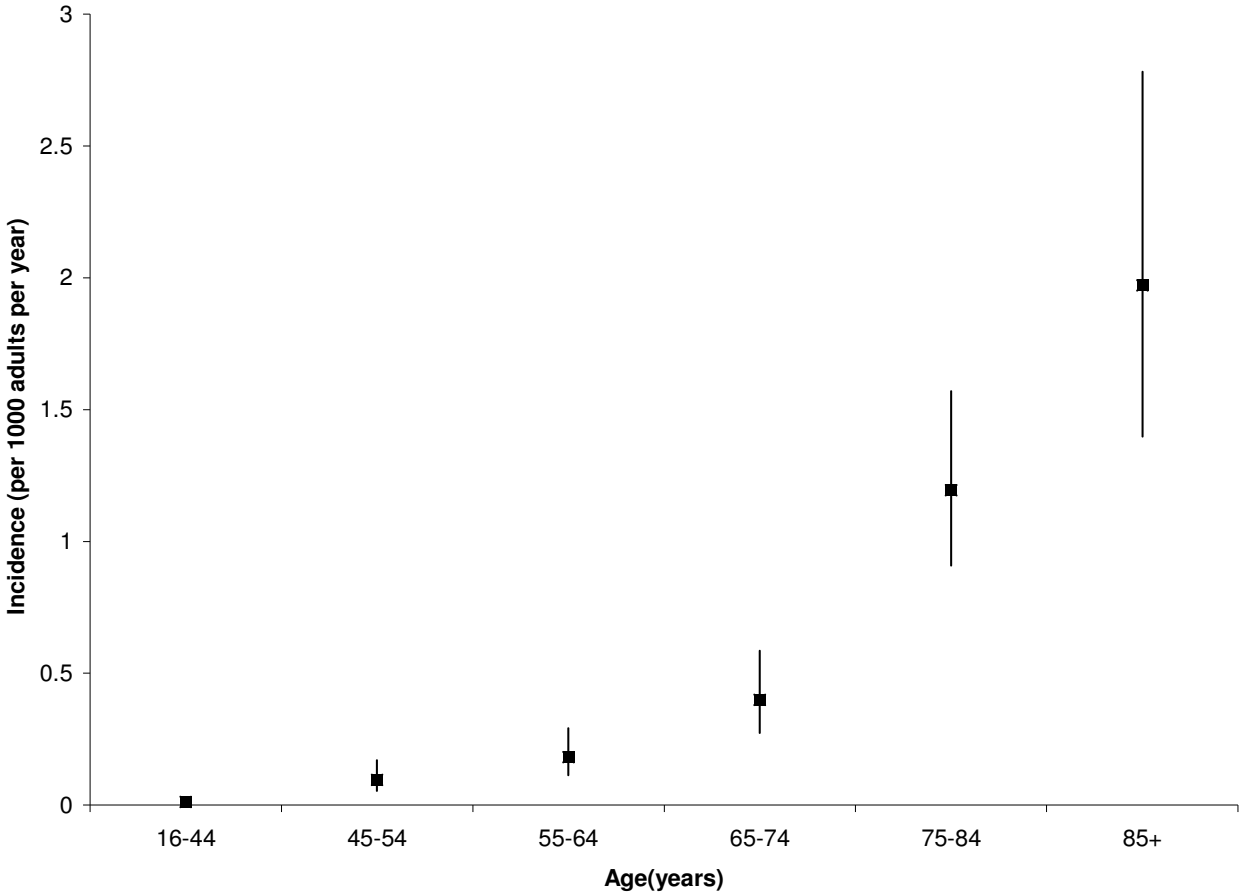
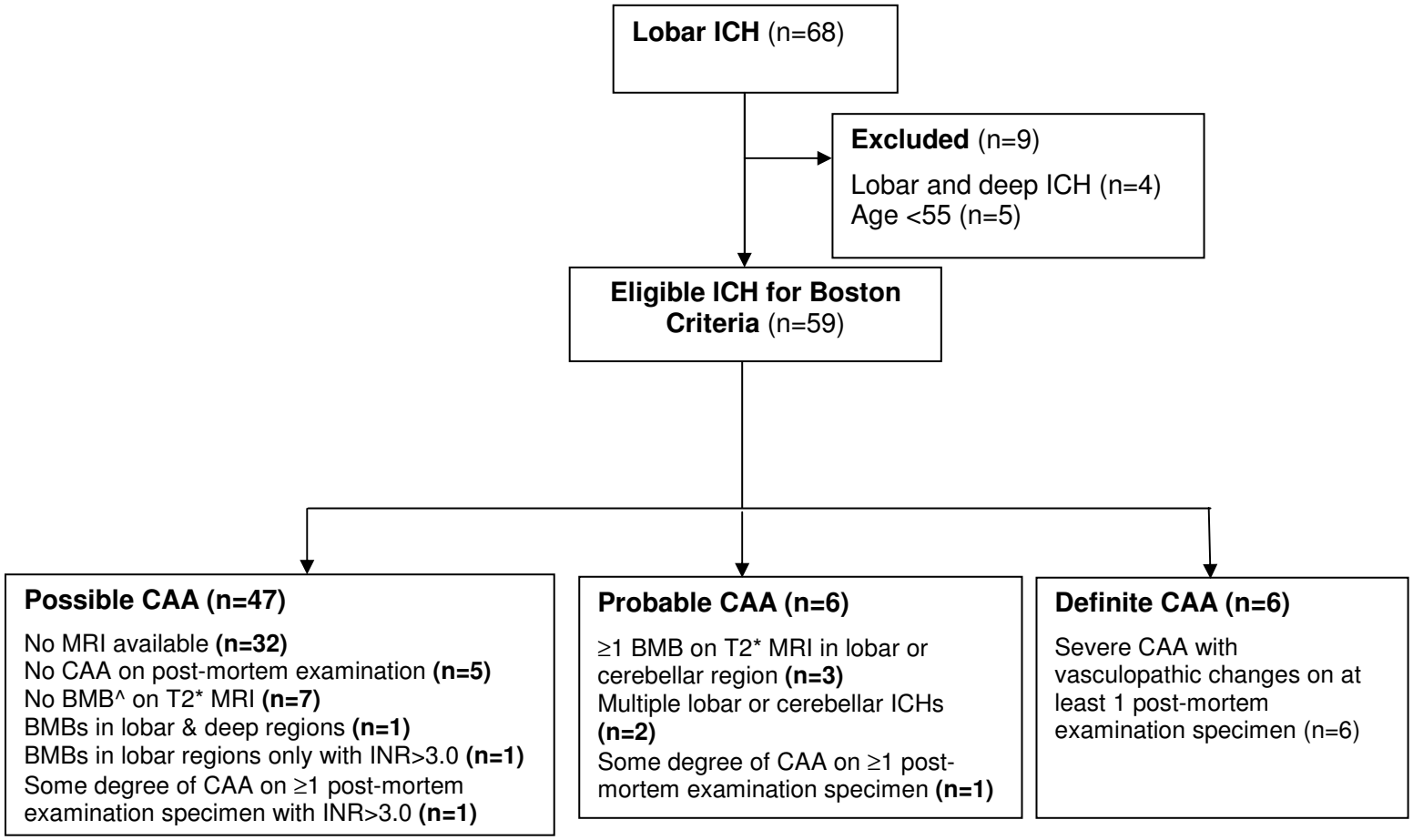


Figure 26 Flowchart of the modified Boston criteria applied to our cohort of first-ever lobar ICHs



Chapter 6 A cross-sectional study of magnetic resonance imaging features in adults with lobar vs. non-lobar intracerebral haemorrhage

Chapter contents

6 A cross-sectional study of magnetic resonance imaging features in adults with lobar vs. non-lobar intracerebral haemorrhage

6.1 Introduction

The neuroimaging correlates of small vessel disease include BMBs, WMH and enlarged perivascular spaces (EPVS) [Pantoni 2010;Doubal et al. 2010].

BMBs in a lobar distribution have been associated with lobar ICH whereas supratentorial deep BMBs (in the basal ganglia or thalamus) have been associated with ICH in deep regions of the brain [Lee et al. 2004]. Non-lobar BMBs (both in supratentorial deep and infratentorial regions) have been associated with hypertension in population-based studies [Vernooij et al. 2008]. Lobar BMBs are associated with apolipoprotein ε4 allele, which is in turn associated with CAA [Greenberg et al. 1995;Vernooij et al. 2008]. This may support lobar and non-lobar ICH having different underlying causes.

There has been little research into the relationship between distribution of WMH and ICH location. WMH are independently associated with BMBs [Yamada et al. 2012]. Although ICH has been associated with higher WMH volumes in comparison to other stroke subtypes [Rost et al. 2010], previous studies comparing WMH in lobar vs. deep ICH have had conflicting results, with both more severe occipital predominant WMH in lobar ICH in comparison to deep ICH [Zhu et al. 2012] and no difference in WMH volume [Smith et al. 2010] reported.

Evidence from tracer studies suggests that perivascular spaces facilitate the drainage of solutes and interstitial fluid from the brain parenchyma [Weller et al. 2009a]. Lobar (cortical) arterioles do not have a perivascular space whereas deep arterioles in the basal ganglia have an expandable perivascular space [Weller, Boche, & Nicoll 2009]. The more effective drainage of solutes such as Aβ from deep regions (in comparison to lobar) may offer some explanation for why CAA is preferentially seen in the lobar regions of the brain. Both basal ganglia EPVS and non-lobar BMBs are independently associated with lacunar stroke [Vernooij et al. 2008] . Since lacunar strokes and deep

intracerebral haemorrhage occur in similar locations and share similar risk factors [Lammie 2002], the distribution of EPVS might also differ in lobar vs. non-lobar ICH.

In a population-based study of ICH, I therefore sought to:

- ascertain the proportion of participants with at least one BMB in lobar and non-lobar ICH;
- compare the distribution of BMBs in lobar and non-lobar ICH and
- compare the distribution and severity of WMH, EPVS and atrophy in lobar and non-lobar ICH.

6.2 Methods

6.2.1 Participants

Participants recruited into the LINCHPIN study (an ethically-approved interventional study of patients with spontaneous primary ICH), underwent MRI. This chapter describes the findings of participants who underwent MRI in the first two years of the study (1st June 2010 – 31st May 2012). The MRI inclusion and exclusion criteria and MRI procedure are described in Chapter 3.

6.2.2 Data collection

Data were collected on an MRI rating proforma using standard definitions (Chapter 3 and Appendix). I rated all brain scans blind to the participants' names and clinical characteristics. A consultant neuroradiologist with a special interest in stroke rated BMBs on all scans. The BMB analysis uses BMBs categorised as 'certain' according to BOMBS [Cordonnier et al. 2009] by a neuroradiologist. I have used my ratings for the analyses of WMH, EPVS and atrophy.

6.2.3 Statistical analysis

I compared features of small vessel disease in participants with lobar and non-lobar ICH. I used parametric statistics for between group comparisons when the data had a normal distribution and log-transformed variables or used non-parametric statistics when it did not. If an estimated cell count was less than five, I used the Fisher's exact test. I conducted all statistical analyses using Stata version 11.1 (StataCorp LP, Texas, USA). All p values are two-sided.

6.3 Results

6.3.1 LINCHPIN MRI scans

6.3.1.1 Participant flowchart

Figure 27 on page 164 illustrates the recruitment of participants for the LINCHPIN MRI study from 1st June 2010-31st May 2012. 80 participants consented to an MRI brain scan of which 20 were too unwell to tolerate a scan, six were unable to tolerate MRI because of agitation or claustrophobia, in three incorrect sequences were obtained and a further one was excluded because her MRI scan identified a secondary cause for the ICH (a cavernous malformation). 50 participants (17% of those who were eligible for an MRI scan) entered the analysis of which 25 had lobar ICH. 46 participants were scanned using a 1.5T GE Signal LX EchoSpeed scanner (GE, Milwaukee, WI, USA) and the remainder were scanned using a 1.5T Philips Gyroscan Intera scanner (Philips Ltd, Best, The Netherlands.) The mean interval between the ICH date and scan date was 85 days (SD 52 days) and this did not differ between those with lobar and non-lobar ICH (77 days, SD=11 vs. 93 days, SD=10; p=0.30.)

6.3.1.2 Characteristics of participants who underwent MRI vs. those who did not

Table 9 on page 160 shows the characteristics of participants who had an MRI scan in year 1 vs. those who did not for those in the first year of the study only (1st June 2010-31st May 2011). Those who had an MRI scan were younger and had higher admission GCS scores, smaller ICHs and less frequent extension of the ICH into the ventricles.

6.3.1.3 Baseline characteristics of LINCHPIN MRI participants

The characteristics of participants who underwent MRI are given in Table 10 on page 161. There was no difference between participants with lobar ICH and non-lobar ICH in any demographic or clinical characteristics.

6.3.1.4 Microbleeds in lobar and non-lobar ICH

24 people (48%, 95% CI 35-62%) had ≥ one BMB and this did not differ between lobar (10/25; 40%, 95% CI 23-59%) and non-lobar ICH (14/25; 56%, 95% CI 37-73%; p=0.26). There was no difference in the total number of certain BMBs and the number of certain lobar BMBs between those with lobar and non-lobar ICH. Participants with non-lobar ICH seemed to have a greater number of non-lobar BMBs compared to

those with lobar ICH (p=0.052) (Table 11 on page 162). This was unchanged in a sensitivity analysis in which all non-lobar BMBs (both certain and uncertain) were included. The proportion of participants with ≥ one non-lobar BMB was greater in those with non-lobar ICH. 13 participants (26%; 95% CI 16-40%) had BMBs in both lobar and non-lobar distributions and the proportions of participants with both lobar and non-lobar BMBs did not differ between those with lobar and non-lobar ICH.

6.3.1.5 White matter hyperintensities, atrophy and EPVS in lobar and non-lobar ICH

There was no difference in the distribution or severity of deep and periventricular WMH, deep and cortical atrophy and basal ganglia and centrum semiovale EPVS between the lobar and non-lobar ICH groups (Table 11 on page 162 & Table 12 on page 163). Hippocampal EPVS seemed more frequent in the lobar group but this was not statistically significant (p=0.050).

6.4 Summary

- The proportion of participants with one or more non-lobar BMB was significantly higher in participants with non-lobar ICH.
- There was no difference in the severity or distribution of lobar BMBs, WMH, EPVS and atrophy between lobar and non-lobar ICH.

6.5 Discussion

6.5.1 Strengths and limitations of the study

The study meets the recently established STandards for ReportIng Vascular changes on neuroImaging (STRIVE) [Wardlaw et al. 2013] in that I have reported:

(i) the proportions of participants with vascular risk factors and their method of measurement (Chapters 2 & 5);

(ii) the interval between the ICH date and date of scan;

(iii) the procedure for image acquisition including the MRI sequences used, the use of two MRI scanners and their characteristics and the acquisition parameters on both scanners (Chapter 3);

(iv) that scans were viewed blinded to participants' clinical details and

(v) the rating scales used for rating deep and periventricular WMH, basal ganglia, centrum semiovale and hippocampal EPVS, atrophy and BMBs.

Nevertheless, this is a small study which should be viewed as a preliminary assessment of small vessel disease in participants with ICH for future larger studies. Only 17% of those eligible to have an MRI scan were scanned, and those who had an MRI scan were younger and had less severe ICHs than those who did not. Therefore these participants are unlikely to be representative of all those with ICH.

6.5.2 Neuroimaging correlates of small vessel disease in lobar and non-lobar ICH

6.5.2.1 Brain microbleeds

The proportion of participants with ≥ one BMB in our study is similar to previous studies [Cordonnier, Wardlaw, & Al-Shahi Salman 2007]. I demonstrated that non-lobar BMBs were commoner in participants with non-lobar ICH but did not show any difference in total or lobar BMBs between lobar and non-lobar ICH.

The finding that non-lobar BMBs were commoner in those with non-lobar ICHs is consistent with previous studies showing that supratentorial deep BMBs are more common in those with deep ICH [Lee et al. 2004;Smith et al. 2010]. Although this supports the theory of non-lobar BMBs and non-lobar ICHs having a similar underlying pathology, 26% participants had both lobar and non-lobar BMBs and this is comparable to a previous study which examined the distribution of BMBs (although not cerebellar BMBs) in participants with ICH [Smith et al. 2010]. Lobar BMBs and ICHs are commonly thought to be due to CAA and non-lobar BMBs and ICHs to be due to hypertension [Greenberg et al. 2009]. However, in view of the substantial proportion of participants with a mixed distribution of BMBs this dichotomisation may be too simplistic.

6.5.2.2 Lack of association between lobar BMBs and lobar ICH

Several factors require consideration when explaining why I did not demonstrate that lobar BMBs were commoner in lobar ICH.

Firstly, our cohort was small and therefore may have lacked power to detect a difference.

Secondly, I used two MRI scanners, and although the scans contained the same sequences it was not possible for the parameters to be identical.

Thirdly, the detection of BMBs is dependent on imaging characteristics such as the magnetic field strength, pulse sequence and spatial resolution [Greenberg et al. 2009]. A higher field strength [Stehling et al. 2008] or the use of susceptibility-weighted imaging (which enhances T2* effects to increase signal drop out and detection of BMBs) [Greenberg et al. 2009] may have increased the detection of lobar BMBs. However, without radio-pathological correlation of presumed BMBs to confirm their histology, it may be that these techniques lead to greater sensitivity at the cost of specificity for BMB detection. A recent study which correlated BMBs and 'mini-bleeds' (areas of haemosiderin of 200-500μm) on 7.0T post-mortem MRI with their histological appearances showed that although the sensitivity of MRI for haemosiderin detection ranged from 85-96%, the specificity was 38-50% [De Reuck J et al. 2011]. Perforating vessels or perivascular iron deposits were frequently mistaken for BMBs. I restricted the analysis of BMBs to those classified as 'certain' according to BOMBS to increase the specificity of BMB detection and internal validity of the findings but it is notable that the findings remained consistent in a sensitivity analysis in which both certain and uncertain BMBs were included.

Lastly, it is possible that lobar BMBs were missed although all scans were rated by a neuroradiologist with a special interest in stroke and extensive previous experience in rating BMBs.

The incidence rate of new BMBs following symptomatic ICH is as yet unknown. In a study of 26 participants with probable or possible CAA according to the Boston criteria, 12 (46%) developed new BMBs during follow up (median interval between baseline and follow up scan 1.1 years) [Chen et al. 2006]. Although I attempted to arrange scans within three months of the participant's ICH, the interval between the ICH and the scan

was frequently substantially longer, due to the participant being too unwell soon after their ICH to either tolerate the scan or give informed consent. In some patients the scan was intentionally delayed allowing greater resorption of blood to look for a secondary cause of the ICH. Importantly, the interval between the ICH and scan date did not differ significantly between the lobar and non-lobar ICH groups so it is unlikely that this affected the findings.

6.5.2.3 White matter hyperintensities

I did not demonstrate any difference in the distribution of periventricular and deep WMH in participants with lobar and non-lobar ICH.

The study was most likely underpowered to detect a difference if one truly exists. A future study in a larger cohort would have greater power to detect a difference in WMH distribution in ICH subtypes after adjusting for other covariates including age, gender and risk factors for stroke such as hypertension and diabetes. Previous studies have examined both WMH distribution [Zhu et al. 2012] and WMH volume [Smith et al. 2010;Rost et al. 2010]. The automated techniques previously used for measuring WMH volume in this context [Smith et al. 2010] such as Freesurfer (http://surfer.nmr.mgh.harvard.edu), have not been validated against either histological derived or manually measured values and therefore the accuracy and reliability of these techniques are as yet unknown [Gronenschild et al. 2012].

6.5.2.4 Enlarged perivascular spaces and atrophy

I did not demonstrate any difference in either the prominence of basal ganglia or centrum semiovale EPVS or distribution of atrophy in participants with lobar and non-lobar ICH.

Although hippocampal EPVS were more common in the lobar ICH group, the relevance of this finding in a small sample is unclear. 'Severe' (>40) centrum semiovale EPVS have been associated with probable or possible CAA-related ICH according to the Boston criteria [Charidimou et al. 2013c]. In a recent study, centrum semiovale EPVS were more common in pathologically-proven CAA compared to ICH unrelated to CAA [Charidimou et al. 2013b], although the study was small and used a combination of sampling methods to assess for CAA, potentially leading to misclassification of CAA-related ICH. Although I rated EPVS (rather than a neuroradiologist) inter-observer

agreement for centrum semiovale EPVS was fair (κ=0.30; Chapter 3). 17/50 (34%) participants had moderate or severe WMH which may have made the severity and distribution of co-existing EPVS difficult to determine [Wardlaw et al. 2013]. I may have lacked sufficient power to detect a difference in EPVS distribution between lobar and non-lobar ICH if one does exist.

Lobar ICH has been associated with dementia which in turn, was associated with atrophy in one hospital based study [Cordonnier et al. 2010b]. Since dementia is more common in lobar ICH (Chapter 5), the prevalence or severity of atrophy might also be expected to be greater in those with lobar ICH.

It would be of interest to repeat the study in a larger cohort to determine if the distribution of EPVS and atrophy differs and correlate radiological with pathological findings.

6.5.3 Future directions

Although this is a small, preliminary study of small vessel disease markers in ICH, it indicates that questions regarding the significance of these markers remain unanswered.

In particular, the sensitivity and specificity of strictly lobar BMBs for pathologically proven CAA should be determined in a larger cohort of participants with ICH.

Given the difficulty distinguishing BMBs from their mimics on imaging, pathological correlation of presumed BMBs observed either on ante mortem and/or post-mortem MRI will be invaluable to confirm them either as true BMBs containing haemosiderin or mimics such as pseudoaneurysms or perforating vessels [Shoamanesh, Kwok, & Benavente 2011] and determine the underlying histology. There have only been six radio-pathological correlation studies of BMBs involving a total of 44 patients [Tanaka et al. 1999;Fazekas et al. 1999;Kikuta et al. 2007;Tatsumi, Shinohara, & Yamamoto 2008;Schrag et al. 2010;De Reuck J et al. 2011] of which four correlated pathological findings with post-mortem rather than ante mortem MRI [Fazekas et al. 1999;Tatsumi, Shinohara, & Yamamoto 2008;Schrag et al. 2010;De Reuck J et al. 2011]. Three further studies examined the pathology of BMBs [Dichgans et al. 2002;Fisher et al. 2010] and so-called 'microscopic haemorrhages' [Tanskanen et al. 2012] without radiological correlation. Post-mortem MRI for BMBs would overcome the technical difficulties of using MRI in patients with ICH who are clinically unwell, facilitating larger and more

generalisable samples of participants. However, a lack of studies correlating ante mortem with post-mortem BMB appearances means that the effect of a protracted terminal phase of death on BMB formation is as yet unknown and informed consent for whole brain donation (rather than tissue samples only) would also be mandatory.

Given the increasing use of radio-labelled ligands such as ^{11}C-Pittsburgh Compound B (PiB) for the in-vivo detection of cerebral β- amyloid using PET imaging both in patients with ICH and Alzheimer's dementia [Ly et al. 2010], a future study could also compare strictly lobar BMBs seen on MRI vs. PiB vs. pathologically proven CAA (as the gold standard) to assess the diagnostic accuracy of these different methods for CAA detection.

A future priority is also to develop a larger population-based study to increase the power to detect differences in WMH, EPVS and atrophy distribution between lobar and non-lobar ICH if they truly exist. It would be important to continue using standardised definitions of these variables to increase external validity and to compare findings between different cohorts and quantify inter-observer agreement between raters with varying levels of expertise.

Table 9 Baseline characteristics of participants who had an MRI scan vs. those who did not in the first year of the study (1st June 2010-31st May 2011)

	MRI (n=28)	No MRI (n=113)	p value
Sex (male), %	13 (46)	51 (45)	0.90
Age (years); median (IQR)	72 (59-79)	79 (68-85)	0.003
History of hypertension			
Yes (n,%)	16 (57)	75 (66)	0.55
History of diabetes*			
Yes (n,%)	3 (11)	13 (12)	1.00
History of smoking*			
Never (n, %)	11 (39)	50 (44)	
Ex-smoker (n, %)	9 (32)	42 (38)	
Current (n, %)	8 (29)	20 (18)	0.56
Alcohol consumption**			
median (IQR)	5 (1-30)	2 (0-13)	0.04
Admission GCS score***			
mean (SD)	14 (2)	11 (4)	<0.001
ICH volume (ml)			
median (IQR)	12 (4-22)	24 (8-56)	0.009
Intraventricular extension****			
Yes (n,%)	8 (29)	63 (56)	0.01

*data missing in one case
**data missing in nine cases
***data missing in five cases
****data missing in four cases

Table 10 Baseline characteristics of 50 participants with lobar and non-lobar ICH who underwent MRI

	Any lobar ICH (n=25)	Non-lobar ICH (n=25)	p value
Sex (male), %	12 (48)	14 (56)	0.57
Age (years); median (IQR)	72 (15)	70 (14)	0.14
Previous stroke			
ICH Yes (n, %)	2 (8)	2 (8)	1.00
Ischaemic stroke			
Yes (n, %)	2 (8)	4 (16)	0.67
History of hypertension			
Yes (n, %)	12 (48)	17 (68)	0.15
History of diabetes			
Yes (n, %)	1 (4)	2 (8)	1.00
History of smoking			
Never (n, %)	9 (36)	11 (44)	
Ex-smoker (n, %)	8 (32)	10 (40)	
Current (n, %)	8 (32)	4 (16)	0.43
Alcohol consumption median (IQR)	6 (19)	5 (28)	0.87
Anticoagulant use			
Yes (n, %)	3 (12)	3 (12)	1.00

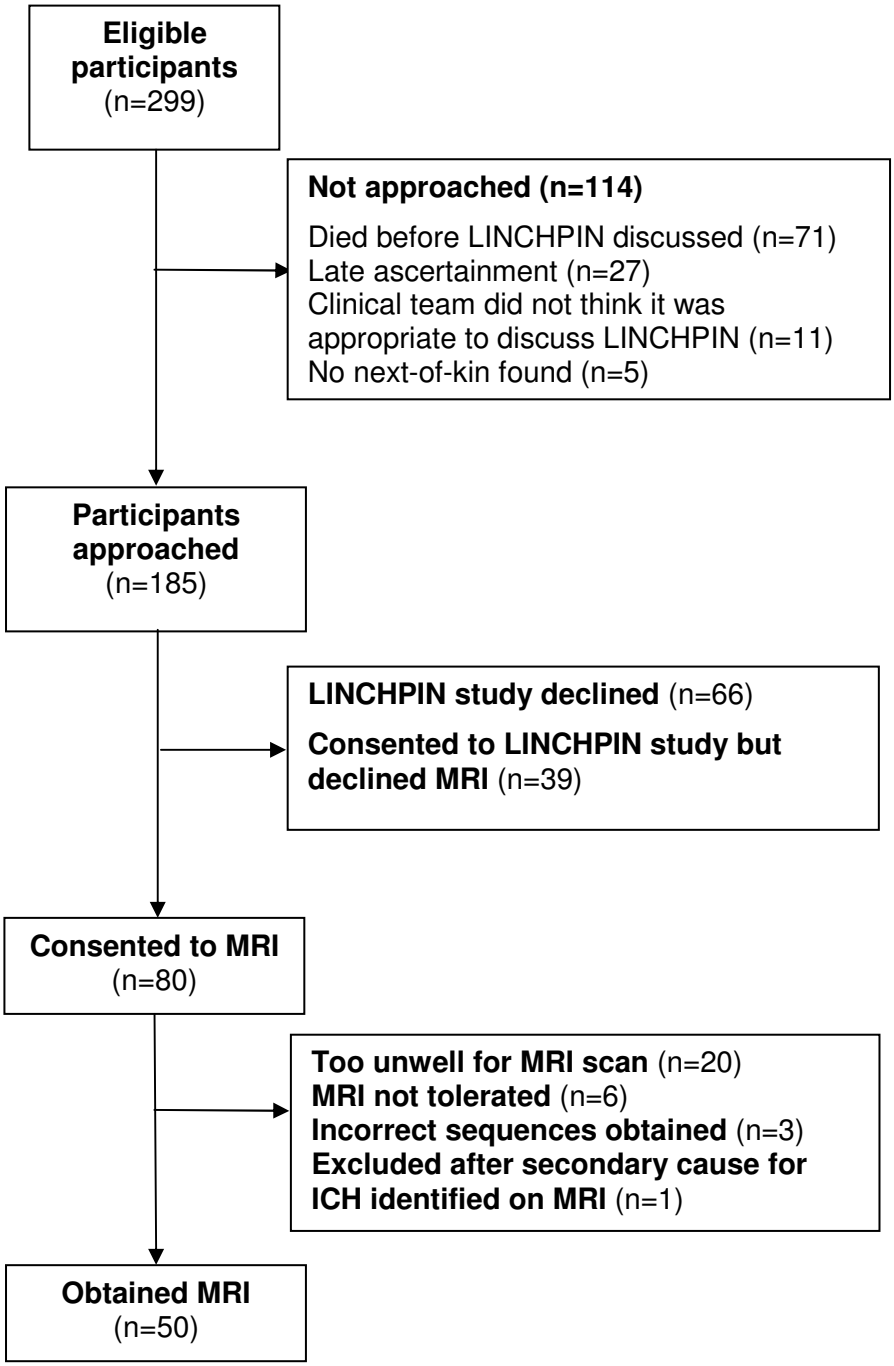
Table 11 Comparison of microbleeds, white matter hyperintensities and atrophy in participants with lobar and non-lobar ICH

	Any lobar ICH (n=25)	Non-lobar ICH (n=25)	p value
BMBs; median (IQR)			
Total	0 (3)	1 (6)	0.28
Lobar	0 (2)	0 (1)	0.80
Non-lobar	0 (0)	1 (5)	0.05
≥1 BMB -any location (n,%)	10 (40)	14 (56)	0.26
≥1 Lobar BMB	8 (32)	10 (40)	0.56
≥1 Non-lobar BMB	6 (24)	13 (52)	0.04
Lobar and non-lobar BMB	4 (16)	9 (36)	0.20
Deep WMH (n,%)			
None	6 (24)	4 (16)	
Mild	9 (36)	14 (56)	
Moderate	10 (40)	7 (28)	0.49
Periventricular WMH (n,%)			
None	8 (32)	4 (16)	
Mild	8 (32)	13 (52)	
Moderate	9 (36)	7 (28)	
Severe	0 (0)	1 (4)	0.25
Cortical atrophy (n,%)			
None	1 (4)	1 (4)	
Mild	9 (36)	15 (60)	
Moderate	14 (56)	9 (36)	
Severe	1 (4)	0 (0)	0.23
Deep atrophy (n,%)			
None	2 (8)	1 (4)	
Mild	12 (48)	15 (60)	
Moderate	10 (40)	8 (32)	
Severe	1 (4)	1 (4)	0.83

Table 12 Comparison of Enlarged Perivascular Spaces in participants with lobar and non-lobar ICH

	Any lobar ICH (n=25)	Non-lobar ICH (n=25)	p value
Basal ganglia EPVS (n,%)			
None	8 (32)	7 (28)	
<10	13 (52)	10 (40)	
11-20	4 (16)	6 (24)	
21-40	0 (0)	2 (8)	0.50
Centrum semiovale EPVS (n,%)			
None	0 (0)	4 (16)	
<10	10 (40)	6 (24)	
11-20	13 (52)	14 (56)	
21-40	2 (8)	1 (4)	0.15
Hippocampal EPVS (n,%)			
None	0 (0)	4 (16)	
<10	25 (100)	20 (80)	
11-20	0 (0)	1 (4)	0.05

Figure 27 Flowchart of participants recruited in the LINCHPIN MRI study from 1st June 2010 to 31st May 2012



Chapter 7 Results of a community-based study of intracerebral haemorrhage-death, prognosis and survival outcomes

Chapter contents

7 Results of a community-based study of intracerebral haemorrhage-death, prognosis and survival outcomes

7.1 Introduction

Studies of case fatality and disease outcomes require comprehensive case ascertainment and pre-specified standard definitions (of both the disease being studied and any outcomes) to increase external validity (Chapter 2). The study should be prospective with outcomes being assessed equally between groups to avoid information bias [Grimes & Schulz 2002a], and there should be at least 80% follow-up to minimise loss to follow-up bias [Wells et al. 2002].

In the context of ICH, the aim of such a study would be to describe case fatality and level of disability at standard time points after the inception point and determinants of prognosis, leading to the refinement of existing prognostic models and potentially the selection of subgroups of participants who may benefit from interventions and might be entered into a randomised controlled trial. In the longer term, the study would describe the incidence of both haemorrhagic and vaso-occlusive outcomes following an ICH, since survivors of ICH have risk factors for both haemorrhagic and vaso-occlusive outcomes [Ariesen et al. 2003], with comparison of outcomes stratified by ICH location.

Studies of prognosis after ICH [Portenoy et al. 1987;Franke et al. 1992;Lisk et al. 1994;Tuhrim et al. 1995;Fogelholm, Avikainen, & Murros 1997;Hemphill et al. 2001;Ariesen et al. 2005;Weimar, Benemann, & Diener 2006;Flaherty et al. 2006;Ruiz-Sandoval et al. 2007;Rost et al. 2008;El-Senousey et al. 2010;Stein et al. 2010;Mittal & Lele 2011;Chen et al. 2011;Nag et al. 2012;Li et al. 2012], have identified various determinants of outcome. These have included demographic and clinical variables such as age at diagnosis of ICH [Lisk et al. 1994;Hemphill et al. 2001;Weimar, Benemann, & Diener 2006;Flaherty et al. 2006;Ruiz-Sandoval et al. 2007;Rost et al. 2008;El-Senousey et al. 2010], diabetes [Flaherty et al. 2006;Chen et al. 2011], pre-ICH cognitive impairment [Rost et al. 2008], anticoagulation use [Flaherty et al. 2006], pulse pressure [Tuhrim et al. 1995], GCS score on admission [Portenoy et al. 1987;Franke et al. 1992;Lisk et al. 1994;Tuhrim et al. 1995;Hemphill et al. 2001;Ruiz-Sandoval et al.

2007;Rost et al. 2008;El-Senousey et al. 2010;Chen et al. 2011], brainstem reflexes [Mittal & Lele 2011], NIHSS score [Weimar, Benemann, & Diener 2006] and blood glucose [Fogelholm, Avikainen, & Murros 1997;Li et al. 2012]. ICH features which help to determine outcome include its volume [Portenoy et al. 1987;Franke et al. 1992;Lisk et al. 1994;Tuhrim et al. 1995;Hemphill et al. 2001;Ruiz-Sandoval et al. 2007;Rost et al. 2008;Chen et al. 2011], presence of intraventricular [Portenoy et al. 1987;Hemphill et al. 2001;Ruiz-Sandoval et al. 2007;Stein et al. 2010;Chen et al. 2011] or subarachnoid [Fogelholm, Avikainen, & Murros 1997] extension, ICH location [Tuhrim et al. 1995;Hemphill et al. 2001;Ruiz-Sandoval et al. 2007;Rost et al. 2008], midline shift [Nag et al. 2012] and hydrocephalus [Ariesen et al. 2005;Stein et al. 2010]. However, all of the studies listed above except one [Fogelholm, Avikainen, & Murros 1997] have used hospital based cohorts and many have been retrospective [Portenoy et al. 1987;Hemphill et al. 2001;Stein et al. 2010;Chen et al. 2011;Li et al. 2012]. The ICH score [Hemphill et al. 2001] is a prognostic score for determining 30 day case fatality following ICH and comprises age, GCS score on admission, ICH location (supratentorial vs. infratentorial), intraventricular extension and ICH volume. It is the only score for modelling prognosis after ICH which has been externally validated, albeit using a hospital-based cohort [Hemphill, Farrant, & Neill 2009].

Although infratentorial ICH is known to have a higher risk of death [Hemphill et al. 2001;Flaherty et al. 2006], the majority of ICHs are supratentorial. Determining whether supratentorial ICH location influences outcome may help to guide treatment options. Lobar ICH may have a better prognosis in comparison to ICH in other locations [Juvela 1995;Garibi et al. 2002;Rost et al. 2008;Zia et al. 2009] but the results of studies conflict [Castillo et al. 1994;Nilsson et al. 2002;Inagawa et al. 2003;Rodriguez-Luna et al. 2011;Nag et al. 2012;D'Amore et al. 2013;Palm et al. 2013].

I therefore sought to describe 30 day and one year case fatality in a prospectively recruited population based cohort of participants following first-ever ICH, validate the ICH score and determine whether ICH location affected case fatality after adjusting for other known prognostic factors. I will also describe the incidence of haemorrhagic and vaso-occlusive events during one year follow up, stratified by ICH location.

7.2 Methods

7.2.1 Case fatality at one month and one year

I used multiple overlapping sources of follow up to determine whether a participant had died and if applicable, their date of death. I used the following sources:

(i) the hospital electronic patient records system which records whether a patient has died and clinical letters pertaining to their most recent admission;

(ii) the participant's GP, who completed an annual questionnaire which requests confirmation that the participant is still alive;

(iii) death certificates obtained from the General Register Office for Scotland and

(iv) the Office of the Procurator Fiscal which records information relating to any sudden deaths later found to be as a result of ICH at post-mortem examination.

7.2.1.1 The influence of ICH location on case fatality

Based on existing knowledge regarding prognostic factors following ICH, I selected *a priori* five variables (variables comprising the ICH score [Hemphill et al. 2001] and prior anticoagulant use [Stead et al. 2010]) in addition to whether the haemorrhage involved a lobar region of the brain or not (Section 1.1.5.4, page 37) to examine their influence on case fatality.

I applied the ICH score to our cohort to assess how well it discriminated between those who died and those who survived in the first 30 days following ICH.

7.2.1.2 One year modified Rankin scale, recurrent ICH and vaso-occlusive events

I used information from questionnaires (Appendix), sent annually to participants' GPs to obtain the level of disability one year after their ICH using the modified Rankin scale and assess whether they had suffered from the following conditions over the subsequent year:

(i) Myocardial infarction (MI)-defined as clinical symptoms accompanied where available by electrocardiographic and biochemical cardiac marker changes or confirmed at post-mortem examination [Mendis et al. 2010].

(ii) Venous thromboembolism-defined as the occurrence of symptomatic DVT or PE confirmed by imaging [National Institute for Health and Clinical Excellence (NICE) 2010].

(iii) TIA-defined as rapidly developing clinical signs of focal disturbance of cerebral function lasting less than 24 hours with no alternative explanation [Thorvaldsen et al. 1997].

(iv) Ischaemic stroke- defined as rapidly developing clinical signs of focal (or global) disturbance of cerebral function lasting for at least 24 hours, with no alternative explanation [Thorvaldsen et al. 1997].

(v) Recurrent ICH – confirmed by imaging, which I reviewed with a neuroradiologist or at post-mortem examination.

If a GP was unable to complete the modified Rankin scale [van Swieten et al. 1988], I contacted participants and completed the modified Rankin scale using a semi-structured telephone interview [Wilson et al. 2002]. I corroborated information received from questionnaires with information from participants and from the hospital electronic records system (which records all in-patient and out-patient appointments to hospitals serving the Lothian health board) to validate all outcome events.

7.2.2 Statistical analysis

7.2.2.1 Logistic regression model for predictors of case fatality

I entered ICH location (any lobar ICH vs. non-lobar ICH) and covariates into two logistic regression models in which the dependent variables were death within 30 days and one year following the index ICH. I checked for variables which substantially changed the effect estimate by using the `chest` command in Stata which selects variables in a stepwise fashion depending on the size of adjusted and unadjusted effect estimates [Wang 2007]. The variable which is included first produces the largest change in effect estimate and the selection of variables continues till all are included in the model. I assessed the performance of the logistic regression model and checked that its assumptions were met as follows:

(i) In logistic regression, the log odds of the outcome (dependent variable) is modelled as the linear combination of the predictor (independent) variables. This assumes

that there is no specification error; that is I have not included any variables that should not be in the model and have also included all relevant variables and should not be able to find any other statistically significant predictors except by chance. I used the Stata command `linktest` to check that this assumption was met.

(ii) I tested the calibration of the model using a likelihood ratio test which examined the predictive value of the model with these predictors in comparison to a null model with no predictors. I also used Hosmer and Lemeshow's goodness of fit test which divides subjects into deciles based on predicted probabilities of case fatality and calculates a χ^2 based upon observed and expected frequencies of deaths in each decile. A p value of >0.05 indicates that there is no significant difference between the observed and model-predicted values, implying that the model fits the data adequately.

7.2.2.2 Validation of the ICH score

I entered the variables comprising the ICH score into a regression model in which the dependent variable was 30 day case fatality and used the `roctab` command in Stata to plot a receiver operator curve and measure the area under the curve (AUROC). An AUROC of 0.5 indicates that the discrimination of the ICH score is no better than chance and an AUROC of 1.0 indicates that the ICH score discriminates perfectly.

7.2.2.3 Survival analysis

I calculated the total person-years of follow up using the sum of follow-up times irrespective of whether an event took place or whether the person was censored. I quantified the completeness of follow-up data by calculating the sum of follow-up times (days) divided by the sum of potential follow-up times which could have been obtained before death or the end of the one year follow-up period [Clark, Altman, & De Stavola 2002]. I used Kaplan-Meier survival curves and life tables with log rank tests to analyse follow up data for outcome events during one year follow-up and used Cox regression if proportional hazards assumptions were satisfied [Bradburn et al. 2003]. Survival analyses of the time to an event started on the date of the ICH and ended either on the date of the first event or on the date of censoring if no event occurred. I censored follow-up on the date of death or one year after their ICH depending on which occurred first.

7.2.2.4 Other analyses

I used parametric statistics for between group comparisons when the data had a normal distribution and log-transformed variables or used non-parametric statistics when it did not. If an estimated cell count was less than five, I used the Fisher's exact test. I conducted all statistical analyses using Stata version 11.1 (StataCorp LP, Texas, USA). All p values are two-sided.

7.3 Results

7.3.1 Prognosis after first-ever spontaneous primary ICH

7.3.1.1 Completeness of follow up

I obtained complete data regarding survival at one month and one year and dates of death, where applicable. I obtained the level of disability at one year as measured by the modified Rankin scale for 124 out of 128 participants (97%). I obtained 63 person-years of follow up for all vaso-occlusive and ICH outcomes (97% completeness).

7.3.1.2 Case fatality in the first 30 days and at one year

In the first 30 days following a first-ever spontaneous primary ICH, 55 adults (43%, 95% CI 35-52%) died. The proportions did not differ between lobar and non-lobar ICH (lobar 32/68 (47%); non-lobar 23/60 (38%); χ^2 =0.99, p=0.32). Figure 28 on page 188 shows a Kaplan-Meier of survival time in the first 30 days following first-ever spontaneous primary ICH in participants with lobar and non-lobar ICH. There was no difference in survival between those with lobar and non-lobar ICH (χ^2 =0.69, p=0.40).

By one year, 72 adults (56%, 95% CI 48-65%) had died and the proportions did not differ between lobar and non-lobar ICH (lobar 39/68 (57%); non-lobar 33/60 (55%); χ^2 =0.07, p=0.79). The primary causes of death as listed in Section 1A on their death certificates are shown in Table 13 on page 183. The majority of participants died as a result of their ICH although three different ICD-10 codes were used to classify ICH: 'intracerebral haemorrhage,' 'intracranial haemorrhage' and 'stroke, not classified as haemorrhage or infarction.'

7.3.1.3 Impact of ICH location on one month case fatality

Table 14 on page 184 shows the logistic regression model of predictors of death in the first month following a first-ever spontaneous primary ICH. 122 participants entered the model.

Older age, lower GCS scores on admission, larger ICH volumes on the diagnostic CT brain scan and ICH in a non-lobar location were significant predictors of one month case fatality in the crude model. The odds of death decreased by 81% (95% CI 31-95%) if a haemorrhage involved a lobar location. In a sensitivity analysis restricted to adults with supratentorial ICH (n= 110; Table 15 on page 184), the impact of lobar ICH was no longer significant (OR 0.26, 95% CI 0.07-1.03; p=0.06). There was no statistically relationship between 30 day case fatality and intraventricular haemorrhage or prior use of anticoagulants. The model was well calibrated (Likelihood ratio test $p<0.001$; Hosmer and Lemeshow's goodness of fit test $\chi^2=3.79$, $p=0.88$) implying that there was no statistically significant difference between the observed values in the dataset and the values predicted by the model and there was no evidence of a specification error.

7.3.1.4 Influence of ICH location on one year case fatality

Table 16 on page 185 shows the logistic regression model of predictors of death in the first year following a first-ever spontaneous primary ICH.

Older age, lower GCS scores on admission, larger ICH volumes on brain imaging and non-lobar ICH appeared again as significant predictors of one year case fatality in the crude model. Lobar ICH was associated with a lower odds of death at one year and this was unchanged when the model was restricted to adults with solely supratentorial ICH; (OR 0.28, 95% CI 0.09-0.88; p=0.03, Table 17 on page 185).

When the model was checked for the effect of covariates on the relationship between lobar ICH and death, the addition of ICH volume to the model significantly reduced the odds of death in the first year after a lobar ICH; suggesting that the association seen between non-lobar ICH and death was likely to be because of ICH volume. None of the other variables substantially changed the effect estimate. When ICH volume was divided into three categories (0-29, 30-59 and 60-90ml) and used to stratify ICH location, a lower proportion of lobar ICHs were dead at one year in every volume category; and in the 30-59ml category this difference was statistically significant (lobar

6/12 (50%) vs. non-lobar 10/11 (91%); χ^2 =4.54, p=0.04; Table 18 on page 185). The model was well calibrated (Likelihood ratio test p<0.001; Hosmer and Lemeshow's goodness of fit test χ^2 =4.43, p=0.82) with no evidence of a specification error.

7.3.1.5 Validation of the ICH score

Table 19 on page 186 shows that the percentage of deaths in the first 30 days following a first-ever spontaneous primary ICH increased from 5% with an ICH score of 0 to 100% with a score of 5. Table 20 on page 186 shows the logistic regression model of 30 day case fatality using the ICH score. Decreasing GCS score on admission, ICH volume (≥30ml) and infratentorial ICH were associated with death. The AUROC was 0.85 (95% CI 0.78-0.91) indicating that the ICH score discriminated well between survivors and deaths from first-ever spontaneous ICH within the first 30 days (Figure 29 on page 189).

7.3.1.6 Level of disability at one year following first-ever spontaneous primary ICH

Figure 30 on page 190 shows the modified Rankin scores at one year from 124 participants with first-ever spontaneous primary ICH. The distribution of modified Rankin scores is similar in participants with lobar and non-lobar ICH. At one year, 107 (86%) adults were dead or dependent (modified Rankin scale score ≥ 3). This did not vary by ICH location (lobar 58/66 (88%) vs. non-lobar 49/58 (84%); χ^2 =0.30, p=0.58).

7.3.1.7 Vaso-occlusive events and recurrent ICH following first-ever spontaneous primary ICH

Figure 31 on page 191 shows a Kaplan-Meier curve of the time to a composite outcome of vaso-occlusive events or recurrent ICH in participants with lobar and non-lobar ICH. There was no difference in the time to events observed in the lobar and non-lobar ICH groups (χ^2 =0.73, p=0.39).

7.3.1.8 Recurrent ICH following first-ever spontaneous primary ICH

Figure 32 on page 192 shows a Kaplan-Meier curve of the time to recurrent ICH in 128 participants with first-ever spontaneous primary ICH. There were four ICHs, all of which were lobar and occurred exclusively in survivors of lobar ICH (annual risk of recurrent ICH after lobar ICH 11.8%, 95% CI 4.6% to 28.5% vs. annual risk after non-lobar ICH 0%). All cases were spontaneous primary definite ICHs confirmed by

imaging. There was a statistically significant excess of recurrent ICH in participants with lobar ICH (χ^2=4.10, p=0.04).

7.3.1.9 Ischaemic stroke, TIA and myocardial infarction following first-ever spontaneous primary ICH

Figure 33 on page 193 shows a Kaplan-Meier curve of the time to first ischaemic stroke, TIA or MI in 128 participants with first-ever spontaneous primary ICH. There were seven events (Table 21 on page 187) (annual risk 10.8%, 95% CI 5.2%-21.7%) and the annual risk did not differ between adults with lobar (annual risk 9.9%, 95% CI 3.3%-27.8%) and non-lobar ICH (annual risk 11.7%, 4.4%-29.0%) (χ^2=0.10, p=0.76).

7.3.1.10 Venous thromboembolism following first-ever spontaneous primary ICH

Figure 34 on page 194 shows a Kaplan-Meier curve of the time to venous thromboembolism in 128 participants with first-ever spontaneous primary ICH. Four events (one PE and three DVTs) occurred (annual risk 6.1%, 95% CI 2.3% -15.5%) and the annual risk did not differ between adults with lobar (annual risk 6.7%, 95% 1.7%-24.4%) and non-lobar ICH (annual risk 5.7%, 95% CI 1.5%-21.1%) (χ^2=0.00, p=1.00).

7.3.1.11 Recurrent ICH vs. vaso-occlusive events

There were 10 vaso-occlusive events and the risk of vaso-occlusive events did not differ between adults with lobar (16.2%, 95% CI 7.0% to 34.8%) and non-lobar ICH (14.4%, 95% CI 6.1% to 31.9%; p=1.00). The annual risk of any vaso-occlusive event, regardless of ICH location was 15.1% (95% CI 8.3% to 26.6%). For all adults, regardless of ICH location, vaso-occlusive events seemed to be at least as frequent as recurrent ICH in the first year (Figure 35 on page 195; hazard ratio 2.66, 95% CI 0.83-8.48, p=0.08).

7.4 Summary

- Case fatality at one month was 43% and at one year was 56%.
- Lobar ICH was independently associated with a lower odds of death in the first year after first-ever spontaneous primary ICH after adjusting for other covariates.
- The ICH score discriminated well between those who survived and died in the first 30 days following a first-ever spontaneous primary ICH.

- There was a higher risk of recurrent ICH in the lobar ICH group.
- There was no difference in the risk of vaso-occlusive events observed in the lobar and non-lobar ICH groups.
- Regardless of ICH location, there was a non-significant excess of vaso-occlusive events in comparison to recurrent ICH in the first year.

7.5 Discussion

7.5.1 Strengths of the study

This study has a prospective population-based design and uses multiple overlapping sources of follow-up. It is the first study to externally validate the ICH score to determine one year survival in a population-based cohort.

7.5.2 Limitations of the study

The study has a short follow up period and small number of outcome events making it difficult to establish the recurrence risks of outcome events precisely. I did not adjust for premorbid conditions which might affect outcome [Bar & Hemphill, III 2011] although since most demographic and clinical characteristics in both lobar and non-lobar ICH groups were similar (Chapter 5), it is unlikely that this affected the findings. Similarly, I did not adjust for the presence of subarachnoid extension [Maas et al. 2013a] as this is associated with a larger ICH volume which is likely to be the primary determinant of poor outcome [Chen et al. 2013]. I only categorised the presence or absence of intraventricular haemorrhage rather than determining the extent of it (Section 7.5.6, page 181) [Hwang et al. 2012]. I assessed intraventricular haemorrhage on the first (diagnostic) CT and therefore may have missed patients who had delayed intraventricular haemorrhage [Maas et al. 2013b].

7.5.3 Case fatality after first-ever spontaneous primary ICH

7.5.3.1 Ascertainment of deaths

The 30 day and one year case fatality rates in our study following spontaneous primary ICH were 43% (95% CI 35-52%) and 56% (95% CI 48-65%) respectively.

The WHO MONICA project (World Health Organisation Monitoring Trends and Determinants in Cardiovascular Disease) which examined trends in cardiovascular and stroke epidemiology prospectively in different populations specified indicators of study quality in a stroke incidence study [Asplund et al. 1995]. Although the generalisability of these criteria to our study is somewhat limited since MONICA studies included both ischaemic stroke and subarachnoid haemorrhage in their cohorts, the case fatality outcomes in our study fulfil certain indicators:

(i) The one month case fatality rate in our cohort is similar to other studies of ICH [van Asch et al. 2010]. Therefore, assuming that our population is similar to others in which previous ICH incidence studies have been conducted and that no changes in either population structure or ICH prognosis have happened over time, it is unlikely that I have significantly under-ascertained either fatal or non-fatal ICHs.

(ii) The proportion of fatal cases occurring outside of hospital in relation to all stroke deaths is used to estimate the completeness of data on fatal out-of-hospital events and although the proportion may vary between populations it is likely to be at least 10%. The proportion in my cohort is 7% (95% CI 3-16%).

(iii) The proportion of fatal cases examined by a physician before death or subjected to post-mortem examination is used as an indication of the accuracy of the clinical diagnosis of stroke. Of all definite and suspected ICH cases, 93% met this criterion.

7.5.3.2 Predictors of one month and one year case fatality

Although there was no difference in crude survival over one year, after adjusting for other covariates, lobar ICH was strongly associated with survival in the first year. Lobar ICH location appeared to be associated with a lower odds of death in the first 30 days but this was not statistically significant in a sensitivity analysis restricted to supratentorial ICH. Lobar ICH has been associated with a better outcome in a population-based study [Zia et al. 2009], but not in two others [Nilsson et al. 2002;Inagawa et al. 2003] which may reflect confounding by other factors which influence prognosis after ICH such as intraventricular haemorrhage [Hemphill et al. 2001].

Although lobar ICHs were larger than non-lobar ICHs they may be associated with a better outcome in comparison to ICH in other locations since they are less likely to cause hydrocephalus [Diringer, Edwards, & Zazulia 1998] and are also associated with dementia which in turn is associated with cortical atrophy [Cordonnier et al. 2010b], which may protect against mass effect caused by lobar ICH. The finding that moderately sized lobar ICHs in our cohort were significantly more likely to survive in the first year than similar sized non-lobar ICHs is compatible with anecdotal clinical observations of small infratentorial ICHs causing significant impairment because of their tendency to rupture into the ventricular system or cause brainstem compression.

I did not demonstrate the same association in the first 30 days following ICH (Table 14 on page 184 and Table 15 on page 184), which might be explained by the smaller sample size once infratentorial ICHs were removed with a consequent reduction in power.

Anticoagulation use and intraventricular haemorrhage were not associated with one month or one year case fatality. Only 14% of the cohort used anticoagulant medications at the time of their ICH and the study may have lacked sufficient power to demonstrate an association between anticoagulant use and case fatality. The ICH score simply categorises intraventricular haemorrhage as presence or absence rather than determining the extent of intraventricular haemorrhage and this may have obscured an association if one did exist in our cohort (Section 7.5.6, page 181).

7.5.3.3 Statistical considerations

I examined several methods to determine the influence of ICH location on prognosis.

I calculated a Mantel-Haenzel odds ratio for the likelihood of death stratified by ICH location and adjusted for pre-specified covariates. However, continuous variables had to be stratified within relatively wide bands and the number of variables that could be adjusted for was limited because of the generation of too many strata, raising the possibility of residual confounding between the outcome and exposure variable within strata [McNamee 2005] . A Cox proportional hazards regression model of the time to death in lobar vs. non-lobar ICH assumes that the hazard ratio (the relative risk of death in both groups) is the same at all times during follow up [Bewick, Cheek, & Ball 2004]. Although I generated a multivariable Cox regression model, when I tested the proportional hazards assumption using Schoenfeld residuals, the model did not meet

this assumption. This was consistent with a Kaplan-Meier curve of survival following first-ever primary ICH which showed crossing of survival lines for lobar and non-lobar ICH indicating that the assumption was unmet (Figure 28 on page 188).

I was limited in the number of variables that I could include in a multivariable analysis by the rule of 10 outcome events per predictor variable. I used nine events per variable since in logistic regression five to nine outcome events per variable is valid, especially if variables are selected *a priori* and the associations found are plausible in the context of current knowledge [Vittinghoff & McCulloch 2007].

7.5.4 ICH score

I demonstrated that the ICH score [Hemphill et al. 2001] discriminated well when using it to predict 30 day case fatality. Age and intraventricular haemorrhage were not significant predictors of mortality in the regression model and this may be due to the loss of statistical power from age being used as a categorical variable (rather than continuous variable as in the previous models) and the study being underpowered given the small sample size.

The performance of the ICH score might also be improved by assessing intraventricular haemorrhage according to a validated score rather than merely the presence or absence of it (Section 7.5.6, page 181).

7.5.5 Recurrent ICH and vaso-occlusive events after first-ever spontaneous primary ICH

7.5.5.1 Ascertainment of events

There are various complexities in ascertaining events. Some participants may not seek medical attention; they may fail to recognise the significance of symptoms [Kainth et al. 2004;Sprigg et al. 2009] particularly if they have cognitive impairment or the symptoms resolve quickly and are therefore perceived as benign. Older age and female sex have also been associated with delays to presentation [Moser et al. 2006]. Since the majority of our cohort had significant persisting neurological deficits following their ICH, it may also have been difficult to ascertain a recurrent stroke given their baseline level of impairment. However, since the participants' level of dependency following ICH in our cohort led to many being looked after in nursing homes or by trained carers, I would anticipate that carers were alert to the possibility of subsequent medical problems.

Moreover, since ICH, MI and PE are causes of sudden death some may have been misclassified if a participant died suddenly without investigation. Even if a diagnosis of ICH is confirmed by imaging or post-mortem examination, it may still be misclassified as a 'stroke, not specified as haemorrhage or infarction' or as 'intracranial haemorrhage' (Table 13, page 183). However, I obtained all GP records and, if available, hospital records of participants who died to corroborate information recorded on their death certificate and determine the likelihood of such a diagnosis. By reviewing records of all calls made to the Office of the Procurator Fiscal, I was also able to review the circumstances of sudden deaths in our cohort where the Procurator Fiscal was contacted.

The study could be improved by using a second clinician to independently classify outcomes blinded to the location of a participant's ICH and their clinical history. Interviewing all participants and/or their carer individually would also reduce the chance of outcomes being undetected, although this was beyond the scope of our study and might have led to ascertainment bias and reduced the external validity of the findings by influencing participants' health-seeking behaviour.

7.5.5.2 ICH recurrence

I demonstrated a significantly higher risk of recurrent ICH in the lobar ICH group with a tendency for the recurrent ICHs to be lobar and this is consistent with other studies [Bailey et al. 2001a].

The recurrence rate in our cohort is higher than that observed in a previous population based study [Flynn et al. 2010] and comparable to other studies [Passero et al. 1995;Bailey et al. 2001a;Yen et al. 2007] which makes it less likely that I have missed recurrent ICHs and supports the notion that different vasculopathies underlie lobar and non-lobar ICH.

The study benefited from its population-based design with both hospital and community-based methods of ascertaining follow-up events and validation of events by review of medical records and diagnostic imaging by a neuroradiologist. Other studies have placed more reliance upon hospital records and discharge data [Flynn et al. 2010;Chong et al. 2012] and/or surveillance of established stroke registries to ascertain outcomes [Hanger et al. 2007;Zia et al. 2009;Flynn et al. 2010]. I have maintained the

external validity of the study by only including symptomatic ICHs in contrast to other studies which also classified new BMBs seen on MRI as recurrent ICH [Viswanathan et al. 2006].

The study is limited by the short follow-up period and small cohort, which makes it difficult to establish the recurrence risk of ICHs precisely. A longer follow-up period in a larger cohort would allow me to establish whether the absence of recurrence in the non-lobar ICH group has merely occurred by chance (which seems likely).

The increased risk of recurrence of lobar ICHs supports the existing theory that lobar and non-lobar ICH may have different causes. Since hypertension has a greater role in causing non-lobar ICH [Jackson & Sudlow 2006], then prescribing of antihypertensive medications for secondary prevention of an ICH may be expected to have a greater effect on reducing non-lobar rather than lobar ICH. However, this has not as yet been demonstrated, with prescribing of antihypertensives in one study found to reduce the incidence of both ICHs attributed to CAA and hypertension [Arima et al. 2010], although since ICHs were classified according to their presumed cause instead of by location, misclassification of the outcome may have occurred. In the future I could investigate whether the prescribing of antihypertensive medications for secondary prevention differentially influences the risk of lobar vs. non-lobar ICH although a large sample would be needed and the study would be vulnerable to the biases affecting non-randomised observational studies.

7.5.5.3 Vaso-occlusive events

I did not demonstrate any difference in the risk of vaso-occlusive events between participants with lobar and non-lobar ICH. I found survivors of spontaneous ICH to be at similar risk of both recurrent ICH and vaso-occlusive events, which creates dilemmas regarding the prescription of antithrombotic medications following ICH.

The recurrence rates of ischaemic stroke are comparable to those of other population-based studies [Hanger et al. 2007;Zia et al. 2009] and the proportion of participants who had a MI or venous thromboembolism is similar to a previous retrospective single centre hospital based study [Goldstein et al. 2009]. Strengths of this study include the prospective ascertainment of outcomes and population-based design. However, I would need to follow a larger number of participants up for a longer period of time to

increase the number of outcome events detected and determine if a true difference exists between the risk of vaso-occlusive events and recurrent ICH following a first-ever ICH and whether the risk of vaso-occlusive events differs by ICH location. If the risk of vaso-occlusive events is similar to the risk of recurrent ICH following first-ever ICH this may have implications for restarting antithrombotic medications following an ICH especially since patients with ICH may also have risk factors for vaso-occlusive events such as hypertension. Further investigation of the effects of antithrombotic medications according to the underlying vasculopathy are required in observational studies [Biffi et al. 2010a]. This clinical dilemma is also being explored in a randomised controlled trial of restarting vs. avoiding antithrombotic medications in survivors of spontaneous ICH who were taking these medications prior to their ICH (www.RESTARTtrial.org, ISRCTN71907627).

7.5.6 Future directions

This study indicates that unanswered questions remain regarding prognosis and the risk of haemorrhagic and vaso-occlusive outcomes after ICH.

The study needs replication in a larger cohort to determine whether the association between lobar ICH and one year case fatality is consistent. In a larger population, I would also investigate residual confounding by other known adverse prognostic variables such as 'Do Not Resuscitate' (DNR) orders [Zahuranec et al. 2007] and hydrocephalus [Diringer, Edwards, & Zazulia 1998]. Larger studies would also be able to investigate biomarkers of the underlying vasculopathy such as BMBs and superficial siderosis [Smith et al. 2004;Park et al. 2013;Charidimou et al. 2013a], which require further investigation in adequately powered studies to determine whether they are predictors and surrogate markers of recurrent ICH.

Since lobar ICH has a higher risk of recurrence [Bailey et al. 2001a], secondary prevention (such as reducing alcohol intake [Ariesen et al. 2003] and blood pressure [Arima et al. 2010]) may be particularly relevant to this group of patients.

It is interesting that I did not show any association between intraventricular haemorrhage and case fatality. As mentioned in Section 7.5.3.2, page 176 measuring the extent of intraventricular haemorrhage might have improved the predictive value of the regression model even further. The extent of intraventricular haemorrhage may be

measured by three validated scales: the Graeb [Graeb et al. 1982], IVH [Hallevi et al. 2009] and Le Roux [LeRoux et al. 1992] scales. Each relies upon assessors to judge the extent of filling of each of the ventricles with blood on CT brain imaging as a percentage of the space within the ventricle and in the case of the IVH score the presence of hydrocephalus is an additional parameter in determining a final score. They have been shown in hospital based studies to have predictive value in determining case fatality following spontaneous ICH [Hwang et al. 2012] and increase the specificity of the ICH score [Hallevi et al. 2009]. A future study might validate these scores in our population based cohort and assess whether integration of this variable and ICH location (lobar vs. non-lobar) improves the calibration of the ICH score.

The ICH score has also been used to predict disability at one year after ICH in a hospital based cohort where disability was assessed either by semi-structured interview or using medical records (in 9% of the cohort) [Hemphill, Farrant, & Neill 2009]. It would be useful to externally validate the use of the ICH score for this purpose in a population-based cohort.

Vaso-occlusive events are of special significance in patients with ICH since their occurrence leads to the clinical dilemma of treatment with antithrombotic medications vs. the risk of further ICH. In our cohort there were small but appreciable numbers of these outcomes. Since antithrombotic medications are a relative contraindication following ICH [Keir et al. 2002] but those with ICH often have risk factors for vaso-occlusive events, it is important to determine the risk of recurrent ICH and vaso-occlusive events after an ICH. Although there is some evidence that the incidence of vaso-occlusive events is at least as high as that of recurrent ICH after spontaneous primary ICH [Flynn et al. 2010], two hospital based studies have shown a higher incidence of ICH [Viswanathan et al. 2006;Chong et al. 2012]. I would aim to recruit more participants and continue prospective follow-up of participants for several years to increase the number of outcome events observed and determine the incidence rates of both recurrent ICH and vaso-occlusive events.

Table 13 Causes of death listed in Section 1A of the death certificate in 72 participants who died in the first year following first-ever spontaneous primary ICH

Primary cause of death	Frequency (%)
Intracerebral haemorrhage	46 (64)
Intracranial haemorrhage (non-traumatic), unspecified	7 (10)
Stroke, not specified as haemorrhage or infarction	2 (3)
Bronchopneumonia	13 (19)
Acute myocardial infarction	1 (1)
Infarcted bowel	1 (1)
Malignant neoplasm of biliary tract	1 (1)
Oesophageal varices with bleeding	1 (1)

Table 14 Logistic regression model of predictors of death in the first 30 days after first-ever spontaneous primary ICH

Variable	OR, 95% CI	p
Age (years)	1.04 (1.00-1.09)	0.047
Glasgow coma scale	0.70 (0.58-0.84)	<0.001
ICH volume (ml)	1.06 (1.03-1.09)	<0.001
Lobar ICH	0.19 (0.05-0.69)	0.012
Presence of intraventricular extension	1.36 (0.41-4.51)	0.613
Anticoagulant use	2.29 (0.43-12.20)	0.331

Table 15 Logistic regression model of predictors of death in the first 30 days after first-ever spontaneous primary supratentorial ICH

Variable	OR, 95% CI	p
Age (years)	1.05 (1.00-1.10)	0.045
Glasgow coma scale	0.73 (0.60-0.89)	0.002
ICH volume (ml)	1.06 (1.03-1.10)	<0.001
Lobar ICH	0.26 (0.07-1.03)	0.06
Presence of intraventricular extension	1.33 (0.37-4.80)	0.663
Anticoagulant use	0.77 (0.09-6.80)	0.814

Table 16 Logistic regression model of predictors of death in the first year after first-ever spontaneous primary ICH

Variable	OR, 95% CI	p value
Age (years)	1.07 (1.03-1.12)	0.001
Glasgow coma scale	0.80 (0.67-0.95)	0.013
ICH volume (ml)	1.05 (1.02-1.08)	0.001
Lobar ICH	0.21 (0.07-0.64)	0.006
Presence of intraventricular extension	0.64 (0.23-1.80)	0.430
Anticoagulant use	2.22 (0.46-10.65)	0.319

Table 17 Logistic regression model of predictors of death in the first year after first-ever spontaneous primary supratentorial ICH

Variable	OR, 95% CI	p
Age (years)	1.07 (1.02-1.11)	0.002
Glasgow coma scale	0.83 (0.69-1.01)	0.06
ICH volume (ml)	1.05 (1.02-1.08)	0.001
Lobar ICH	0.28 (0.09-0.88)	0.03
Presence of intraventricular extension	0.66 (0.23-1.91)	0.448
Anticoagulant use	1.44 (0.24-8.63)	0.688

Table 18 Number of deaths in the first year after first-ever primary ICH, stratified by ICH volume

ICH volume (ml)	Lobar ICH		Non-lobar ICH	
	n	Deaths in first year, n (%)	n	Deaths in first year, n (%)
0-29	31	10 (32)	46	20 (43)
30-59*	12	6 (50)	11	10 (91)
60-90	22	20 (91)	2	2 (100)

*p=0.04

Table 19 Deaths in the first 30 days after first-ever spontaneous primary ICH according to ICH score

ICH score	n (%)	Deaths in first 30 days n,(%; 95% CI)
0	21 (17)	1 (5; 1-22)
1	33 (27)	5 (15; 7-31)
2	26 (21)	13 (50; 32-68)
3	22 (18)	12 (55; 35-73)
4	12 (10)	11 (92; 65-99)
5	8 (7)	8 (100; 68-100)

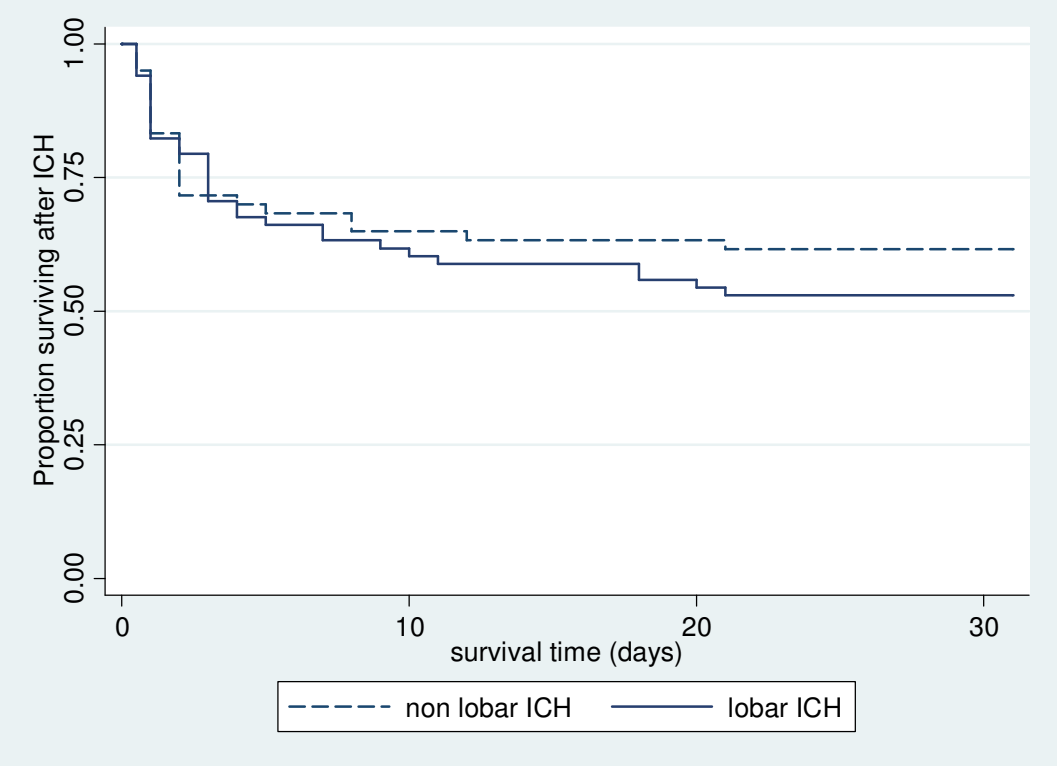
Table 20 Logistic regression model of 30 day case fatality using the ICH score after first-ever spontaneous primary supratentorial ICH

Variable	OR, 95% CI	p
Age (≥80 years)	1.91 (0.71-5.17)	0.20
Glasgow coma scale		
13-15	1.00	
5-12	6.25 (2.18-17.87)	0.001
3-4	24.18 (2.58-226.50)	0.005
ICH volume (≥ 30ml)	5.75 (1.88-17.56)	0.002
Infratentorial ICH	7.13 (1.57-32.49)	0.011
Presence of intraventricular extension	1.19 (0.41-3.44)	0.753

Table 21 Frequency of myocardial infarction, TIA and ischaemic strokes observed in 128 participants following first-ever spontaneous primary ICH

	Lobar ICH (n=68)	Non-lobar ICH (n=60)
Myocardial infarction	0	2
TIA	2	1
Ischaemic stroke	1	1

Figure 28 Kaplan-Meier curve of survival time following first-ever spontaneous primary ICH



	Number at risk (number of deaths during subsequent 10 day period)			
Lobar	68 (26)	42 (4)	38 (2)	36
Non-lobar	60 (21)	39 (1)	38 (1)	37

Figure 29 Receiver operator curve for ICH score applied to 122 participants with first-ever spontaneous primary ICH

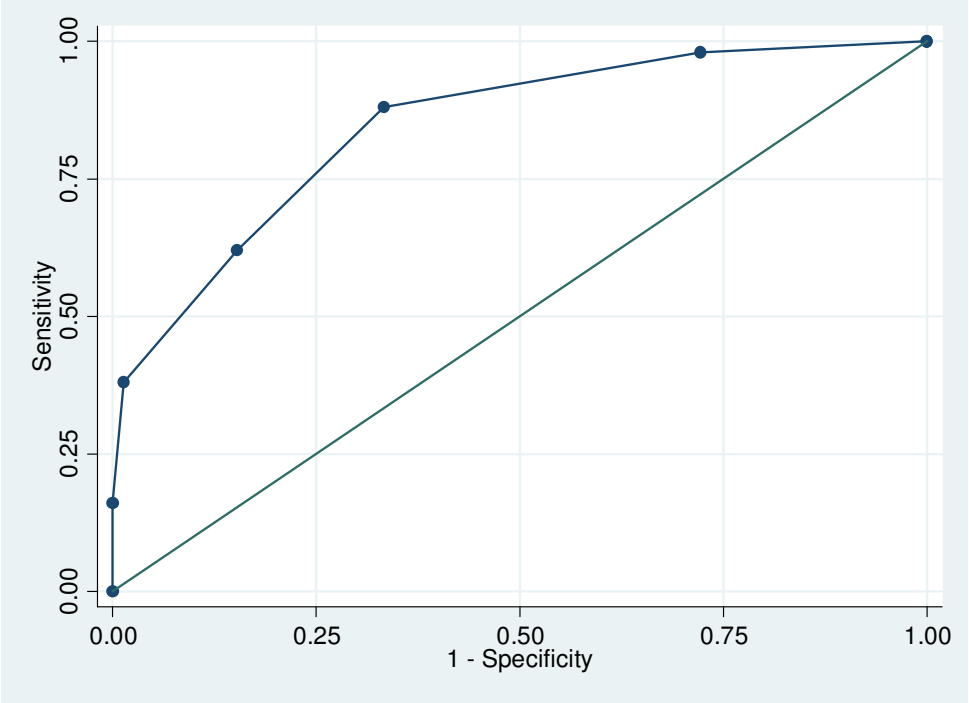


Figure 30 Bar chart showing modified Rankin scale scores of 124 participants one year after first-ever spontaneous primary ICH

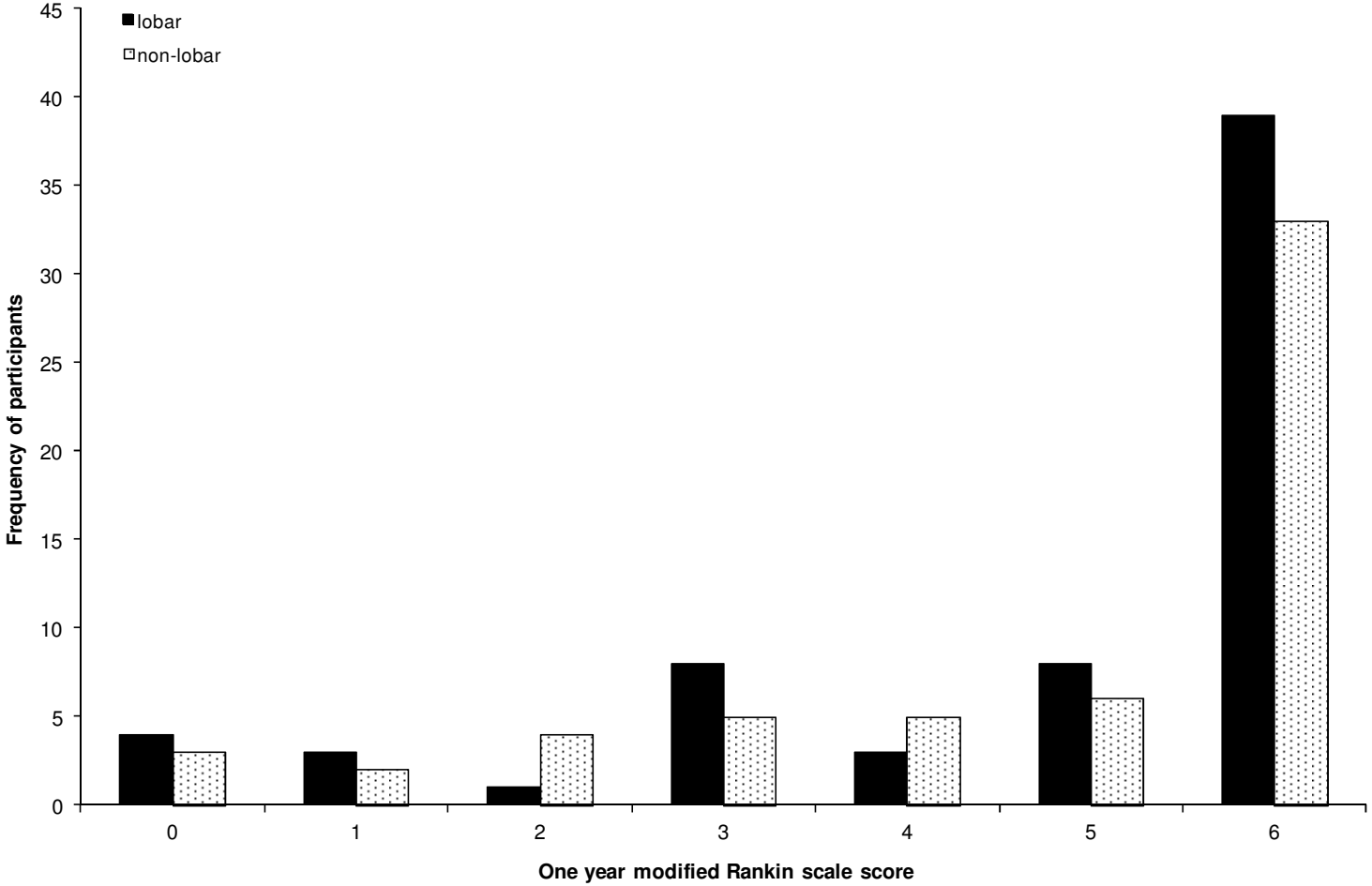
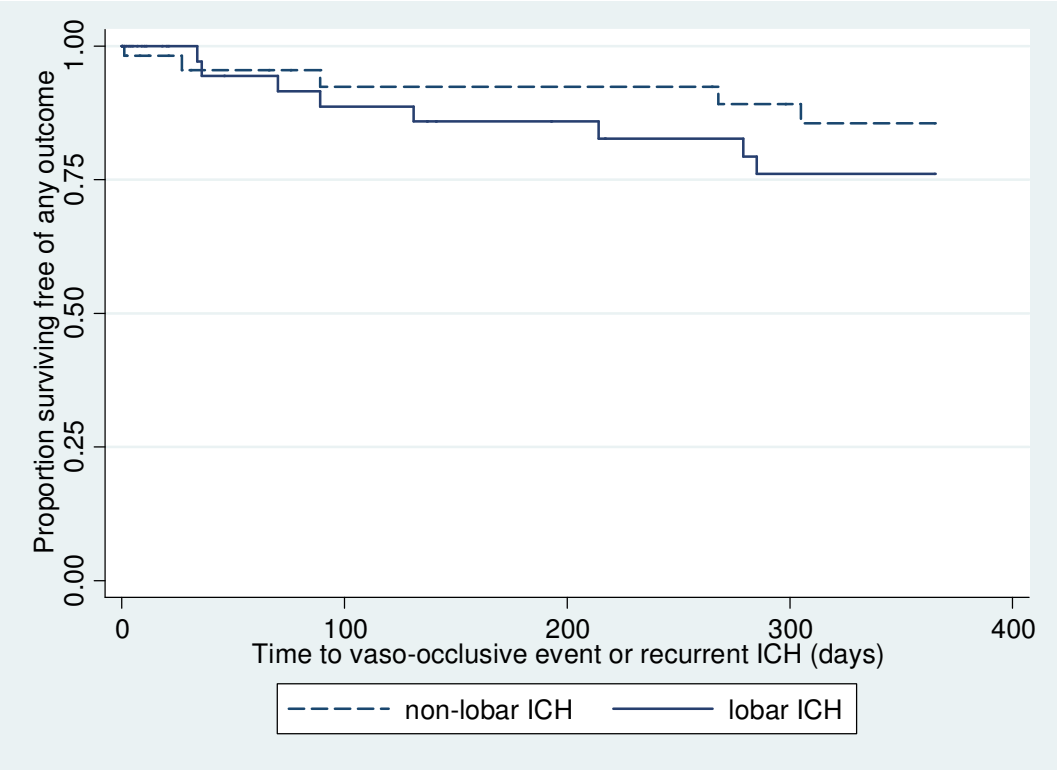
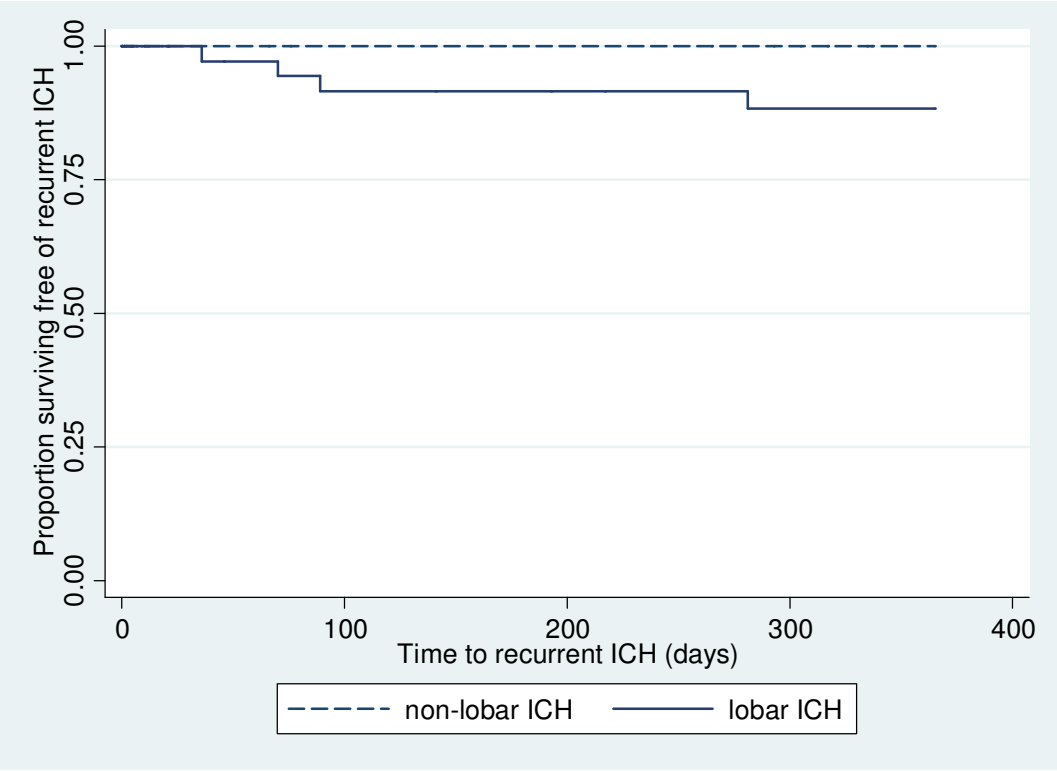


Figure 31 Kaplan-Meier curve of time to any vaso-occlusive event or recurrent ICH following first-ever spontaneous primary ICH, stratified by ICH location



	Number at risk (number of events during each 100 day interval)			
Lobar	68 (4)	31 (1)	27 (3)	23 (0)
Non-lobar	60 (3)	30 (0)	30 (1)	25 (1)

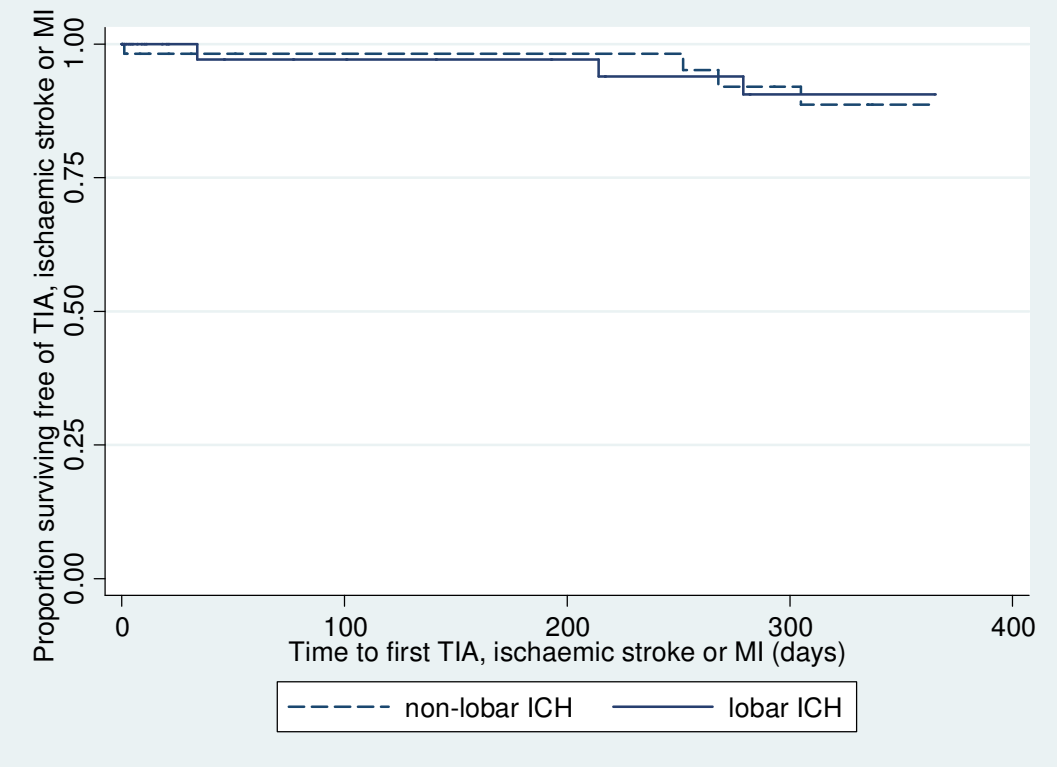
Figure 32 Kaplan-Meier curve of time to recurrent ICH following first-ever spontaneous primary ICH



Number at risk (number of events during each 100 day interval)

Lobar	68 (3)	32 (0)	29 (1)	27 (0)
Non-lobar	60 (0)	33 (0)	33 (0)	29 (0)

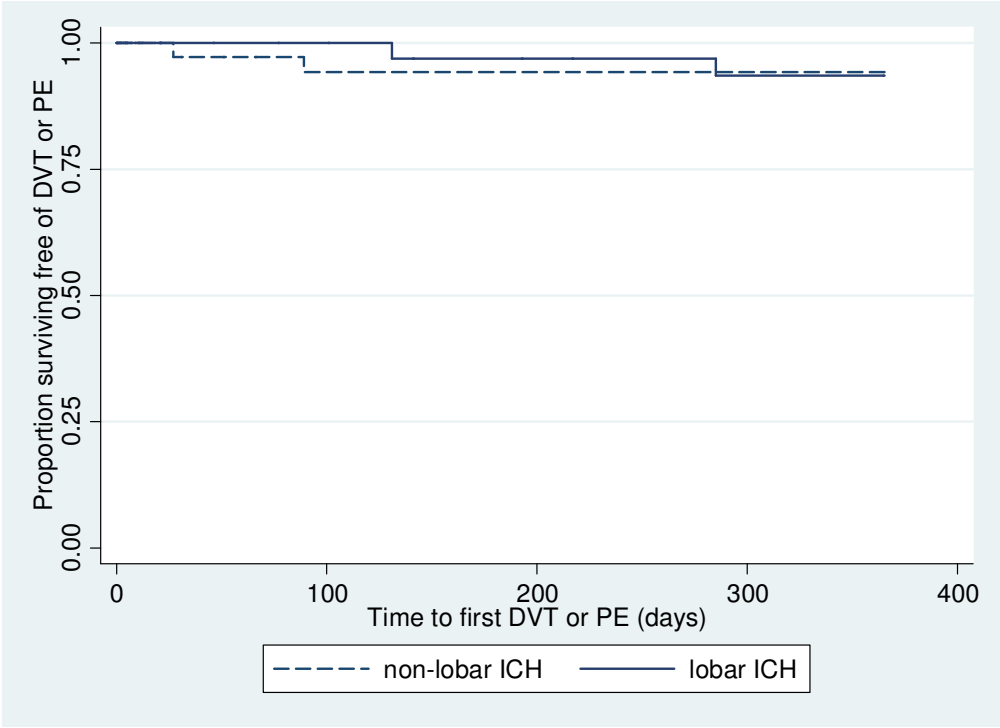
Figure 33 Kaplan-Meier curve of time to first-ever TIA, ischaemic stroke or myocardial infarction following first-ever spontaneous primary ICH



Number at risk (number of events during each 100 day interval)

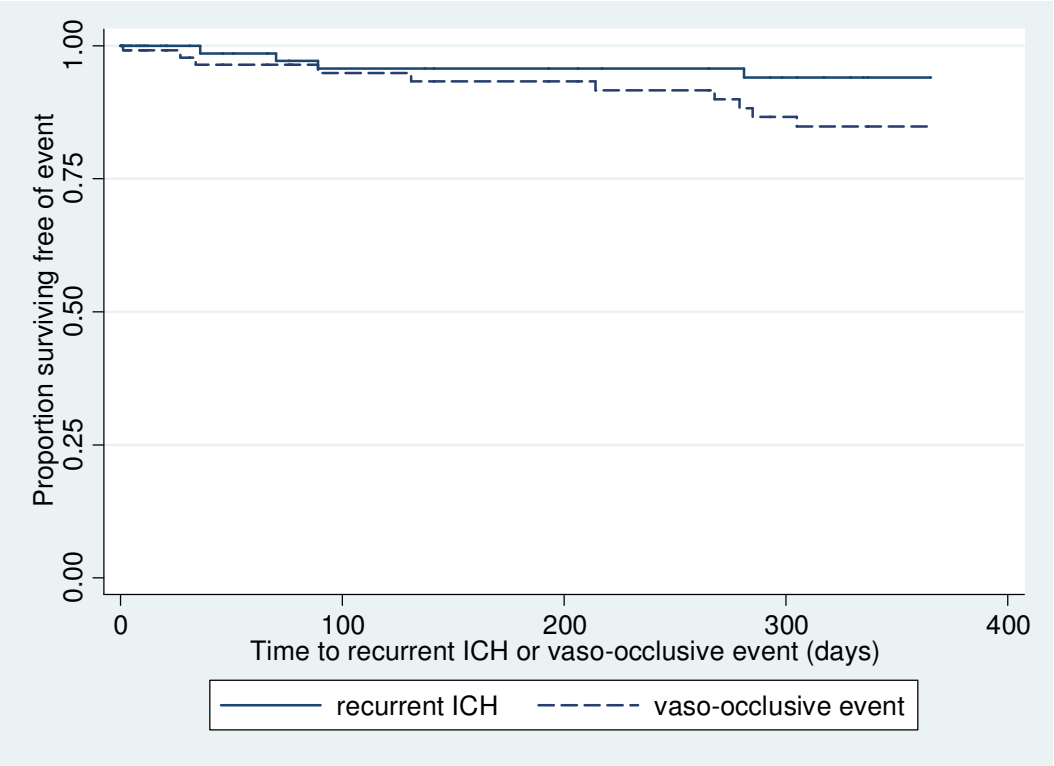
Lobar	68 (1)	32 (0)	28 (2)	24 (0)
Non-lobar	60 (1)	32 (0)	32 (2)	26 (1)

Figure 34 Kaplan-Meier curve of time to DVT or PE following first-ever spontaneous primary ICH



	Number at risk (number of events during each 100 day interval)			
Lobar	68 (0)	33 (1)	28 (1)	26 (0)
Non-lobar	60 (2)	31 (0)	31 (0)	27 (0)

Figure 35 Kaplan-Meier curve of time to recurrent ICH or vaso-occlusive event following first-ever spontaneous primary ICH



	Number at risk (number of events during each 100 day interval)			
Recurrent ICH	128 (3)	65 (0)	62 (1)	56 (0)
Vaso-occlusive event	128 (4)	63 (1)	58 (4)	49 (1)

Chapter 8 A cross-sectional study of apolipoprotein E genotypes in lobar vs. non-lobar intracerebral haemorrhage

Chapter contents

8 A cross-sectional study of apolipoprotein E genotypes in lobar vs. non-lobar intracerebral haemorrhage

8.1 Introduction

The three common alleles of the apolipoprotein E gene, ε2, ε3 and ε4 lead to single amino acid changes in the apolipoprotein E protein [Verghese, Castellano, & Holtzman 2011]. The ε4 allele increases the risk of pathologically-proven CAA in a dose-dependent manner [Rannikmae et al. 2013b]. The ε4 allele has also been associated with an increased risk of lobar and supratentorial deep ICH in comparison to unaffected controls [Biffi et al. 2010b] and it may also contribute to an increased risk of death after ICH [Martinez-Gonzalez & Sudlow 2006] although the results of studies conflict [Biffi et al. 2011a]. The ε2 allele has been associated with an increased risk of haematoma expansion [Brouwers et al. 2012] and death [Biffi et al. 2011a] after lobar ICH.

In the following chapter I will:

- describe the baseline characteristics of participants who underwent genotyping in the first two years of the study (1st June 2010 – 31st May 2012);
- describe the proportion of participants with ε2 and ε4 alleles stratified by ICH location who underwent genotyping in the first two years of the study (1st June 2010 – 31st May 2012) and
- compare the outcomes (death in the first 30 days following an ICH and death or dependency in the first year after an ICH) for those who possessed at least one ε2 or ε4 allele vs. those who did not, for those recruited in the first year of the study (1st June 2010-31st May 2011).

8.2 Methods

8.2.1 Participants

Participants recruited into the LINCHPIN study (an ethically-approved interventional study of patients with spontaneous primary ICH), underwent apolipoprotein E genotyping. The inclusion and exclusion criteria, genotyping procedure and phenotype

definition are described in Chapter 4. The definitions of baseline characteristics are described in Chapter 2.

I applied the modified Boston criteria for CAA [Knudsen et al. 2001;Linn et al. 2010] by integrating MRI brain and post-mortem examination findings where available, to the cohort to describe the frequency of possible, probable and definite CAA-related ICH as described in Chapter 5. I compared the proportion of participants with either an ε2 or ε4 allele in the definite or probable CAA-related ICH group vs. the possible CAA-related ICH group.

For participants in the first year cohort, I assessed outcomes (death in the first 30 days following an ICH and death or dependency in the first year after an ICH) as described in Chapter 7. I examined the proportion of participants with either an ε2 or ε4 allele who died in the first 30 days following an ICH vs. those who did not. Similarly, I compared the proportion of participants with either an ε2 or ε4 allele who died in the first year after an ICH or were dependent (modified Rankin scale 3-5) [Rankin 1957] one year after an ICH.

8.2.2 Statistical analysis

I used parametric statistics for between group comparisons when the data had a normal distribution and log-transformed variables or used non-parametric statistics when it did not. If an estimated cell count was less than five, I used the Fisher's exact test. I conducted all statistical analyses using Stata version 11.1 (StataCorp LP, Texas, USA). All p values are two-sided.

8.3 Results

8.3.1 Study participants

Of the 90 participants, 48 had lobar ICH. 84 participants had a first-ever ICH. Three participants had recurrent lobar ICH and three had recurrent non-lobar ICH. The baseline characteristics of 84 participants with first-ever lobar and non-lobar ICH are shown in Table 22 on page 206. There were no significant differences between participants with lobar and non-lobar ICH in any baseline demographic or clinical characteristics. One participant with recurrent non-lobar ICH was of Chinese ethnic

origin and the remainder were Caucasian. Dementia was more common in those with lobar ICH, although this difference was not statistically significant. The genotype frequencies are given in Table 3 and Table 4 on page 116.

8.3.2 Proportion of participants with at least one ε2 allele in first-ever lobar vs. non-lobar ICH

There were 81 participants with first-ever ICH for whom the presence of an ε2 allele could be determined (Table 4 on page 116). 11 out of 42 participants (26%; 95% CI 15-41%) with first-ever lobar ICH had one ε2 allele in comparison to five out of 39 participants (13%; 95% CI 6-27%) with non-lobar ICH (χ^2= 2.28, p=0.13). No participants possessed an ε2ε2 genotype.

8.3.3 Proportion of participants with at least one ε4 allele in first-ever lobar vs.non-lobar ICH

There were 81 participants with first-ever ICH for whom the presence of an ε4 allele could be determined (Table 4 on page 116). 18 out of 44 participants (41%; 95% CI 28-56%) with first-ever lobar ICH had at least one ε4 allele in comparison to seven out of 37 participants (19%; 95% CI 10-34%) with non-lobar ICH (χ^2= 4.56, p=0.03). Three participants with first-ever lobar ICH had an ε4ε4 genotype compared to none in the non-lobar ICH group but this difference was not statistically significant (p=0.25).

8.3.4 Application of the modified Boston criteria to adults with first-ever spontaneous primary lobar ICH

Two out of 45 participants (4%) with first-ever lobar ICH had an ICH involving both the lobar and deep regions of the brain and were therefore not eligible for classification under the modified Boston criteria. A further three participants (7%) were aged under 55 years old and the genotype of one participant (2%) was undetermined. Of the remaining 39 participants, 33 (85%) had undergone either MRI (n=22) or post-mortem examination (n=11). Five had definite CAA-related ICH according to the modified Boston criteria and eight had probable CAA-related ICH.

8.3.5 Proportion of participants with at least one ε2 allele in probable or definite CAA vs. possible CAA

Two out of 13 participants (15%; 95% CI 4-42%) with probable or definite CAA-related ICH had an ε2 allele in comparison to eight out of 26 participants (31%; 17-50%) with possible CAA-related ICH. There was no association between the presence of an ε2 allele and probable or definite CAA-related ICH ((χ^2=1.08, p=0.30).

8.3.6 Proportion of participants with at least one ε4 allele in probable or definite CAA vs. possible CAA

Possession of at least one ε4 allele was more frequent in the probable and definite CAA group (7/13 (54%); 95% CI 29-77%) vs. the possible CAA group (8/26 (31%); 95% CI 17-50%), although this difference was not significant (χ^2=1.95, p=0.16.)

8.3.7 Possession of at least one ε2 allele and death or dependency

49 participants were recruited in the first year of the study (1st June 2010-31st May 2011), all of whom had outcome data. The presence of at least one ε2 allele could be determined in 48 participants.

8.3.7.1 ε2 allele and death in the first 30 days following a first-ever ICH

The possession of at least one ε2 allele was not associated with death in the first 30 days following a first-ever ICH (has at least one ε2 allele 1/8 (13%); 95% CI 2-47% vs. does not have an ε2 allele 8/40 (20%); 95% CI 11-35%; $\chi^2$0.25, p=0.62). In an analysis stratified by ICH location the results were unchanged.

8.3.7.2 ε2 allele and death or dependency in the first year following a first-ever ICH

The possession of at least one ε2 allele was not associated with death or dependency (modified Rankin scale 3-5) at one year (has at least one ε2 allele 5/8 (63%); 95% CI 31-86% vs. does not have an ε2 allele 29/40 (73%); 95% CI 57-84%; $\chi^2$0.32, p=0.57). In an analysis stratified by ICH location the results were unchanged.

8.3.8 Possession of at least one ε4 allele and death or dependency

The presence of at least one ε4 allele could be determined in 47 participants.

8.3.8.1 ε4 allele and death in the first 30 days following a first-ever ICH

The possession of at least one ε4 allele was not associated with death in the first 30 days following a first-ever ICH (has at least one ε4 allele 5/18 (28%); 95% CI 13-51% vs. does not have an ε4 allele 4/29 (14%); 95% CI 6-31%; $\chi^2$1.40, p=0.24). In an analysis stratified by ICH location the results were unchanged.

8.3.8.2 ε4 allele and death or dependency in the first year following a first-ever ICH

Possession of at least one ε4 allele was more frequent in those who were either dead or dependent (modified Rankin scale 3-5) at one year; (has at least one ε4 allele 15/18 (84%); 95% CI 61-94% vs. does not have an ε4 allele 18/29 (62%); 95% CI 44-77%) but this difference was not statistically significant ($\chi^2$2.40, p=0.12). In an analysis stratified by ICH location the results were unchanged.

8.4 Summary

- The ε4 allele was significantly more common in participants with lobar ICH in comparison to those with non-lobar ICH.
- There was no difference in the frequency of the ε2 allele between those with lobar and non-lobar ICH.
- The ε4 allele was more frequent in those participants with probable or definite CAA-related ICH in comparison to those with possible CAA-related ICH according to the modified Boston criteria for CAA but the difference was not significant.
- The presence of at least one ε2 allele or ε4 allele was not associated with death in the first 30 days following a first-ever ICH or death or dependency at one year.

8.5 Discussion

8.5.1 Strengths of the study

The study meets criteria for genetic association studies including [Dichgans & Markus 2005;Little et al. 2009]:

(i) establishment of the hypothesis to be tested *a priori*;

(ii) consistent definitions of baseline characteristics of participants (Chapter 2);

(iii) a population-based design;

(iv) careful phenotyping of lobar and non-lobar ICH by a neuroradiologist with a special interest in stroke who was blind to the participant's genotype and

(v) reporting of baseline characteristics and outcome events for all participants.

Both comparison groups were drawn from the same population and did not differ in either their ethnic composition or other baseline characteristics. Since allele frequencies may differ by ethnicity; for example, the frequency of apolipoprotein ε4 alleles is lower in Asians [Tzourio et al. 2008], ethnic differences between comparison groups may lead to differences in allele frequencies and disease risks, giving rise to confounding (otherwise known as population stratification [Little et al. 2009]). It is unlikely that population stratification has affected the findings of this study.

8.5.2 Limitations of the study

This is a small genetic association study of the apolipoprotein E gene and ICH phenotypes. It should be viewed as a precursor to future larger studies using samples from the LINCHPIN study.

8.5.3 Study findings

8.5.3.1 ε2 and ε4 alleles and ICH location

The ε4 allele was significantly more common in participants with lobar ICH in comparison to those with non-lobar ICH.

This is consistent with the findings of other studies including a meta-analysis of 2189 ICH cases and 4041 controls which showed an association between the ε4 and ε2 alleles and lobar ICH in comparison to controls without a past history of ICH or dementia [Biffi et al. 2010b]. This meta-analysis also demonstrated an association between the ε4 allele and both deep and brainstem ICH. If these are true associations, then the association observed in my study between the ε4 allele and lobar ICH might have been stronger had I used non-ICH controls as the comparison group, rather than participants with non-lobar ICH. Although dementia appeared to be more frequent in those with lobar ICH in the cohort it is unlikely that dementia (through its association with CAA) is

acting as a confounder of the association between the ε4 allele and lobar ICH, since CAA is associated with the ε4 allele independent of its association with dementia [Rannikmae et al. 2013b]. However, the association between CAA and lobar ICH needs further exploration in demented and non-demented individuals.

I did not demonstrate a statistically significant difference in the proportion of participants possessing an ε2 allele in the lobar ICH (vs. non-lobar ICH) group or a difference in the proportions of participants with an ε2 or ε4 allele when those with probable and definite CAA-related ICH according to the modified Boston criteria [Knudsen et al. 2001;Linn et al. 2010] were compared to those with possible CAA-related ICH. Given the small number of participants, it is likely that the study was underpowered to detect a difference if one did exist. A recent meta-analysis [Biffi et al. 2010b] which found that ε2 and ε4 alleles were associated with probable and definite CAA according to the modified Boston criteria [Knudsen et al. 2001;Linn et al. 2010] used age-matched controls without a history of ICH as a comparison group. Since I used those with possible CAA-related ICH as a comparison group, any association may have been underestimated given the higher underlying prevalence of ε2 and ε4 alleles in those with lobar ICH in comparison to controls without ICH [Sudlow et al. 2006;Biffi et al. 2010b].

Since the Boston criteria for possible, probable and definite CAA rely upon integrating a radiological description of lobar ICH with MRI findings of BMBs or superficial siderosis and/or pathological findings, participants may have been misclassified if:

(i) they had a single lobar ICH and did not undergo either MRI or post-mortem examination in which case they could only be classified as having 'possible' CAA;

(ii) BMBs were incorrectly assessed on MRI or

(iii) CAA was missed at post-mortem examination.

The majority (85%) of participants had undergone either MRI or post-mortem examination. A neuroradiologist reviewed all imaging (Chapter 3) and an experienced neuropathologist used an extensive post-mortem examination sampling protocol (Appendix) and rated CAA blind to the participant's genotype. These factors mitigate against misclassification of participants, although given the paucity of radio-pathological

correlation studies of BMBs, pathological confirmation of CAA remains the gold standard.

8.5.3.2 ε2 and ε4 alleles and death or dependency

I did not demonstrate an association between the presence of at least one ε2 or ε4 allele and death or dependency in the whole cohort or when subdivided by ICH location. The study may have been underpowered to detect an association if one does exist. A recent study found that carriers of the ε2 allele (but not the ε4 allele) who had lobar ICH had larger ICH volumes, poorer functional outcomes (assessed by telephone using the modified Rankin scale at 90 days) and increased mortality [Biffi et al. 2011a]. There was no such association for deep ICH [Biffi et al. 2011a]. In one other study the ε4 allele seemed to be associated with an increased likelihood of death or dependency following ICH although the association was not statistically significant (OR 1.38; 95% CI 0.99-1.92) [Martinez-Gonzalez & Sudlow 2006]. The mechanism by which either or both of these alleles might influence outcome is unclear and needs further exploration in large population-based studies using assessment of outcomes blinded to genotype at a standard interval following ICH.

8.5.4 Future research

Although the association between the ε4 allele and lobar ICH demonstrated in this small study is meaningful (in view of the adequate study quality and similar findings in other larger independent populations), the biological mechanism underlying the association between ε2 and ε4 alleles and lobar ICH remains unclear. A common hypothesis is that the ε4 allele increases the deposition of Aβ in the vessel wall and ε2 promotes the vasculopathic changes that lead to rupture [McCarron et al. 1999b]. However, an individual patient data meta-analysis of participants with pathologically proven CAA (without ICH) in comparison to controls did not show any association between the ε2 allele and vasculopathy [Rannikmae et al. 2013a]. Further studies of ICH which correlate genotyping information with pathological findings may clarify the mechanism behind the association of these alleles and lobar ICH.

The severity of CAA is associated with the number of ε4 alleles in patients with Alzheimer's dementia but a dose response relationship is yet to be established in those

with ICH. When analysing data in genetic association studies several different genetic models exist [Lewis 2002]. In studies of apolipoprotein E the dominant model is most commonly used which presupposes that carrying the ε4 allele confers an increased risk of disease and therefore the ε2/ε4, ε3/ε4 and ε4/ε4 genotypes are pooled giving a two by two table in which both comparison groups are classed as ε4+ or ε4 -. An additive model presupposes an increased risk of r for ε2/ε4 and ε3/ε4 genotypes and 2r for an ε4/ε4 genotype. There is some evidence to support a dose response relationship since an additive genetic model in one study provided a more accurate prediction of disease status (lobar ICH vs. control) in comparison to the commonly used dominant model [Biffi et al. 2010b].

The role of apolipoprotein E in non-lobar ICH is as yet unknown. Despite one study showing an association between the ε4 allele and brainstem and deep ICH [Biffi et al. 2010b], and a further study showing an association between both ε2 and ε4 alleles and supratentorial deep ICH [Tzourio et al. 2008], other studies have failed to show an association between these alleles and non-lobar ICH [Sudlow et al. 2006;Peck et al. 2008]. Future studies should explore this, especially given the inclusion of cerebellar BMBs in the Boston criteria for 'probable' CAA [Knudsen et al. 2001]. Given the low incidence of infratentorial ICH a multicentre collaborative approach is likely to be required with meticulous phenotyping of cases.

Table 22 Baseline characteristics for 84 participants with first-ever ICH

	Any lobar (n=45)	Non-lobar ICH (n=39)	p value
Sex (male); (%)	18 (40)	21 (54)	0.20
Age (years); median (IQR)	77 (69-81)	73 (59-77)	0.12
History of hypertension			
Yes (n, %)	27 (60)	26 (67)	0.53
History of diabetes			
Yes (n, %)	2 (4)	5 (13)	0.24
History of dementia			
Yes (n, %)	8 (18)	2 (5)	0.10
History of smoking			
Never (n, %)	18 (40)	16 (41)	
Ex-smoker (n, %)	16 (36)	17 (44)	
Current (n, %)	11 (24)	6 (15)	0.55
Premorbid medication			
Antiplatelet use (n, %)	17 (38)	12 (31)	0.50
Anticoagulant use (n, %)	7 (16)	2 (5)	0.17

Section D The pathology of intracerebral haemorrhage in the Lothian region of Scotland

Chapter 9 The association between cerebral amyloid angiopathy and intracerebral haemorrhage: a systematic review and meta-analysis

Chapter contents

9 The association between cerebral amyloid angiopathy and intracerebral haemorrhage: a systematic review and meta-analysis

9.1 Introduction

During the last decade spontaneous (non-traumatic) ICH accounted for ~10% of strokes in high income countries and ~20% of strokes in low/middle income countries, where the one month case fatalities were 25–35% and 30-48% respectively [Feigin et al. 2009]. Although time trends have varied between regions, the case fatality one month after ICH has remained ~40% across the globe during the last few decades [van Asch et al. 2010].

Understanding that systemic arterial hypertension is the strongest modifiable risk factor for ICH led to trials of secondary prevention with antihypertensive drugs [Ariesen et al. 2003;Chapman et al. 2004] which appear to improve outcome for survivors of ICH (regardless of its location)[Arima et al. 2010]. Further improvements in outcome could arise from better understanding the causes of ICH. Because survivors of lobar ICH appear to be at a higher risk of recurrent ICH than survivors of deep ICH [Bailey et al. 2001b], the causes of lobar ICH are of particular interest.

The deposition of β-amyloid peptide in the media of cortical and leptomeningeal arteries, arterioles, and capillaries – now known as cerebral amyloid angiopathy (CAA) – was first identified in the early twentieth century [Oppenheim 1909;Scholz 1938]. CAA is quite prevalent in cognitively unimpaired elderly people and even more prevalent in those with dementia [Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) 2001].

Since the late 1970s influential case series have suggested that CAA may cause lobar ICH [Jellinger 1977;Okazaki, Reagan, & Campbell 1979]. However, in everyday clinical practice, [Cordonnier et al. 2010a] CAA is often inferred to be the cause of lobar or cerebellar ICH, especially if the patient is elderly, the ICHs are recurrent or multifocal, or haem-sensitive GRE MRI sequences demonstrate at least one lobar brain BMB [Knudsen et al. 2001].

However, the strength of the overall association between CAA and ICH remains to be precisely quantified (in lobar and cerebellar locations in particular, but also in deep locations [Ritter et al. 2005]), so I performed a systematic review and meta-analysis of published neuropathological studies. I aimed to take account of three potential confounding factors that should be described, and preferably controlled or adjusted for in comparisons of cases and controls: firstly, patients' ages, given the increasing CAA prevalence with age [Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) 2001]; secondly, cognitive impairment (and its severity), given the greater prevalence of CAA in those with cognitive impairment than those without dementia; [Masuda et al. 1988;Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) 2001] and thirdly, patients' racial origins, given racial differences in the prevalence of apolipoprotein E polymorphisms which have been associated with ICH due to CAA [Corbo & Scacchi 1999;Biffi et al. 2010b] and racial differences in the proportion of ICH in a lobar location [Lavados et al. 2010].

9.2 Methods

9.2.1 *Search strategy and selection criteria*

In April 2011 I searched Ovid Medline (1950-) and Embase (1980-) using comprehensive electronic search strategies (Table 23 on page 218 and Table 24 on page 219). I searched the bibliographies of relevant publications and Google scholar for other papers citing each included paper. I also searched the tables of contents of several journals (The Journal of Neurology, Neurosurgery and Psychiatry; The Lancet Neurology; Annals of Neurology; Brain; Lancet; Neurology; and Stroke) since 2005 and our personal files.

9.2.2 *Eligibility criteria*

Studies were eligible for inclusion if they had diagnosed CAA on pathological examination of a brain biopsy or at post-mortem examination and quantified the prevalence of CAA in patients with spontaneous (non-traumatic) ICH as well as a group of patients without ICH.

9.2.3 *Data collection*

I screened all titles and abstracts for eligibility, removed duplicates and read the full text of articles that were potentially eligible for inclusion. Eligible studies were read in full by myself and Professor Salman who extracted data independently on: study design, the types of cases and controls, methods of assessment and grading of CAA, the prevalence of CAA in cases and controls, and whether any confounders were accounted for. We resolved disagreements by discussion.

If pertinent study attributes or data were unavailable or unclear in an eligible publication, I sought clarification from the authors by post and email. I also sought individual patient data from included publications – either in person, or by email and post – in order to stratify or adjust the analyses for the potential confounding variables of age and co-morbid cognitive impairment, as well as to explore the strength of the association between ICH and CAA according to neuropathological severity of CAA; unfortunately, only one study provided these data [Ritter et al. 2005], precluding an individual patient data meta-analysis.

9.2.4 *Methodological assessment*

I was guided in my assessment of the methodological quality of the included studies by the Newcastle-Ottawa scale [Wells et al. 2002], which uses eight items to judge the quality of case-control studies on their selection of study groups, the comparability of their cases and controls, and their ascertainment of cases' and controls' exposure (i.e. to CAA). If a study fulfils the criteria for an item a score of one point is allocated, with the exception of comparability which can score up to two points, resulting in a maximum score of nine points.

9.2.5 *Statistical analysis*

If I identified multiple publications relating to the same cohort, I included the largest study. For each study, I determined the numbers of cases and controls and the prevalence of CAA in each group. I sought to stratify analyses by ICH location (deep, lobar, cerebellar, or all locations grouped together), age, neuropathological ratings of CAA severity, and racial origin of the participants. I meta-analysed the data in StatsDirect statistical software version 2.7.8, using a random effects model with DerSimonian-Laird weights, quantified the strength of any association using the odds

ratio (OR) and its associated 95% CI, and assessed inconsistency with the I-squared (I^2) statistic. In a separate sensitivity analysis, I tested whether the restriction of our analysis to studies explicitly stating that all ICH were non-traumatic changed the direction or strength of the association between CAA and ICH in all locations. In sub-group analyses, I examined the strength of the relationship between CAA and cases with lobar ICH in studies where the average ages of cases and controls were comparable versus those where average ages of cases and controls were dissimilar or unknown, and in studies in which participants were Asian versus those in which they were not.

9.3 Results

The search strategies identified 1,824 articles, of which 19 appeared to be eligible (Figure 36 on page 223). I excluded nine studies because they provided insufficient data to quantify the numbers of cases and controls with and without CAA in seven studies [Tomonaga 1981;Yamada et al. 1987;Yamanouchi, Shimada, & Kuramoto 1990;Itoh et al. 1993;Ellis et al. 1996;Itoh & Yamada 1997;Jellinger 2006], cases were selected only if they were affected by CAA [Vonsattel et al. 1991], and the remaining study reported data already included in this systematic review [Jellinger, Lauda, & Attems 2007]. I included ten neuropathological studies involving 481 cases and 3,219 controls from China [Ng, Leung, & Wong 1991;Xu, Yang, & Wang 2003], Japan [Masuda et al. 1988;Ishihara et al. 1991], Chile [Cartier et al. 1999], India [Badhe, Mehta, & Desai 2005], Australia [Mastaglia et al. 2003] and Europe [Ritter et al. 2005;Attems, Lauda, & Jellinger 2008;Guidoux 2008].

9.3.1 *Critical appraisal*

I compared included studies to the ideal design for a study of the association between CAA and lobar ICH (Table 25 on page 220), and rated them using the Newcastle-Ottawa scale (Table 26 on page 221)[Wells et al. 2002].

9.3.2 *Selection of study groups*

Studies' ascertainment of cases ranged from highly representative samples of deaths in a community to consecutive, randomly sampled, or selected hospital autopsies (Table 26 on page 221). Eight studies clearly defined their cases as having spontaneous ICH [Ishihara et al. 1991;Ng, Leung, & Wong 1991;Cartier et al. 1999;Xu, Yang, & Wang

2003;Ritter et al. 2005;Badhe, Mehta, & Desai 2005;Attems, Lauda, & Jellinger 2008;Guidoux 2008] and the corresponding author confirmed that ICH cases were spontaneous in another study [Mastaglia et al. 2003]. Five studies stated that the ICH had been clinically symptomatic [Ng, Leung, & Wong 1991;Cartier et al. 1999;Ritter et al. 2005;Badhe, Mehta, & Desai 2005;Guidoux 2008]. Two studies included both first-ever and recurrent ICH [Attems, Lauda, & Jellinger 2008;Guidoux 2008], but the remainder did not specify the inception point for ICH cases. Only five studies described the ages of included cases [Ishihara et al. 1991;Cartier et al. 1999;Ritter et al. 2005;Attems, Lauda, & Jellinger 2008;Guidoux 2008]. Three studies did not systematically specify ICH locations [Ng, Leung, & Wong 1991;Xu, Yang, & Wang 2003;Badhe, Mehta, & Desai 2005] but the remainder did albeit with different categories (Table 26 on page 221). Furthermore, studies varied in their definitions of 'lobar' ICH, including: ICH that had originated in the cerebellum [Guidoux 2008], cortex or subarachnoid space [Ishihara et al. 1991]; subcortical, cortical or in the insular cortex closely related to the basal ganglia [Ritter et al. 2005]; and in another, lobar ICH was distinguished from multiple cortico-subcortical ICH [Attems, Lauda, & Jellinger 2008].

9.3.3 *Comparability of cases and controls*

All but one study [Badhe, Mehta, & Desai 2005] described ascertainment of controls from the same population as the cases (consecutive hospital post-mortem examination controls [Ng, Leung, & Wong 1991;Mastaglia et al. 2003;Xu, Yang, & Wang 2003;Attems, Lauda, & Jellinger 2008], selected hospital post-mortem examination controls, [Ishihara et al. 1991;Cartier et al. 1999;Ritter et al. 2005;Guidoux 2008] and community controls [Masuda et al. 1988]), such that the controls could have been cases had they been affected by ICH (Table 26 on page 221). In three studies, 46-82% of the control groups had ischaemic stroke [Ishihara et al. 1991;Ritter et al. 2005;Badhe, Mehta, & Desai 2005]. Considering potential confounding factors, two studies included controls with dementia diagnosed on clinical and neuropathological grounds [Xu, Yang, & Wang 2003;Attems, Lauda, & Jellinger 2008], only five studies described the average ages of their cases [Ishihara et al. 1991;Cartier et al. 1999;Ritter et al. 2005;Attems, Lauda, & Jellinger 2008;Guidoux 2008] and only four studies described the average ages of their controls [Ishihara et al. 1991;Cartier et al. 1999;Ritter et al. 2005;Guidoux 2008],

but just three studies accounted for confounding by matching the ages of cases and controls within five years [Cartier et al. 1999;Ritter et al. 2005;Guidoux 2008].

9.3.4 *Ascertainment of cases' and controls' exposures*

All studies but one [Guidoux 2008] assessed cases and controls for CAA in the same way (Table 27 on page 222), but only one study [Cartier et al. 1999] reported that the assessment was blinded (although the nature of blinding was unclear). The extent of sampling varied between studies (Table 27 on page 222). Eight studies used Congo Red staining to detect CAA [Masuda et al. 1988;Ishihara et al. 1991;Ng, Leung, & Wong 1991;Cartier et al. 1999;Xu, Yang, & Wang 2003;Ritter et al. 2005;Badhe, Mehta, & Desai 2005;Guidoux 2008], one of which also used immunohistochemistry in every case [Xu, Yang, & Wang 2003]. The rating of CAA severity involved a variety of rating scales – many of which were bespoke being devised by the authors themselves (Table 27 on page 222) – and CAA severity in cases and controls was seldom quantified in every patient. Following communication with the corresponding author of one study [Ritter et al. 2005], I established that only four studies described whether CAA was specifically present in the vessels adjacent to the ICH (so that lobar ICH could be attributed to lobar CAA) [Masuda et al. 1988;Ishihara et al. 1991;Ng, Leung, & Wong 1991;Xu, Yang, & Wang 2003].

9.3.5 *Association between CAA and ICH*

Our meta-analyses (Figure 37 on page 224) did not reveal an association between CAA and ICH in any location in all ten studies (OR 1.21, 95% CI 0.87 to 1.68, I-squared 29%;), nor in a sensitivity analysis omitting one study that might have included traumatic ICH (OR 1.19, 95% CI 0.84 to 1.67) [Masuda et al. 1988]. There was not a significant association between CAA and deep ICH (OR 0.81, 95% CI 0.30 to 2.19; five studies, I-squared 58%) [Masuda et al. 1988;Cartier et al. 1999;Mastaglia et al. 2003;Ritter et al. 2005;Attems, Lauda, & Jellinger 2008] or cerebellar ICH, although there were only eight cases of cerebellar ICH (OR 2.05, 95% CI 0.55 to 7.63; four studies, I-squared 0%) [Masuda et al. 1988;Mastaglia et al. 2003;Xu, Yang, & Wang 2003;Attems, Lauda, & Jellinger 2008]. However, CAA was more prevalent in lobar ICH cases (54/105, 51%) in comparison to controls (1,119/2,629, 43%) (OR 2.21 95% CI 1.09 to 4.45; six studies, I-squared 40%) [Masuda et al. 1988;Ishihara et al. 1991;Cartier et al. 1999;Mastaglia et al. 2003;Ritter et al. 2005;Attems, Lauda, & Jellinger

2008]. In sub-group analyses of the association between CAA and lobar ICH, the association remained in the three studies where cases' and controls' average ages were comparable (OR 3.24, 95% CI 1.02 to 10.26),14;31;32 but not in those where ages were dissimilar or unknown (OR 1.58, 95% CI 0.91 to 2.77) [Masuda et al. 1988;Mastaglia et al. 2003;Attems, Lauda, & Jellinger 2008]. I could not demonstrate that the association between CAA and lobar ICH was different in studies of Asian patients (OR 3.02, 95% CI 0.44 to 20.77) [Masuda et al. 1988;Ishihara et al. 1991] or patients of other ethnic origins (OR 1.89, 95% CI 0.86 to 4.15) [Cartier et al. 1999;Mastaglia et al. 2003;Ritter et al. 2005;Attems, Lauda, & Jellinger 2008].

9.4 Summary

- In a systematic review and meta-analysis of ten neuropathological cross-sectional or case-control studies involving 481 cases and 3,219 controls, I found a significant association between CAA and lobar ICH, but not with ICH in other locations.
- There was wide variation in the methodological quality of included studies.
- Strategies for both brain sampling and CAA detection varied between studies.

9.5 Discussion

9.5.1 *Strengths of the study*

This systematic review and meta-analysis benefited from thorough ascertainment of pertinent studies, comprehensive critical appraisal to determine their inclusion, clarification by correspondence with study authors, and a large number of cases and controls in our analyses.

9.5.2 *Limitations of the study*

The systematic review was somewhat limited by the variable quality of included studies. Unfortunately, only three of the included studies assessed the association of CAA and ICH having taken other competing risk factors for ICH into account [Ishihara et al. 1991;Ritter et al. 2005;Guidoux 2008] and just two studies described the influence on the association of other potential effect modifiers (Alzheimer-type pathology [Attems, Lauda, & Jellinger 2008] and antithrombotic drugs [Ritter et al. 2005]).

9.5.3 *Study findings*

It is reassuring that our finding of an overall association between lobar ICH and CAA was confirmed by the three studies in which minimal confounding by patient age was evident [Ishihara et al. 1991;Mastaglia et al. 2003;Ritter et al. 2005].

This association between CAA and lobar ICH might have been even stronger had the included studies accounted for potential confounding factors (for example, age, severity of cognitive impairment, ethnic origin, and possibly prior ischaemic stroke [Cadavid et al. 2000]), included a consistent definition of 'lobar' ICH [Biffi et al. 2010b] and focussed on the prevalence of severe CAA (and other vasculopathic features, such as microaneurysms) in the blood vessels that were anatomically related to the ICH. The association might also have been stronger had the cases been selected according to the Boston diagnostic criteria, which 'definitely' attribute the cause of lobar ICH to CAA if there is pathological evidence of severe CAA with vasculopathy at post-mortem [Millar, Wardlaw, & Lammie 1999;Knudsen et al. 2001;Linn et al. 2010].

The Boston criteria for 'probable CAA' have an excellent specificity and therefore do not misclassify people who have lobar ICH without underlying severe CAA (100%, 95% CI 77% to 100%), [Knudsen et al. 2001] but the sensitivity of these criteria for 'probable CAA' was 44% (95% CI 28% to 62%) and their negative predictive value was 39% (95% CI 22 to 58), because more than half the people with lobar ICH and severe CAA were not identified by the 'probable' criteria [Knudsen et al. 2001].

Both systematic use of GRE MRI to identify brain BMBs and the inclusion of superficial siderosis in the Boston criteria have improved their diagnostic accuracy [Linn et al. 2010], but false positives and false negatives still exist and the role of other degrees of CAA severity in causing lobar ICH remains to be clarified, given that the studies in this meta-analysis were unable to do so.

Further confirmation of the direction of this association between CAA and lobar ICH, and exploration of the strength of the association, could only arise from further research with an ideal study design (Table 25 on page 220), given that an individual patient data meta-analysis was impossible.

9.5.4 *Future directions*

The prevalence of CAA in patients with lobar ICH and the strength of the overall association between CAA and lobar ICH (Figure 37 on page 224), as well as the diagnostic accuracy of the Boston criteria for ICH due to CAA, [Knudsen et al. 2001;Linn et al. 2010] is consistent with CAA being one of several potential causes of lobar ICH in the elderly. Although I have demonstrated an association between CAA and lobar ICH, this does not necessarily imply causation.

Of Sir Austin Bradford Hill's nine criteria [Hill 1965] that would support an association being causal, CAA is a plausible cause of lobar ICH [Weller, Boche, & Nicoll 2009], but further work is required to more reliably establish the association's strength, demonstrate its consistency and evaluate its biological gradient [Hill 1965]. If the methodological problems noted above are addressed and cases of lobar ICH are carefully phenotyped (according to their history of transient neurological events and cognitive impairment, and the presence of strictly lobar brain BMBs on GRE MRI and superficial siderosis [Linn et al. 2010]), then the strength of the association between CAA and lobar ICH would be likely to be much stronger. However, understanding whether milder degrees of CAA are associated with lobar ICH is also important to investigate the biological gradient and explore whether there are interactions with CAA of milder severity that might precipitate ICH.

Future research should include well-designed case-control and cohort studies to explore the CAA-ICH association (and its effect modifiers) [Schulz & Grimes 2002], individual patient data meta-analyses of comparable studies, further comparisons of the sensitivity and specificity of different methods of CAA detection (such as Congo Red staining versus immunohistochemistry [Haglund & Englund 2002]), and the development and validation of a unified rating scale for CAA distribution and severity [Greenberg et al. 2004].

Table 23 Ovid Medline search strategy

	Search term(s)
1	Stroke/
2	Cerebrovascular Disorders/
3	exp basal ganglia cerebrovascular disease/ or exp brain ischemia/ or carotid artery diseases/ or carotid artery thrombosis/ or carotid stenosis/ or exp intracranial arterial diseases/ or exp "intracranial embolism and thrombosis"/ or exp intracranial hemorrhages/ or exp brain infarction/ or hypoxia-ischemia, brain/
4	((brain$ or cerebr$ or cerebell$ or cortical or vertebrobasil$ or hemispher$ or intracran$ or intracerebr$ or infratentorial or supratentorial or mca$ or middle cerebr$ or anterior circulation or posterior circulation or basal ganglia or parenchyma$ or brain?stem or posterior fossa or ganglion$ or thalam$ or cortical) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypox$ or obstruction or vasculopathy)).tw.
5	((lacunar or cortical) adj5 infarct$).tw.
6	((brain$ or cerebr$ or cerebell$ or intracerebr$ or intracran$ or parenchyma$ or intraventricular or infratentorial or supratentorial or basal gang$ or ganglion$ or putaminal or putamen or posterior fossa or brain?stem or intra?axial or lobar or deep or thalam$ or cortical or superficial or vertebrobasil$ or front$ or tempor$ or pariet$ or occipit$) adj5 (haemorrhage$ or hemorrhage$ or haematoma$ or hematoma$ or bleed$)).tw.
7	((h?emorrhag$ or isch?emi$) adj6 (stroke$ or cerebrovasc$ or cerebr?vasc$ or cerebral vasc$ or brain vasc$ or cva$ or apoplex$ or attack$ or event$ or insult$)).tw.
8	1 or 2 or 3 or 4 or 5 or 6 or 7
9	exp Pathology, Clinical/
10	exp Amyloid beta-Protein/ or exp Amyloid/ or exp Amyloid beta-Protein Precursor/
11	exp Cerebral Amyloid Angiopathy/
12	exp Congo Red/
13	(cerebral amyloid angiopathy or congophil$ or congo?red or amyloid$ or A?beta or beta?amyloid).tw.
14	10 or 11 or 12 or 13
15	(patholog$ or post?mortem$ or autops$ or necrops$ or biops$ or tissue$ or histo?patholog$ or neuro?patholog$ or clinic?patholog$).tw.
16	9 or 15
17	8 and 14 and 16
18	limit 17 to humans

Table 24 Ovid Embase search strategy

	Search term(s)
1	cerebrovascular disease/
2	basal ganglion hemorrhage/ or cerebral artery disease/ or cerebrovascular accident/ or stroke/ or vertebrobasilar insufficiency/ or exp carotid artery disease/ or exp brain hematoma/ or exp brain hemorrhage/ or brain infarction/ or brain infarction size/ or brain stem infarction/or cerebellum infarction/ or exp brain ischemia/ or exp occlusive cerebrovascular disease/ or cerebellum injury/ or exp carotid artery/
3	((h?emorrhag$ or isch?emi$) adj6 (stroke$ or cerebrovasc$ or cerebr?vasc$ or cerebral vasc$ or brain vasc$ or cva$ or apoplex$ or attack$ or event$ or insult$)).tw.
4	((brain$ or cerebr$ or cerebell$ or cortical or vertebrobasil$ or hemispher$ or intracran$ or intracerebr$ or infratentorial or supratentorial or mca$ or middle cerebr$ or anterior circulation or posterior circulation or basal ganglia or parenchyma$ or brain?stem or posterior fossa or ganglion$ or thalam$ or cortical) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypox$ or obstruction or vasculopathy)).tw.
5	((lacunar or cortical) adj5 infarct$).tw.
6	((brain or cerebr$ or cerebell$ or intracerebr$ or intracran$ or parenchyma$ or intraventricular or infratentorial or supratentorial or basal gang$ or ganglion$ or putaminal or putamen or posterior fossa or brain?stem or intra?axial or lobar or deep or thalam$ or cortical or superficial or vertebrobasil$ or front$ or tempor$ or pariet$ or occipit$) adj5 (haemorrhage$ or hemorrhage$ or haematoma$ or hematoma$ or bleed$)).tw.
7	1 or 2 or 3 or 4 or 5 or 6
8	"amyloid beta protein [1-42]"/ or exp amyloid/ or "amyloid beta protein [1-40]"/ or exp amyloid precursor protein/ or exp amyloid beta protein/
9	exp vascular amyloidosis/
10	exp congo red/
11	(cerebral amyloid angiopathy or congophil$ or congo?red or amyloid$ or A?beta or beta?amyloid).tw.
12	8 or 9 or 10 or 11
13	exp pathology/
14	(pathолog$ or post?mortem$ or autops$ or necrops$ or biops$ or tissue$ or histo?patholog$ or neuro?patholog$ or clinic?patholog$).tw.
15	13 or 14
16	7 and 12 and 15
17	limit 16 to human

Table 25 Ideal design of a pathological study of the association between CAA & spontaneous ICH[Wells et al. 2002;Schulz & Grimes 2002]

Selection of cases and controls

- Representative sample of cases, with ascertainment clearly defined
- Independent validation of case diagnosis
    - clinical features described
    - incident cases of ICH, recruited at a specified inception point, described in relation to the time of neuropathological examination
    - spontaneous and traumatic ICH distinguished
    - first-ever and recurrent ICH distinguished
    - radiological confirmation of ICH diagnosis and its anatomical distribution, using a standardised classification of lobar vs. deep ICH (whose inter-observer reliability has been assessed)
- Appropriate controls
    - Derived from the same population as cases
    - Ascertained in the same way as the cases
    - Without a history of ICH (if cases were first-ever diagnoses)
- Quantification of eligible cases and controls not included or omitted from analyses

Assessment of CAA

- Pathologically confirmed either at tissue biopsy or at post-mortem examination
- Detected and rated blind to relevant clinical information
- Methods of tissue preparation, staining and analysis identical for all cases and controls
- Rated according to a standard or externally validated rating scale, and severity specified
- Anatomical location specified in relation to ICH

Reporting and analysis

- Presentation of summary data, stratified by ICH location, age of person, past history of cognitive impairment
- Cases and controls matched for major confounders (i.e. age and past history of cognitive impairment), or confounders adjusted for in the analysis

Table 26 Characteristics of the selection and comparability of cases and controls in the included studies

Study	Study design	Cases					Controls			
		Spontaneous haemorrhage?	Clinically symptomatic?	ICH described on imaging?	ICH location(s)	Age , years (mean /range)	Co-morbidities	Age, years (mean / range)	Accounted for confounders?	Newcastle-Ottawa scale quality score
Masuda 1988	>80% community deaths, age >40 yrs, Japan	?	?	×	D, L, IT	?	O	?	×	6
Ishihara 1991	Selected hospital autopsies, Japan	S	?	×	D+IT, D+L, L	72	IS, O	75	×	4
Ng 1991	Consecutive hospital autopsies, age >40yrs, Hong Kong	S	✓	×	?	?	?	?	×	5
Cartier 1999	Selected hospital autopsies, Chile	S	✓	×	L, IT, BG	65	O	64	Age	5
Mastaglia 2003	Consecutive hospital autopsies, mean age 75 years, Australia	S	?	×	C, EC, L,	?	IS, ?	?	×	4
Xu 2003	Hospital autopsies, mean age 78yrs, China	S	?	×	?	?	?, D	?	×	4
Badhe 2005	Randomly selected autopsies, aged >70yrs, India	S	✓	×	?	?	IS, O	?	×	4
Ritter 2005	Selected consecutive hypertensive hospital autopsies, Hungary	S	✓	✓	D+IT, L	69	IS, O	73	Age	8
Attems 2008	Consecutive hospital autopsies, Austria	S	?	×	D, H, L, IT	62-96	?, D	?	×	5
Guidoux 2008	Selected consecutive hospital autopsies, France	S	✓	×	D+B, L+C	76	?	77	Age	6

✓ - criterion met, × - criterion not met, ? – unknown, ICH = ICH

ICH location: BG – basal ganglia, C – cerebellum, D – deep (basal ganglia/thalamus), EC – external capsule, H – hemispheric, IT – infratentorial (cerebellum or brain stem), L – lobar, D+B – deep includes brainstem, D+L – involving both deep and lobar locations, D+IT – deep includes infratentorial, L+C – lobar includes cerebellum

Control type: D – dementia (diagnosed according to either clinical or pathological criteria), IS – ischaemic stroke, O – other

Table 27 Characteristics of the assessment of exposure in the included studies

Study	Number of tissue blocks examined	Locations examined in cases	Locations examined in controls	CAA detection: Congo Red stain	CAA detection: Immuno-histochemistry	Rating of severity of CAA
Masuda 1988	6	BG, HC, I[$], L	BG, HC, L	✓	×	Bespoke
Ishihara 1991	?	B, C, GM, I, L	B, C, GM, L	✓	S	Bespoke
Ng 1991	?	I,?	HC, L*	✓	×	Vinters [Vinters & Gilbert 1983]
Cartier 1999	?	B, BG, C, L^	B,BG,C, L^	✓	×	Presence/ absence
Mastaglia 2003	2-6	I, L**	I, L**	×	✓	Bespoke
Xu 2003	?	I[$],?	?	✓	✓	Bespoke
Badhe 2005	10-12	BG, C, HC, L	BG, C, HC, L	✓	×	Vinters [Vinters & Gilbert 1983]
Ritter 2005	4-5	BG, C, I, L***	BG, C, L***	✓	×	Vonsattel [Vonsattel et al. 1991]
Attems 2008	?	B, BG, C, L****	B, BG, C, L****	×	✓	Olichney [Olichney et al. 1995] & bespoke
Guidoux 2008	3-5[§]	?	B, BG, C, HC, L*****	✓[§]	✓[¶]	Presence/ absence

✓ - criterion met, × - criterion not met, ? – unknown, S- some
B – brainstem, BG – basal ganglia, C – cerebellum, GM – central grey matter, HC – hippocampus, I – site of ICH, L – lobar (every lobe unless * - Parieto-occipital only, ** - frontal, temporal and parietal only, *** - Fronto-parietal and occipital only, **** - Frontal, temporal and occipital only, ***** - temporal and occipital only, ^ - not specified)
[$]- Presence of CAA specified in vessels at the site of the ICH in CAA-positive cases, but unclear whether this was done for *all* cases
[§] - cases, but not controls, [¶] - controls, but not cases

Figure 36 Selection of studies included in the systematic review

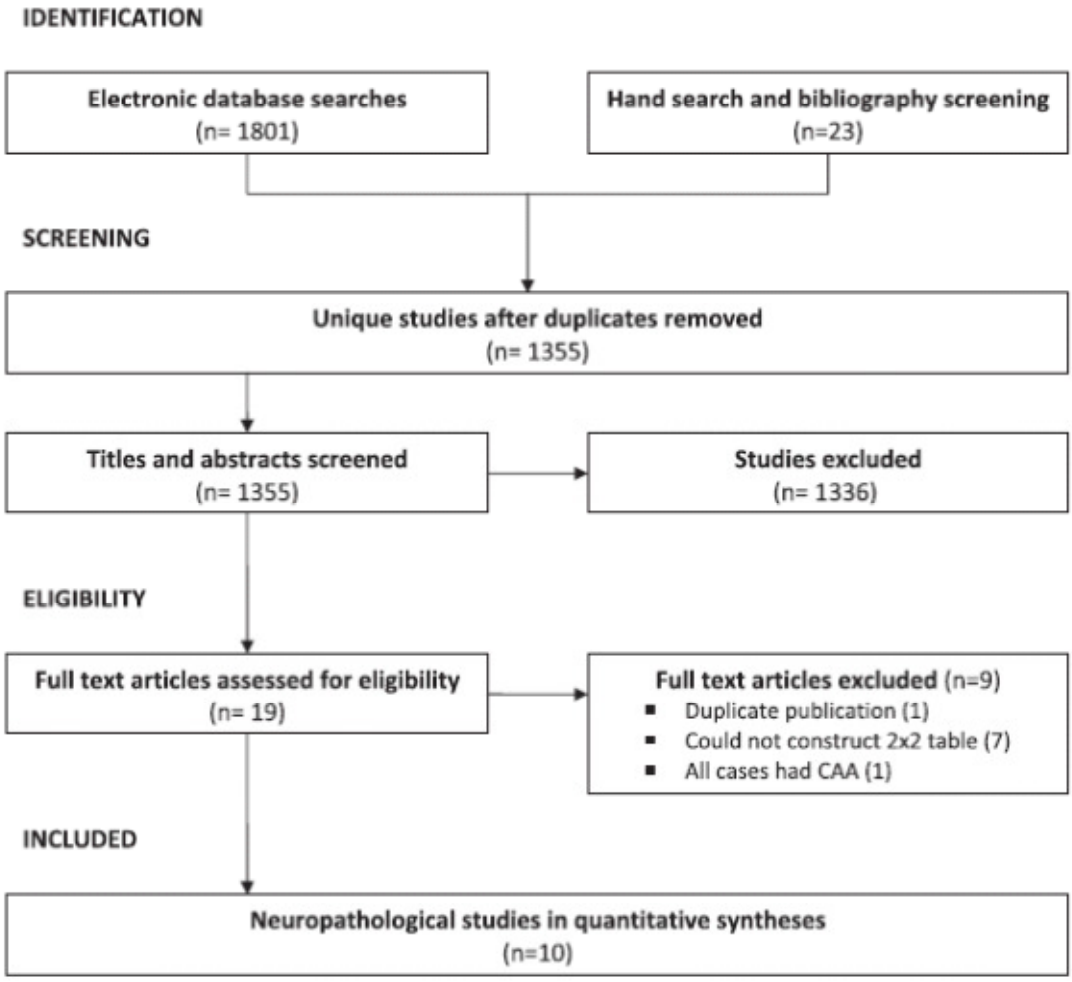
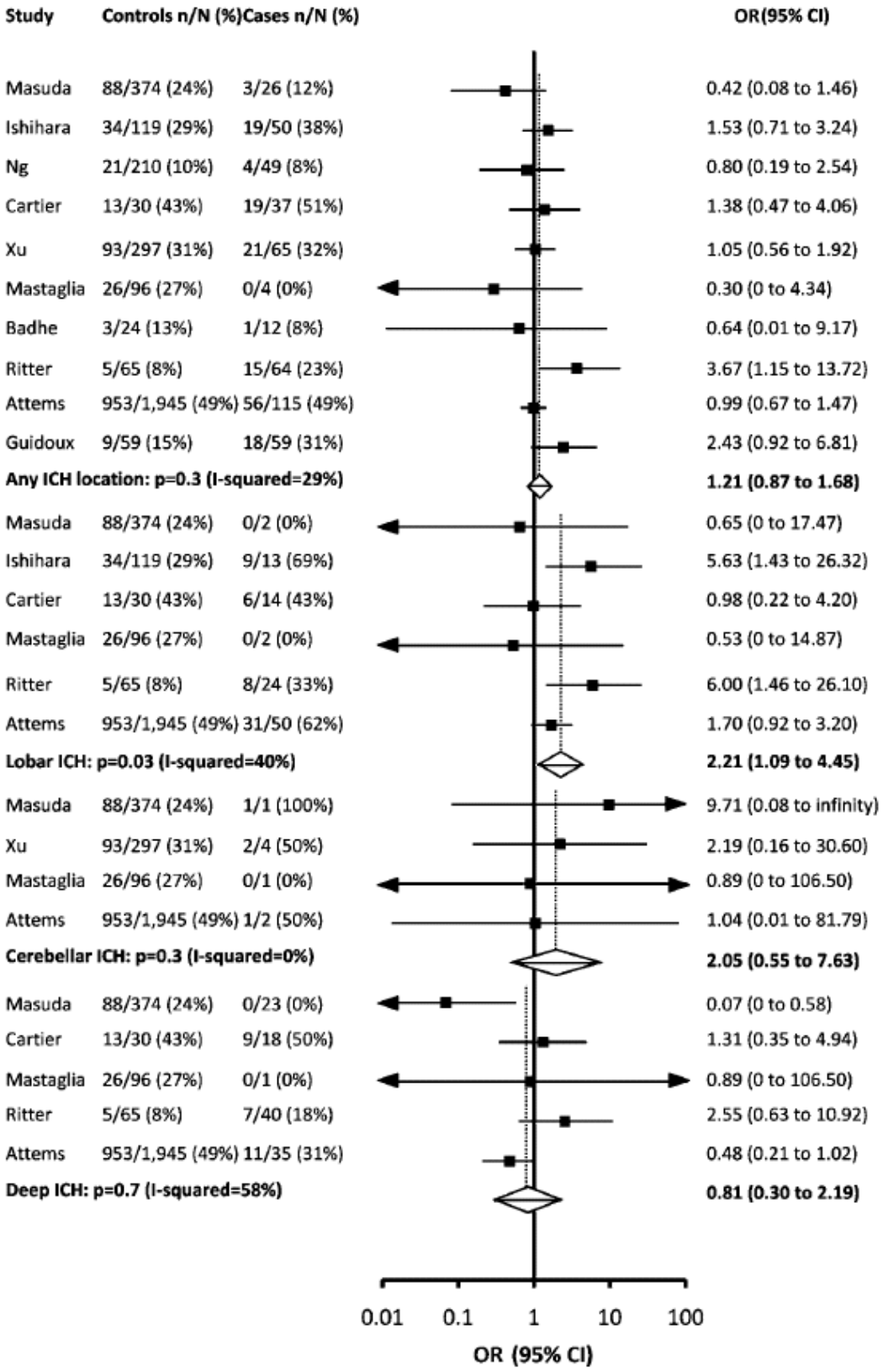


Figure 37 OR meta-analysis: CAA prevalence in cases with ICH (stratified by location) vs. controls Squares are point estimates of the studies, error bars are 95% CIs and diamonds represent pooled summary estimates (whose width is their 95% CI)



Chapter 10 A cross-sectional study of imaging and pathology findings in adults with lobar intracerebral haemorrhage

Chapter contents

10 A cross-sectional study of imaging and pathology findings in patients with lobar intracerebral haemorrhage

10.1 Introduction

My application of the modified Boston criteria for CAA [Knudsen et al. 2001;Linn et al. 2010] to a community-based cohort of participants with ICH (Chapter 5), showed that all participants with a single, first-ever lobar ICH who were unsuitable for an MRI brain looking for BMBs or superficial siderosis [Linn et al. 2010] were classified as having 'possible' CAA; which may not be useful for clinicians who wish to determine the likelihood of a patient having CAA ante-mortem. Since 47% of all participants with lobar ICH were unsuitable for MRI in our cohort (Chapter 6), the use of the criteria was limited.

Although amyloid positron emission tomography (PET) imaging using Pittsburgh Compound B might also help to detect CAA ante-mortem, a recent small study which compared 11 persons with probable CAA according to the Boston criteria vs. nine healthy age-matched controls without a pathological gold standard, showed that despite reasonable sensitivity (91%, 95% CI 62-98%), its specificity for CAA was limited (55%, 95% CI 27-81%) reflecting positivity among healthy elderly controls with probable asymptomatic early Alzheimer's dementia pathology [Baron et al. 2014].

Given that CT is as good as MRI in the diagnosis of acute ICH [Brazzelli et al. 2009], determining whether any radiological features of the ICH on CT discriminate between CAA-related lobar ICH and other lobar ICH, might improve the sensitivity of the Boston criteria, enabling an ICH to be attributed to CAA without requiring pathological confirmation, which would be valuable in low-middle income countries in which ICH incidence is increasing [Feigin et al. 2014].

10.1.1 *Ideal study design*

The ideal study design would be a radio-pathological diagnostic test accuracy study [Bossuyt et al. 2003;Whiting et al. 2003] of the sensitivity and specificity of CT imaging features of ICH for the detection of pathologically-proven CAA-related lobar ICH. In

such a study, CT imaging variables potentially associated with lobar ICH could be selected using different strategies, including:

a) Selection *a priori* based upon existing literature or clinical reasoning.
b) Selecting variables which are reliably and accurately measured, discriminate between participants, are independent of each other to avoid collinearity and have minimal missing data since missing values may relate to participant or disease characteristics and could lead to bias [Lewis 2007;Bouwmeester et al. 2012].
c) Selecting variables which are statistically significant in univariate analyses although this may be problematic if the sample is small and there are many candidate variables [Bouwmeester et al. 2012]. A general rule is that there should be 'ten events per variable' [Peduzzi et al. 1996] but if the number of variables tested is large relative to the sample size, overfitting may occur; that is the model uses more variables than is necessary and produces optimistic prediction results that are not replicated in the wider population [Babyak 2004].
d) Selection using a multivariable analysis commonly using a stepwise selection algorithm, either forwards or backwards [Altman & Royston 2000]. Forwards selection starts with no variables in the model and enters variables in order of their strength of association with the outcome, checking their p value when added to the model and continuing till the fit of the model is optimised whilst backwards selection reverses this procedure. This method is fully automated and therefore convenient [Altman & Royston 2000] and still requires in general, a minimum of ten events per variable.

The study group for a comparative diagnostic test accuracy study can be assembled using a cross-sectional sample, or either retrospectively or prospectively in a cohort study. Ideally, the study sample would be assembled prospectively, without selection bias, at a uniform inception point in the disease to give a sample representative of the population of patients from which they were selected with consistent definitions of radiological features, pathological (outcome) variables and confounders and a description of how any missing data were accounted for [Grimes & Schulz 2002b;von Elm E. et al. 2007]. The outcome would be 'CAA-related ICH' but although this is a commonly used term, it can be difficult to infer that CAA has caused an ICH (Section 10.1.3, page 229).

10.1.2 *Systematic review*

I systematically reviewed Ovid Medline from 1950 and Embase from 1980 to identify diagnostic test accuracy studies of imaging features of lobar ICH to distinguish those due to pathologically confirmed CAA and those not, studies that described the association of imaging features with lobar ICH due to pathologically confirmed CAA versus lobar ICH due to other causes, and lastly case series describing imaging features of patients with lobar ICH due to pathologically confirmed CAA. I also searched the bibliographies of relevant studies and Google scholar for citations of each included study.

I did not identify any diagnostic test accuracy studies or comparative association studies, and only found case series. Excluding case series with less than five participants [Patel et al. 1984;Finelli, Kessimian, & Bernstein 1984;Sobel et al. 1985;Andoh et al. 1989], case series that did not report both radiological and pathological findings for lobar ICH [Ishii, Nishihara, & Horie 1984;Knudsen et al. 2001;Tang et al. 2013] and case series that used MRI [Linn et al. 2010], there have been 13 case series describing 255 patients with pathologically proven CAA-related supratentorial lobar ICH (Table 28 on page 264) [Wagle, Smith, & Weiner 1984;Brown, Coates, & Gilbert 1985;Cosgrove et al. 1985;Yong et al. 1992;Wakai, Kumakura, & Nagai 1992;Minakawa et al. 1995;Millar, Wardlaw, & Lammie 1999;Izumihara et al. 1999;Lang et al. 2001;Oide et al. 2003;Chen et al. 2004;Patel et al. 2009;Hirohata et al. 2010].

10.1.2.1 Critical appraisal of studies

All studies have been hospital-based and retrospective, bar one which selected consecutive patients from a prospectively recruited cohort [Patel et al. 2009]. Only three studies stated the time interval between the ICH symptom onset and scan [Wagle, Smith, & Weiner 1984;Yong et al. 1992;Minakawa et al. 1995], which may affect the radiological appearances of an ICH (Chapter 2). The authors of one study stated that imaging interpretation was performed 'without knowledge of clinical information, including radiology reports' [Patel et al. 2009] but no other studies described whether imaging was interpreted blind to clinical or pathological information.

Studies have lacked clear descriptions of radiological variables thought to be related to CAA such as an 'irregular' or 'lobulated' ICH [Yong et al. 1992;Izumihara et al.

1999;Lang et al. 2001], ICH in a 'superficial location' [Wagle, Smith, & Weiner 1984] or 'periventricular white matter lucency' [Millar, Wardlaw, & Lammie 1999].

The outcome (pathologically proven 'CAA-related' ICH) was explicitly defined in only one study [Patel et al. 2009] which categorised an ICH as CAA-related according to the Boston criteria (which require severe CAA to be present with vasculopathy in at least one vessel in biopsy or post-mortem specimen(s)for a diagnosis of 'definite' CAA-related ICH [Vonsattel et al. 1991;Knudsen et al. 2001]).

10.1.2.2 Findings of studies

Studies have described various imaging features of pathologically-proven CAA-related lobar ICH (Table 29 on page 265) including most commonly: multiple ICHs, extension of the ICH into the subarachnoid space and an 'irregular' or 'lobulated' ICH.

10.1.3 *What is 'CAA-related' ICH?*

10.1.3.1 CAA rating scales

From my systematic review of neuropathological case control studies of CAA and ICH (Chapter 9), I knew that although multiple different scales for rating CAA exist, which encompass various pathological features of CAA (Figures 38-40, pages 277-279), none have been independently externally validated for use in patients with ICH. A recent proposal [Alafuzoff et al. 2009] to standardise the rating of β-amyloid deposits achieved good inter-rater agreement in the assessment of CAA but neuropathologists rated only the presence or absence of CAA in specimens and its type rather than the severity of CAA.

The various CAA rating scales are described below:

Vinters *et al.* [Vinters & Gilbert 1983] have previously rated CAA symmetrically from each lobe and both hippocampi as 1+ if only one or two vessels had evidence of CAA, 2+ if three to five vessels had CAA and 3+ if six or more vessels had CAA with a score of 3+ interpreted as having severe CAA. Only parenchymal vessels were considered (not leptomeningeal) and the rating of CAA severity for each region was based solely on the number of vessels involved rather than the extent of CAA deposition within the walls of blood vessel(s). A brain was considered to have 'severe' CAA if at least one of the ten regions sampled was given a rating of 3+.

Vonsattel *et al.'s* scale [Vonsattel et al. 1991], which was also used in the formation of the Boston criteria [Knudsen et al. 2001], uses ratings of CAA in either a single leptomeningeal or cortical vessel to infer the severity of CAA for the entire brain. Severity of CAA is classified as mild (when it is restricted to a rim around normal or atrophic smooth muscle fibres), moderate (when the tunica media is replaced by amyloid and is thicker than normal) or severe (when there is fragmentation of the vessel wall and at least one focus of perivascular leakage of blood or haemosiderin.) If there is evidence of one vessel with 'severe' CAA, the entire brain is rated as having CAA.

Olichney *et al.'s* scale [Olichney et al. 1996] incorporates the changes seen in both leptomeningeal and cortical vessels in a single specimen to give an overall grade of CAA severity for a brain where: 0-no evidence of CAA in the leptomeningeal or superficial cortical vessels; 1-traces of CAA seen in either the leptomeningeal or cortical vessels; 2-some vessels in the leptomeninges or cortex had amyloid deposits; 3-widespread amyloid in many leptomeningeal and superficial cortical vessels; 4-as for 3 but with the addition of dysphoric changes in which amyloid extends from the blood vessel into the adjacent parenchyma.

Ellis *et al.* devised a scale to rate CAA semi-quantitatively in the parenchymal and meningeal vessels of five brain regions: the frontal, parietal and temporal lobes, hippocampus and entorhinal cortex [Ellis et al. 1996]. Their scale, used as part of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria, rates CAA in each region as 0-no CAA, 1-sparse or mild CAA, 2-moderate CAA or 3-frequent or severe CAA. The CAA scores for each region are either summed to give an 'overall' CAA score or averaged over the five cerebral regions examined per subject to give a mean CAA score. Both types of outcome measure have been used in their cohort [Ellis et al. 1996].

Thal *et al.* [Thal et al. 2002a] sampled the occipital lobe, temporal lobe and entorhinal cortex and rated CAA overall as either mild – where CAA is restricted to the blood vessels of one given region (for example, the occipital lobe) and is restricted to small deposits next to the smooth muscle cells or severe – where CAA is seen in numerous vessels in many regions and there is replacement of portions of the tunica media of the blood vessel wall by Aβ. CAA was also classified as type one or type two (in which type

one CAA indicated that CAA was present in capillaries vs. type two in which CAA was not present).

Attems *et al.'s* scale [Attems, Jellinger, & Lintner 2005] rates leptomeningeal and cortical vessels separately for each lobe of the brain where 0-no Aβ positive vessels, 1-mild (scattered positivity in a few vessels), 2-moderate (scattered positivity in many vessels or strong positivity in a few vessels), 3-severe (strong positivity in many vessels), 4- severe with vasculopathic changes in cortical vessels. The overall score for CAA severity is the mean score for all regions.

Allen *et al.* [Allen et al. 2013] recently used a modified version of the scale devised by Attems *et al.* [Attems, Jellinger, & Lintner 2005]; by removing the grade 4 rating (and rating all such cases as grade 3) and recording the presence and severity (0-none, 1-mild, 2-moderate, 3-severe) of capillary CAA deposition.

The Bristol rating scale [Love et al. 2014] requires a neuropathologist to provide separate ratings of CAA severity for each brain region leading to an overall rating of CAA for the entire brain which is a composite measure of the severity of leptomeningeal, parenchymal, capillary CAA and vasculopathic changes in each lobe of the brain (Table 6 on page 144). It is the only scale which allows evaluation of each aspect of CAA per lobe and other commonly used scales could also be derived from it.

10.1.3.2 Attributing an ICH to CAA is complex

ICH is classed as probable 'CAA-related' ICH (with supporting pathology) according to the modified Boston criteria if there is pathologically-proven CAA of any severity in a single specimen taken from the evacuated haematoma or a cortical biopsy in a person with ICH [Knudsen et al. 2001]. This assumes that CAA will be distributed uniformly in different regions of the brain and that its presence, in the absence of secondary causes, indicates that it is the likely cause of the ICH.

However, CAA is known to co-exist with other small vessel diseases such as arteriolosclerosis neither of which are necessary or sufficient causes for an ICH. CAA may also be patchy and segmental and may vary in presence and severity between lobes of the brain [Allen et al. 2013]. It is also a common incidental finding in the elderly [Masuda et al. 1988] and those with dementia [Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) 2001]. Therefore

the presence of CAA does not signify that it is the sole cause of the ICH and conversely, CAA may be absent in a specimen because of sampling variation.

This problem was highlighted by one study which examined the sensitivity and specificity of cortical biopsy for the diagnosis of CAA [Greenberg & Vonsattel 1997]. In seven brains all of which had been previously assigned a global CAA severity rating of either mild, moderate or severe (two of which had 'CAA-related' ICH) new biopsy sized specimens were prepared, stained with Congo Red and rated blind to the brain from which each specimen was taken. If the previous global CAA severity rating is taken as the reference standard, the new specimens had 33% sensitivity (95% CI 19-52%) for detecting mild CAA and 69% sensitivity (95% CI 54-81%) for detecting moderate-severe CAA. 52% specimens sampled from brains with mild CAA were rated as not having CAA and in the two brains with ICH attributed to CAA, CAA severity ranged from mild in 2% of specimens to severe with evidence of fibrinoid necrosis in 46% of specimens.

10.1.3.3 CAA with vasculopathy may be related to ICH

Possible indicators of CAA severity are: the extent of leptomeningeal vessel involvment, the extent of parenchymal vessel involvement, the presence of capillary CAA and the presence of vasculopathic changes. Vasculopathic changes may manifest as one or more of the following: fibrinoid necrosis, microaneurysm formation, 'double barrelling' (fragmentation) of the vessel wall and perivascular blood leakage (Figure 4 on page 55)[Attems 2005].

Because of the paucity of well conducted neuropathological case control studies of CAA and ICH (Chapter 9) and the lack of standardised externally validated rating scales for rating CAA, it remains unclear which markers of CAA severity are most closely related to ICH. When examining brains with CAA, fibrinoid necrosis is more common in those with ICH compared to those without, although its specificity for ICH is 69% (95% CI 42-87%), since it is also found in brains with moderate-severe CAA but without ICH [Greenberg & Vonsattel 1997].

Because of the lack of diagnostic test accuracy studies, and in view of the limitations of the existing literature, I performed a radio-pathological study nested within a prospective inception cohort study of first-ever lobar ICH, using clearly pre-defined

clinical, imaging and pathological characteristics and blinded rating in order to ascertain the association and diagnostic accuracy of CT-based imaging characteristics of lobar ICH with pathologically proven CAA that was likely to have contributed to causing the lobar ICH.

10.2 Methods

10.2.1 *Participants*

Participants were recruited into the LINCHPIN study (an ethically-approved study of patients with spontaneous primary ICH) and underwent a research post-mortem examination limited to the brain.

10.2.1.1 Inclusion criteria

Participants were eligible for a post-mortem examination limited to the brain in the LINCHPIN study if they met all of the following inclusion criteria:

- They had a first-ever or recurrent spontaneous primary lobar ICH during 1st June 2010-31st May 2012.
- They were resident in the Lothian region at the time of their ICH.
- The diagnosis of ICH was confirmed using CT imaging.
- They (or their nearest relative) gave written informed consent to a post-mortem examination limited to the brain only after death.

10.2.1.2 Exclusion criteria

Participants were excluded if they met any of the following exclusion criteria:

- They were known to have had a secondary ICH before death or were found to have a secondary cause of ICH at post-mortem examination.
- They died before diagnostic CT brain imaging could be performed.
- They had had a previous spontaneous primary ICH (since I aimed to obtain participants with first-ever ICH and therefore, a uniform inception point) as CAA is likely to be gradually deposited over time.

10.2.2 *Data collection*

I collected data on baseline demographic and clinical characteristics as outlined in Chapter 2. I collected the following information: demographic variables – age and sex; clinical variables – whether the ICH was first-ever or recurrent, history of hypertension, dementia, ischaemic stroke and prior use of antithrombotic medications. I collected information regarding the date of the ICH, the date of the diagnostic CT scan and date of the post-mortem examination.

10.2.3 *Imaging*

10.2.3.1 Development of a CT rating proforma

From imaging variables identified in previous radio-pathological studies of pathologically proven CAA-related lobar ICH (Table 29 on page 265), I selected both imaging features of ICH and features of surrounding brain parenchyma which might plausibly be associated with CAA. The final list of included variables and a consensus definition of each variable was decided upon after discussion with two consultant neuroradiologists (Professor J Wardlaw, University of Edinburgh and Professor P White, University of Newcastle-upon-Tyne) and a consultant neurologist (Professor R Salman, University of Edinburgh) with special interests in stroke (Appendix). Each variable is described below, guided by the STRIVE guidelines if applicable [Wardlaw et al. 2013].

10.2.3.2 Included variables and their definitions

(i) Multiple ICH: An ICH was multiple if several unconnected acute ICHs of a similar age (based on attenuation), separated by unaffected brain parenchyma, were present on the diagnostic brain imaging obtained at presentation. 'Acute' ICH was determined radiologically by the presence of a hyperdense area in the brain parenchyma.

(ii) Intraventricular extension: An ICH extended into the ventricles if any blood was present within the ventricles of the brain

(iii) Subarachnoid extension: An ICH extended into the subarachnoid space if any blood was present within the subarachnoid compartment of the brain. If subarachnoid blood was present, the rater indicated if this was adjacent to the

ICH (and therefore likely resulted from direct rupture of the ICH into the subarachnoid space) since if it was not adjacent to the ICH it might have been due to blood simply re-circulating, for example through the ventricles into the subarachnoid spaces of the contralateral hemisphere.

(iv) Subdural extension: An ICH extended into the subdural space if any acute blood was present within the subdural compartment of the brain without any clinical or radiological evidence of trauma.

(v) Presence of a blood or fluid level: The rater looked for the presence of a fluid level within each haematoma, excluding any intraventricular fluid level.

(vi) 'Irregular' or 'lobulated' ICH: Since the terms 'irregular' and 'lobulated' have been used interchangeably in previous studies, I combined these terms into a single imaging characteristic. An ICH was irregular if it had an 'uneven' margin or had one or more lobules arising from it, and was therefore lobulated (Figure 41 on page 280).

(vii) 'Finger-like' protrusions: If the ICH was irregular, the rater then assessed whether it had one or more finger-like protrusions which extended to the cortex since protrusions to the cortex or ICH in a 'superficial' location have been described in relation to CAA [Wagle, Smith, & Weiner 1984;Brown, Coates, & Gilbert 1985;Yong et al. 1992].

(viii) Variable density of ICH: An ICH was of 'variable' density if the blood within any single ICH was of high but not uniform attenuation (Figure 42 on page 281). The ICH appeared 'patchy' or 'dilute' in contrast to an ICH in which the blood appeared to be of uniform whiteness on CT imaging.

(ix) Old vascular lesions: Old strokes were classified as either old infarcts (cortical, striatocapsular, borderzone, lacune, brainstem or cerebellar) or as probable haemorrhages (which were either cortical or subcortical) [Wardlaw et al. 2007].

(x) WMH: WMH (periventricular lucencies) were rated using the van Swieten scale [van Swieten et al. 1990] in which 0-no lucency, 1- lucency restricted to region adjoining ventricles and 2-lucency covering entire region from lateral ventricle to cortex.

(xi) Atrophy: Both central (deep) and cortical atrophy were rated using a template

validated in previous observational studies of stroke [Farrell et al. 2009].

10.2.3.3 Excluded variables

I did not include the following variables which were identified in previous radio-pathological studies of pathologically proven CAA-related lobar ICH (Table 29 on page 265):

(i) 'Surrounding oedema' since the presence of oedema will vary depending on the interval between ICH onset and imaging as the haematoma matures and clot retraction ensues giving rising to serum extrusion and vasogenic oedema [Parizel et al. 2001;Venkatasubramanian et al. 2011].

(ii) 'High density of blood sedimenting posteriorly' as this may depend on the head position of the patient and whether there is a fluid level within a haematoma in which case the denser posterior sediment may correspond to settled blood cells [Parizel et al. 2001].

10.2.3.4 ICH volume

I measured ICH volume on the first CT brain scan following the participant's presentation with ICH (the diagnostic scan) using Quantomo computerised planimetry software (Cybertrial Inc, Calgary, Canada) [Kosior et al. 2011]. Since subarachnoid extension is associated with a larger ICH volume [Chen et al. 2013], ICH volume may confound any association between subarachnoid extension and CAA-related lobar ICH.

10.2.3.5 Rating of CT scans

A neuroradiologist with a special interest in stroke used only the diagnostic unenhanced CT scan (five mm slice thickness) to rate the imaging features, blinded to the clinical details and post-mortem examination findings, without any other imaging studies performed subsequently. A neuroradiologist classified an ICH as 'strictly' lobar if it was confined to lobar regions of the brain without any involvement of the basal ganglia, internal or external capsule or thalamus.

10.2.4 *Pathology*

10.2.4.1 Ethical approval

In Spring 2010, I sought ethical approval for the LINCHPIN study from the Scotland A Research Ethics Committee which assesses studies involving adults lacking mental capacity. I received ethical approval (Reference Number: 10/MRE00/23) in April 2010. I also received approval from the NHS Lothian Research and Development office (Reference Number: 2010/W/NEU/04).

10.2.4.2 Statutory requirements relating to consent for post-mortem examination

In accordance with the Adults with Incapacity (Scotland) Act 2000 [Department of Health (Scotland) 2006], I deemed an individual as lacking mental capacity if they were unable to do any of the following as a result of mental disorder or of inability to communicate because of the physical disability or neurological impairment:

- act;
- make decisions;
- communicate decisions;
- understand decisions or
- retain the memory of decisions.

I assessed the patient and liaised with the clinical team looking after the patient to facilitate this decision.

If the patient was deemed to lack the mental capacity to be able to decide whether to participate in the LINCHPIN study, and their clinical condition made it unlikely that they would regain mental capacity, I sought written informed authorisation from the patient's nearest relative or their legal representative, in accordance with the statutory requirements of the Human Tissue (Scotland) Act 2006 [Department of Health (Scotland) 2006] which requires authorisation (consent) to be sought before the use of a deceased person's organs, tissues or cells for medical research.

'Authorisation' equates to the term 'consent' which is used in the Human Tissue Act (2004) [Department of Health 2004] and indicates that people have the right to express, during their lifetime, their wishes about what should happen to their bodies after death

in the expectation that those wishes will be respected [Department of Health (Scotland) 2006].

I determined which relative was the 'nearest' using the hierarchy of authorisation specified by the Human Tissue (Scotland) Act 2006, in which relatives are ranked in order of priority. A spouse or civil partner is considered to be the nearest relative, followed in descending order of priority by a partner for over six months, child, parent, sibling, grandparent, grandchild, uncle or aunt, cousin, niece or nephew and a friend of long standing is considered the lowest ranking individual. Where the patient's nearest relative had given consent and a participant subsequently regained mental capacity, I sought written informed consent from the participant.

10.2.4.3 Consent process

Seeking consent for brain donation for research purposes is a complex process involving consideration of numerous factors such as when post-mortem examination should be discussed, how consent should be sought and how information should be provided.

I liaised with the clinical team looking after the patient to decide if or when it would be appropriate to approach the patient or their family. There were various factors to consider when making this decision, including:

- **The clinical condition of the patient**. If a patient was unlikely to survive their ICH, post-mortem examination would need to be discussed sooner than if the patient was stable. If a patient was recovering but had impairments in speech or language resulting from their ICH, discussion of post-mortem examination might be delayed till their deficits allowed them to improve in order to express their own wishes.
- **Findings of other medical investigations**. If a patient was known to have a secondary ICH prior to death they would not be eligible for the LINCHPIN study.
- **Care discussions** between the clinical team looking after the patient and the patient or their family; including the management of secondary complications of ICH such as hydrocephalus or pneumonia and placing Do Not Attempt Resuscitation orders. I ensured that I only discussed post-mortem examination after the medical management of the participant had been decided upon and this decision had been

communicated to the participant or their relatives. I approached discussion of post-mortem examination in a sensitive manner and avoided causing confusion amongst relatives that could result from post-mortem examination being discussed at the same time as potentially therapeutic interventions for the ICH.

- **The availability of relatives**. Whilst I tried to discuss consent for post-mortem examination in person, occasionally, for example, if the nearest relative lived a long distance away, I discussed consent by phone.
- **The involvement of other departments** such as the Office of the Procurator Fiscal or the NHS Blood and Transplant team. In Scotland, the Office of the Procurator Fiscal will investigate any sudden, unexplained, unexpected or accidental death and the Procurator Fiscal may decide that a fiscal (coroner's) post-mortem examination is indicated. In the event that an ICH was found at a fiscal post-mortem examination, I sought consent from the patient's family for additional brain tissue samples to be taken at the time of the post-mortem examination and retained for research purposes.

After discussion with the clinical team, I typically sought consent in person, tailoring information to the participant's wishes and frequently discussing brain donation issues on several occasions with different family members to ensure that all concerns were addressed.

Where a patient lacked mental capacity I discussed brain donation in a separate quiet room. I provided every participant or their nearest relative with a study information leaflet outlining the post-mortem examination process (Appendix). The information leaflet had been reviewed by members of the Division of Clinical Neurosciences, Edinburgh and two patient representatives, both of whom had suffered ICH.

Whenever possible I sought to give the nearest relative at least 24 hours to decide whether they wished to consent to brain donation and if this was not possible, I provided relatives with a telephone number that they could contact out-of-hours with their decision.

The post-mortem examination discussion covered a range of issues including those generic to research studies and information and concerns specific to brain donation.

I discussed the following generic issues [Samarasekera et al. 2013]:

- the voluntary nature of participation in the LINCHPIN study;
- the right to withdraw consent at any time;
- the need for written informed consent prior to taking brain tissue samples without which brain tissue would not be taken and
- the maintenance of the donor and the family's confidentiality at all times.

I discussed the following issues surrounding brain donation:

- the purpose of the LINCHPIN study and the need for brain tissue;
- what the brain tissue retained would be used for, both now and in the future;
- the need for demographic and clinical information pertaining to the donor;
- the retention of brain tissue samples (not the whole brain) in a post-mortem examination limited to the brain;
- the process of retrieving brain tissue, explaining that post-mortem examination would be conducted by a neuropathologist with a special interest in stroke;
- the timing and location of the post-mortem examination, including the need for transfer of the patient's body from their place of death to a specified mortuary if applicable;
- practical arrangements that the LINCHPIN study puts in place to ensure that a donor's ante-mortem wishes are fulfilled;
- provision of contact details for the participant or their relatives and
- provision of post-mortem examination results for relatives who wanted further information.

I addressed donors' and relatives' concerns as appropriate; for example:

- disagreement with previously expressed wishes of the donor;
- disfigurement to the donor's body resulting from tissue retrieval;
- delay to the donor's funeral as a consequence of brain donation;
- financial costs incurred as a result of brain donation or
- whether any other organs would be removed.

If the participant consented to post-mortem examination, they completed the LINCHPIN post-mortem examination consent form (Appendix) and copies of the form were given to the participant and filed in their hospital medical records. If a

nearest relative gave assent, they completed the LINCHPIN post-mortem examination consent form for adults with incapacity (Appendix). At the time of death, I met the donor's family to complete the NHS Lothian 'Authorisation for the Hospital Post-mortem examination on an Adult who left no formal authorisation' which seeks the authorisation of the nearest relative for a limited post-mortem examination of the head in which brain tissue samples are retained and used for teaching, audit and research purposes (Appendix).

Copies of both the research and NHS Lothian forms were required by the neuropathologist before a post-mortem examination could be performed. Meeting the donor's family after death also provided an opportunity to address any outstanding questions which might have arisen and ensure that the timing of the post-mortem examination would be co-ordinated with the family's funeral arrangements.

10.2.4.4 Requirements after obtaining consent

If I obtained post-mortem examination consent, I ensured that everyone involved in the participant's care (including nursing staff, medical staff, the participant's GP, their residential or nursing home if applicable) was aware of this so that their ante-mortem wishes could be fulfilled at the time of death. I also gave the participant or their nearest relative a study 'donor card' containing a contact number in case of death. In addition, I requested that the participant's GP inserted a computerised alert into their primary care records which would appear if the record was opened to indicate that the participant had given consent to brain donation.

If a relative had wished to be informed about the results of the post-mortem examination, I contacted them, usually by letter, when results were available (typically three to four months after the post-mortem examination). I informed relatives of macroscopic and histological findings. Genetic tests were not done. Relatives were invited to contact me at any time if they had further questions or comments.

10.2.4.5 Sampling protocol at post-mortem examination

After making an incision through the skull the brain was removed. A neuropathologist with a special interest in stroke sliced the brain into one centimetre thick coronal sections and took one cm^3 samples according to a comprehensive sampling protocol (Appendix). Each lobe, the basal ganglia, thalamus and cerebellum were sampled

symmetrically from each hemisphere and a single sample was taken from the midbrain, pons, medulla and cerebellar vermis; producing 32 blocks in total.

10.2.4.6 Sample processing

Each tissue sample was placed in a plastic cassette. The cassettes were placed in 10% formalin solution at room temperature and fixed for at least 24 hours. No frozen sections were taken. To produce paraffin blocks, each sample was dehydrated by being placed in a series of alcohol-based solutions of varying concentrations before being embedded in paraffin wax. Once paraffin blocks were formed they were sliced into sections of ten micrometer thickness and placed on Superfrost® slides.

10.2.4.7 CAA assessment

CAA was assessed by immunohistochemistry. We used a monoclonal mouse antibody to human β-amyloid, (Clone 6F/3D, Dako, Copenhagen) at a concentration of 1:100. The procedure used 95% formic acid for pretreatment of paraffin-embedded, formalin-fixed sections to improve amyloid immunoreactivity and immunolabelling, followed by staining using Novolink™ Polymer Detection Kit RE7280/K (Leica Biosystems, Wetzlar, Germany.)

10.2.4.8 CAA rating

The neuropathologist rated CAA using the Bristol rating scale [Love et al. 2014] (Table 6 on page 144) which rates parenchymal and meningeal CAA from 0 to 3 where: 0 – CAA absent, 1 –scant deposition, 2-some circumferential amyloid, 3-widespread circumferential amyloid; capillary CAA as either 0 (absent) or 1 (present) and vasculopathy where 0-absent, 1-vasculopathy affecting occasional vessels and 2-vasculopathy affecting many vessels. The neuropathologist was unblinded to ICH location (lobar) but blinded to other imaging features of the ICH and the study hypothesis.

10.2.5 *Analysis: CAA-related ICH*

From my review of CAA rating scales (Section 10.1.3.1, page 229), it was apparent that inferring an ICH as 'CAA-related' is complex. Previous rating scales have tended to label an ICH as 'CAA-related' if CAA is present in one or more tissue samples taken from anywhere in the brain although the distribution and severity of CAA may vary

between lobes of the brain [Greenberg & Vonsattel 1997;Allen et al. 2013] and CAA coexists with other small vessel diseases.

The Bristol rating scale [Love et al. 2014] was validated in persons with and without Alzheimer's dementia and examined the frequency and severity of CAA in different lobes and its association with apolipoprotein E genotype. As yet, the scale has not been validated for use in ICH and there is no particular 'cut-off' above which an ICH would classified as 'CAA-related.'

When forming a definition of 'CAA-related ICH' I considered the following factors:

- The proximity of CAA to the ICH as ICH is a localised pathology and to infer an ICH as 'CAA-related', the CAA should be found within the vicinity of the ICH [Samarasekera, Smith, & Al-Shahi Salman 2012].
- The severity of CAA; for which I considered parenchymal or leptomeningeal CAA of grades 2 (some Aβ deposition) or 3 (widespread Aβ deposition) to be severe.
- Presence of vasculopathy as vasculopathy would be expected to be present in CAA-related ICH.

10.2.5.1 Primary outcome

I defined CAA-related lobar ICH as the presence of severe CAA (parenchymal or meningeal CAA of at least grade 2 or above) with vasculopathy (of at least grade 1), both occurring in at least one of the lobes affected by the ICH.

10.2.5.2 Secondary outcomes

I defined CAA-related lobar ICH in four other ways:

(i) Severe CAA (parenchymal or meningeal CAA of at least grade 2 or above) with vasculopathy (of at least grade 1), both occurring in the same hemisphere as the ICH (although not in the same lobe(s) as the ICH).

(ii) Severe CAA (parenchymal or meningeal CAA of at least grade 2 or above) without vasculopathy (grade 0), occurring in the same lobe(s) or in the same hemisphere as the ICH.

(iii) Any degree of CAA in the same hemisphere as the ICH.

(iv) Any degree of CAA occurring anywhere in the brain.

10.2.6 *Analysis: Distribution of CAA in lobar ICH*

Since each autopsied brain was symmetrically sampled from each lobe (Appendix), in pre-specified analyses I aimed to:

- Determine the frequency of CAA deposition in each lobe in both the hemispheres affected and unaffected by ICH since CAA may preferentially deposit in the occipital lobes [Johnson et al. 2007];
- Compare the severity of parenchymal and leptomeningeal CAA in lobe(s) affected by ICH vs. the contralateral lobe(s) to ascertain if CAA is symmetrically distributed;
- Compare the frequency of vasculopathy in lobe(s) affected by ICH vs. the contralateral lobe(s) since vasculopathy might be more commonly seen in the lobe(s) affected by ICH;
- Compare the severity of parenchymal CAA with leptomeningeal CAA in each lobe to assess if they are associated;
- Determine whether vasculopathy is associated with severe CAA (defined as parenchymal or meningeal CAA of at least grade 2 or above) in the same lobe of the brain and
- Determine if capillary CAA is associated with severe CAA or vasculopathy in the same lobe of the brain.

10.2.6.1 CAA severity sum score

To obtain a measure of the overall CAA severity, I calculated a CAA severity score by summing the components of the Bristol scale in all brain regions sampled. Previously the components of the scale have been used separately to assess their association with apolipoprotein E genotype but not combined to assess overall CAA severity. The maximum CAA score per lobe is nine, giving a maximum sum score of 72. I assessed whether CAA severity was associated with age or a past history of dementia since I hypothesised that CAA would be more severe with any of these variables.

10.2.7 *Univariate analyses*

10.2.7.1 Demographic and clinical characteristics of CAA-related and CAA-unrelated lobar ICH

I compared baseline demographic and clinical variables in participants with the primary outcome of CAA-related lobar ICH vs. those who with lobar ICH unrelated to CAA. I compared the time interval between the date of onset of symptoms and the date of the diagnostic CT scan and the interval between the date of the scan and post-mortem examination in the two groups since these intervals may affect the radiological or pathological appearances of the ICH.

10.2.7.2 Imaging characteristics of CAA-related and CAA-unrelated lobar ICH

I pre-specified the imaging characteristics that I would compare between those with and without the primary outcome of CAA-related lobar ICH. In two pre-specified sensitivity analyses, I firstly compared the imaging characteristics between those with CAA-related and CAA-unrelated lobar ICH in the entire cohort where those with a CAA-related lobar ICH met either the primary outcome measure or secondary outcome measures. Secondly, I restricted the analysis to those with strictly lobar ICH and compared the imaging characteristics in those with CAA-related ICH vs. CAA-unrelated lobar ICH in this group.

Since this study is limited by a small sample size (Section 10.3, page 246), I initially selected variables *a priori* after discussion with a neuroradiologist who was blinded to the imaging and pathology data.

I excluded the following variables:

- Atrophy, WMH (periventricular white matter lucency), old vascular lesions (old cerebral infarction)-since these variables were not features of the ICH but other features of the brain parenchyma and the aim of the study was to determine whether any ICH features differentiated between CAA-related ICH and other ICH.
- I assessed ICH volume as a potential confounder of an association between subarachnoid extension and CAA-related lobar ICH, since subarachnoid extension is associated with a larger ICH volume [Chen et al. 2013] but did not include ICH volume as a candidate variable for discriminating between lobar ICH related and unrelated to CAA.

The variables selected should not have the same value for all participants and therefore I reviewed the imaging data blinded to the pathology results and excluded variables which were either very frequent or infrequent and were therefore unlikely to discriminate between participants (Section 10.3.2.2, page 249).

10.2.8 *Statistical analysis*

I used parametric statistics for between group comparisons when the data had a normal distribution and log–transformed variables or used non-parametric statistics when it did not. If an estimated cell count was less than five, I used the Fisher's exact test. I used Spearman's correlation coefficient to assess the relationship between parenchymal and leptomeningeal CAA in different lobes of the brain and assess whether the distribution of CAA was symmetrical in both hemispheres.

I intended to choose imaging variables which differed between CAA-related lobar and CAA-unrelated lobar ICH in the univariate analyses to enter a logistic regression model of imaging features predictive of CAA-related lobar ICH, with the number of variables determined by the frequency of CAA-related lobar ICH in the sample. I conducted all statistical analyses using Stata version 11.1 (StataCorp LP, Texas, USA). All p values are 2-sided.

10.3 Results

The following section describes descriptive analyses of CAA in participants with first-ever lobar ICH and univariate analyses of CAA-related lobar ICH vs. CAA-unrelated lobar ICH. Chapter 11 describes the participants who gave consent for post-mortem examination in comparison to the remainder of the cohort.

10.3.1 *Descriptive analyses*

10.3.1.1 Baseline characteristics of participants

Of 36 potentially eligible participants with lobar ICH, 33 (92%) had first-ever ICH and formed the analysis group. 15 (45%) were males and the median age was 81 years (IQR 77-86 years). 22 (67%) had a past history of hypertension, 10 (30%) had a prior history of dementia and seven (21%) had a prior history of ischaemic stroke. 19 (58%) were taking antiplatelet medications and three (9%) were taking anticoagulant medications at

the time of their ICH. Table 30 on page 266 describes the locations of ICHs in all 33 participants.

10.3.1.2 Primary and secondary outcome measures

Nine (27%) had no evidence of parenchymal or leptomeningeal CAA. Table 31 on page 267 shows the scores for each component of the Bristol scale in the lobes affected by ICH for the remaining 24 participants.

12 participants met the primary outcome measure for CAA-related ICH.

The remaining 12 participants fulfilled the criteria for a secondary outcome measure. Three fulfilled (i): severe CAA (parenchymal or meningeal CAA of at least grade 2 or above) with vasculopathy (of at least grade 1), both occurring in the same hemisphere as the ICH (although not in the same lobe(s) as the ICH). Eight fulfilled (ii): severe CAA (parenchymal or meningeal CAA of at least grade 2 or above) without vasculopathy (grade 0), occurring in the same lobe(s) or in the same hemisphere as the ICH.

One fulfilled (iii): Any degree of CAA in the same hemisphere as the ICH.

Participants who fulfilled the primary outcome measure for CAA-related lobar ICH had significantly higher total CAA scores (n=12, median 54, IQR 49-58) than those who met a secondary outcome measure for CAA-related lobar ICH (n=12, median 33, IQR 21-35; $p<0.001$).

10.3.1.3 Distribution of CAA in every lobe

The frequency of CAA of any severity was 22/33 (67%) in the frontal and temporal lobes and 24/33 (73%) in the parietal and occipital lobes and did not differ according to whether the lobe was in the hemisphere affected or unaffected by ICH (Figure 43 on page 282).

10.3.1.4 Distribution of parenchymal and leptomeningeal CAA and vasculopathy between lobes affected and unaffected by ICH in participants with first-ever lobar ICH

The presence or absence of CAA of any degree in a lobe(s) affected by ICH was concordant with the presence or absence of CAA in the corresponding contralateral lobe(s) (Table 32 on page 269).

To assess CAA severity, I used a combined sum score for parenchymal and leptomeningeal CAA. The severity of CAA in the lobe(s) affected by the ICH was positively correlated with the contralateral corresponding lobe (frontal lobes (n=22) ρ=0.96; p<0.001; temporal lobes (n=6) ρ=1.00; p<0.001; parietal lobes (n=16) ρ=0.97; p<0.001.) Only two participants had an ICH affecting the occipital lobes and I could not test for an association.

The presence or absence of vasculopathy of any degree in a lobe(s) affected by ICH was concordant with the presence or absence of vasculopathy in the corresponding contralateral lobe(s) in frontal and parietal regions (Table 33 on page 269). Only six participants had an ICH affecting their temporal lobes and only two participants had an ICH affecting their occipital lobes, precluding firm conclusions.

10.3.1.5 Relationship between parenchymal and leptomeningeal CAA and vasculopathy

The severity of parenchymal CAA was closely correlated with that of leptomeningeal CAA in all lobes of the brain, whether in the hemisphere affected or unaffected by the ICH (Table 34 on page 270).

In both the affected and unaffected hemispheres the presence of severe CAA (defined as parenchymal or leptomeningeal CAA of at least grade 2 or above) was associated with vasculopathy in all lobes bar the occipital lobes, where there was a similar tendency which failed to reach statistical significance (Table 35 on page 271).

Severe CAA was seen without vasculopathy in 81/264 (31%) of lobes but vasculopathy only occurred in four lobes (2%) in which severe CAA was absent.

10.3.1.6 Association between CAA, vasculopathy and capillary CAA

Capillary CAA was most frequently seen in the occipital lobes (19/66 (29%)) followed by the parietal (13/66(20%)), temporal (10/66 (15%)) and frontal lobes (8/66(12%)) [χ^2=6.81, p=0.08]. Severe CAA was seen without capillary CAA in 115/264 (44%) of lobes but capillary CAA only occurred in one lobe (0.4%) in which severe CAA was absent.

Using a sum score for parenchymal and leptomeningeal CAA in each lobe, whether in the hemisphere affected or unaffected by ICH the presence of capillary CAA was associated with significantly more severe CAA in the same lobe (Table 36 on page 272).

Of 264 lobes, in 31 (12%) both vasculopathy and capillary CAA were present, in 158 (60%) both were absent, vasculopathy occurred in the absence of capillary CAA in 56 (21%) lobes and the converse happened in 19 (7%) lobes (Table 37 on page 273).

Examination of each lobe of the affected and unaffected hemispheres revealed that capillary CAA was associated with vasculopathy in the occipital lobes of both hemispheres (Table 37 on page 273) but not in other lobes.

10.3.1.7 CAA total score and relationship with age and dementia

I used a total score for CAA comprising all elements of the rating scale. In 33 participants with first-ever lobar ICH, there was no relationship between the CAA total score and age (p=0.69) or a prior history of dementia (dementia: median CAA score 34, IQR 29-50 vs. no dementia: median CAA score 33, IQR 0-53; p=0.52).

10.3.2 *Comparison of CAA-related lobar and CAA-unrelated lobar ICH*

10.3.2.1 CAA-related lobar and CAA-unrelated lobar ICH

Although there tended to be more males, and fewer participants with a prior history of hypertension at the time of their ICH in the CAA-related ICH group, there were no statistically significant differences between the demographic and clinical characteristics of those with CAA-related lobar and CAA-unrelated lobar ICH defined as per the primary outcome measure (Table 38 on page 274). The interval between the date of the ICH and brain imaging and between the date of imaging and post-mortem examination was similar in both groups (Table 39 on page 275).

10.3.2.2 Imaging features in the cohort

I excluded the following variables since they varied little between participants:

- single vs. multiple ICH (31 participants had single ICHs),
- subdural haemorrhage (only three participants had subdural haemorrhage in association with their ICH),

- presence of a blood or fluid level within the largest ICH (only five participants had a fluid level) and
- an ICH with an irregular border (29 out of 33 participants had an irregular haematoma.)

The final candidate variables selected for comparison between participants with CAA-related lobar and CAA-unrelated lobar ICH were: intraventricular extension of the main (largest) haematoma, subarachnoid extension, variable density within the ICH and an irregular or lobulated ICH with one or more finger-like protrusions to the cortex (Table 39 on page 275). I included the latter variable in place of an ICH with an irregular border, since 19 participants had an ICH with one or more finger-like protrusions to the cortex (all of whom also were also had an irregular ICH).

10.3.2.3 CAA–related lobar ICH vs. CAA-unrelated lobar ICH defined as per primary outcome measure

12 participants met the primary outcome measure for CAA-related ICH. There were no statistically significant differences in imaging variables between those with CAA-related lobar and CAA-unrelated lobar ICH (Table 39 on page 275).

10.3.2.4 CAA-related lobar ICH defined as per primary and secondary outcome measures vs. CAA-unrelated lobar ICH

24 participants met either the primary outcome measure for CAA-related lobar ICH or one of the secondary outcome measures.

In a sensitivity analysis comparing the imaging features of those with CAA-related lobar ICH as per this definition (n=24) vs. the remainder (n=9), the results were unchanged with no differences between CAA-related lobar ICH vs. CAA-unrelated lobar ICH in intraventricular haemorrhage (n=15 (63%) vs. n=4 (44%); χ^2=0.87, p=0.35), subarachnoid haemorrhage (n=19 (79%) vs. n=6 (67%); p=0.65), variable density within ICH (n=15 (63%) vs. n=5 (56%); p=1.00), or irregular or lobulated ICH with ≥one finger like protrusion to cortex (n=14 (58%) vs. n=4 (44%); p=0.69).

10.3.2.5 CAA-related and CAA-unrelated strictly lobar ICH

17 participants had a strictly lobar ICH (that is, an ICH confined to lobar regions of the brain) of which eight (47%) met the primary outcome measure for CAA-related lobar ICH.

In a sensitivity analysis comparing the imaging features of CAA-related strictly lobar ICH vs. the remainder in this subgroup, the presence of subarachnoid haemorrhage seemed to be more frequent in those with CAA–related strictly lobar ICH (n=8, 100%) vs. CAA-unrelated strictly lobar ICH (n=5, 56%) p=0.08) but the results were otherwise unchanged (Table 40 on page 276). The sensitivity of subarachnoid haemorrhage for the presence of CAA was 100% (95% CI 63-100%) and the specificity was 44% (95% CI 14-79%).

Since none of the imaging variables differed between the two groups, I did not perform a logistic regression model.

10.4 Summary

- Of 33 participants with first-ever lobar ICH, 9 participants (27%) did not have any CAA in their cerebral hemispheres.
- The presence of CAA or vasculopathy and the severity of CAA in a lobe affected by ICH was concordant with that of the corresponding contralateral unaffected lobe.
- The severity of parenchymal CAA was positively correlated with leptomeningeal CAA in all lobes of the brain, whether affected or unaffected by ICH.
- Severe CAA was associated with vasculopathy.
- Capillary CAA was associated with more severe CAA in the same lobe, whether affected or unaffected by ICH.
- Capillary CAA was associated with vasculopathy in the occipital lobes of both hemispheres but not in the other lobes of the brain.
- The total CAA score was not associated with participant age or a prior history of dementia.
- No imaging variables were statistically significantly associated with CAA-related lobar ICH.

- Subarachnoid extension tended to be more frequent in those with CAA-related *strictly* lobar ICH vs. CAA-unrelated *strictly* lobar ICH, with a sensitivity of 100% and specificity of 44% for pathologically –proven CAA-related strictly lobar ICH.

10.5 Discussion

This study is a preliminary radio-pathological study of CAA-related lobar ICH and its findings should be viewed as a precursor to larger studies.

10.5.1 *Strengths of the study*

Strengths of the study include its prospective population-based design with the reference standard (pathologically-proven CAA-related lobar ICH) defined using a comprehensive sampling protocol (Appendix) by a consultant neuropathologist, who, although he could not be blinded to ICH location, was blinded to other imaging features of the ICH and assessed CAA in all specimens using immunohistochemistry. Imaging features were classified independently by a consultant neuroradiologist blinded to the reference standard and I selected imaging features for comparison between CAA-related and CAA-unrelated lobar ICH without knowledge of pathology results on the basis of previous studies and those variables whose values were not very common or very rare amongst participants.

10.5.2 *Limitations of the study*

The study is limited by its small sample size and it may therefore lack power to demonstrate differences between certain imaging features of CAA-related lobar ICH in comparison to CAA-unrelated lobar ICH if they truly exist. All pathology ratings were done by a single experienced neuropathologist without an inter-rater comparison. Although I pre-specified analyses, I nevertheless performed multiple comparisons, increasing the likelihood of a statistically significant result occurring by chance. Since this was a post-mortem examination-based study, study participants had severe strokes as shown by their large ICH volumes (Chapter 11). They may therefore be less representative of all patients with lobar ICH. Participant selection was partly based upon their ability to have the index test (CT imaging.) Since 98% of the cohort described in the first year of the study (Chapter 5) underwent a diagnostic CT brain scan, it is unlikely that this has been a significant source of selection bias. This is the

first study to use the Bristol rating scale in adults with ICH and the validity and reliability of the rating scale has not been assessed in this group, nor is it clear how its constituent ratings should be used (Section 10.5.5.1, page 259).

10.5.3 *Study findings from descriptive pathological analyses*

10.5.3.1 CAA was absent in 27% of the cohort

It is interesting that 9 (27%) of those with first-ever lobar ICH did not have any evidence of CAA in their hemispheres, four of whom had strictly lobar ICH.

Although misclassification of ICH location is possible, I think it is unlikely that this has occurred since all ICHs were classified by a neuroradiologist with a special interest in stroke. It is unlikely that CAA was missed in the supratentorial regions given the comprehensive sampling undertaken and the use of immunohistochemistry. There have been very few studies comparing the sensitivity of different techniques for detection of CAA but in one study of ten samples rated by both Congo Red staining and immunohistochemistry, immunohistochemistry was superior in that it detected CAA in two specimens in which Congo Red staining had been scarce, giving Congo Red a sensitivity of 71% (95% CI 36-92%) for the detection of CAA [Haglund & Englund 2002]. Ratings of the infratentorial regions were not available at the time of analysis but it is unlikely that this would have altered these findings since CAA is thought to deposit in the supratentorial regions first, possibly in the occipital lobes [Johnson et al. 2007] and even if CAA had been present in the infratentorial regions, the ICHs would not have been classified as 'CAA-related' according to the pre-specified definition of 'CAA-related ICH.'

This finding is of note, since it indicates that all lobar ICH cannot be thought of as 'possible CAA-related' ICH and further work is needed to determine the frequency of underlying causes of spontaneous primary lobar ICH.

10.5.3.2 CAA and vasculopathy are symmetrically distributed

My systematic review of case-control and cross-sectional pathological studies of CAA and ICH revealed that no previous studies had systematically assessed for CAA in all lobes of both cerebral hemispheres [Samarasekera, Smith, & Al-Shahi Salman 2012]. When studying a localised pathology such as ICH, it is of interest to determine whether

the distribution of CAA is symmetrical, since if not, this may provide clues as to why haemorrhage as occurred in one particular region. I did not find any differences in the frequency or severity of CAA deposition or frequency of vasculopathy in lobes affected by ICH vs. the contralateral lobe or frequency of vasculopathy supporting existing theories that CAA may result from diffusely compromised drainage of Aβ along perivascular pathways as arteries and arterioles stiffen with age, leading to vascular Aβ accumulation within the brain [Weller, Boche, & Nicoll 2009].

It is unclear why those with lobar ICH bleed in one hemisphere but not the other. Potential explanations include an interaction with other small vessel diseases such as arteriolosclerosis which might be more severe on the affected side, or an ICH may develop at the site of prior BMB [Gurol et al. 2012].

10.5.3.3 Vasculopathy is associated with severe CAA

The severity of parenchymal CAA was correlated with leptomeningeal CAA and severe CAA was associated with vasculopathy.

In patients with Alzheimer's dementia, CAA may deposit in the leptomeningeal vessels first before progressing to parenchymal vessels [Thal et al. 2003]. It is unknown whether a similar pattern occurs in those without dementia. I did not see patients with leptomeningeal CAA who lacked parenchymal CAA possibly because those with evidence of CAA had CAA at a more advanced stage when both leptomeningeal and parenchymal vessels might be affected.

The finding that severe CAA was associated with vasculopathy in the same lobe is in keeping with what is known about the development of CAA, since vasculopathy (fibrinoid necrosis, microaneurysm formation, vessel wall fragmentation and perivascular leakage) is seen in the 'end-stages' of CAA [Attems 2005]. However lobar ICH occurred without vasculopathy any of the same lobe(s) as the ICH in 11/24 (46%) participants who had any CAA present, suggesting that vasculopathy is not a necessary condition for ICH to occur. One possible explanation is misclassification of vasculopathy as absent, particularly in larger ICHs which disrupt tissue architecture, but if vasculopathy was missed in the affected hemisphere, given the symmetrical distribution of vasculopathy (Section 10.5.3.2, page 253) I would expect vasculopathy to be present in the contralateral hemisphere and none of these had vasculopathy in the

unaffected hemisphere. Alternatively, other small vessel diseases such as arteriolosclerosis or genetic factors such as presence of the apolipoprotein ε2 or ε4 alleles may contribute to the risk of ICH with severe CAA.

10.5.3.4 Capillary CAA is associated with severe CAA

Lobes with capillary CAA had significantly more severe CAA than lobes without capillary CAA, suggesting that capillary CAA is associated with severe CAA.

The significance of capillary CAA in adults with ICH is as yet unknown. Previous hospital –based post-mortem examination studies have suggested that capillary CAA may be a type of CAA distinct from parenchymal or leptomeningeal CAA, since Aβ1-42 is found in capillary CAA within the vessel wall in contrast to Aβ1-40 in larger vessels [Thal et al. 2002a;Richard et al. 2010]. Moreover, no difference in either CAA severity [Thal et al. 2002a;Attems, Lintner, & Jellinger 2004;Oshima et al. 2006] or age [Thal et al. 2002a] has been noted between those with and without capillary CAA, although those with capillary CAA might be expected to be older if capillary CAA was a progression from non-capillary (parenchymal or leptomeningeal) CAA.

However, these findings might be due to a) the participants selected since the studies listed above have included only 121 participants, included participants only with capillary CAA [Richard et al. 2010] and either not described participants' clinical phenotypes [Oshima et al. 2006] or used adults with and without dementia rather than adults with ICH [Thal et al. 2002a;Attems, Lintner, & Jellinger 2004;Richard et al. 2010], or b) sampling error since CAA was sampled solely from the frontal lobe [Attems, Lintner, & Jellinger 2004], occipital lobe [Oshima et al. 2006] or the temporal and occipital lobes [Thal et al. 2002a;Richard et al. 2010].

Both in my cohort and in some previous studies [Thal et al. 2002a;Oshima et al. 2006;Richard et al. 2010;Allen et al. 2013], capillary CAA has only occurred in the presence of parenchymal or leptomeningeal CAA. Moreover, the apolipoprotein ε4 allele is associated both with severe CAA [Rannikmae et al. 2013b] and capillary CAA [Richard et al. 2010;Allen et al. 2013], and for these reasons capillary CAA may be a manifestation of severe CAA.

10.5.3.5 Capillary CAA is associated with vasculopathy in the occipital lobes

When all lobes were combined, capillary CAA was associated with vasculopathy, but on examination of each lobe, this association remained in the occipital lobes but not in other regions of the brain. However, only rarely did capillary CAA occur without vasculopathy (Section 10.3.1.6, page 248) and it may be that given the small sample, the study lacked sufficient power to demonstrate an association between CAA and vasculopathy in each lobe. Given that capillary CAA seems to be associated with severe CAA (Section 10.5.3.4, page 255), it is plausible that capillary CAA might be associated with vasculopathy.

10.5.3.6 No association between total CAA score and age or dementia

The study may have lacked sufficient power to demonstrate an association between total CAA score and these variables, since the sample size was small and the age distribution of participants was narrow (median age 81 years; IQR 77-86 years.) I classified a person as having dementia if this was listed in their medical (GP & hospital) records or if the IQCODE score was ≥64 [Jorm 1994], but since only 37% participants in the first year of the cohort had an IQCODE (Chapter 5) I may have classified some participants with dementia incorrectly as not having dementia.

This is the first study to use the Bristol rating scale for rating CAA in adults with ICH and there is no established method as yet for using the scale for this purpose.

Although using a total CAA score is one method of quantifying overall CAA, it assumes firstly, that the response categories for each item are integers with equal differences between levels, which is not the case. The difference between 'zero' – no vasculopathy and 'one' –vasculopathy affecting occasional vessels may be more important than the difference between 'one' and 'two' where two- vasculopathy affecting many vessels. Secondly, it assumes that the differences have equal meaning – a scale difference of, for example five points, has the same meaning across all items in the scale [Hobart 2003] and lastly, that the scale is unidimensional; that is, all scale items measure the same characteristic, in this case CAA severity.

10.5.3.7 CAA-related lobar ICH

The prevalence of CAA-related lobar ICH in the cohort was 36% (95% CI 22-53%) which is lower than the 51% (95% CI 42-61%) prevalence of CAA in cases of lobar

ICH from my systematic review of hospital-based cross-sectional and case-control neuropathological studies of ICH with varied methods of CAA assessment (Chapter 9). I prospectively recruited participants in a population-based design and used a stringent definition of CAA-related lobar ICH which required evidence of severe CAA, with vasculopathy to be present in at least one of the lobes affected by the ICH. This may have led to a reduction in sensitivity (cases of CAA-related ICH incorrectly classified as CAA-unrelated ICH) at the expense of maintaining specificity. However, when determining the true prevalence of CAA-related ICH in the cohort, specificity appears to be more important than sensitivity, since small losses in specificity may grossly over-inflate the prevalence estimate whereas small losses in sensitivity appear to have only modest effects on prevalence estimates [Copeland et al. 1977].

10.5.4 *Study findings from univariate analyses-Radiological features did not differ between the CAA-related ICH and CAA-unrelated ICH groups*

I did not identify any differences in the radiological features of CAA-related lobar ICH vs. CAA-unrelated lobar ICH. This might be due to the study's small sample size leading to a lack of power to detect differences between the groups if they do exist, the impact of measured and unmeasured variables on imaging features, the definitions of imaging features or the definitions of outcome.

10.5.4.1 Impact of measured and unmeasured variables

Radiological features of ICH are influenced by several factors including ICH volume and the time interval between symptom onset and imaging. Intraventricular extension is associated with larger ICH volumes [Hallevi et al. 2008] and may be delayed [Maas et al. 2013b], so might be missed if a patient is scanned soon after symptom onset. The density of a haematoma also varies with time; being hyperdense in the acute stage and increasing as clot retraction occurs, but becoming hypodense in the subacute stage and in those with anaemia [Kidwell & Wintermark 2008]. In this cohort, neither ICH volume nor the time interval between symptom onset and imaging differed between the CAA-related and CAA-unrelated ICH groups. Intraventricular extension may be associated with white matter hyperintensities [Kim et al. 2013] which I did not assess and may be difficult to assess because of disruption of the brain parenchyma by the ICH. Similarly haematoma density may be lower in those with anaemia (which I did not assess) or if the haematoma arises from multiple foci [Barras et al. 2009]. Therefore

ICH appearances might have been influenced by these unmeasured variables which affected the findings.

10.5.4.2 Definitions of imaging features

Previous studies have used various definitions of haematoma shape and density.

Radio-pathological studies of CAA-related lobar ICH have either not defined the terms used to describe ICH shape [Yong et al. 1992;Izumihara et al. 1999] or used pictorial representations [Wagle, Smith, & Weiner 1984;Brown, Coates, & Gilbert 1985;Minakawa et al. 1995;Lang et al. 2001]. ICH shape has been most frequently labelled as either round or irregular [Minakawa et al. 1995], sometimes with additional use of the term 'lobulated' [Izumihara et al. 1999;Lang et al. 2001] and inter-observer agreement has not been quantified.

Other studies of ICH have used a variety of descriptive terms, including ICH classed as round (with round and smooth margins), irregular (with irregular, multinodular margins) or separated (with a fluid level in the cavity) [Fujii et al. 1994], round to ellipsoid, irregular (with frayed margins) or multinodular to separated [Huttner et al. 2006], round or irregular [Miyahara, Murata, & Abe 2007;Sheth et al. 2010] and regular vs. irregular defined using a five point categorical scale [Barras et al. 2009]. In one study which measured inter-rater agreement using the kappa statistic, agreement was substantial [Sheth et al. 2010], in two others raw agreement ranged from 85% [Barras et al. 2009] to 93% [Huttner et al. 2006] and in the remainder agreement was not assessed [Fujii et al. 1994;Miyahara, Murata, & Abe 2007].

Variable density has previously been rated using a five-point categorical scale in which grade one indicated a homogenous ICH and grades two to five indicated progressively increasing degrees of heterogeneity. There was moderate inter-rater agreement (κ=0.61) [Barras et al. 2009].

I sought to clearly describe these terms by classifying an ICH as either irregular or not and either of uniform or variable density (Figure 41 on page 280 and Figure 42 on page 281).

I used a binary classification as the aim of the study was to assess for easily applicable CT imaging features of ICH which might differentiate CAA-related from other lobar ICH. I assessed both whether an ICH was irregular and whether there were finger-like

protrusions to the cortex since ICH in a 'superficial location' has been a noted feature in CAA-related lobar ICH [Wagle, Smith, & Weiner 1984;Yong et al. 1992] and CAA may deposit in the leptomeningeal and neocortical vessels first [Thal et al. 2003]. Although a neuroradiologist reviewed all brain imaging, imaging features are subject to inter-observer variation. In a future study I would assess inter-observer variation not only of haematoma shape and density but also other imaging features such as ICH location.

10.5.4.3 Definition of outcome

Although the stringent definition of CAA-related ICH used may have led to differential misclassification of the outcome (by making it more likely that ICHs related to CAA were classified as unrelated to CAA), a sensitivity analysis which included both ICHs meeting the primary and secondary outcome measures for CAA-related lobar ICH, did not alter the findings.

10.5.4.4 Subarachnoid haemorrhage may be more common in strictly lobar ICH

Subarachnoid extension of the ICH tended to be more frequent in the CAA-related strictly lobar ICH group vs. CAA-unrelated strictly lobar ICH although this was not statistically significant and needs further exploration in a larger cohort. Subarachnoid extension might be seen in CAA-related ICHs if leptomeningeal and cortical vessels are more vulnerable to CAA deposition than other vessels [Thal et al. 2003] and bleed preferentially but subarachnoid extension is also more common in lobar ICH (Chapter 5).

If subarachnoid haemorrhage is a marker for CAA-related lobar ICH it could improve the sensitivity of the Boston criteria [Knudsen et al. 2001] which have excellent specificity but only moderate sensitivity for the ante-mortem diagnosis of CAA-related ICH (Section 10.5.5.6, page 263).

10.5.5 *Future directions*

This study raises interesting questions regarding the assessment of CAA in pathological studies and its role in ICH.

10.5.5.1 Assessment of the Bristol rating scale

A pathological scale should measure what it claims to (be valid), should be reproducible (reliable) and for pathological scores, particularly when scale items are summed to

obtain a total score, all items included in the scale should measure the same characteristic [Hobart et al. 2007;Coste et al. 2014]. If items measuring different characteristics are included in the scale, then more than one trait is being assessed and a sum score is not valid.

The Delphi-style survey [Linstone & Turoff 1975] used in the formation of the Bristol scale [Love et al. 2014] is one approach to gaining content validity. The scale has reasonable external construct validity (that is, it behaves as predicted when tested with a different variable) since the presence of CAA, measured by the Bristol scale, was strongly associated with apolipoprotein ε2 and ε4 genotype when tested in persons with dementia although this is still to be tested in those with ICH. Internal construct validity (the extent to which items on the scale expected to relate to each other, do so statistically) is satisfactory as shown by the positive correlations between parenchymal and leptomeningeal CAA and severe CAA and vasculopathy.

A future study could assess convergent and discriminant construct validity [Hobart et al. 2007]. The former might be assessed by applying different scales measuring CAA to the cohort to determine if the Bristol scale correlates with scales which measure similar features. The validity of using the CAA sum score as a measure of total CAA severity could also be tested by comparing CAA sum scores from the Bristol scale[Love et al. 2014] with other CAA scales which also use a summary measure of individual components to measure CAA severity [Ellis et al. 1996;Attems, Jellinger, & Lintner 2005] rather than inferring CAA severity for an entire brain from a single tissue sample[Vonsattel et al. 1991;Olichney et al. 1996].

Divergent construct validity might be assessed by applying scales measuring a different construct such as arteriolosclerosis to the sample and comparing scores to check that the scores from the two scales do not correlate.

Inter-rater reliability of the Bristol scale was good when tested on samples from 20 persons with Alzheimer's dementia and five controls [Love et al. 2014]. I have not assessed inter-rater reliability in this study and a future study should do this, using samples from both ICH brains and controls and a pathologist blind to case/control status and sample location.

10.5.5.2 Assessment of imaging features

A future study should assess the inter-rater reliability of imaging variables using two neuroradiologists blinded to the pathological findings. Definitions of haematoma density and ICH shape have varied in earlier studies (Section 10.5.4, page 257) and may be more likely to be subject to inter-rater variation. Although there has been reasonable agreement regarding the classification of ICH location [Wermer et al. 2002;Battathiri et al. 2003], even with larger haematomas [Wermer et al. 2002], previous studies have classified ICH location as either lobar or deep and a future study should clearly define ICH location and examine inter-rater agreement for classifying ICHs as lobar, deep or mixed since some haematomas will inevitably involve both the lobar and deep regions of the brain (Chapter 5).

10.5.5.3 Assessment of other small vessel diseases

Given that only 36% lobar ICHs are explained by CAA, other small vessel diseases probably contribute to lobar ICH.

Studies should assess the prevalence and severity of other small vessel diseases, especially arteriolosclerosis in lobar ICH. CAA may be a risk factor for ischaemic stroke [Cadavid et al. 2000] and microinfarcts are associated with severe CAA [Soontornniyomkij et al. 2010] but the role of CAA in their formation is yet to be established. It has been suggested that CAA leads to cerebral hypoperfusion and infarcts [Okamoto et al. 2012] but microinfarcts do not invariably correlate with CAA [Kovari et al. 2013] and the role of arteriolosclerosis is unclear. Since participants with lobar ICH also have vascular risk factors (Chapter 5), co-existing arteriolosclerosis may modify the effect of CAA and influence the risk of ICH.

10.5.5.4 Dementia

Clinico-pathological studies of pre-morbid dementia and lobar ICH are scarce. There have been no prospective population-based studies although one recent hospital-based prospective study of pre-stroke dementia and ICH included five post-mortem examination cases of lobar ICH all of which had either stage V or VI Braak [Braak et al. 1996] Aβ pathology [Cordonnier et al. 2010b].

Ten neuropathological studies (of 141 participants) have examined the prevalence of CAA in lobar ICH and assessed Alzheimer's disease pathology. The prevalence of

Alzheimer's pathology in CAA-related lobar ICH has varied from 37% [Gilles et al. 1984] to ~ 70% [Yoshimura et al. 1992;Itoh et al. 1993] to ~100% [Jellinger 1977;Vinters & Gilbert 1983;Ishii, Nishihara, & Horie 1984]. Two studies did not state the prevalence of parenchymal Aβ in those with lobar ICH [Tomonaga 1981;Attems, Lauda, & Jellinger 2008] and in two others no cases of lobar ICH had parenchymal Aβ[Cartier et al. 1999;Mastaglia et al. 2003].

A larger population-based study would determine the prevalence of pre-existing dementia in persons with lobar ICH (and ICH in other locations) and might demonstrate an association between a prior history of dementia and CAA severity, as this is plausible given the established association between CAA and Alzheimer's dementia [Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) 2001]. The study should also assess parenchymal Aβ deposition (plaques), neurofibrillary tangles and vascular pathologies which contribute to dementia and death [Matthews et al. 2009] since this may provide clues as to the presumed causes of cognitive decline in persons with ICH and correlate parenchymal Aβ with CAA severity to assess if this differs according to apolipoprotein E genotype, since senile plaque density may increase with possession of an ε4 allele [Rebeck et al. 1993] although studies conflict [Allen et al. 2013]. By comparing these pathologies in demented vs. undemented persons it might be possible to assess the relative contributions of different pathologies to dementia in persons with lobar ICH.

10.5.5.5 Apolipoprotein E gene

The ε4 allele of the apolipoprotein E gene has a dose-dependent relationship with pathologically-proven CAA [Rannikmae et al. 2013b], but both the ε2 and ε4 alleles are associated with lobar ICH [Biffi et al. 2010b]. The commonly proposed hypothesis is that ε4 promotes the deposition of CAA but the ε2 allele promotes progression to vasculopathy that leads to vessel rupture and ICH [McCarron et al. 1999a].

However, a recent individual patient data meta-analysis of 497 adults without ICH (50% with dementia, 20% not demented and 30% dementia status unknown) with pathologically-proven CAA showed an association between the ε4 allele and vasculopathy but no such association with the ε2 allele although the number of participants with the ε2 allele was small [Rannikmae et al. 2013a].

A future study should compare the prevalence of ε2 and ε4 alleles in persons with CAA-related lobar ICH (that is, severe CAA with vasculopathy meeting the primary outcome measure) in comparison to those with lobar ICH not related to CAA to determine whether the ε2 allele is more frequent in those with vasculopathy and confirm the presence of a dose dependent relationship between the ε4 allele and CAA severity in persons with ICH. Given the low prevalence of the ε2 allele, a multi-centre approach may be necessary.

Since capillary CAA is associated with the ε4 allele in persons with Alzheimer's dementia [Thal et al. 2002a;Allen et al. 2013], the study should assess whether the same association is seen in persons with ICH.

10.5.5.6 Modification of diagnostic criteria for CAA

The eventual aim of this study would be to improve the diagnostic utility of the Boston criteria for the ante-mortem diagnosis of CAA. Currently the Boston criteria have excellent specificity (100% 95% CI 77-100%) so they do not misclassify those without CAA as having CAA, but their sensitivity is 44% (95% CI 28-62%), so are liable to classify those with CAA as not having a CAA-related lobar ICH.

For the diagnostic criteria to be widely applicable in clinical settings, they need to comprise standards that have been tested and validated independently in population-based cohorts representative of the patients likely to develop ICH, use technology that is applicable in lower-income countries where MRI is likely to be less accessible and lead to diagnoses that are meaningful to be able to guide prognosis and treatment for an individual patient [Brayne 2014]. Given the widespread use of CT in both high and low-middle income countries, integration of any CT imaging variables which reliably differentiate between CAA-related lobar ICH and other lobar ICH would guide clinicians and limit unnecessary investigations into other causes of ICH.

A larger study would have greater power to determine if any of the four candidate imaging variables are actually associated with CAA-related lobar ICH and would be able to test other variables such as subdural extension which have been observed with CAA-related lobar ICH in previous studies (Table 29 on page 265).

Table 28 Characteristics of radio-pathological studies of CAA-related lobar ICH (excluding studies of <five participants)

Study (year)	Number of participants with both CT & pathologically-proven CAA-related ICH	Age range (years)	First-ever ICH?	Interval between symptom onset and diagnostic CT scan	Interval between diagnostic CT & post-mortem or biopsy	CAA sampling: Post-mortem/ biopsy	CAA detection: Congo Red (CR) or Immunohistochemistry (IHC)
Wagle (1984)	7	62-84	6	0-5 weeks	?	5/2	CR
Brown (1985)	12	62-89	7	?	?	7/5	CR
Cosgrove (1985)	7	58-88	?	?	?	7/0	CR
Wakai (1992)	6	65-76	5	?	0- ≥15 days	0/6	CR & IHC (on those positive with CR)
Yong (1992)	6	54-86	?	0- ≥2 weeks	?	0/6	CR & IHC
Minakawa (1995)	10	61-80	?	1-48 hours	0-2 days	0/10	CR
Izumihara (1999)	37	61-91	26	?	?	0/37	CR & IHC
Miller (1999)	7	60-86	6	?	1-120 days	7 /0	?
Lang (2001)	41	53-90	?	?	?	0/41	CR
Oide (2003)	64	61-91	42	?	?	12/52	CR & IHC
Chen (2004)	5	65-83	?	?	?	0/5	CR
Patel (2009)	12	?	?	?	?	2/10	CR or IHC
Hirohata (2010)	41	55-85	?	?	?	9/32	CR & IHC

Table 29 Imaging (CT) characteristics observed with pathologically proven CAA-related lobar ICH

Frequency (%) of characteristic in sample; ● = characteristic observed with CAA-related lobar ICH but frequency not stated

	Sample size	Multiple ICH	Intraventricular extension	Subarachnoid extension	Subdural extension	Blood or fluid level within a haematoma	Variable density within a haematoma	High density of blood sedimenting posteriorly	Superficial location	Irregular border	Lobulated ICH	Surrounding oedema	Periventricular white matter lucency	Atrophy
Wagle (1984)	7		4 (57)	3 (43)					3 (43)	6 (86)		6 (86)		
Brown (1985)	12		6 (50)							7 (58)				
Cosgrove (1985)	7		3 (43)	6 (86)	3 (43)									
Wakai (1992)	6			6 (100)							6 (100)			
Yong (1992)	6			●					●	●				
Minakawa (1995)	10		6 (60)	9 (90)		7 (70)				9 (90)				
Izumihara (1999)	37	5 (14)	5 (14)	31(84)	9 (24)					8 (22)	23 (62)			
Millar (1999)	7	5 (71)	5 (71)	5 (71)		3 (43)	5 (71)	●	5 (71)		7 (100)		5 (71)	
Lang (2001)	41	7 (17)	10 (24)	26 (64)						15 (37)	17 (41)			
Oide (2003)	64	6 (9)	10 (16)	53 (83)									27(42)	
Chen (2004)	5		4 (80)											
Patel (2009)	12				8(67)									
Hirohata (2010)	41	●	●	●										

Table 30 Lobes affected by ICH in 33 participants with first-ever lobar ICH

Lobes (s) involved by ICH	Frequency
Frontal	13
Frontal-parietal	4
Frontal-parietal-temporal	2
Frontal-temporal	3
Parietal	8
Parietal-temporal	1
Parietal-occipital	1
Occipital	1

Table 31 Scores for each component of the Bristol scale in 24 participants with first-ever lobar ICH who had any CAA present in their brains
pCAA= parenchymal CAA, lcaa=leptomeningeal CAA, vasc=vasculopathy, capCAA=capillary CAA

Case	Hemisphere (Right [R] or Left [L]) and Lobe(s) involved	Frontal				Temporal				Parietal				Occipital			
		pCAA	ICAA	vasc	capCAA	pCAA	ICAA	vasc	capCAA	pCAA	ICAA	vasc	capCAA	pCAA	ICAA	vasc	capCAA
1	R parietal									3	3	1	1				
2	L frontal	3	3	1	0												
3	R frontal	2	2	1	0												
4	L frontal	3	3	1	0												
5	R fronto-parietal	3	2	1	0					3	3	0	1				
6	L frontal	3	3	1	0												
7	L fronto-temporal	3	3	1	0	2	3	0	0								
8	R frontal	3	3	1	1												
9	L fronto-parieto-temporal	0	0	0	0	1	2	0	0	1	2	0	0				
10	R frontal	2	2	0	0												
11	R parietal									3	3	2	1				
12	L frontal	2	3	2	0												

Case	Hemisphere (Right [R] or Left [L]) and Lobe(s) involved	Frontal				Temporal				Parietal				Occipital			
		pCAA	ICAA	vasc	capCAA	pCAA	ICAA	vasc	capCAA	pCAA	ICAA	vasc	capCAA	pCAA	ICAA	vasc	capCAA
13	R parieto-occipital									1	1	0	0	2	1	0	0
14	L parietal									1	1	0	0				
15	R frontal	1	2	0	0												
16	R frontal	0	1	0	0												
17	L fronto-temporal*	0	0	0	0	0	0	0	0								
18	L parietal									1	1	1	0				
19	R frontal	1	3	0	0												
20	R frontal	3	3	2	1												
21	L occipital													1	3	0	1
22	R frontal	3	3	1	0												
23	L fronto-parietal	3	2	0	0					1	3	0	0				
24	L parietal									3	3	0	0				

*CAA present in left parietal and left occipital lobe

Table 32 Frequency of CAA of any severity in lobes affected and unaffected by ICH in 33 participants with first-ever lobar ICH

Lobe	CAA concordant		CAA discordant	
	CAA present in corresponding lobes of both hemispheres	CAA absent in corresponding lobes of both hemispheres	CAA Affected hemisphere ✓ Unaffected hemisphere ✖	CAA Affected hemisphere ✖ Unaffected hemisphere ✓
Frontal (n=22)	15	7	0	0
Parietal (n=16)	9	7	0	0
Temporal (n=6)	2	4	0	0
Occipital (n=2)	2	0	0	0

Table 33 Frequency of vasculopathy of any severity in lobes affected and unaffected by ICH in 33 participants with first-ever lobar ICH

Lobe	Vasculopathy concordant		Vasculopathy discordant	
	Vasculopathy present in corresponding lobes of both hemispheres	Vasculopathy absent in corresponding lobes of both hemispheres	Vasculopathy Affected hemisphere ✓ Unaffected hemisphere ✖	Vasculopathy Affected hemisphere ✖ Unaffected hemisphere ✓
Frontal (n=22)	10	12	0	0
Parietal (n=16)	3	13	0	0
Temporal (n=6)	0	5	0	1
Occipital (n=2)	0	2	0	0

Table 34 Correlation between parenchymal and leptomeningeal CAA in cerebral hemispheres affected and unaffected by ICH in 33 participants with first-ever lobar ICH

Lobe	Affected hemisphere		Unaffected hemisphere	
	Correlation Coefficient	p value	Correlation Coefficient	p value
Frontal	0.85	<0.001	0.86	<0.001
Parietal	0.85	<0.001	0.89	<0.001
Temporal	0.85	<0.001	0.83	<0.001
Occipital	0.79	<0.001	0.72	<0.001

Table 35 Presence of severe CAA (parenchymal or leptomeningeal CAA of at least grade 2 or above) and vasculopathy in each lobe in 33 participants with first-ever lobar ICH with OR (95% confidence intervals) for the odds of vasculopathy in the presence of severe CAA

Lobe	Hemisphere	Severe CAA ✓ Vasculopathy ✓	Severe CAA ✖ Vasculopathy ✖	Severe CAA ✓ Vasculopathy ✖	Severe CAA ✖ Vasculopathy ✓	OR (95% CI)	p value
Frontal	Affected	13	12	8	0	18.0 (1.9-167.0)	0.01
	Unaffected	12	13	7	1	22.3 (2.4-208.8)	0.007
Parietal	Affected	9	12	11	1	9.8 (1.1-90.6)	0.04
	Unaffected	9	13	10	1	11.7 (1.3-108.2)	0.03
Temporal	Affected	10	12	10	1	12.0 (1.3-110.5)	0.03
	Unaffected	10	12	11	0	10 (1.9-92.0)	0.04
Occipital	Affected	10	11	12	0	8.3 (0.9-76.1)	0.06
	Unaffected	10	11	12	0	8.3 (0.9-76.1)	0.06

Table 36 Comparison of parenchymal and leptomeningeal CAA severity in lobes with and without capillary CAA in 33 participants with first-ever lobar ICH

Lobe	Hemisphere	Sum score for parenchymal and leptomeningeal CAA; median (IQR)		p value
		Lobes with capillary CAA	Lobes without capillary CAA	
Frontal	Affected	6 (6-6)	3 (0-5)	0.02
	Unaffected	6 (6-6)	4 (0-5)	0.005
Parietal	Affected	6 (5-6)	3 (0-5)	0.005
	Unaffected	5 (4-6)	2 (0-5)	0.06
Temporal	Affected	6 (6-6)	3 (0-4)	0.03
	Unaffected	6 (3-6)	3 (0-5)	0.06
Occipital	Affected	6 (5-6)	3 (0-4)	<0.001
	Unaffected	6 (5-6)	3 (0-5)	<0.001

Table 37 Capillary CAA and vasculopathy in each lobe in 33 participants with first-ever lobar ICH

Lobe	Hemisphere	capillary CAA ✓ Vasculopathy ✓	capillary CAA ✖ Vasculopathy ✖	capillary CAA ✓ Vasculopathy ✖	capillary CAA ✖ Vasculopathy ✓	OR (95% CI)	p value
Frontal	Affected	3	19	1	10	5.7 (0.5-62.2)	0.15
	Unaffected	3	19	1	10	5.7 (0.5-62.2)	0.15
Parietal	Affected	4	20	3	6	4.4 (0.8-25.7)	0.10
	Unaffected	3	20	3	7	2.9 (0.5-17.6)	0.26
Temporal	Affected	3	20	2	8	3.7 (0.5-26.8)	0.19
	Unaffected	3	21	2	7	4.5 (0.6-32.7)	0.14
Occipital	Affected	6	19	4	4	7.1 (1.4-37.6)	0.02
	Unaffected	6	20	3	4	10.0 (1.7-57.7)	0.01

Table 38 Baseline clinical characteristics of 33 participants with first-ever lobar ICH

	CAA-related lobar ICH (n=12)	CAA-unrelated lobar ICH (n=21)	p value
Sex (male), (%)	8 (67)	7 (33)	0.08
Age (years); median (IQR)	80 (77-84)	82 (79-87)	0.58
History of hypertension			
Yes (n, %)	6 (50)	16 (76)	0.13
History of dementia			
Yes (n, %)	4 (33)	6 (29)	1.00
History of ischaemic stroke			
Yes (n, %)	3 (25)	4 (19)	0.69
Premorbid medication			
Antiplatelet use (n, %)	5 (42)	14 (66)	0.16
Anticoagulant use (n, %)	0 (0)	3 (14)	0.28

Table 39 Baseline radiological characteristics of 33 participants with first-ever lobar ICH

	CAA-related lobar ICH(n=12)	CAA-unrelated lobar ICH (n=21)	p value
Interval between ICH date and first CT scan (days); median (IQR)	0 (0-1)	1 (0-1)	0.56
Interval between date of first CT scan and post-mortem examination (days); median (IQR)	50 (11-370)	9 (6-33)	0.13
Strictly lobar ICH (vs. mixed) Yes (n, %)	8 (66)	9 (43)	0.29
Multiple ICH; Yes (n, %)	1(8)	1(5)	1.00
Intraventricular haemorrhage Yes (n, %)	9(75)	10 (48)	0.16
Subarachnoid haemorrhage Yes (n, %)	11 (92)	14 (67)	0.21
Subdural haemorrhage Yes (n, %)	1 (8)	2 (10)	1.00
ICH volume; median (IQR)	68 (29-86)	57 (35-108)	0.85
Presence of a blood/fluid level within the largest ICH Yes (n, %)	1 (8)	4 (19)	0.63
ICH with an irregular border Yes (n, %)	11 (92)	18 (86)	1.00
Irregular or lobulated ICH with ≥1 finger like protrusion to cortex; Yes (n, %)	7 (58)	11(52)	0.74
Variable density within ICH Yes (n, %)	6 (50)	14 (67)	0.35

Table 40 Comparison of radiological characteristics in 17 participants with strictly lobar ICH

	CAA-related strictly lobar ICH (n=8)	CAA-unrelated strictly lobar ICH (n=9)	p value
Intraventricular haemorrhage			
Yes (n, %)	5 (63)	5 (56)	1.00
Subarachnoid haemorrhage			
Yes (n, %)	8 (100)	5 (56)	0.08
Irregular or lobulated ICH with ≥1 finger like protrusion to cortex; Yes (n, %)	3 (38)	3 (33)	1.00
Variable density within ICH			
Yes (n, %)	4 (50)	4 (44)	1.00

Figure 38 Tissue section stained with haematoxylin and eosin, showing cortical arterioles with eosinophilic amyloid deposits within the vessel walls (arrows)

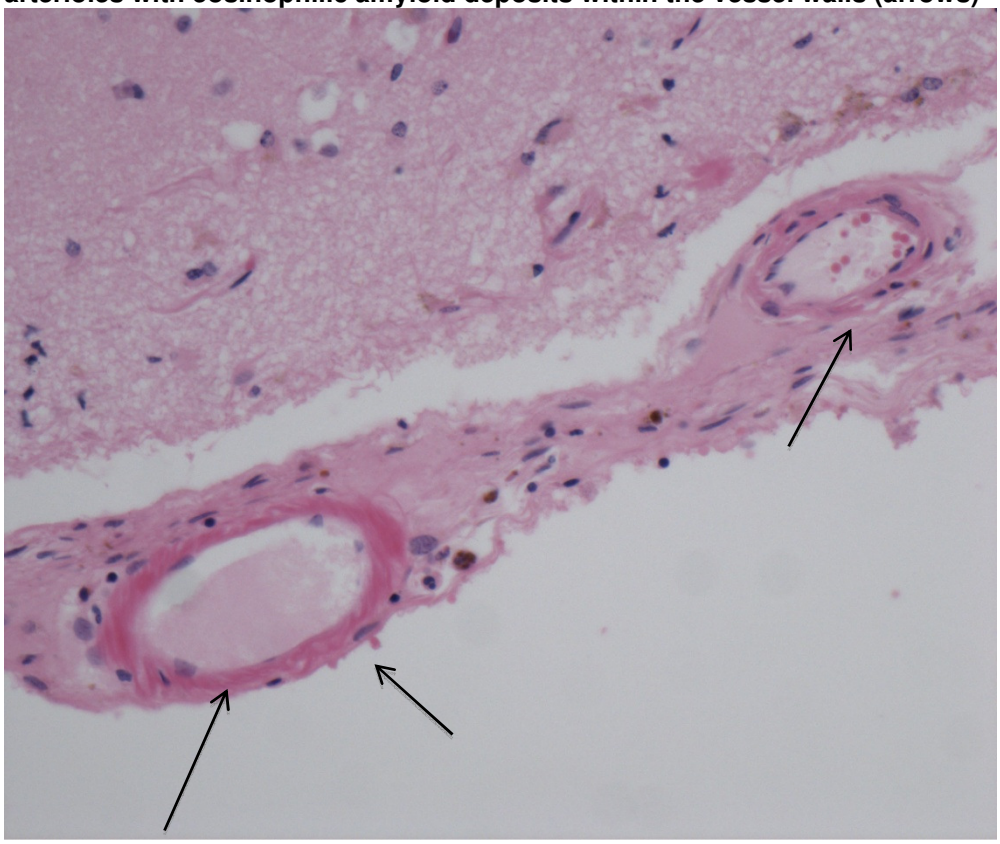


Figure 39 Tissue section stained with haematoxylin and eosin showing cortical arterioles with double barrelling, indicative of severe CAA (arrows)

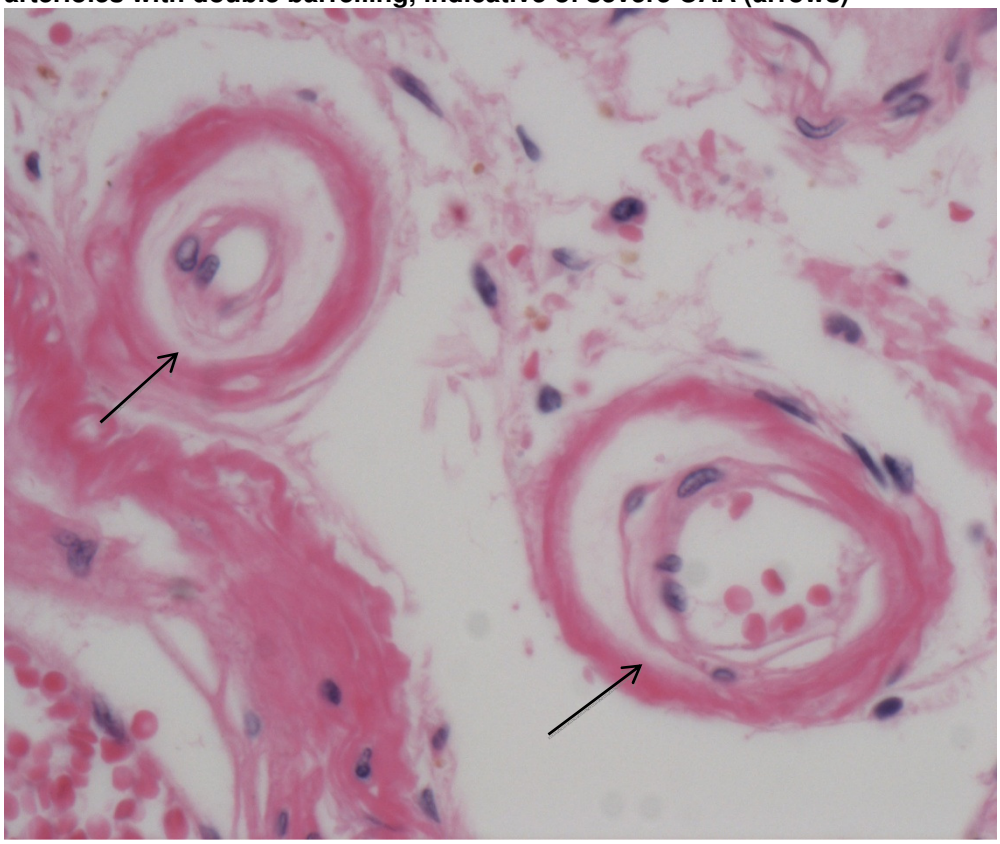


Figure 40 Tissue section immunohistochemically stained with antibodies to amyloid-beta showing small leptomeningeal arterioles with dense deposits of amyloid-beta within their walls (arrows)

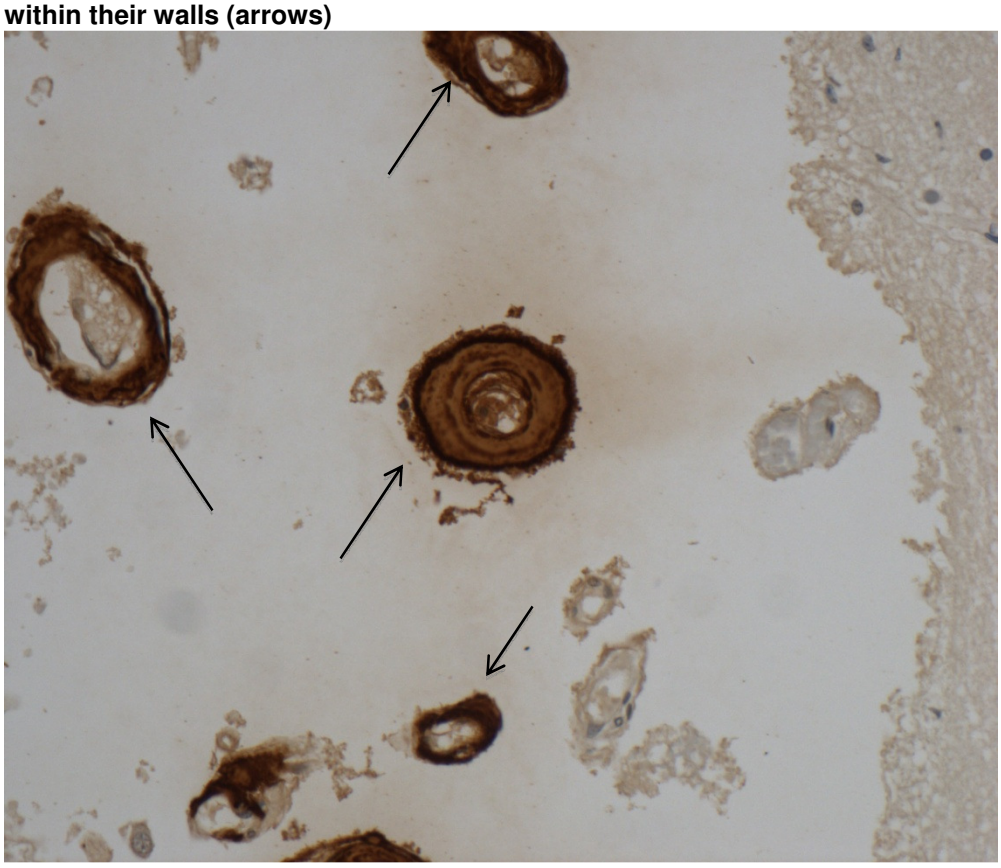


Figure 41 Irregular or lobulated ICH

A & B ICH has an irregular border with finger-like protrusions to the cortex
C & D ICH has an irregular border without finger-like protrusions to the cortex
E & F ICH has a regular border without finger-like protrusions to the cortex

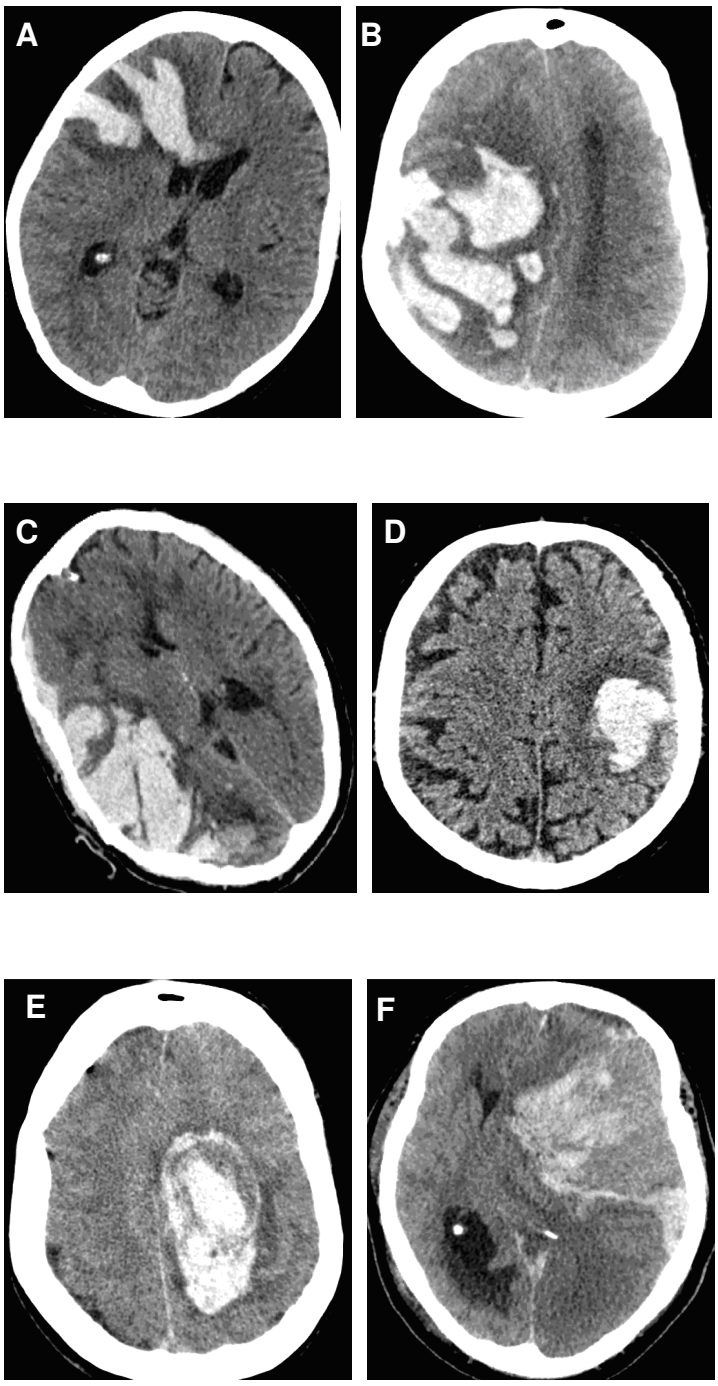


Figure 42 Variable density within an ICH

A,B,C Variable density with an ICH. ICH appears ‘patchy’ or ‘dilute’
D,E Blood within any single ICH is of uniform attenuation on CT

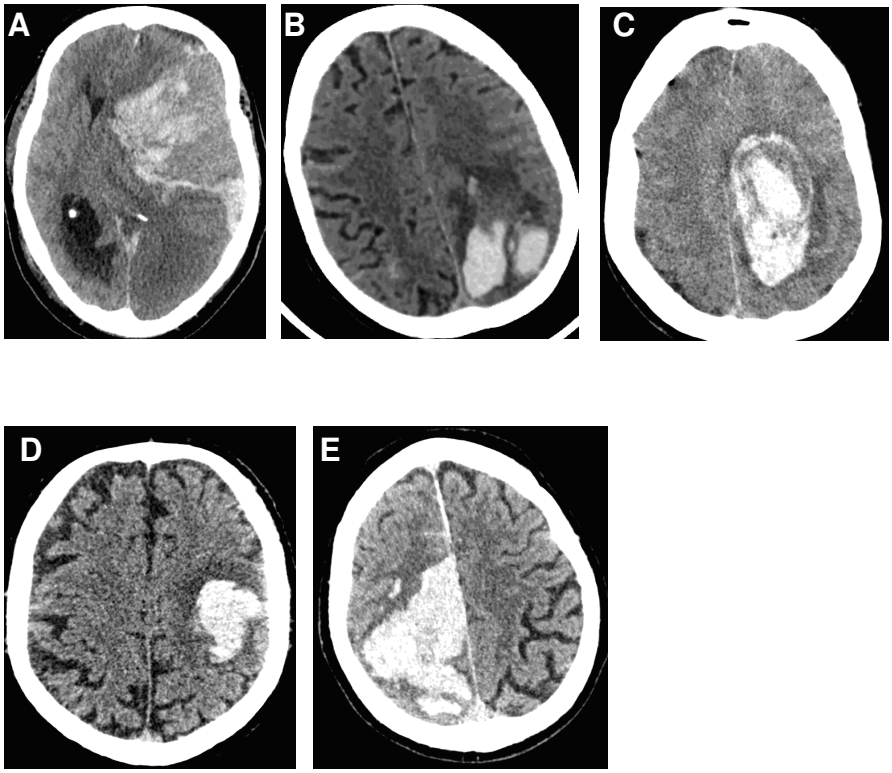


Figure 43 Histogram of frequency of CAA deposition of any severity per lobe in 33 participants with first-ever lobar ICH (grouped by hemisphere affected or unaffected by ICH)

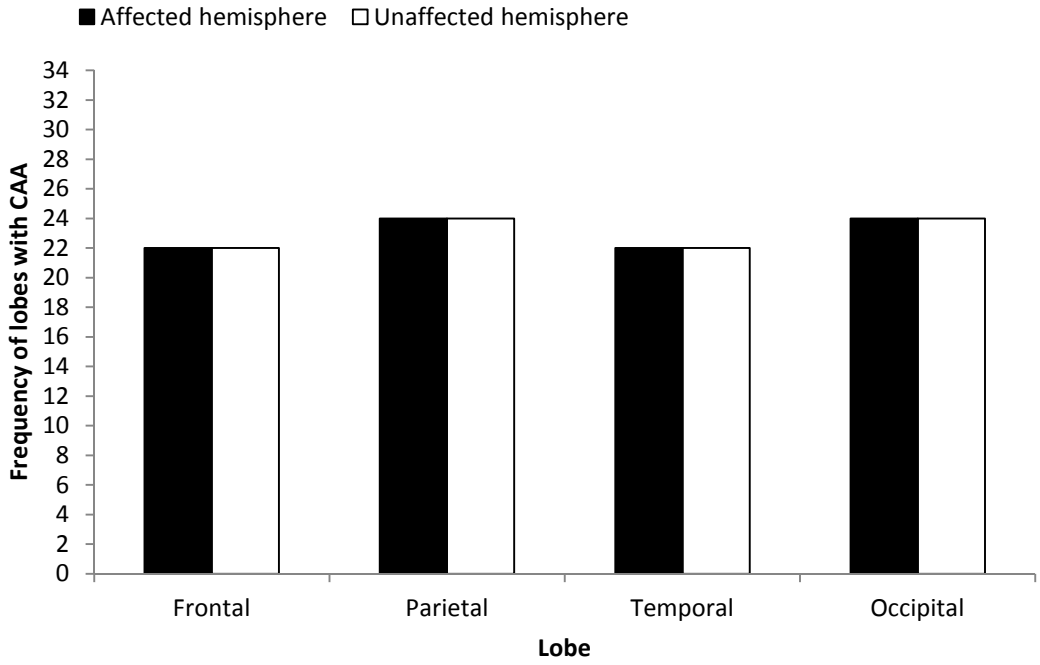
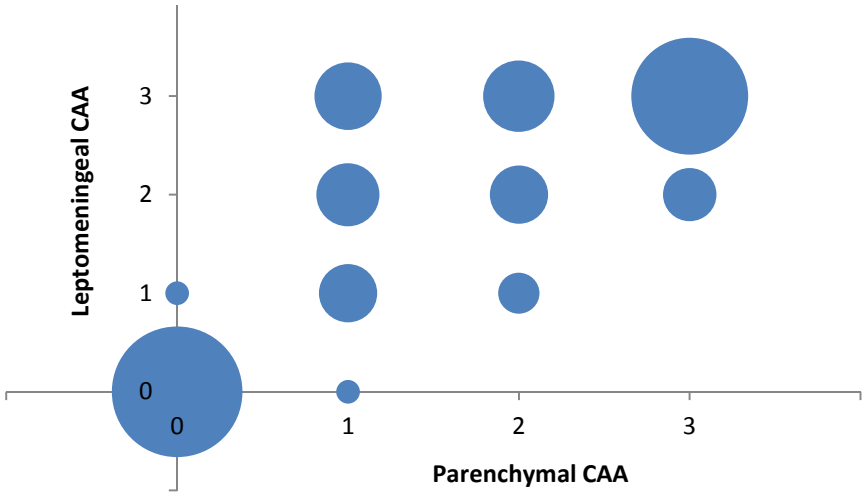
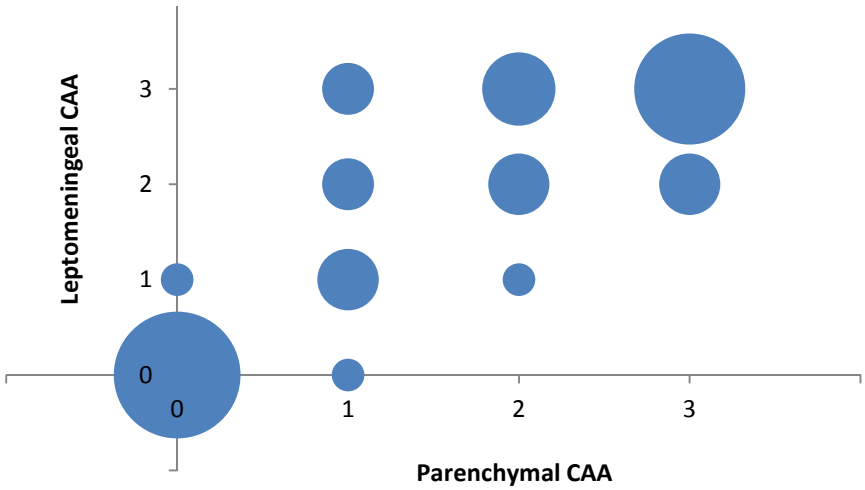


Figure 44 Bubble scatterplot of scores for parenchymal and leptomeningeal CAA (according to the Bristol rating scale) in each lobe of a) hemisphere affected by ICH and b) hemisphere unaffected by ICH in 33 participants with first-ever lobar ICH
(the size of the bubble is proportional to the percentage share of the sample at a particular point)

A) Affected hemisphere



B) Unaffected hemisphere



Chapter 11 Consent for brain tissue donation after intracerebral haemorrhage: a community-based study

Chapter contents

11 Consent for brain tissue donation after intracerebral haemorrhage: a community-based study

11.1 Introduction

ICH is a devastating form of stroke which has an increasing incidence with age (Chapter 3). Given the ageing population in the UK and other developed countries [Di Carlo A. 2009], its incidence is likely to increase.

Despite increasing understanding of factors which contribute to ICH [Ariesen et al. 2003], the extent of the role of CAA and its interaction, if any, with other risk factors such as hypertension remains unknown. The application of the modified Boston criteria [Knudsen et al. 2001;Linn et al. 2010] in my community-based cohort showed that the ante-mortem diagnosis of CAA remains difficult (Chapter 5) and CAA can only be diagnosed with certainty using pathological specimens obtained at biopsy or post-mortem examination.

Research using brain tissue samples both in the UK and elsewhere has been hampered by declining post-mortem examination rates [Kretzschmar 2009] and various organ retention scandals. In the UK this came to light during an inquiry into paediatric heart surgery in the Bristol Royal Infirmary [Kennedy 2001] when it was disclosed that organs had been retained as part of the diagnostic process without the knowledge or informed consent of relatives. Subsequent inquiries in Liverpool [Redfern 2001] and Scotland [Mclean 2003] led to the formation of the Human Tissue Act (2004) [Department of Health 2004] and Human Tissue (Scotland) Act 2006 [Department of Health (Scotland) 2006] which made seeking written informed consent for the retention and use of human tissue samples for research purposes mandatory.

There have been 11 studies of brain donation for research which have reported the proportion of participants giving consent (Figure 45 on page 305). They have either sought control brains or focused on neurological disorders [King, Smith, & Jobst 1993;Stevens 1998;Kaye et al. 1999;Schmitt et al. 2001;Millar et al. 2007;Garrick et al. 2009;Danner, Darnell, & McGuire 2011;Angelini et al. 2011;Kuhta et al. 2011;Jefferson et al. 2011;Harris, Kiger, & Counsell 2012]. Two were set in the UK and predated the

organ retention scandals [King, Smith, & Jobst 1993;Stevens 1998]. Six were studies of healthy ageing and dementia [King, Smith, & Jobst 1993;Stevens 1998;Kaye et al. 1999;Schmitt et al. 2001;Danner, Darnell, & McGuire 2011;Jefferson et al. 2011], three involved participants with chronic neurological disorders (movement disorders [Kuhta et al. 2011;Harris, Kiger, & Counsell 2012] and pontine glioma [Angelini et al. 2011]) and two studies involved participants who had a sudden death [Millar et al. 2007;Garrick et al. 2009]. Consent proportions have ranged from 21-96%.

There have been no previous studies of consent in the setting of ICH. It is also unclear whether any demographic characteristics of participants or factors related to the consent process are associated with obtaining consent for brain donation. Studies of organ donation for transplant have identified the timing of the request and whether the request is made 'collaboratively' by both the organ donation and clinical teams as being predictors of obtaining consent [Simpkin et al. 2009].

In this first community-based study of brain donation for ICH, I sought to ascertain the proportion giving consent, whether any characteristics of participants, their ICH or the consent process were associated with consent and whether those approached to give consent, those who consented and those who eventually became brain donors were representative of the entire cohort of patients with ICH. I also identified reasons cited by participants for consenting or not consenting to brain donation.

11.2 Methods

11.2.1 *Proportion giving consent to brain donation for research*

I used the first complete year of the study (1st June 2010-31st May 2011) to determine the proportions of those with spontaneous primary ICH who were approached to consider brain donation, those who were not approached and of those approached, the proportion who consented to brain donation vs. those who did not.

11.2.2 *Comparison of adults approached vs. those not approached to consider brain donation*

I compared the baseline demographic and clinical characteristics of adults with ICH who were approached to consider donation (either directly if they had mental capacity

or via their nearest relative if they lacked mental capacity) vs. those who were not approached.

Demographic characteristics were the sex, age, ethnicity, level of education and socioeconomic status (using residential postcode as a surrogate marker) of participants. The level of education was defined as either basic (school only) or further/higher education (college, apprenticeships or university).

To assess socioeconomic status I used the Scottish Index of Multiple Deprivation (http://www.scotland.gov.uk/Topics/Statistics/SIMD/BackgroundMethodology; 2012) which ranks regions by postcode from the most deprived (rank one) to the least deprived (rank 20). The level of deprivation is a combination of 38 indicators across the following domains: income, employment, health, education, skills and training, housing, geographic access and crime.

Clinical characteristics were markers of ICH severity including GCS on admission, the presence of intraventricular extension, ICH location (lobar vs. non-lobar), ICH volume calculated using Quantomo computerised planimetry software (Cybertrial Inc, Calgary, Canada) [Kosior et al. 2011] and mean arterial pressure (diastolic blood pressure+1/3rd pulse pressure) [Gauer 1960].

11.2.3 *Comparison of those who consented to brain donation vs. those who did not*

I compared demographic and ICH characteristics (GCS on admission, intraventricular extension and ICH volume) in those who consented vs. those who did not. Based on existing knowledge from studies of organ donation for transplantation on features of the consent process which may influence whether consent is obtained [Simpkin et al. 2009], I selected *a priori* four variables to compare between groups: whether a member of the clinical team or the research team first discussed post-mortem examination, the interval between the date of the ICH and the date post-mortem examination was first discussed, the hospital the patient was admitted to and whether consent was sought from the patient or their nearest relative.

11.2.4 *Comparison of those who consent to brain donation vs. the remainder of the cohort*

I compared demographic and clinical characteristics of those with ICH who consented to brain donation vs. those who did not. The characteristics assessed were those listed in Section 11.2.2, page 286.

11.2.5 *Reasons cited for consenting or not consenting to brain donation*

I contemporaneously documented the discussions that I had with participants or their nearest relatives. If a participant or their relative declined to give consent, I explored their underlying reasons using open questions. I reviewed these conversations to identify the main themes which influenced participants' decisions and noted the frequency of each cited theme.

11.2.6 *Comparison of donors vs. the remainder of the cohort*

I compared demographic and ICH characteristics (GCS on admission, intraventricular extension and ICH volume) in donors vs. other participants to assess how representative donors were of the entire cohort of participants with ICH.

11.2.7 *Statistical analysis*

I calculated 95% CIs around incidence estimates using the Wilson –score interval [Newcombe 1998]. I used parametric statistics for between group comparisons when the data had a normal distribution and log –transformed variables or used non-parametric statistics when it did not. If an estimated cell count was less than five I used the Fisher's exact test. I conducted all statistical analyses using Stata version 11.1 (StataCorp LP, Texas, USA). All p values are 2-sided.

11.3 Results

11.3.1 *Participant flowchart*

Figure 46 on page 306 illustrates the identification of adults with spontaneous primary ICH (who were eligible for brain donation) from 1st June 2010-31st May 2011.

Of 141 adults, 45 (32%) were not approached, leaving 96 adults of whom 47 (49%; 95% CI 39-59%) consented to brain tissue donation in case of death. The most frequent

reason that participants were not approached was that they had died before being ascertained (n=24; 53%). 10 participants (22%) were ascertained late (at least two months after the date of their ICH) when it was no longer considered appropriate to approach participants to request brain donation. Two participants (4%) were not approached because the clinical team advised that their families would be too upset by a request for brain donation. I did not approach one participant (2%) because although her family had consented to organ donation for transplant they had specified to the organ donation co-ordinator that they would not want samples to be taken for educational or research purposes. Figure 47 on page 307 is a diagram (to scale) of the cohort and those who were approached to consider brain donation, those who consented and those who became donors.

11.3.2 *Comparison of adults approached vs. those not approached to consider brain donation*

Table 41 on page 299 shows the characteristics of adults approached to consider brain donation (n=96) vs. those not approached to consider brain donation (n=45). Adults who were not approached were older than those approached but this difference was not statistically significant. Other baseline demographic characteristics were similar in both groups. Adults not approached had significantly lower conscious levels on admission, higher mean arterial pressures and larger ICHs. Of those who died (n=86), adults who were not approached had a significantly shorter interval between the date of their ICH and death (approached: median (days) 19; IQR (days) 3-265 vs. not approached: median: 2; IQR 1-4; p=<0.001).

11.3.3 *Comparison of adults who consent to brain donation vs. those who decline consent*

Table 42 on page 300 shows the patient and ICH characteristics and features of the consent process in those who consent to brain donation vs. those who declined to consent. Although there was a higher proportion who had reached further or higher education in the group who gave consent, this difference was not statistically significant. The only characteristic of the consent process which differed between the groups was the site of hospital admission with the highest consent proportion noted at Western General Hospital, Edinburgh.

11.3.4 *Comparison of those who consented to brain donation vs. the remainder of the cohort*

Table 43 on page 301 shows the characteristics of those who consented to brain donation (n=47) vs. the remainder of the cohort (n=94). There were no significant differences in either demographic or clinical characteristics.

11.3.5 *Reasons cited for giving and declining consent for brain donation*

Table 44 on page 302 lists the main themes cited by participants or their nearest relatives when consenting to brain donation. The median number of themes cited was 1; (IQR 1-1). The most frequent reason given was the potential benefit as a result of their donation to others with the same condition.

Table 45 on page 303 lists the main themes cited by participants or their nearest relatives when not giving consent. The median number of themes cited per person was 1; IQR (1-1). The most frequent reason given was that the person asked did not feel able to make a decision.

11.3.6 *Explanations given for consenting to brain donation*

The following qualitative extracts are taken from conversations with participants or relatives who consented. They highlight themes cited in Section 11.3.5, page 290 and the role of personal experiences in deciding whether to consent.

11.3.6.1 Potential benefit to others with the same condition

Daughter-in-law of a 92 year old lady with a non-lobar ICH: 'I have tried to balance the wishes of my mother-in-law who used to be a nurse with her very private nature…..She had been a prisoner-of-war in Russia and after that was taken to Czechoslovakia and I think she had very traumatic experiences. She refused to let us or the nurses in the home help with personal care……I have to not think about what a post-mortem might involve and instead think about the potential benefit to other people.'

Wife of a 79 year old man with a lobar ICH: 'My husband didn't want to complete the NHS Donor card when he renewed his driver's licence because he thought that he would be 'shuffled off' if he was then admitted to hospital and he was very ill – but now

that that has happened, we know that was wrong and he would be supportive of this….as long as it helps others.'

11.3.6.2 Potential benefit to others/perception of body as a physical shell

Wife of a 54 year old man with a lobar ICH: 'If you're dead, you're dead and your body may as well benefit someone else.'

11.3.6.3 Wish to participate in research, repay previous medical care

Wife of a 75 year old man with a non-lobar ICH: 'My husband would have wanted to participate in research and had said that if this ever happened then they 'could have whatever they want.' I have had a liver transplant and would not be here had it not been for advances in medical care and donations of others.'

11.3.7 *Explanations given for not consenting to brain donation*

The following qualitative extracts are taken from conversations with nearest relatives who did not give consent and highlight themes cited in Section 11.3.5, page 290:

11.3.7.1 Nearest relative unable to decide

Sons and partner of a 60 year old man with a deep ICH: 'I would want him to be able to make the decision for himself….We've never talked about studies…….I wouldn't want him to be a guinea pig.'

11.3.7.2 Perceived invasiveness of brain donation

Son of a 68 year old lady with a lobar ICH: 'My mother has arrived in this world whole and should leave intact.'

Husband of a 81 year old lady with lobar ICH: 'I think that she might think it was too invasive. She had refused to take Aricept for dementia because the doctors said that she would need a permanent pacemaker and she did not want an operation.'

Husband of a 58 year old lady with a lobar ICH: 'I know you say that you cannot tell but I am sure I could tell if she had a post-mortem. One of my relatives did and afterwards the shape of his nose had changed. I think she has been through enough.'

11.3.7.3 Consent was incompatible with prior wishes

Son of a 80 year old lady with a deep ICH: 'My parents talked about this (post-mortem) when Dad was alive because post-mortem was mentioned. He had Alzheimer's disease…but they felt that it was too intrusive.'

11.3.7.4 Conflict in family

Nephew of a 82 year old man with a lobar ICH: 'I understand why research is important but some of the family are saying that because X did not carry a donor card, he should be 'left whole' and the post-mortem should not be done.'

11.3.8 *Comparison of donors vs. the rest of the cohort*

Table 46 on page 304 shows the baseline demographic and clinical characteristics of donors (n=24) vs. the remainder of the cohort (n=117; comprising those who were not approached to consider brain donation, those who did not consent and those who gave consent and are alive.) Although donors tended to be older, the difference was not statistically significant. Donors had a lower conscious level on admission and larger ICHs. Seven donors survived for longer than three months after their ICH (date of ICH to death (days); mean 415; SD 240.) In a sensitivity analysis restricted to donors who died within three months of their ICH the results were unchanged.

11.4 Summary

- During 1st June 2010-31st May 2011, of 96 adults with spontaneous primary ICH approached to consider brain donation, 47 (49%) consented.
- Adults not approached to consider brain donation were likely to have more extensive ICHs in comparison to those approached as indicated by their lower conscious level on admission, larger ICH volumes and higher mean arterial pressures.
- There were no significant differences in the demographic or ICH characteristics of those who consented vs. those who declined brain donation.
- The proportion of adults who consented to brain donation differed by hospital site.
- Those who consented to brain donation were similar to the remainder of the cohort in both their demographic and ICH characteristics.

- Donors were similar to non-donors in their demographic characteristics but were likely to have more extensive ICHs as indicated by their lower conscious levels on admission, larger ICH volumes and increased frequency of intraventricular extension of the ICH.

11.5 Discussion

11.5.1 *Strengths of the study*

This study is the first to report the proportions consenting to brain donation for research purposes in a community-based cohort of participants with ICH. Patients with an ante-mortem diagnosis of ICH would not routinely undergo post-mortem examination unless unanswered clinical questions existed regarding the cause or management of their ICH.

The community-based design increases the generalisability of the findings and permits an evaluation of the representativeness of those who were approached to consider brain donation, those who consented and those who eventually became brain donors in comparison to the remainder of the cohort. The study has also benefitted from clear definitions and unbiased and comprehensive ascertainment of exposure variables. Donors were well phenotyped since the clinical history was ascertained prospectively prior to the outcome (obtaining consent) and ICH-related variables were ascertained blind to the outcome.

11.5.2 *Weaknesses of the study*

The study took place in a predominantly white population in South East Scotland. It was likely that the study was underpowered to detect if ethnicity influenced the decision to donate and I was also unable to examine the role of different cultural beliefs on donation. I sought consent for brain tissue samples to be taken from the brain for research purposes (without retention of the entire brain.) Although there is some evidence that participants may be more likely to consent to retention of tissue samples rather than their brain [Millar et al. 2007], I was unable to explore this in our cohort. However, sampling of brain tissue is in any case likely to be preferable to whole brain retention since it avoids the tissue degradation associated with repeated freeze-thaw cycles [Monoranu et al. 2009].

I did not explore any demographic or cultural factors pertaining to nearest relatives although these may influence participants' perceptions of brain donation. In addition, the study was largely quantitative in nature and studying the influence of cultural and spiritual beliefs and personal experience on donation is likely to be more suited to qualitative methodologies.

11.5.3 *Comparison with other studies*

11.5.3.1 Proportion consenting to brain donation for research purposes

49% participants consented to a research post-mortem examination limited to the brain. The consent proportion in my cohort is comparable to other studies of brain donation in different settings (Figure 45 on page 305).

Two studies; one in participants with dementia [King, Smith, & Jobst 1993] and the other in the setting of sudden death [Millar et al. 2007], reported significantly higher consent proportions of >95%. The study of participants with dementia [King, Smith, & Jobst 1993] predated the much publicised organ retention scandals which led to a cultural shift in attitudes to organ retention for educational and research purposes and may have contributed to a decline in post-mortem examination rates [Burton & Underwood 2003]. In the setting of sudden death [Millar et al. 2007], nearest relatives may be more able to envisage consenting to additional brain tissue samples being taken for research when a fiscal post-mortem examination will already be required.

11.5.3.2 Comparison of characteristics of participants approached vs. those not approached

Adults who were not approached to consider brain donation had more extensive ICH and a shorter time interval between the ICH and death, which is consistent with the most frequent reason for a lack of approach being death before ascertainment. Nevertheless, adults who consented to post-mortem examination were similar in both demographic and ICH characteristics to those who either declined consent or were not approached (Table 41 on page 299 and Table 42 on page 300) and were therefore representative of the cohort. Other studies have been hospital-based [Angelini et al. 2011;Kuhta et al. 2011] or used previously established research registries [King, Smith, & Jobst 1993;Stevens 1998;Kaye et al. 1999;Schmitt et al. 2001;Danner, Darnell, & McGuire 2011;Jefferson et al. 2011] and none have reported whether the characteristics

of participants who were approached differed from others, making their findings potentially vulnerable to selection bias.

11.5.3.3 Comparison of adults who consent to brain donation vs. those who do not

There was no difference in demographic characteristics between adults who consented and those who declined donation. There was a non-significant excess of adults who reached further or higher education in the group who consented and the study may have lacked sufficient power to detect a difference if one does exist. Although other studies of brain donation have been heterogeneous both in terms of their settings and donor populations, they have similarly not shown any influence of donor sex [Stevens 1998;Garrick et al. 2009;Kuhta et al. 2011;Jefferson et al. 2011], age [Stevens 1998;Garrick et al. 2009;Jefferson et al. 2011], level of education [Stevens 1998;Jefferson et al. 2011] or socioeconomic status [Stevens 1998] on the decision to consent.

There was no difference between groups regarding the person making the approach or the timing of it. Since a joint approach by the clinical and research teams was rare in this cohort, it may be that there was insufficient power to demonstrate a difference. One other study of sudden death found that the longer the interval between death and the request for brain donation, the more likely that consent would be given [Garrick et al. 2009]. A systematic review of studies of organ donation for transplant identified that the timing of the approach, in particular separation of the request from the notification of brainstem death influenced consent [Simpkin et al. 2009]. Although the timing of the approach may influence the likelihood of consent, this might interact with other factors which are difficult to measure quantitatively such as the expertise of the person making the request, their interpersonal skills and the donor's perceived satisfaction with clinical care [Simpkin et al. 2009].

Interestingly the only potentially modifiable feature of the consent process which differed between groups was the admitting hospital. The consent proportion was highest at the Western General Hospital. The research team was based at this hospital and one possible explanation is that this allowed closer liaison with the clinical team to optimise the timing of any approach.

11.5.3.4 Reasons for brain donation

These findings are in keeping with other studies in which donors have commonly reported altruistic motivations including a desire to help others [King, Smith, & Jobst 1993;Stevens 1998;Garrick et al. 2009;Danner, Darnell, & McGuire 2011;Kuhta et al. 2011;Harris, Kiger, & Counsell 2012] or support research [Stevens 1998;Millar et al. 2007;Danner, Darnell, & McGuire 2011;Angelini et al. 2011;Jefferson et al. 2011] or express gratitude for medical care received [Stevens 1998;Garrick et al. 2009;Kuhta et al. 2011]. The perception of the body as a mere physical shell has also been reported by relatives of donors with dementia who, observing the donor's gradual deterioration, perceived a dualistic separation of the donor's mind and body which encouraged them to authorise donation as they viewed the body at the time of death as bearing no resemblance to the relative they knew [King, Smith, & Jobst 1993].

11.5.3.5 Reasons for not consenting to brain donation

The most frequently cited reason for not giving consent in this cohort was that donors or their nearest relatives felt unable to make a decision, which was related in part to the distress caused by a diagnosis of ICH and a lack of clarity regarding what the donor's wishes would have been. This has not been cited in other studies of brain donation which have predominantly been conducted in either healthy participants or those with chronic neurological diseases where the decision regarding brain donation does not usually have to be made rapidly or unexpectedly. Other reasons cited by our cohort are in keeping with previous studies of donation for research and transplantation which have identified previously expressed donor wishes [Barber et al. 2006], the perceived invasiveness of the procedure [Angelini et al. 2011;Jefferson et al. 2011], conflict within the family [Stevens 1998;Schmitt et al. 2001;Millar et al. 2007;Garrick et al. 2009] spiritual beliefs [Stevens 1998;Angelini et al. 2011;Kuhta et al. 2011;Jefferson et al. 2011] and unmet clinical expectations [Austrom et al. 2011] as barriers to donation.

11.5.3.6 Comparison of donors vs. the remainder of the cohort

Donors had more extensive ICH in comparison to the remainder of the cohort. This is unsurprising since although those who consented are similar in both demographic and ICH characteristics to those who did not, those with less severe ICH will survive and would therefore only become donors over several years. Other studies have not

compared the characteristics of those who actually donated brain tissue to the remainder of the sample. Although donors are therefore not representative of the entire cohort in terms of ICH severity, since ICH carries an early case fatality (Chapter 7), they are likely to be representative of those who die early and for whom any advances have most benefit.

11.5.4 *Meaning of study*

A major dilemma continues to be how to increase the number of brains donated for research. This is acknowledged as a priority by the UK Medical Research Council which in 2009 set up the UK Brain Banks Network with the aim of connecting the UK's 12 brain banks to provide samples for researchers and tackle the shortage of brains donated for research [Medical Research Council 2009]. This study demonstrates the feasibility of seeking consent for brain donation in ICH, a condition which affects 10000 adults per year in the UK [Lovelock, Molyneux, & Rothwell 2007].

Although reasons for refusing consent which were likely irreversible predominated in our cohort, potentially modifiable reasons included not knowing what the participant's wishes would have been and dissatisfaction with medical care. Conversely, being a registered organ donor has been associated with consent in other studies [Glaw et al. 2009;Sundqvist, Garrick, & Harding 2010] since brain donation was viewed as being consistent with previously expressed wishes. It is essential to increase public awareness of organ donation for transplant and research with the aim of empowering people to reach their own decisions and communicate this to relatives when they still have the mental capacity to do so [Sanner 2006].

The lack of association between demographic characteristics and consent in this and other studies suggests that deciding whether to consent may be related to personal beliefs and unquantifiable aspects of the consent process. Researchers or clinicians should avoid presuming on the basis of demographic variables that either consent will not be obtained or asking will cause upset [Burton & Underwood 2007;Medical Research Council 2009].

11.5.5 *Future directions*

Future studies should examine brain donation in different populations for other neurological diseases. Qualitative studies should explore the influence of personal

beliefs and experiences on the decision to consent. It would be useful to evaluate nearest relatives experiences of the consent and donation process to assess whether their expectations were met, whether they had any regrets regarding donation and any improvements that could be made.

Table 41 Baseline characteristics of adults with spontaneous ICH approached and not approached to consider brain donation

	Adults approached to consider brain donation (n=96)	Adults not approached to consider brain donation (n=45)	p value
Sex male, (%)	45 (47)	20 (44)	0.79
Age (years); mean (SD)	73 (14)	77 (13)	0.18
Ethnicity non-white, (%)	3 (3)	1 (2)	0.76
Socio-economic status (postcode rank)	12 (7-18)	11 (6-16)	0.31
Admission GCS score* median (IQR)	14 (10-15)	6 (3-13)	<0.001
Mean arterial pressure** (mmHg) mean (SD)	116 (24)	128 (29)	0.02
Lobar ICH location	49 (51)	24 (53)	0.80
Intraventricular extension^	46 (48)	25 (56)	0.23
ICH volume^ (ml); median (IQR)	20 (6-41)	36 (12-69)	0.04

*missing in five cases; three of whom were not admitted to hospital
**missing in 13 cases
^not applicable in four cases in which the diagnosis was confirmed at post-mortem examination

Table 42 Demographic and clinical variables and characteristics of the consent process in adults who give consent to brain tissue donation and those who decline donation

	Adults who consented to brain donation (n=47)	Adults who declined brain donation (n=49)	p value
Non-modifiable patient characteristics			
Sex male, (%)	26 (55)	19 (39)	0.10
Age (years); mean (SD)	73 (13)	73 (14)	0.96
Ethnicity non-white, (%)	1 (2)	2 (4)	0.58
Socio-economic status (postcode rank)	13 (6-19)	11 (7-16)	0.25
Donor education*; further or higher, (%)	13 (28)	7 (14)	0.11
Non-modifiable ICH characteristics			
Admission GCS score median (IQR)	13 (10-15)	14 (12-15)	0.07
Intraventricular extension** Yes (n, %)	24 (51)	22 (45)	0.48
ICH volume** (ml);median (IQR)	21 (6-45)	15 (6-39)	0.25
Modifiable characteristics of the consent process			
Approached by clinical team and researcher (vs. researcher alone)	3 (6)	1 (2)	0.29
Interval between date of ICH and date post-mortem examination discussed (days)	2 (1-4)	2 (1-5)	0.75
Hospital			
Western General hospital	30 (64)	18 (37)	
Royal Infirmary	13 (28)	20 (41)	
St John's hospital	3 (6)	9 (18)	
Rehabilitation wards	1 (2)	2 (4)	0.02
Approached nearest relative (vs. participant)	32 (68)	32 (65)	0.58

*missing in two cases
** not applicable in 1 case of ICH which was diagnosed at post-mortem examination

Table 43 Demographic and clinical characteristics in those who consented to brain donation vs. remainder of the cohort

	Adults consented to brain donation (n=47)	Adults not consented to brain donation (n=94)	p value
Sex male, (%)	26 (55)	39 (41)	0.12
Age (years); mean (SD)	73 (13)	75 (14)	0.55
Ethnicity non-white, (%)	1 (2)	3 (6)	1.00
Socio-economic status			
(postcode rank)	13 (6-19)	11 (6-16)	0.14
Admission GCS score*			
median (IQR)	13 (10-15)	13 (8-15)	0.47
Mean arterial pressure** (mmHg)			
mean (SD)	119 (24)	119 (27)	0.96
Lobar ICH location	27 (57)	46 (49)	0.34
Intraventricular extension^	24 (51)	47 (50)	0.94
ICH volume^ (ml);median (IQR)	21 (6-46)	21 (7-50)	0.99

*missing in five cases; three of whom were not admitted to hospital
**missing in 13 cases
^not applicable in four cases in which the diagnosis was confirmed at post-mortem examination

Table 44 Reasons given for consent to brain donation

Reasons given for donation (most common first)	Frequency
Potential benefit to others with the same condition	21
Wished to participate in a research study	18
Perception of body as merely a physical shell	6
Offer an explanation for the intracerebral haemorrhage	5
Consistent with prior wish to donate body to medical science	2
Wish to repay medical care provided	2
No objection to research post-mortem examination	2
Potential benefit to patient from participation in a research study	1
No reason given	1

Table 45 Reasons given for not consenting to brain donation

Reasons given for refusal to consent	Frequency
Nearest relative unable to decide	12
- too upset by diagnosis of intracerebral haemorrhage	5
- Did not know what the potential donor's wishes would be	2
- No reason given	5
Did not wish to be involved in a research study	8
Consent was incompatible with previously expressed wishes	8
Brain donation is 'too invasive' or 'not something they wished to put their next-of-kin through'	4
Conflict in family regarding post-mortem examination decision	5
Dislike the idea of brain tissue donation	4
Consent was incompatible with spiritual or religious beliefs	3
Dissatisfaction with medical care	1
Dissatisfaction with previous discussion regarding organ donation	1
No reason given	6

Table 46 Demographic and clinical variables in donors and non-donors

	Donors (n=24)	Non-donors (n=117)	p value
Sex (male), (%)	14 (58)	51 (44)	0.19
Age (years); mean (SD)	78 (10)	73 (14)	0.10
Ethnicity (non-white); (%)	0 (0)	4 (3)	0.47
Socio-economic status (postcode rank)	12 (5-19)	12 (7-17)	0.95
GCS on admission* median (IQR)	12 (9-13)	14 (9-15)	0.07
Intraventricular extension**; n (%)	16 (70)	55 (48)	0.06
ICH volume** median (IQR)	36 (22-85)	17 (5-48)	0.002

* missing in five cases, three of whom were not admitted to hospital
**missing in four cases which were diagnosed at post-mortem examination

Figure 45 Proportions of participants giving consent (and 95% confidence intervals) in studies of brain donation for research with donor characteristics and mode of approach (n=number of people giving consent to brain donation, N=sample size)

Study	Donor population	Donor age,years (mean/range)	Initial approach	n/N
Sudden deaths				
Millar (2007)	Controls, CNS disorders, UK	1-89	Nurse phone call	107/111
Garrick (2009)	Controls, CNS disorders, Aus.	18-85	Nurse phone call	107/200
Neurological disorders				
Angelini (2011)	Pontine glioma, Canada	0-16.9	Consultation	10/21
Kuhta (2011)	Movement disorders, USA	66	Questionnaire	114/203
Harris (2012)	Parkinson's disease, UK	?	?	14/40
Healthy ageing & dementia				
King (1993)	Alzheimer's disease study, UK	?	Consultation	250/260
Stevens (1998)	MRC CFAS participants, UK	67-100	Letter	193/594
Kaye (1999)	Healthy adults, Oregon, USA	65-102	Consultation	65/140
Schmitt (2001)	Healthy adults, USA	>60	Letter	186/870
Danner (2011)	Healthy Africa-Americans, USA	≥65	Interview	13/25
Jefferson (2011)	Alzheimer disease registry, USA	76	?	296/464

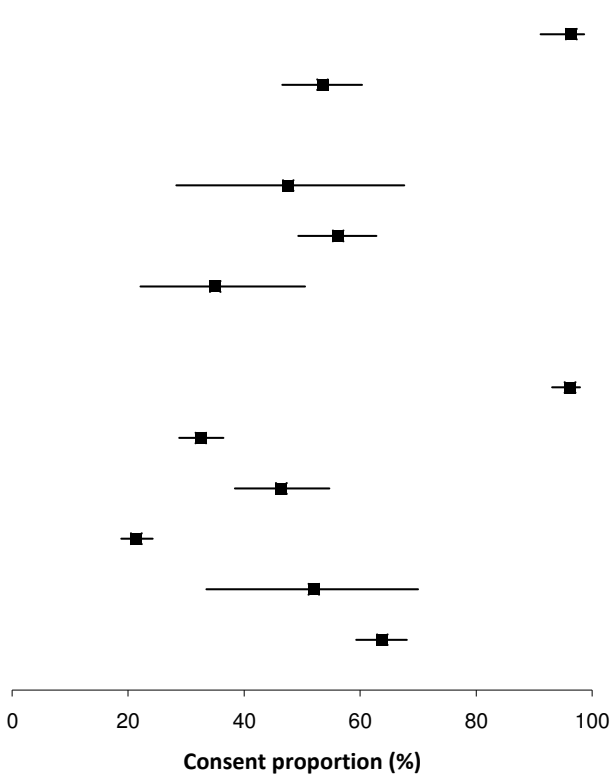


Figure 46 Flowchart of participants with spontaneous primary ICH eligible to participate in the LINCHPIN study from 1st June 2010-31st May 2011

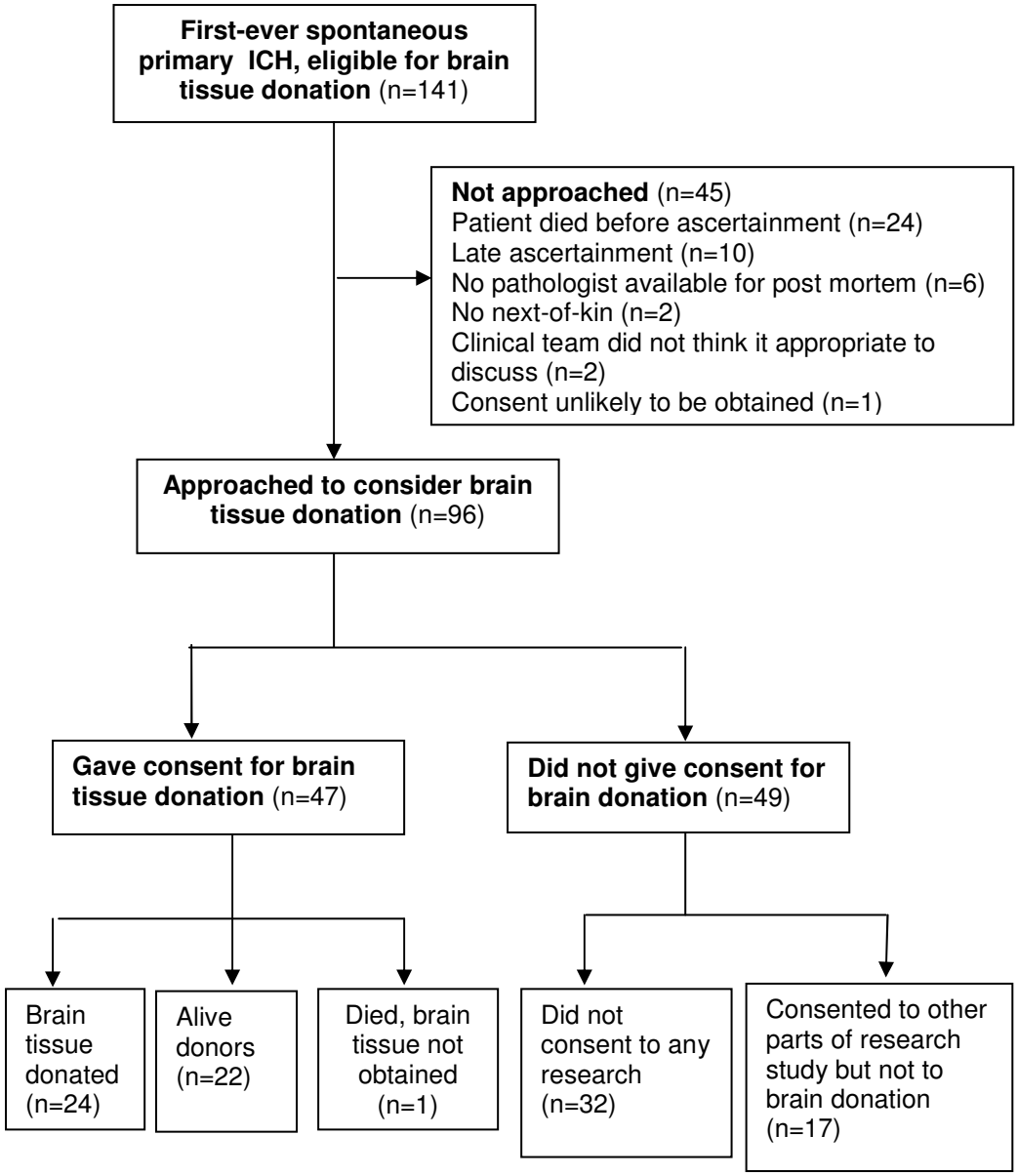
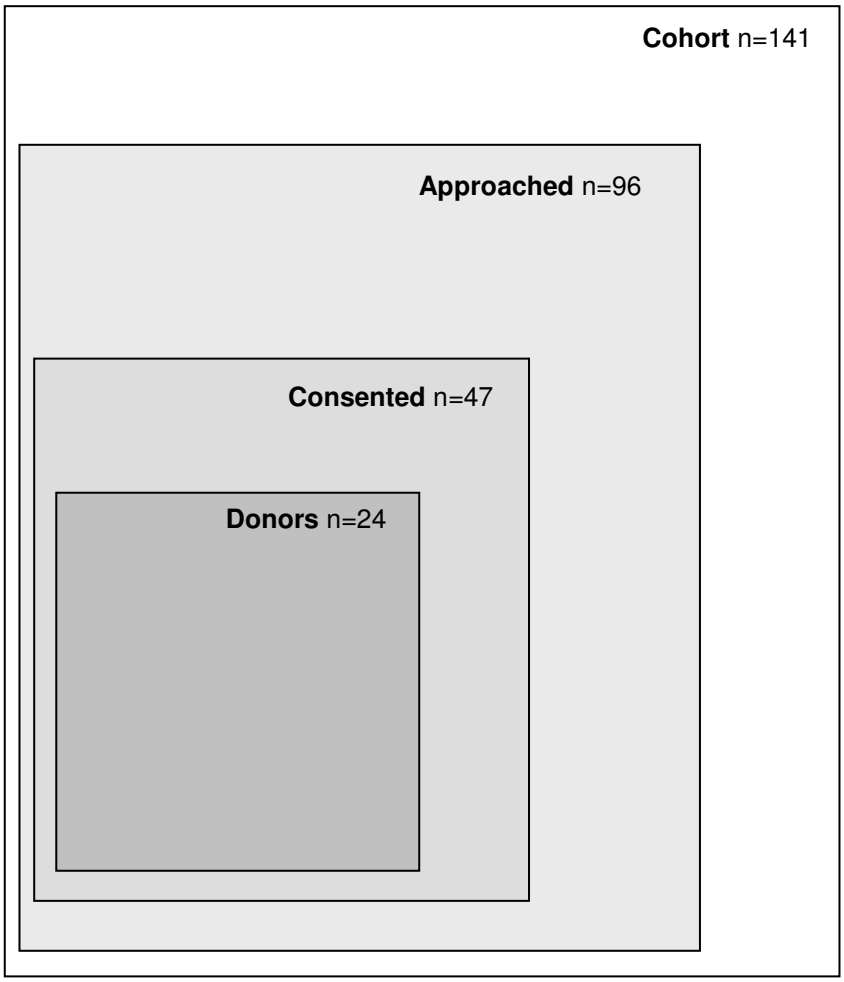


Figure 47 Diagram of cohort and those who were approached, gave consent and became donors



Section E Conclusions

Chapter 12 Conclusions

Chapter contents

12 Conclusions

12.1 Main findings of thesis

12.1.1 *Methods of a prospective community-based study of ICH*

- I have set up a community-based, prospective incidence and longitudinal cohort study of spontaneous primary ICH which uses multiple sources of case ascertainment and follow-up.
- The study includes adults aged 16 years or over at the time of their ICH, resident in the region served by the NHS Lothian Health board and diagnosed between 1st June 2010-1st June 2016.
- I confirmed the diagnosis of ICH by review of diagnostic imaging or post-mortem examination reports.
- I ascertained cases of suspected ICH in which the history was suggestive of ICH but the diagnosis of ICH was unconfirmed before or after death.

12.1.2 *Methods of a cross-sectional study of MRI brain imaging features in lobar vs. non-lobar ICH*

- Inter-observer agreement when rating WMH, EPVS and atrophy on both the training scans and LINCHPIN MRI scans predominantly ranged from fair to moderate.
- Inter-observer agreement when rating the presence of zero vs. ≥ one BMB on LINCHPIN MRI scans was similar to the inter-observer agreement achieved for training scans and ranged from moderate to substantial.

12.1.3 *Methods of a cross-sectional study of apolipoprotein E genotypes in lobar vs.non-lobar ICH*

- The genotyping process for apolipoprotein E fulfilled pre-specified quality control measures.
- The genotype frequencies for SNPs rs429358 and rs7412 conformed to Hardy-Weinberg equilibrium.

- There was no difference in the proportion of participants with an undetermined genotype between the lobar and non-lobar ICH groups.

12.1.4 *Results of a community-based, cross-sectional study of ICH-incidence and baseline characteristics*

- During 1st June 2010-31st May 2011 the crude incidence of spontaneous definite ICH was 0.24 per 1000 per year (95% CI 0.21 to 0.27) and the incidence of first-ever spontaneous primary definite ICH was 0.18 per 1000 per year (95% CI 0.16 to 0.22) and did not vary by ICH location.
- Lobar ICHs were more likely to have a past history of dementia in comparison to non-lobar ICH, but there were no other differences in pre-ICH characteristics between lobar and non-lobar ICH.
- Lobar ICHs were associated with a lower GCS score on admission.
- Lobar ICHs were larger and associated with extension of the ICH into the subarachnoid and subdural compartments of the brain.
- 12 out of 59 participants with first-ever lobar ICH eligible to be considered under the modified Boston criteria, fulfilled the criteria for definite or probable CAA-related ICH.

12.1.5 *A cross-sectional study of MRI features in adults with lobar vs. non-lobar ICH*

- The proportion of participants with one or more non-lobar BMB was significantly higher in participants with non-lobar ICH.
- There was no difference in the severity or distribution of lobar BMBs, WMH, EPVS and atrophy between lobar and non-lobar ICH.

12.1.6 *Results of a community-based study of ICH-death, prognosis and survival outcomes*

- Case fatality at one month was 43% and at one year was 56%.
- Lobar ICH was independently associated with a lower odds of death in the first year after first-ever spontaneous primary ICH after adjusting for other covariates.
- The ICH score discriminated well between those who survived and died in the first 30 days following a first-ever spontaneous primary ICH.
- There was a higher risk of recurrent ICH in the lobar ICH group.

- There was no difference in the risk of vaso-occlusive events observed in the lobar and non-lobar ICH groups.
- Regardless of ICH location, there was a non-significant excess of vaso-occlusive events in comparison to recurrent ICH in the first year.

12.1.7 *A cross-sectional study of apolipoprotein E genotypes in lobar vs. non-lobar ICH*

- The ε4 allele was significantly more common in participants with lobar ICH in comparison to those with non-lobar ICH.
- There was no difference in the frequency of the ε2 allele between those with lobar and non-lobar ICH.
- The ε4 allele was more frequent in those participants with probable or definite CAA-related ICH in comparison to those with possible CAA-related ICH according to the modified Boston criteria for CAA but the difference was not significant.
- The presence of at least one ε2 allele or ε4 allele was not associated with death in the first 30 days following a first-ever ICH or death or dependency at one year.

12.1.8 *The association between cerebral amyloid angiopathy and intracerebral haemorrhage-systematic review and meta-analysis*

- In a systematic review and meta-analysis of ten neuropathological cross-sectional or case-control studies involving 481 cases and 3,219 controls, I found a significant association between CAA and lobar ICH, but not with ICH in other locations.
- There was wide variation in the methodological quality of included studies.
- Strategies for both brain sampling and CAA detection varied between studies.

12.1.9 *A cross-sectional study of imaging and pathology findings in adults with lobar ICH*

- Of 33 participants with first-ever lobar ICH, nine participants (27%) did not have any CAA in their cerebral hemispheres.
- The presence of CAA or vasculopathy and the severity of CAA in a lobe affected by ICH was concordant with that of the corresponding contralateral unaffected lobe.
- The severity of parenchymal CAA was positively correlated with leptomeningeal CAA in all lobes of the brain, whether affected or unaffected by ICH.

- Severe CAA was associated with vasculopathy.
- Capillary CAA was associated with more severe CAA in the same lobe, whether affected or unaffected by ICH.
- Capillary CAA was associated with vasculopathy in the occipital lobes of both hemispheres but not in the other lobes of the brain.
- The total CAA score was not associated with participant age or a prior history of dementia.
- No imaging variables were statistically significantly associated with CAA-related lobar ICH.
- Subarachnoid extension tended to be more frequent in those with CAA-related strictly lobar ICH vs. CAA-unrelated strictly lobar ICH, with a sensitivity of 100% and specificity of 44% for pathologically –proven CAA-related strictly lobar ICH.

12.1.10 *Consent for brain tissue donation after ICH-a community-based study*

- During 1st June 2010-31st May 2011, of 96 adults with spontaneous primary ICH approached to consider brain donation, 47 (49%) consented.
- Adults not approached to consider brain donation were likely to have more extensive ICHs in comparison to those approached as indicated by their lower conscious level on admission, larger ICH volumes and higher mean arterial pressures.
- There were no significant differences in the demographic or ICH characteristics of those who consented vs. those who declined brain donation.
- The proportion of adults who consented to brain donation differed by hospital site.
- Those who consented to brain donation were similar to the remainder of the cohort in both their demographic and ICH characteristics.
- Donors were similar to non-donors in their demographic characteristics but were likely to have more extensive ICHs as indicated by their lower conscious levels on admission, larger ICH volumes and increased frequency of intraventricular extension of the ICH.

12.2 Implications for routine practice

The incidence estimates of first-ever spontaneous primary definite ICH provide confirmation that ICH incidence increases with age which is of significance when planning healthcare provision for an ageing population.

My first-ever validation of the ICH score in a community-based study shows that the ICH score discriminates well between those who survive and those who do not in the first 30 days following an ICH, providing clinicians with valuable prognostic information.

Since adults with lobar ICH have a lower odds of death at one year despite larger ICHs but are also at higher risk of ICH recurrence in comparison to those with non-lobar ICH, secondary prevention with antihypertensive medications is of particular relevance to this patient group. Following an ICH, survivors are at risk of both recurrent ICH and vaso-occlusive events underlining the need for randomised controlled trials to resolve the uncertainty about whether to restart antithrombotic medications after ICH.

My systematic review of neuropathological case control studies of CAA and ICH confirmed an association between CAA and lobar ICH but the association was not as strong as one might expect if CAA was the sole cause of lobar ICH. This was supported by the radio-pathological study of lobar ICH, in which 27% of adults with lobar ICH did not have any evidence of CAA in their cerebral hemispheres. Clinicians should be aware that other factors, such as arteriolosclerosis may contribute to lobar ICH.

The study of consent for research post-mortem examination indicates that adults with ICH and their relatives are willing to consider consenting to brain donation if this is discussed sensitively giving families sufficient time for questions and concerns to be explored. Although I was seeking consent for a post-mortem examination for research purposes which was limited to the brain only, the findings may be applicable to clinicians seeking consent for post-mortem examination to ascertain the cause of a patient's death.

12.3 Implications for future research

12.3.1 *Epidemiology*

Population-based studies of follow-up after ICH are scarce [Poon, Fonville, & Al-Shahi Salman 2013]. One future aim would be to quantify follow-up outcomes (both vaso-occlusive events and recurrent ICH) after first-ever spontaneous ICH, stratified by ICH location, to confirm a) whether the risk of recurrent ICH is higher in the lobar ICH group, b) whether the risk of recurrent ICH varies over time and c) whether the risks of vaso-occlusive events and recurrent ICH remain similar in both lobar and non-lobar ICH. It would be of interest to explore whether levels of certain biomarkers such as copeptin [Yu et al. 2014] or plasma Aβ [Marti-Fabregas et al. 2014] can help to predict prognosis.

There has only been one population-based study of long term prognosis after ICH which followed up 172 adults surviving at least one year after ICH onset for 13 years and did not report any differential effect of ICH location on survival [Hansen et al. 2013]. A long term follow up study should provide further information regarding both functional outcome and survival, explore whether any baseline factors affect long term survival after ICH and whether survival varies over time.

12.3.2 *Imaging*

Studies of ICH require a consistent definition of ICH location. The two previous studies of inter-rater reliability of ICH location classified <100 supratentorial ICHs as either lobar or deep without a category for 'mixed' ICH [Wermer et al. 2002;Battathiri et al. 2003] although some ICHs will inevitably involve both lobar and deep regions. It would be important to examine inter-rater reliability on a larger sample, rating supratentorial ICH as lobar, deep or mixed and using a variety of raters with different levels of radiology experience.

Given the limited number of radiopathological studies of BMBs, a future study would aim to correlate microbleeds seen on MRI with their pathological appearances. To overcome the logistical difficulties of either stroke patients being too unwell to have an MRI brain or a long time interval between those able to have MRI brain and death, post-mortem MRI might be used.

Centrum semiovale EPVS may be a marker of CAA [Charidimou et al. 2013c]. A future study might confirm this association using a larger sample of participants with CAA-related ICH vs. ICH unrelated to CAA, potentially using Pittsburgh compound B positron emission tomography for in vivo detection of CAA [Park et al. 2013].

12.3.3 *Genetics*

The apolipoprotein ε4 allele is associated with microbleeds in a lobar distribution both in the general population and persons with dementia [Schilling et al. 2013;Loehrer et al. 2014]. Possession of two ε4 alleles is associated with increasing WMH burden in demented and non-demented individuals [Godin et al. 2009;Schilling et al. 2013]. The presence and strength of an association between apolipoprotein E and WMH in persons with ICH is yet to be determined.

Future genetic studies might explore the relationship between apolipoprotein E and both WMH and EPVS to determine whether the spatial distribution varies according to genotype and therefore whether these neuroimaging correlates might be imaging markers of CAA.

12.3.4 *Pathology*

One of the initial aims of my PhD was to conduct a pathological case control study of CAA and ICH. Unfortunately, this did not happen because it was not possible to rate control brains. Although disappointing, this is one of the aims which I still intend to fulfil which would provide information regarding the strength of the association between CAA and lobar ICH, confirm that the association which I noted in my systematic review (Chapter 9) is consistent and assess whether there is a dose-response gradient; for example, whether individuals with multiple or recurrent ICHs have more severe CAA. The paucity of pathological case control studies means that data regarding the presence and strength of any association between CAA and supratentorial deep or cerebellar ICH is also yet to be determined. This is relevant since cerebellar ICH is permitted in the category of 'probable CAA' (without supporting pathology) in the Boston criteria for the diagnosis of CAA-related ICH[Knudsen et al. 2001]. Since cerebellar ICH is infrequent (10 (7%) in my cohort of first-ever ICH), a multicentre approach would be ideal. However, other groups examining CAA, such as Boston, have

reported different sampling and rating methods making it more difficult to combine their samples with our cohort.

The role of other small vessel diseases in ICH is yet to be explored. The commonly accepted hypothesis is that CAA is implicated in lobar ICH and arteriolosclerosis in non-lobar ICH although since both lobar and non-lobar ICH share common risk factors such as hypertension (Chapter 5) and the recurrence risk of both is reduced with secondary prevention with antihypertensive medications [Arima et al. 2010], this division may be too simplistic.

I would aim to examine the severity of arteriolosclerosis in persons with lobar and non-lobar ICH vs. age-matched controls to determine whether the strength of the association differs according to ICH location. Any association between arteriolosclerosis and ICH might be stronger in those with non-lobar ICH given the purported role of hypertension in supratentorial deep ICH. Since CAA is thought to result from age-related stiffening of arterial walls leading to decreased propulsion of solutes along perivascular lymphatic pathways and a consequent failure of Aβ elimination from the brain [Weller et al. 2009b], it would be of interest to determine if the severity of arteriolosclerosis and CAA are correlated in persons with ICH and whether arteriolosclerosis might modify the strength of the association between CAA and lobar ICH.

As alluded to in Chapter 10, little is known about the nature of dementia in persons with ICH. A future study should examine the prevalence of pre-ICH cognitive impairment and dementia in persons with ICH and correlate pre-ICH dementia status with neuropathology at post-mortem examination including the presence of neuritic plaques, neurofibrillary tangles, Lewy bodies, multiple infarcts and hippocampal atrophy [Matthews et al. 2009]. Comparison of neuropathological substrates by lobar and non-lobar ICH location, may shed light on the causes of pre-stroke dementia in ICH and the vasculopathies underlying lobar and non-lobar ICH.

The eventual aim would be to develop clinical trials for potential treatments for CAA. Interventions might reduce Aβ production or enhance Aβ clearance. A phase 2 trial for an anti-Aβ monoclonal antibody (Ponezumab) has begun (Study Evaluating the Safety, Tolerability and Efficacy of PF-04360365 in Adults with Probable Cerebral Amyloid

Angiopathy, NCT01821118) but no phase 3 trials have been conducted as yet. Further knowledge about the contribution of different vasculopathies to ICH will allow potential interventions to be targeted more appropriately at subgroups of ICH survivors.

Section F References and Appendices

References

Al-Shahi Salman, R., Labovitz, D. L., & Stapf, C. 2009, "Spontaneous intracerebral haemorrhage", *BMJ*, vol. 339, p. b2586.

Alafuzoff, I., Thal, D. R., Arzberger, T., Bogdanovic, N., Al-Sarraj, S., Bodi, I., Boluda, S., Bugiani, O., Duyckaerts, C., Gelpi, E., Gentleman, S., Giaccone, G., Graeber, M., Hortobagyi, T., Hoftberger, R., Ince, P., Ironside, J. W., Kavantzas, N., King, A., Korkolopoulou, P., Kovacs, G. G., Meyronet, D., Monoranu, C., Nilsson, T., Parchi, P., Patsouris, E., Pikkarainen, M., Revesz, T., Rozemuller, A., Seilhean, D., Schulz-Schaeffer, W., Streichenberger, N., Wharton, S. B., & Kretzschmar, H. 2009, "Assessment of beta-amyloid deposits in human brain: a study of the BrainNet Europe Consortium", *Acta Neuropathologica*, vol. 117, no. 3, pp. 309-320.

Allen, N., Robinson, A. C., Snowden, J., Davidson, Y. S., & Mann, D. M. 2013, "Patterns of cerebral amyloid angiopathy define histopathological phenotypes in Alzheimer's disease", *Neuropathol Appl.Neurobiol.*

Altman, D. G. & Royston, P. 2000, "What do we mean by validating a prognostic model?", *Stat.Med*, vol. 19, no. 4, pp. 453-473.

Anderson, C. S., Chakera, T. M., Stewart-Wynne, E. G., & Jamrozik, K. D. 1994, "Spectrum of primary intracerebral haemorrhage in Perth, Western Australia, 1989-90: incidence and outcome", *J.Neurol Neurosurg.Psychiatry*, vol. 57, no. 8, pp. 936-940.

Andoh, Y., Wakai, S., Nagai, M., Honma, K., & Fujihara, S. 1989, "[Lobar intracerebral hemorrhage secondary to cerebral amyloid angiopathy: a clinicopathologic study of three operated cases]", *No To Shinkei*, vol. 41, no. 12, pp. 1217-1223.

Angelini, P., Hawkins, C., Laperriere, N., Bouffet, E., & Bartels, U. 2011, "Post mortem examinations in diffuse intrinsic pontine glioma: challenges and chances", *Journal of Neuro-Oncology*, vol. 101, no. 1, pp. 75-81.

Arboix, A. & Grive, E. 2012, "Intracerebral Haemorrhage: Influence of Topography of Bleeding on Clinical Spectrum and Early Outcome," in *Neuroimaging Methods*, P. Bright, ed., InTech, pp. 277-292.

Ariesen, M. J., Algra, A., van der Worp, H. B., & Rinkel, G. J. 2005, "Applicability and relevance of models that predict short term outcome after intracerebral haemorrhage", *J.Neurol Neurosurg.Psychiatry*, vol. 76, no. 6, pp. 839-844.

Ariesen, M. J., Claus, S. P., Rinkel, G. J. E., & Algra, A. 2003, "Risk Factors for Intracerebral Hemorrhage in the General Population", *Stroke*, vol. 34, no. 8, pp. 2060-2065.

Arima, H., Tzourio, C., Anderson, C., Woodward, M., Bousser, M. G., MacMahon, S., Neal, B., & Chalmers, J. 2010, "Effects of perindopril-based lowering of blood pressure on intracerebral hemorrhage related to amyloid angiopathy: the PROGRESS trial", *Stroke*, vol. 41, no. 2, pp. 394-396.

Arvanitakis, Z., Leurgans, S. E., Wang, Z., Wilson, R. S., Bennett, D. A., & Schneider, J. A. 2011, "Cerebral amyloid angiopathy pathology and cognitive domains in older persons", *Ann.Neurol*, vol. 69, no. 2, pp. 320-327.

Asplund, K., Bonita, R., Kuulasmaa, K., Rajakangas, A. M., Feigin, V., Schaedlich, H., Suzuki, K., Thorvaldsen, P., & Tuomilehto, J. 1995, "Multinational Comparisons of Stroke Epidemiology (the WHO MONICA project)", *Stroke*, vol. 26, no. 3, pp. 355-360.

Attems, J. 2005, "Sporadic cerebral amyloid angiopathy: pathology, clinical implications, and possible pathomechanisms", *Acta Neuropathologica*, vol. 110, no. 4, pp. 345-359.

Attems, J., Jellinger, K. A., & Lintner, F. 2005, "Alzheimer's disease pathology influences severity and topographical distribution of cerebral amyloid angiopathy", *Acta Neuropathologica*, vol. 110, no. 3, pp. 222-231.

Attems, J., Lauda, F., & Jellinger, K. A. 2008, "Unexpectedly low prevalence of intracerebral hemorrhages in sporadic cerebral amyloid angiopathy: an autopsy study", *Journal of Neurology*, vol. 255, no. 1, pp. 70-76.

Attems, J., Lintner, F., & Jellinger, K. A. 2004, "Amyloid beta peptide 1-42 highly correlates with capillary cerebral amyloid angiopathy and Alzheimer disease pathology.[Erratum appears in Acta Neuropathol (Berl). 2004 May;107(5):479-80]", *Acta Neuropathologica*, vol. 107, no. 4, pp. 283-291.

Austrom, M. G., Dickinson, S. L., Denny, S. S., Matthews, B. R., Gao, S., & Lu, Y. 2011, "Frontotemporal dementia caregivers and researchers: partnering for brain donation", *American Journal of Alzheimers Disease and Other Dementias*, vol. 26, no. 6, pp. 477-483.

Babyak, M. A. 2004, "What you see may not be what you get: a brief, nontechnical introduction to overfitting in regression-type models", *Psychosom.Med*, vol. 66, no. 3, pp. 411-421.

Badhe, P. B., Mehta, N. K., & Desai, A. P. 2005, "Cerebral Amyloid Angiopathy: an Indian Study", *Bombay Hospital Journal*, vol. 47, no. 1.

Bailey, R. D., Hart, R. G., Benavente, O., & Pearce, L. A. 2001a, "Recurrent brain hemorrhage is more frequent than ischemic stroke after intracranial hemorrhage", *Neurology*, vol. 56, no. 6, pp. 773-777.

Bailey, R. D., Hart, R. G., Benavente, O., & Pearce, L. A. 2001b, "Recurrent brain hemorrhage is more frequent than ischemic stroke after intracranial hemorrhage", *Neurology*, vol. 56, no. 6, pp. 773-777.

Banks, W. 2009, "Characteristics of compounds that cross the blood-brain barrier", *BMC Neurology*, vol. 9, no. Suppl 1, p. S3.

Bar, B. & Hemphill, J. C., III 2011, "Charlson comorbidity index adjustment in intracerebral hemorrhage", *Stroke*, vol. 42, no. 10, pp. 2944-2946.

Barber, K., Falvey, S., Hamilton, C., Collett, D., & Rudge, C. 2006, "Potential for organ donation in the United Kingdom: audit of intensive care records", *BMJ*, vol. 332, no. 7550, pp. 1124-1127.

Baron, J. C., Farid, K., Dolan, E., Turc, G., Marrapu, S. T., O'Brien, E., Aigbirhio, F. I., Fryer, T. D., Menon, D. K., Warburton, E. A., & Hong, Y. T. 2014, "Diagnostic utility of amyloid PET in cerebral amyloid angiopathy-related symptomatic intracerebral hemorrhage", *J Cereb Blood Flow Metab*, vol. 34, no. 5, pp. 753-758.

Barras, C. D., Tress, B. M., Christensen, S., MacGregor, L., Collins, M., Desmond, P. M., Skolnick, B. E., Mayer, S. A., Broderick, J. P., Diringer, M. N., Steiner, T., & Davis, S. M. 2009, "Density and shape as CT predictors of intracerebral hemorrhage growth", *Stroke*, vol. 40, no. 4, pp. 1325-1331.

Battathiri, P. S., Gregson, B., Prasad, K. S., Mitchell, P., Soh, C., Mitra, D., Gholkar, A., & Mendelow, A. 2003, "Reliability assessment of computerized tomography scanning measurements in intracerebral hematoma", *Neurosurgical Focus*, vol. 15, no. 4, p. E6.

Bejot, Y., Cordonnier, C., Durier, J., Aboa-Eboule, C., Rouaud, O., & Giroud, M. 2013, "Intracerebral haemorrhage profiles are changing: results from the Dijon population-based study", *Brain*, vol. 136, no. Pt 2, pp. 658-664.

Berlit, P. & Tornow, K. 1994, "Outcome of intracerebral hemorrhage: clinical and CT findings in 326 patients", *European Journal of Neurology*, vol. 1, no. 1, pp. 29-34.

Bewick, V., Cheek, L., & Ball, J. 2004, "Statistics review 12: survival analysis", *Crit Care*, vol. 8, no. 5, pp. 389-394.

Bhopal, R. 2002, "Concepts of Epidemiology," Oxford University Press, Oxford, p. 317.

Biffi, A., Anderson, C. D., Jagiella, J. M., Schmidt, H., Kissela, B., Hansen, B. M., Jimenez-Conde, J., Pires, C. R., Ayres, A. M., Schwab, K., Cortellini, L., Pera, J., Urbanik, A., Romero, J. M., Rost, N. S., Goldstein, J. N., Viswanathan, A., Pichler, A., Enzinger, C., Rabionet, R., Norrving, B., Tirschwell, D. L., Selim, M., Brown, D. L., Silliman, S. L., Worrall, B. B., Meschia, J. F., Kidwell, C. S., Broderick, J. P., Greenberg, S. M., Roquer, J., Lindgren, A., Slowik, A., Schmidt, R., Woo, D., & Rosand, J. 2011a, "APOE genotype and extent of bleeding and outcome in lobar intracerebral haemorrhage: a genetic association study", *Lancet Neurol*, vol. 10, no. 8, pp. 702-709.

Biffi, A., Halpin, A., Towfighi, A., Gilson, A., Busl, K., Rost, N., Smith, E. E., Greenberg, M. S., Rosand, J., & Viswanathan, A. 2010a, "Aspirin and recurrent intracerebral hemorrhage in cerebral amyloid angiopathy", *Neurology*, vol. 75, no. 8, pp. 693-698.

Biffi, A., Sonni, A., Anderson, C. D., Kissela, B., Jagiella, J. M., Schmidt, H., Jimenez-Conde, J., Hansen, B. M., Fernandez-Cadenas, I., Cortellini, L., Ayres, A., Schwab, K., Juchniewicz, K., Urbanik, A., Rost, N. S., Viswanathan, A., Seifert-Held, T., Stoegerer, E. M., Tomas, M., Rabionet, R., Estivill, X., Brown, D. L., Silliman, S. L., Selim, M., Worrall, B. B., Meschia, J. F., Montaner, J., Lindgren, A., Roquer, J., Schmidt, R., Greenberg, S. M., Slowik, A., Broderick, J. P., Woo, D., & Rosand, J. 2010b, "Variants at APOE influence risk of deep and lobar intracerebral hemorrhage", *Ann.Neurol*, vol. 68, no. 6, pp. 934-943.

Biffi, A., Cortellini, L., Nearnberg, C. M., Ayres, A. M., Schwab, K., Gilson, A. J., Rost, N. S., Goldstein, J. N., Viswanathan, A., Greenberg, S. M., & Rosand, J. 2011b, "Body Mass Index and Etiology of Intracerebral Hemorrhage", *Stroke*.

Bilbao, G., Garibi, J., Pomposo, I., Pijoan, J. I., Carrasco, A., Catalan, G., & Gonzalez, S. 2005, "A prospective study of a series of 356 patients with supratentorial spontaneous intracerebral haematomas treated in a Neurosurgical Department", *Acta Neurochir.(Wien.)*, vol. 147, no. 8, pp. 823-829.

Boonyakarnkul, S., Dennis, M., Sandercock, P., Bamford, J., Burn, J., & Warlow, C. 1993, "Primary Intracerebral Haemorrhage in the Oxfordshire Community Stroke Project", *Cerebrovascular Diseases*, vol. 3, no. 6, pp. 343-349.

Bornebroek, M., Haan, J., Maat-Schieman, M. L., Van Duinen, S. G., & Roos, R. A. 1996, "Hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D): I- A review of clinical, radiologic and genetic aspects", *Brain Pathol.*, vol. 6, no. 2, pp. 111-114.

Borthwick, S., Campbell, C., Cant, B., Chalmers, C., Dennis, M., Flaig, R., Leiper, C., Lowe, G., McDowall, M., Muir, R., & Stott, D. 2005, *National Report on Stroke Services in Scottish Hospitals 2004/2005 Scottish Stroke Care Audit*.

Bossuyt, P. M., Reitsma, J. B., Bruns, D. E., Gatsonis, C. A., Glasziou, P. P., Irwig, L. M., Moher, D., Rennie, D., de Vet, H. C., & Lijmer, J. G. 2003, "The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration", *Ann.Intern.Med*, vol. 138, no. 1, pp. W1-12.

Bouwmeester, W., Zuithoff, N. P., Mallett, S., Geerlings, M. I., Vergouwe, Y., Steyerberg, E. W., Altman, D. G., & Moons, K. G. 2012, "Reporting and methods in clinical prediction research: a systematic review", *PLoS Med*, vol. 9, no. 5, pp. 1-12.

Braak, H., Braak, E., Yilmazer, D., de Vos, R. A., Jansen, E. N., & Bohl, J. 1996, "Pattern of brain destruction in Parkinson's and Alzheimer's diseases", *J Neural Transm.*, vol. 103, no. 4, pp. 455-490.

Bradburn, M. J., Clark, T. G., Love, S. B., & Altman, D. G. 2003, "Survival analysis Part III: multivariate data analysis -- choosing a model and assessing its adequacy and fit", *Br.J.Cancer*, vol. 89, no. 4, pp. 605-611.

Braffman, B. H., Zimmerman, R. A., Trojanowski, J. Q., Gonatas, N. K., Hickey, W. F., & Schlaepfer, W. W. 1988, "Brain MR: pathological correlation with gross and

histopathology. 1. Lacunar infarction and Virchow-Robin spaces.", *American Journal of Roentgenology*, vol. 151, pp. 551-588.

Brayne, C. 2014, "A population perspective on the IWG-2 research diagnostic criteria for Alzheimer's disease", *Lancet Neurol*, vol. 13, no. 6, pp. 532-534.

Brazzelli, M., Sandercock, P. A., Chappell, F. M., Celani, M. G., Righetti, E., Arestis, N., Wardlaw, J. M., & Deeks, J. J. 2009, "Magnetic resonance imaging versus computed tomography for detection of acute vascular lesions in patients presenting with stroke symptoms", *Cochrane Database Syst.Rev* no. 4, p. CD007424.

Brennan, P. & Silman, A. 1992, "Statistical methods for assessing observer variability in clinical measures.", *British Medical Journal*, vol. 304, pp. 1491-1494.

Brenner, H. & Kliebsch, U. 1996, "Dependence of weighted kappa coefficients on the number of categories", *Epidemiology*, vol. 7, pp. 199-202.

Broderick, J. B. 1993, "Lobar hemorrhage in the elderly: The undiminishing importance of hypertension", *Stroke*, vol. 24, no. 1, p. 1993.

Brott, T., Thalinger, K., & Hertzberg, V. 1986, "Hypertension as a risk factor for spontaneous intracerebral hemorrhage", *Stroke*, vol. 17, no. 6, pp. 1078-1083.

Brouwers, H. B., Biffi, A., Ayres, A. M., Schwab, K., Cortellini, L., Romero, J. M., Rost, N. S., Viswanathan, A., Greenberg, S. M., Rosand, J., & Goldstein, J. N. 2012, "Apolipoprotein E genotype predicts hematoma expansion in lobar intracerebral hemorrhage", *Stroke*, vol. 43, no. 6, pp. 1490-1495.

Brown, R. T., Coates, R. K., & Gilbert, J. J. 1985, "Radiographic-pathologic correlation in cerebral amyloid angiopathy. A review of 12 patients", *J.Can.Assoc.Radiol.*, vol. 36, no. 4, pp. 308-311.

Burton, J. L. & Underwood, J. 2007, "Clinical, educational, and epidemiological value of autopsy", *Lancet*, vol. 369, no. 9571, pp. 1471-1480.

Burton, J. L. & Underwood, J. C. 2003, "Necropsy practice after the "organ retention scandal": requests, performance, and tissue retention", *J.Clin.Pathol.*, vol. 56, no. 7, pp. 537-541.

Byrt, T., Bishop, J., & Carlin, J. B. 1993, "Bias, prevalence and kappa", *Journal of Clinical Epidemiology*, vol. 46, pp. 423-429.

Cadavid, D., Mena, H., Koeller, K., & Frommelt, R. A. 2000, "Cerebral beta amyloid angiopathy is a risk factor for cerebral ischemic infarction. A case control study in human brain biopsies", *Journal of Neuropathology & Experimental Neurology*, vol. 59, no. 9, pp. 768-773.

Calhoun, M. E., Burgermeister, P., Phinney, A. L., Stalder, M., Tolnay, M., Wiederhold, K. H., Abramowski, D., Sturchler-Pierrat, C., Sommer, B., Staufenbiel, M., & Jucker, M. 1999, "Neuronal overexpression of mutant amyloid precursor protein results in

prominent deposition of cerebrovascular amyloid", *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 24, pp. 14088-14093.

Caplan, L. R. 1992, "Intracerebral haemorrhage", *Lancet*, vol. 14, no. 339 (8794), pp. 656-658.

Carare, R. O., Bernardes-Silva, M., Newman, T. A., Page, A. M., Nicoll, J. A., Perry, V. H., & Weller, R. O. 2008, "Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroimmunology", *Neuropathol.Appl.Neurobiol.*, vol. 34, no. 2, pp. 131-144.

Cartier, L., Araya, F., Avendano, R., Vergara, C., & Garcia, M. 1999, "Hemorragias cerebrales y angiopatía amiloidea", *Revista Medica de Chile*, vol. 127, no. 3, pp. 295-303.

Casanova, M. F. & Araque, J. M. 2003, "Mineralization of the basal ganglia: implications for neuropsychiatry, pathology and neuroimaging", *Psychiatry Research*, vol. 121, pp. 59-87.

Castillo, J., Leira, R., Martinez, F., Corredera, E., Aldrey, J. M., & Noya, M. 1994, "[Prognostic factors in spontaneous intracerebral hemorrhage]", *An.Med Interna*, vol. 11, no. 7, pp. 318-321.

Chalmers, K., Wilcock, G. K., & Love, S. 2003, "APOE epsilon4 influences the pathological phenotype of Alzheimer's disease by favouring cerebrovascular over parenchymal accumulation of A beta protein", *Neuropathology and Applied Neurobiology*, vol. 29, pp. 231-238.

Chapman, N., Huxley, R., Anderson, C., Bousser, M. G., Chalmers, J., Colman, S., Davis, S., Donnan, G., MacMahon, S., Neal, B., Warlow, C., & Woodward, M. 2004, "Effects of a perindopril-based blood pressure-lowering regimen on the risk of recurrent stroke according to stroke subtype and medical history: the PROGRESS Trial", *Stroke*, vol. 35, no. 1, pp. 116-121.

Charcot, J. M. & Bouchard, C. 1868, "Nouvelles recherches sur la pathogenie de l'hemorrhagie cerebrale", *Arch Physiol norm Pathol*, vol. 1, pp. 110-27.

Charidimou, A., Jager, R. H., Fox, Z., Peeters, A., Vandermeeren, Y., Laloux, P., Baron, J. C., & Werring, D. J. 2013a, "Prevalence and mechanisms of cortical superficial siderosis in cerebral amyloid angiopathy", *Neurology*, vol. 81, no. 7, pp. 626-632.

Charidimou, A., Jaunmuktane, Z., Baron, J. C., Burnell, M., Varlet, P., Peeters, A., Xuereb, J., Jager, R., Brandner, S., & Werring, D. J. 2013b, "White matter perivascular spaces: An MRI marker in pathology-proven cerebral amyloid angiopathy?", *Neurology*.

Charidimou, A., Meegahage, R., Fox, Z., Peeters, A., Vandermeeren, Y., Laloux, P., Baron, J. C., Jager, H. R., & Werring, D. J. 2013c, "Enlarged perivascular spaces as a marker of underlying arteriopathy in intracerebral haemorrhage: a multicentre MRI cohort study", *J Neurol Neurosurg.Psychiatry*, vol. 84, no. 6, pp. 624-629.

Charidimou, A., Peeters, A., Fox, Z., Gregoire, S. M., Vandermeeren, Y., Laloux, P., Jager, H. R., Baron, J. C., & Werring, D. J. 2012, "Spectrum of transient focal neurological episodes in cerebral amyloid angiopathy: multicentre magnetic resonance imaging cohort study and meta-analysis", *Stroke*, vol. 43, no. 9, pp. 2324-2330.

Charidimou, A., Peeters, A. P., Jager, R., Fox, Z., Vandermeeren, Y., Laloux, P., Baron, J. C., & Werring, D. J. 2013d, "Cortical superficial siderosis and intracerebral hemorrhage risk in cerebral amyloid angiopathy", *Neurology*, vol. 81, no. 19, pp. 1666-1673.

Chen, C. S., Lo, Y. K., Yan, S. H., Lin, Y. T., Lai, P. H., & Lo, Y. S. 2004, "Lobar cerebral hemorrhage from amyloid angiopathy: clinical, neuroimaging, pathologic and outcome correlations", *Acta Neurol Taiwan.*, vol. 13, no. 1, pp. 14-19.

Chen, G., Arima, H., Wu, G., Heeley, E., Delcourt, C., Zhang, P., Rabinstein, A. A., Robinson, T., Stapf, C., Huang, Y., Song, L., Yang, J., Wang, X., Li, Q., Chen, X., Chalmers, J., & Anderson, C. 2013, "Subarachnoid Extension of Intracerebral Hemorrhage and 90-Day Outcomes in INTERACT2", *Stroke*, vol. 45, pp. 258-260.

Chen, H. S., Hsieh, C. F., Chau, T. T., Yang, C. D., & Chen, Y. W. 2011, "Risk Factors of In-Hospital Mortality of Intracerebral Hemorrhage and Comparison of ICH Scores in a Taiwanese Population", *European Neurology*, vol. 66, no. 1, pp. 59-63.

Chen, Y. W., Gurol, M. E., Rosand, J., Viswanathan, A., Rakich, S. M., Groover, T. R., Greenberg, S. M., & Smith, E. E. 2006, "Progression of white matter lesions and hemorrhages in cerebral amyloid angiopathy", *Neurology*, vol. 67, no. 1, pp. 83-87.

Chen, Y. W., Tang, S. C., Tsai, L. K., Yeh, S. J., Chiou, H. Y., Yip, P. K., & Jeng, J. S. 2012, "Pre-ICH warfarin use, not antiplatelets, increased case fatality in spontaneous ICH patients", *Eur.J.Neurol*, vol. 20, no. 8, pp. 1128-1134.

Chewning, R. H. & Murphy, K. P. 2010, "Computerised tomography and CT angiography in intracerebral haemorrhage," in *Intracerebral haemorrhage*, J. R. Carhuapoma, S. A. Mayer, & D. F. Hanley, eds., Cambridge University Press, Cambridge, pp. 121-124.

Chong, B. H., Chan, K. H., Pong, V., Lau, K. K., Chan, Y. H., Zuo, M. L., Lui, W. M., Leung, G. K., Lau, C. P., Tse, H. F., Pu, J. K., & Siu, C. W. 2012, "Use of aspirin in Chinese after recovery from primary intracranial haemorrhage", *Thromb.Haemost.*, vol. 107, no. 2, pp. 241-247.

Chung, K. K., Anderson, N. E., Hutchinson, D., Synek, B., & Barber, P. A. 2011, "Cerebral amyloid angiopathy related inflammation: three case reports and a review", *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 82, no. 1, pp. 20-26.

Clark, T. G., Altman, D. G., & De Stavola, B. L. 2002, "Quantification of the completeness of follow-up", *Lancet*, vol. 359, no. 9314, pp. 1309-1310.

Clayton, D. G., Walker, N. M., Smyth, D. J., Pask, R., Cooper, J. D., Maier, L. M., Smink, L. J., Lam, A. C., Ovington, N. R., Stevens, H. E., Nutland, S., Howson, J. M., Faham, M., Moorhead, M., Jones, H. B., Falkowski, M., Hardenbol, P., Willis, T. D., &

Todd, J. A. 2005, "Population structure, differential bias and genomic control in a large-scale, case-control association study", *Nat Genet*, vol. 37, no. 11, pp. 1243-1246.

Cloft, H. J., Joseph, G. J., & Dion, J. E. 1999, "Risk of cerebral angiography in patients with subarachnoid hemorrhage, cerebral aneurysm, and arteriovenous malformation: a meta-analysis", *Stroke*, vol. 30, no. 2, pp. 317-320.

Cohen, J. 1960, "A coefficient of agreement for nominal scales", *Education and Psychological Measurement*, vol. 20, pp. 37-46.

Copeland, K. T., Checkoway, H., McMichael, A. J., & Holbrook, R. H. 1977, "Bias due to misclassification in the estimation of relative risk", *Am J Epidemiol.*, vol. 105, no. 5, pp. 488-495.

Corbo, R. M. & Scacchi, R. 1999, "Apolipoprotein E (APOE)allele distribution in the world. Is APOE*4 a `thrifty' allele?", *Annals of Human Genetics*, vol. 63, pp. 301-310.

Cordonnier, C., Wardlaw, J., & Al-Shahi Salman, R. 2007, "Spontaneous brain microbleeds: systematic review, subgroup analyses and standards for study design and reporting", *Brain*, vol. 130, no. Pt 8, pp. 1988-2003.

Cordonnier, C., Klijn, C. J. M., van Beijnum, J., & Al-Shahi Salman, R. 2010a, "Radiological Investigation of Spontaneous Intracerebral Hemorrhage", *Stroke*, vol. 41, no. 4, pp. 685-690.

Cordonnier, C., Leys, D., Dumont, F., Deramecourt, V., Bordet, R., Pasquier, F., & Henon, H. 2010b, "What are the causes of pre-existing dementia in patients with intracerebral haemorrhage?", *Brain*, vol. 133, no. 11, pp. 3281-3289.

Cordonnier, C., Potter, G. M., Jackson, C. A., Doubal, F., Keir, S., Sudlow, C. L. M., Wardlaw, J. M., & Al-Shahi Salman, R. 2009, "Improving Interrater Agreement About Brain Microbleeds - Development of the Brain Observer MicroBleed Scale (BOMBS)", *Stroke*, vol. 40, pp. 94-99.

Cosgrove, G. R., Leblanc, R., Meagher-Villemure, K., & Ethier, R. 1985, "Cerebral amyloid angiopathy", *Neurology*, vol. 35, no. 5, pp. 625-631.

Coste, J., Tissier, F., Pouchot, J., Ecosse, E., Rouquette, A., Bertagna, X., Libe, R., & Viallon, V. 2014, "Rasch analysis for assessing unidimensionality and identifying measurement biases of malignancy scores in oncology. The example of the Weiss histopathological system for the diagnosis of adrenocortical cancer", *Cancer Epidemiol.*

Coughlan, C. M. & Breen, K. C. 2000, "Factors influencing the processing and function of the amyloid beta precursor protein--a potential therapeutic target in Alzheimer's disease?", *Pharmacol.Ther.*, vol. 86, no. 2, pp. 111-145.

Coull, A. J., Silver, L. E., Bull, L. M., Giles, M. F., Rothwell, P. M., & on behalf of the Oxford Vascular (OXVASC) Study 2004, "Direct Assessment of Completeness of Ascertainment in a Stroke Incidence Study", *Stroke*, vol. 35, no. 9, pp. 2041-2045.

Counsell, C., Dennis, M., McDowall, M., & Warlow, C. 2002, "Predicting outcome after acute and subacute stroke: Development and Validation of New Prognostic Models", *Stroke*, vol. 33, pp. 1041-1047.

D'Amore, C., Paciaroni, M., Silvestrelli, G., Agnelli, G., Santucci, P., Lanari, A., Alberti, A., Venti, M., Acciarresi, M., & Caso, V. 2013, "Severity of acute intracerebral haemorrhage, elderly age and atrial fibrillation: Independent predictors of poor outcome at three months", *Eur J Intern.Med.*

Danaei, G., Finucane, M. M., Lin, J. K., Singh, G. M., Paciorek, C. J., Cowan, M. J., Farzadfar, F., Stevens, G. A., Lim, S. S., Riley, L. M., & Ezzati, M. 2011, "National, regional, and global trends in systolic blood pressure since 1980: systematic analysis of health examination surveys and epidemiological studies with 786 country-years and 5.4 million participants", *Lancet*, vol. 377, no. 9765, pp. 568-577.

Danner, D., Darnell, K. R., & McGuire, C. 2011, "African American Participation in Alzheimer's Disease Research that Includes Brain Donation", *American Journal of Alzheimers Disease and Other Dementias*, vol. 26, no. 6, pp. 469-476.

Davenport, R. & Dennis, M. 2000, "Neurological emergencies: acute stroke", *J.Neurol Neurosurg.Psychiatry*, vol. 68, no. 3, pp. 277-288.

Davis, J., Xu, F., Deane, R., Romanov, G., Previti, M. L., Zeigler, K., Zlokovic, B. V., & Van Nostrand, W. E. 2004, "Early-onset and robust cerebral microvascular accumulation of amyloid beta-protein in transgenic mice expressing low levels of a vasculotropic Dutch/Iowa mutant form of amyloid beta-protein precursor", *J.Biol.Chem.*, vol. 279, no. 19, pp. 20296-20306.

De Reuck J, Auger, F., Cordonnier, C., Deramecourt, V., Durieux, N., Pasquier, F., Bordet, R., Maurage, C. A., & Leys, D. 2011, "Comparison of 7.0-T T(2)*-magnetic resonance imaging of cerebral bleeds in post-mortem brain sections of Alzheimer patients with their neuropathological correlates", *Cerebrovasc Dis*, vol. 31, no. 5, pp. 511-517.

De Reuck, J., Deramecourt, V., Cordonnier, C., Auger, F., Durieux, N., Pasquier, F., Bordet, R., Defebvre, L., Caparros-Lefebvre, D., Maurage, C. A., & Leys, D. 2013, "Superficial siderosis of the central nervous system: a post-mortem 7.0-tesla magnetic resonance imaging study with neuropathological correlates", *Cerebrovasc Dis*, vol. 36, no. 5-6, pp. 412-417.

Delgado Almandoz, J. E., Schaefer, P. W., Goldstein, J. N., Rosand, J., Lev, M. H., Gonzalez, R. G., & Romero, J. M. 2010, "Practical scoring system for the identification of patients with intracerebral hemorrhage at highest risk of harboring an underlying vascular etiology: the Secondary Intracerebral Hemorrhage Score", *AJNR Am.J.Neuroradiol.*, vol. 31, no. 9, pp. 1653-1660.

Dennis, M., Bamford, J. M., Molyneux, A. J., & Warlow, C. 1987, "Rapid resolution of signs of Primary Intracerebral Haemorrhage in Computerised Tomograms of the Brain", *British Medical Journal*, vol. 295, no. 6594, pp. 379-381.

Department of Health. Human Tissue Act. UK government . 15-11-2004.
Ref Type: Electronic Citation

Department of Health (Scotland). Human Tissue (Scotland) Act. UK government . 16-3-2006. 25-9-2012.
Ref Type: Electronic Citation

Di Carlo A. 2009, "Human and economic burden of stroke", *Age Ageing*, vol. 38, no. 1, pp. 4-5.

Dichgans, M. & Markus, H. S. 2005, "Genetic association studies in stroke: methodological issues and proposed standard criteria", *Stroke*, vol. 36, no. 9, pp. 2027-2031.

Dichgans, M., Holtmannspötter, M., Herzog, J., Peters, N., Bergmann, M., & Yousry, T. A. 2002, "Cerebral Microbleeds in CADASIL", *Stroke*, vol. 33, no. 1, pp. 67-71.

Dickson, D. W. 2005, "Required techniques and useful molecular markers in the neuropathologic diagnoses of neurodegenerative diseases", *Acta Neuropathologica*, vol. 109, pp. 14-24.

Diringer, M. N., Edwards, D. F., & Zazulia, A. R. 1998, "Hydrocephalus: A Previously Unrecognized Predictor of Poor Outcome From Supratentorial Intracerebral Hemorrhage", *Stroke*, vol. 29, no. 7, pp. 1352-1357.

Doubal, F. N., MacLullich, A. M., Ferguson, K. J., Dennis, M. S., & Wardlaw, J. M. 2010, "Enlarged perivascular spaces on MRI are a feature of cerebral small vessel disease", *Stroke*, vol. 41, no. 3, pp. 450-454.

El-Senousey, M. Y., Rabie, M. O., Elbeshlawy, W. F., & Deewan, K. 2010, "Outcome of spontaneous supratentorial intracerebral haematoma", *Egyptian Journal of Neurology, Psychiatry and Neurosurgery*, vol. 47, no. 2, pp. 241-247.

Ellis, R. J., Olichney, J. M., Thal, L. J., Mirra, S. S., Morris, J. C., Beekly, D., & Heyman, A. 1996, "Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, Part XV", *Neurology*, vol. 46, no. 6, pp. 1592-1596.

Esiri, M. M. & Wilcock, G. K. 1986, "Cerebral amyloid angiopathy in dementia and old age", *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 49, no. 11, pp. 1221-1226.

Farrell, C., Chappell, F., Armitage, P. A., Keston, P., MacLullich, A., Shenkin, S., & Wardlaw, J. M. 2009, "Development and initial testing of normal reference MR images for the brain at ages 65–70 and 75–80 years", *European Radiology*, vol. 19, pp. 177-183.

Faught, E., Peters, D., Bartolucci, A., Moore, L., & Miller, P. C. 1989, "Seizures after primary intracerebral hemorrhage", *Neurology*, vol. 39, no. 8, pp. 1089-1093.

Fazekas, F., Chawluk, J. B., Alavi, A., Hurtig, H. I., & Zimmerman, R. A. 1987, "MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging", *American Journal of Roentgenology*, vol. 149, pp. 351-356.

Fazekas, F., Kleinert, R., Roob, G., Kleinert, G., Kapeller, P., Schmidt, R., & Hartung, H. P. 1999, "Histopathologic Analysis of Foci of Signal Loss on Gradient-Echo T2*-Weighted MR Images in Patients with Spontaneous Intracerebral Hemorrhage: Evidence of Microangiopathy-Related Microbleeds", *American Journal of Neuroradiology*, vol. 20, no. 4, pp. 637-642.

Feigin, V. L. & Carter, K. 2004, "Editorial comment--Stroke incidence studies one step closer to the elusive gold standard?", *Stroke*, vol. 35, no. 9, pp. 2045-2047.

Feigin, V. L., Forouzanfar, M. H., Krishnamurthi, R., Mensah, G. A., Connor, M., Bennett, D. A., Moran, A. E., Sacco, R. L., Anderson, L., Truelsen, T., O'Donnell, M., Venketasubramanian, N., Barker-Collo, S., Lawes, C. M., Wang, W., Shinohara, Y., Witt, E., Ezzati, M., Naghavi, M., & Murray, C. 2014, "Global and regional burden of stroke during 1990-2010: findings from the Global Burden of Disease Study 2010", *Lancet*, vol. 383, no. 9913, pp. 245-254.

Feigin, V. L., Lawes, C. M., Bennett, D. A., Barker-Collo, S. L., & Parag, V. 2009, "Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review", *Lancet Neurol*, vol. 8, no. 4, pp. 355-369.

Feinstein, A. R. & Cicchetti, D. V. 1990, "High agreement but low kappa, I: the problems of two paradoxes.", *Journal of Clinical Epidemiology*, vol. 43, pp. 543-549.

Fieschi, C., Carolei, A., Fiorelli, M., Argentino, C., Bozzao, L., Fazio, C., Salvetti, M., & Bastianello, S. 1988, "Changing prognosis of primary intracerebral hemorrhage: results of a clinical and computed tomographic follow-up study of 104 patients", *Stroke*, vol. 19, no. 2, pp. 192-195.

Finelli, P. F., Kessimian, N., & Bernstein, P. W. 1984, "Cerebral amyloid angiopathy manifesting as recurrent intracerebral hemorrhage", *Arch Neurol*, vol. 41, no. 3, pp. 330-333.

Fisher, C. M. 1971, "Pathological observations in hypertensive cerebral hemorrhage", *J.Neuropathol.Exp.Neurol*, vol. 30, no. 3, pp. 536-550.

Fisher, M., French, S., Ji, P., & Kim, R. C. 2010, "Cerebral microbleeds in the elderly: a pathological analysis", *Stroke*, vol. 41, no. 12, pp. 2782-2785.

Flaherty, M. L., Haverbusch, M., Sekar, P., Kissela, B., Kleindorfer, D., Moomaw, C. J., Sauerbeck, L., Schneider, A., Broderick, J. P., & Woo, D. 2006, "Long-term mortality after intracerebral hemorrhage", *Neurology*, vol. 66, no. 8, pp. 1182-1186.

Flynn, R. W., MacDonald, T. M., Murray, G. D., MacWalter, R. S., & Doney, A. S. 2010, "Prescribing antiplatelet medicine and subsequent events after intracerebral hemorrhage", *Stroke*, vol. 41, no. 11, pp. 2606-2611.

Fogelholm, R., Avikainen, S., & Murros, K. 1997, "Prognostic value and determinants of first-day mean arterial pressure in spontaneous supratentorial intracerebral hemorrhage", *Stroke*, vol. 28, no. 7, pp. 1396-1400.

Franke, C. L., van Swieten, J. C., Algra, A., & van Gijn, J. 1992, "Prognostic factors in patients with intracerebral haematoma", *J.Neurol Neurosurg.Psychiatry*, vol. 55, no. 8, pp. 653-657.

Fryer, J. D., Simmons, K., Parsadanian, M., Bales, K. R., Paul, S. M., Sullivan, P. M., & Holtzman, D. M. 2005, "Human apolipoprotein E4 alters the amyloid-beta 40:42 ratio and promotes the formation of cerebral amyloid angiopathy in an amyloid precursor protein transgenic model", *J.Neurosci.*, vol. 25, no. 11, pp. 2803-2810.

Fujii, Y., Tanaka, R., Takeuchi, S., Koike, T., Minakawa, T., & Sasaki, O. 1994, "Hematoma enlargement in spontaneous intracerebral hemorrhage", *J Neurosurg.*, vol. 80, no. 1, pp. 51-57.

Garibi, J., Bilbao, G., Pomposo, I., & Hostalot, C. 2002, "Prognostic factors in a series of 185 consecutive spontaneous supratentorial intracerebral haematomas", *Br.J Neurosurg.*, vol. 16, no. 4, pp. 355-361.

Garrick, T., Sundqvist, N., Dobbins, T., Azizi, L., & Harper, C. 2009, "Factors that influence decisions by families to donate brain tissue for medical research", *Cell Tissue Bank*, vol. 10, pp. 309-315.

Gauer, O. H. 1960, "Kreislauf des Blutes," in *Lehbuch der Phsiologie des Menchen*, W. Trautwein, O. H. Gauer, & H. P. Koepchen, eds., von Urban und Schwartzenberg, München.

Gean, A. D. "Traumatic Brain Injury: Imaging Update", pp. 15-16.

Gilles, C., Brucher, J. M., Khoubesserian, P., & Vanderhaeghen, J. J. 1984, "Cerebral amyloid angiopathy as a cause of multiple intracerebral hemorrhages", *Neurology*, vol. 34, no. 6, pp. 730-735.

Giroud, M., Creisson, E., Fayolle, H., Andre, N., Becker, F., Martin, D., & Dumas, R. 1995, "Risk factors for primary cerebral hemorrhage: a population-based study--the Stroke Registry of Dijon", *Neuroepidemiology*, vol. 14, no. 1, pp. 20-26.

Giroud, M., Gras, P., Chadan, N., Beuriat, P., Milan, C., Arveux, P., & Dumas, R. 1991, "Cerebral haemorrhage in a French prospective population study", *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 54, no. 7, pp. 595-598.

Glaw, X. M., Garrick, T. M., Terwee, P. J., Patching, J. R., Blake, H., Harper, C., Glaw, X. M., Garrick, T. M., Terwee, P. J., Patching, J. R., Blake, H., & Harper, C. 2009, "Brain donation: who and why?", *Cell & Tissue Banking*, vol. 10, no. 3, pp. 241-246.

Godin, O., Tzourio, C., Maillard, P., Alperovitch, A., Mazoyer, B., & Dufouil, C. 2009, "Apolipoprotein E genotype is related to progression of white matter lesion load", *Stroke*, vol. 40, no. 10, pp. 3186-3190.

Goldstein, J. N., Fazen, L. E., Wendell, L., Chang, Y., Rost, N. S., Snider, R., Schwab, K., Chanderraj, R., Kabrhel, C., Kinnecom, C., FitzMaurice, E., Smith, E. E.,

Greenberg, S. M., & Rosand, J. 2009, "Risk of thromboembolism following acute intracerebral hemorrhage", *Neurocrit.Care*, vol. 10, no. 1, pp. 28-34.

Gorelick, P. B. & Weisman, S. M. 2005, "Risk of hemorrhagic stroke with aspirin use: an update", *Stroke*, vol. 36, no. 8, pp. 1801-1807.

Graeb, D., Robertson, W., Lapointe, J., Nugent, R., & Harrison, P. 1982, "Computed tomographic diagnosis of intraventricular hemorrhage. Etiology and prognosis.", *Radiology*, vol. 143, pp. 91-96.

Graffagnino, C., Herbstreith, M. H., Roses, A. D., & Alberts, M. J. 1994, "A molecular genetic study of intracerebral hemorrhage", *Arch.Neurol*, vol. 51, no. 10, pp. 981-984.

Greenberg, S. M., Briggs, M. E., Hyman, B. T., Kokoris, G. J., Takis, C., Kanter, D. S., Kase, C. S., & Pessin, M. S. 1996, "Apolipoprotein E epsilon 4 is associated with the presence and earlier onset of hemorrhage in cerebral amyloid angiopathy", *Stroke*, vol. 27, no. 8, pp. 1333-1337.

Greenberg, S. M., Finkelstein, S., & Schaefer, P. 1996, "Petechial hemorrhages accompanying lobar hemorrhage: detection by gradient echo MRI", *Neurology*, vol. 46, pp. 1751-1754.

Greenberg, S. M., Gurol, M. E., Rosand, J., & Smith, E. E. 2004, "Amyloid angiopathy-related vascular cognitive impairment", *Stroke*, vol. 35, no. 11 Suppl 1, pp. 2616-2619.

Greenberg, S. M., Rebeck, G. W., Vonsattel, J. P., Gomez-Isla, T., & Hyman, B. T. 1995, "Apolipoprotein E epsilon 4 and cerebral hemorrhage associated with amyloid angiopathy", *Ann.Neurol*, vol. 38, no. 2, pp. 254-259.

Greenberg, S. M. & Vonsattel, J. P. 1997, "Diagnosis of cerebral amyloid angiopathy. Sensitivity and specificity of cortical biopsy", *Stroke*, vol. 28, no. 7, pp. 1418-1422.

Greenberg, S. M., Vernooji, M. W., Cordonnier, C., Viswanathan, A., Al-Shahi Salman, R., Warach, S., Launer, L. J., Van Buchem, M. A., & Breteler, M. M. B. 2009, "Cerebral microbleeds: a guide to detection and interpretation", *Lancet Neurology*, vol. 8, pp. 165-174.

Gregoire, S. M., Chaudary, U. J., Brown, M. M., Yousry, T. A., Kallis, C., Jager, H. R., & Werring, D. J. 2009, "The Microbleed Anatomical Rating Scale (MARS): Reliability of a tool to map brain microbleeds", *Neurology*, vol. 73, pp. 1759-1766.

Grimes, D. A. & Schulz, K. F. 2002a, "Cohort studies: marching towards outcomes", *Lancet*, vol. 359, no. 9303, pp. 341-345.

Grimes, D. A. & Schulz, K. F. 2002b, "Descriptive studies: what they can and cannot do", *Lancet*, vol. 359, no. 9301, pp. 145-149.

Gronenschild, E. H., Habets, P., Jacobs, H. I., Mengelers, R., Rozendaal, N., van, O. J., & Marcelis, M. 2012, "The effects of FreeSurfer version, workstation type, and Macintosh operating system version on anatomical volume and cortical thickness measurements", *PLoS One*, vol. 7, no. 6, p. e38234.

Guidoux, C. 2008, *Causes des Hémorragies Intracérébrales: Etude Neuropathologique,* Docteur en Médecine Docteur en Médecine, Université Paris Val-De-Marne.

Gulliford, M. C., Charlton, J., & Latinovic, R. 2006, "Predictive validity of different definitions of hypertension for type 2 diabetes", *Diabetes Metab Res.Rev*, vol. 22, no. 5, pp. 361-366.

Gurol, M. E., Dierksen, G., Betensky, R., Gidicsin, C., Halpin, A., Becker, A., Carmasin, J., Ayres, A., Schwab, K., Viswanathan, A., Salat, D., Rosand, J., Johnson, K. A., & Greenberg, S. M. 2012, "Predicting sites of new hemorrhage with amyloid imaging in cerebral amyloid angiopathy", *Neurology*, vol. 79, no. 4, pp. 320-326.

Haglund, M. & Englund, E. 2002, "Cerebral amyloid angiopathy, white matter lesions and Alzheimer encephalopathy - a histopathological assessment", *Dementia & Geriatric Cognitive Disorders*, vol. 14, no. 3, pp. 161-166.

Hallevi, H., Albright, K. C., Aronowski, D., Barreto, A. D., Martin-Schild, S., Khaja, A. M., Gonzales, N. R., Illoh, K., Noser, E. A., & Grotta, J. C. 2008, "Intraventricular hemorrhage: Anatomic relationships and clinical implications", *Neurology*, vol. 70, no. 11, pp. 848-852.

Hallevi, H., Dar, N. S., Barreto, A. D., Morales, M. M., Martin-Schild, S., Abraham, A. T., Walker, K. C., Gonzales, N. R., Illoh, K., Grotta, J. C., & Savitz, S. I. 2009, "The IVH score: a novel tool for estimating intraventricular hemorrhage volume: clinical and research implications", *Crit Care Med*, vol. 37, no. 3, pp. 969-74, e1.

Hanger, H. C., Wilkinson, T. J., Fayez-Iskander, N., & Sainsbury, R. 2007, "The risk of recurrent stroke after intracerebral haemorrhage", *J.Neurol Neurosurg.Psychiatry*, vol. 78, no. 8, pp. 836-840.

Hansen, B. M., Nilsson, O. G., Anderson, H., Norrving, B., Saveland, H., & Lindgren, A. 2013, "Long term (13 years) prognosis after primary intracerebral haemorrhage: a prospective population based study of long term mortality, prognostic factors and causes of death", *J Neurol Neurosurg.Psychiatry*, vol. 84, pp. 1150-1155.

Harris, C., Kiger, A., & Counsell, C. 2012, "Attitudes to brain donation for Parkinson's research and how to ask: a qualitative study with suggested guidelines for practice", *Journal of Advanced Nursing* pp. 1-13.

Hawkes, C. A., Hartig, W., Kacza, J., Schliebs, R., Weller, R. O., Nicoll, J. A., & Carare, R. O. 2011, "Perivascular drainage of solutes is impaired in the ageing mouse brain and in the presence of cerebral amyloid angiopathy", *Acta Neuropathol.*, vol. 121, no. 4, pp. 431-443.

Hawley, M. E. & Kidd, K. K. 1995, "HAPLO: a program using the EM algorithm to estimate the frequencies of multi-site haplotypes", *J Hered.*, vol. 86, no. 5, pp. 409-411.

Hemphill, J. C., Bonovich, D. C., Besmertis, L., Manley, G. T., & Johnston, S. C. 2001, "The ICH Score", *Stroke*, vol. 32, no. 4, pp. 891-897.

Hemphill, J. C., Farrant, M., & Neill, T. A. 2009, "Prospective validation of the ICH Score for 12-month functional outcome", *Neurology*, vol. 73, no. 14, pp. 1088-1094.

Herzig, M. C., Van Nostrand, W. E., & Jucker, M. 2006, "Mechanism of cerebral beta-amyloid angiopathy: murine and cellular models", *Brain Pathol.*, vol. 16, no. 1, pp. 40-54.

Herzig, M. C., Winkler, D. T., Burgermeister, P., Pfeifer, M., Kohler, E., Schmidt, S. D., Danner, S., Abramowski, D., Sturchler-Pierrat, C., Burki, K., Van Duinen, S. G., Maat-Schieman, M. L., Staufenbiel, M., Mathews, P. M., & Jucker, M. 2004, "Abeta is targeted to the vasculature in a mouse model of hereditary cerebral hemorrhage with amyloidosis", *Nature Neuroscience*, vol. 7, no. 9, pp. 954-960.

Hill, A. B. 1965, "The environment and disease: association or causation?", *Proceedings of the Royal Society of Medicine* pp. 295-300.

Hirohata, M., Yoshita, M., Ishida, C., Ikeda, S. I., Tamaoka, A., Kuzuhara, S., Shoji, M., Ando, Y., Tokuda, T., & Yamada, M. 2010, "Clinical features of non-hypertensive lobar intracerebral hemorrhage related to cerebral amyloid angiopathy", *Eur.J.Neurol*, vol. 17, no. 6, pp. 823-829.

Hobart, J. 2003, "Rating scales for neurologists", *J Neurol Neurosurg.Psychiatry*, vol. 74 Suppl 4, p. iv22-iv26.

Hobart, J. C., Cano, S. J., Zajicek, J. P., & Thompson, A. J. 2007, "Rating scales as outcome measures for clinical trials in neurology: problems, solutions, and recommendations", *Lancet Neurol*, vol. 6, no. 12, pp. 1094-1105.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., & Cole, G. 1996, "Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice", *Science*, vol. 274, no. 5284, pp. 99-102.

Hu, Y. Z., Wang, J. W., & Luo, B. Y. 2013, "Epidemiological and clinical characteristics of 266 cases of intracerebral hemorrhage in Hangzhou, China", *J Zhejiang Univ Sci B*, vol. 14, no. 6, pp. 496-504.

Huttner, H. B., Steiner, T., Hartmann, M., Kohrmann, M., Juettler, E., Mueller, S., Wikner, J., Meyding-Lamade, U., Schramm, P., Schwab, S., & Schellinger, P. D. 2006, "Comparison of ABC/2 estimation technique to computer-assisted planimetric analysis in warfarin-related intracerebral parenchymal hemorrhage", *Stroke*, vol. 37, no. 2, pp. 404-408.

Hwang, B. Y., Bruce, S. S., Appelboom, G., Piazza, M. A., Carpenter, A. M., Gigante, P. R., Kellner, C. P., Ducruet, A. F., Kellner, M. A., Deb-Sen, R., Vaughan, K. A., Meyers, P. M., & Connolly, E. S., Jr. 2012, "Evaluation of intraventricular hemorrhage assessment methods for predicting outcome following intracerebral hemorrhage", *J.Neurosurg.*, vol. 116, no. 1, pp. 185-192.

Ikram, M. A., Wieberdink, R. G., & Koudstaal, P. J. 2012, "International epidemiology of intracerebral hemorrhage", *Curr.Atheroscler.Rep.*, vol. 14, no. 4, pp. 300-306.

Inagawa, T., Ohbayashi, N., Takechi, A., Shibukawa, M., & Yahara, K. 2003, "Primary intracerebral hemorrhage in Izumo City, Japan: incidence rates and outcome in relation to the site of hemorrhage", *Neurosurgery*, vol. 53, no. 6, pp. 1283-1297.

Ishihara, T., Takahashi, M., Yokota, T., Yamashita, Y., Gondo, T., Uchino, F., & Iwamoto, N. 1991, "The significance of cerebrovascular amyloid in the aetiology of superficial (lobar) cerebral haemorrhage and its incidence in the elderly population", *Journal of Pathology*, vol. 165, no. 3, pp. 229-234.

Ishii, N., Nishihara, Y., & Horie, A. 1984, "Amyloid angiopathy and lobar cerebral haemorrhage", *J Neurol Neurosurg.Psychiatry*, vol. 47, no. 11, pp. 1203-1210.

Itoh, Y. & Yamada, M. 1997, "Cerebral amyloid angiopathy in the elderly: the clinicopathological features, pathogenesis, and risk factors", *Journal of Medical & Dental Sciences*, vol. 44, no. 1, pp. 11-19.

Itoh, Y., Yamada, M., Hayakawa, M., Otomo, E., & Miyatake, T. 1993, "Cerebral amyloid angiopathy: a significant cause of cerebellar as well as lobar cerebral hemorrhage in the elderly", *Journal of the Neurological Sciences*, vol. 116, no. 2, pp. 135-141.

Iwata, N., Tsubuki, S., Takaki, Y., Watanabe, K., Sekiguchi, M., Hosoki, E., Kawashima-Morishima, M., Lee, H. J., Hama, E., Sekine-Aizawa, Y., & Saido, T. C. 2000, "Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition", *Nat Med*, vol. 6, no. 2, pp. 143-150.

Izumihara, A., Ishihara, T., Iwamoto, N., Yamashita, K., & Ito, H. 1999, "Postoperative outcome of 37 patients with lobar intracerebral hemorrhage related to cerebral amyloid angiopathy", *Stroke*, vol. 30, no. 1, pp. 29-33.

Jackson, C., Hutchison, A., Dennis, M., Wardlaw, J. M., Lewis, S. C., & Sudlow, C. L. M. 2009, "Differences between ischaemic stroke subtypes in vascular outcomes support a distinct lacunar ischaemic stroke arteriopathy. A prospective hospital-based study.", *Stroke*, vol. 40, pp. 3679-3684.

Jackson, C. & Sudlow, C. L. M. 2006, "Is hypertension a more frequent risk factor for deep than for lobar supratentorial intracerebral haemorrhage?", *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 77, no. 11, pp. 1244-1252.

Jamieson, E. I., Newman, D., Metcalf, A. K., Naguib, M. F., Saada, J., Potter, J. F., & Myint, P. K. 2012, "Dementia is strongly associated with 90-day mortality in lobar cerebral amyloid angiopathy related intra-cerebral haemorrhage", *Journal of the Neurological Sciences*, vol. 322, no. 1-2, pp. 161-165.

Janssen, P. M., Visser, N. A., Dorhout Mees, S. M., Klijn, C. J. M., Algra, A., & Rinkel, G. J. E. 2010, "Comparison of Telephone and Face-to-Face Assessment of the Modified Rankin Scale", *Cerebrovascular Diseases*, vol. 29, no. 2, pp. 137-139.

Jefferson, A. L., Lambe, S., Cook, E., Pimontel, M., Palmisano, J., & Chaisson, C. 2011, "Factors associated with African American and White elders' participation in a brain donation program", *Alzheimer Disease & Associated Disorders*, vol. 25, no. 1, pp. 11-16.

Jellinger, K. 1977, "Cerebrovascular amyloidosis with cerebral hemorrhage", *J Neurol*, vol. 214, no. 3, pp. 195-206.

Jellinger, K. 2006, "Spatial Distribution of Haemorrhages in Cerebral Amyloid Angiopathy", *Annals of Neurology*, vol. 59, no. 1, p. 215.

Jellinger, K. A., Lauda, F., & Attems, J. 2007, "Sporadic cerebral amyloid angiopathy is not a frequent cause of spontaneous brain hemorrhage", *European Journal of Neurology*, vol. 14, no. 8, pp. 923-928.

Johnson, K. A., Gregas, M., Becker, J. A., Kinnecom, C., Salat, D. H., Moran, E. K., Smith, E. E., Rosand, J., Rentz, D. M., Klunk, W. E., Mathis, C. A., Price, J. C., Dekosky, S. T., Fischman, A. J., & Greenberg, S. M. 2007, "Imaging of amyloid burden and distribution in cerebral amyloid angiopathy", *Ann.Neurol*, vol. 62, no. 3, pp. 229-234.

Jorm, A. F. 1994, "A short form of the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE): development and cross-validation.", *Psychological Medicine*, vol. 24, no. 1, pp. 145-153.

Juvela, S. 1995, "Risk factors for impaired outcome after spontaneous intracerebral hemorrhage", *Arch Neurol*, vol. 52, no. 12, pp. 1193-1200.

Kainth, A., Hewitt, A., Sowden, A., Duffy, S., Pattenden, J., Lewin, R., Watt, I., & Thompson, D. 2004, "Systematic review of interventions to reduce delay in patients with suspected heart attack", *Emergency Medicine Journal*, vol. 21, no. 4, pp. 506-508.

Karabatsou, K. L. 2007, "Cerebral amyloid angiopathy with symptomatic or occult subarachnoid haemorrhage [1]", *European Neurology*, vol. 57, no. 2, pp. 103-105.

Karapanayiotides, T., Piechowski-Jozwiak, B., van, M. G., Bogousslavsky, J., & Devuyst, G. 2004, "Stroke patterns, etiology, and prognosis in patients with diabetes mellitus", *Neurology*, vol. 62, no. 9, pp. 1558-1562.

Kawarabayashi, T., Shoji, M., Sato, M., Sasaki, A., Ho, L., Eckman, C. B., Prada, C. M., Younkin, S. G., Kobayashi, T., Tada, N., Matsubara, E., Iizuka, T., Harigaya, Y., Kasai, K., & Hirai, S. 1996, "Accumulation of beta-amyloid fibrils in pancreas of transgenic mice", *Neurobiol.Aging*, vol. 17, no. 2, pp. 215-222.

Kaye, J. A., Dame, A., Lehman, S., & Sexton, G. 1999, "Factors associated with brain donation among optimally healthy elderly people", *Journals of Gerontology Series A-Biological Sciences & Medical Sciences*, vol. 54, no. 11, p. M560-M564.

Keir, S. L., Wardlaw, J. M., Sandercock, P. A., & Chen, Z. 2002, "Antithrombotic therapy in patients with any form of intracranial haemorrhage: a systematic review of the available controlled studies", *Cerebrovasc Dis*, vol. 14, no. 3-4, pp. 197-206.

Kennedy, I. The report of the public inquiry into children's heart surgery at the Bristol Royal Infirmary 1984-1995: learning from Bristol. Department of Health . 2001. 11-12-2012.
Ref Type: Internet Communication

Kidwell, C. S. & Wintermark, M. 2008, "Imaging of intracranial haemorrhage", *Lancet Neurol*, vol. 7, no. 3, pp. 256-267.

Kikuta, K., Takagi, Y., Nozaki, K., Okada, T., & Hashimoto, N. 2007, "Histological analysis of microbleed after surgical resection in a patient with moyamoya disease", *Neurol Med Chir (Tokyo)*, vol. 47, no. 12, pp. 564-567.

Kim, B. J., Lee, S. H., Ryu, W. S., Kim, C. K., Chung, J. W., Kim, D., Park, H. K., & Yoon, B. W. 2013, "Extents of white matter lesions and increased intraventricular extension of intracerebral hemorrhage", *Crit Care Med*, vol. 41, no. 5, pp. 1325-1331.

Kim, J., Basak, J. M., & Holtzman, D. M. 2009, "The role of apolipoprotein E in Alzheimer's disease", *Neuron*, vol. 63, no. 3, pp. 287-303.

King, E. M.-F. O., Smith, A., & Jobst, K. 1993, "Autopsy: Consent, Completion and Communication in Alzheimer's Disease Research", *Age and Ageing*, vol. 22, pp. 209-214.

Knudsen, K. A., Rosand, J., Karluk, D., & Greenberg, S. M. 2001, "Clinical diagnosis of cerebral amyloid angiopathy: validation of the Boston criteria.", *Neurology*, vol. 56, no. 4, pp. 537-539.

Kosior, J. C., Idris, S., Dowlatshahi, D., Alzawahmah, M., Eesa, M., Sharma, P., Tymchuk, S., Hill, M. D., Aviv, R. I., Frayne, R., & Demchuk, A. M. 2011, "Quantomo: validation of a computer-assisted methodology for the volumetric analysis of intracerebral haemorrhage", *Int.J Stroke*, vol. 6, no. 4, pp. 302-305.

Kothari, R. U., Brott, T., Broderick, J., Barsan, W., Sauerbeck, L. R., Zuccarello, M., & Khoury, J. 1996, "The ABCs of measuring intracerebral hemorrhage volumes.", *Stroke*, vol. 27, no. 8, pp. 1304-1305.

Kottner, J., Audige, L., Brorson, S., Donner, A., Gajewski, B. J., Hrobjartsson, A., Roberts, C., Shoukri, M., & Streiner, D. L. 2011, "Guidelines for Reporting Reliability and Agreement Studies (GRRAS) were proposed", *J Clin Epidemiol.*, vol. 64, no. 1, pp. 96-106.

Kovari, E., Herrmann, F. R., Hof, P. R., & Bouras, C. 2013, "The relationship between cerebral amyloid angiopathy and cortical microinfarcts in brain ageing and Alzheimer's disease", *Neuropathol Appl.Neurobiol*, vol. 39, no. 5, pp. 498-509.

Kretzschmar, H. 2009, "Brain banking: opportunities, challenges and meaning for the future", *Nature Reviews Neuroscience*, vol. 10, no. 1, pp. 70-78.

Kuhta, T., Zadikoff, C., Simuni, T., Martel, A., Williams, K., & Videnovic, A. 2011, "Brain donation--what do patients with movement disorders know and how do they feel about it?", *Parkinsonism & Related Disorders*, vol. 17, no. 3, pp. 204-207.

Kumar, S., Goddeau, R. P., Jr., Selim, M. H., Thomas, A., Schlaug, G., Alhazzani, A., Searls, D. E., & Caplan, L. R. 2010, "Atraumatic convexal subarachnoid hemorrhage: clinical presentation, imaging patterns, and etiologies", *Neurology*, vol. 74, no. 11, pp. 893-899.

Kuramatsu, J. B., Sauer, R., Mauer, C., Lücking, H., Kloska, S. P., Kiphuth, I. C., Staykov, D., Köhrmann, M., & Huttner, H. B. 2011, "Correlation of age and haematoma volume in patients with spontaneous lobar intracerebral haemorrhage", *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 82, no. 2, pp. 144-149.

Labovitz, D. L., Halim, A., Boden-Albala, B., Hauser, W. A., & Sacco, R. L. 2005, "The incidence of deep and lobar intracerebral hemorrhage in whites, blacks, and Hispanics", *Neurology*, vol. 65, no. 4, pp. 518-522.

Laissy, J. P., Normand, G., Monroc, M., Duchateau, C., Alibert, F., & Thiebot, J. 1991, "Spontaneous intracerebral hematomas from vascular causes. Predictive value of CT compared with angiography", *Neuroradiology*, vol. 33, no. 4, pp. 291-295.

Lammie, G. A. 2002, "Hypertensive cerebral small vessel disease and stroke", *Brain Pathol.*, vol. 12, no. 3, pp. 358-370.

Landis, J. R. & Koch, G. G. 1977, "The measurement of observer agreement for categorical data.", *Biometrics*, vol. 33, pp. 159-174.

Lang, E. W., Ren, Y. Z., Preul, C., Hugo, H. H., Hempelmann, R. G., Buhl, R., Barth, H., Klinge, H., & Mehdorn, H. M. 2001, "Stroke pattern interpretation: the variability of hypertensive versus amyloid angiopathy hemorrhage", *Cerebrovascular Diseases*, vol. 12, no. 2, pp. 121-130.

Larrue, V., von Kummer, R., del Zoppo, G., & Bluhmki, E. 1997, "Hemorrhagic transformation in acute ischemic stroke. Potential contributing factors in the European Cooperative Acute Stroke Study", *Stroke*, vol. 28, pp. 957-960.

Lavados, P. M., Sacks, C., Prina, L., Escobar, A., Tossi, C., Araya, F., Feuerhake, W., Galvez, M., Salinas, R., & Alvarez, G. 2010, "Incidence of lobar and non-lobar spontaneous intracerebral haemorrhage in a predominantly Hispanic-Mestizo population-the PISCIS stroke project: a community-based prospective study in Iquique, Chile", *Neuroepidemiology*, vol. 34, no. 4, pp. 214-221.

Lee, E. J. Y. 2002, "The Empty Delta Sign", *Radiology*, vol. 224, no. 3, pp. 788-789.

Lee, S. H., Bae, H. J., Kwon, S. J., Kim, H., Kim, Y. H., Yoon, B. W., & Roh, J. K. 2004, "Cerebral microbleeds are regionally associated with intracerebral hemorrhage", *Neurology*, vol. 62, no. 1, pp. 72-76.

LeRoux, P. D., Haglund, M. M., Newell, D. W., Grady, M. S., & Winn, H. R. 1992, "Intraventricular hemorrhage in blunt head trauma: an analysis of 43 cases", *Neurosurgery*, vol. 31, no. 4, pp. 678-684.

Lewis, C. M. 2002, "Genetic association studies: design, analysis and interpretation", *Brief.Bioinform.*, vol. 3, no. 2, pp. 146-153.

Lewis, S. 2007, "Regression analysis", *Pract.Neurol*, vol. 7, no. 4, pp. 259-264.

Li, Y. F., Luo, J., Li, Q., Jing, Y. J., Wang, R. Y., & Li, R. S. 2012, "A new simple model for prediction of hospital mortality in patients with intracerebral hemorrhage", *CNS Neurosci.Ther.*, vol. 18, no. 6, pp. 482-486.

Linn, J., Halpin, A., Demaerel, P., Ruhland, J., Giese, A. D., Dichgans, M., van Buchem, M. A., Bruckmann, H., & Greenberg, S. M. 2010, "Prevalence of superficial siderosis in patients with cerebral amyloid angiopathy", *Neurology*, vol. 74, no. 17, pp. 1346-1350.

Linn, J., Herms, J., Dichgans, M., Bruckmann, H., Fesl, G., Freilinger, T., & Wiesmann, M. 2008, "Subarachnoid hemosiderosis and superficial cortical hemosiderosis in cerebral amyloid angiopathy", *Ajnr: American Journal of Neuroradiology*, vol. 29, no. 1, pp. 184-186.

Linstone, H. A. & Turoff, M. 1975, *The Delphi method: techniques and applications* Addison-Wesley Publishing Co..

Lipton, R. B., Berger, A. R., Lesser, M. L., Lantos, G., & Portenoy, R. K. 1987, "Lobar vs thalamic and basal ganglion hemorrhage: clinical and radiographic features", *J.Neurol*, vol. 234, no. 2, pp. 86-90.

Lisk, D. R., Pasteur, W., Rhoades, H., Putnam, R. D., & Grotta, J. C. 1994, "Early presentation of hemispheric intracerebral hemorrhage: prediction of outcome and guidelines for treatment allocation", *Neurology*, vol. 44, no. 1, pp. 133-139.

Little, J., Bradley, L., Bray, M. S., Clyne, M., Dorman, J., Ellsworth, D. L., Hanson, J., Khoury, M., Lau, J., O'Brien, T. R., Rothman, N., Stroup, D., Taioli, E., Thomas, D., Vainio, H., Wacholder, S., & Weinberg, C. 2002, "Reporting, appraising, and integrating data on genotype prevalence and gene-disease associations", *Am.J.Epidemiol.*, vol. 156, no. 4, pp. 300-310.

Little, J., Higgins, J. P., Ioannidis, J. P., Moher, D., Gagnon, F., von, E. E., Khoury, M. J., Cohen, B., Davey-Smith, G., Grimshaw, J., Scheet, P., Gwinn, M., Williamson, R. E., Zou, G. Y., Hutchings, K., Johnson, C. Y., Tait, V., Wiens, M., Golding, J., van, D. C., McLaughlin, J., Paterson, A., Wells, G., Fortier, I., Freedman, M., Zecevic, M., King, R., Infante-Rivard, C., Stewart, A., & Birkett, N. 2009, "STrengthening the REporting of Genetic Association Studies (STREGA): an extension of the STROBE statement", *PLoS Med*, vol. 6, no. 2, p. e22.

Loehrer, E., Ikram, M. A., Akoudad, S., Vrooman, H. A., van der, L. A., Niessen, W. J., Hofman, A., & Vernooij, M. W. 2014, "Apolipoprotein E genotype influences spatial distribution of cerebral microbleeds", *Neurobiol Aging*, vol. 35, no. 4, pp. 899-905.

Love, S. 2011, "Autopsy approach to stroke", *Histopathology*, vol. 58, pp. 333-351.

Love, S., Chalmers, K., Ince, P., Esiri, M., Attems, J., Jellinger, K., Yamada, M., McCarron, M., Minett, T., Matthews, F., Greenberg, S., Mann, D., & Kehoe, P. G. 2014,

"Development, appraisal, validation and implementation of a consensus protocol for the assessment of cerebral amyloid angiopathy in post-mortem brain tissue", *Am J Neurodegener.Dis*, vol. 3, no. 1, pp. 19-32.

Lovelock, C. E., Anslow, P., Molyneux, A. J., Byrne, J. V., Kuker, W., Pretorius, P., Coull, A., & Rothwell, P. 2009, "Substantial Observer Variability in the Differentiation Between Primary Intracerebral Hemorrhage and Hemorrhagic Transformation of Infarction on CT Brain Imaging", *Stroke*, vol. 40, pp. 3763-3767.

Lovelock, C. E., Molyneux, A. J., & Rothwell, P. 2007, "Change in incidence and aetiology of intracerebral haemorrhage in Oxfordshire, UK, between 1981 and 2006: a population-based study", *The Lancet Neurology*, vol. 6, no. 6, pp. 487-493.

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S. Y., Alvarado, M., Anderson, H. R., Anderson, L. M., Andrews, K. G., Atkinson, C., Baddour, L. M., Barker-Collo, S., Bartels, D. H., Bell, M. L., Benjamin, E. J., Bennett, D., Bhalla, K., Bikbov, B., Bin, A. A., Birbeck, G., Blyth, F., Bolliger, I., Boufous, S., Bucello, C., Burch, M., Burney, P., Carapetis, J., Chen, H., Chou, D., Chugh, S. S., Coffeng, L. E., Colan, S. D., Colquhoun, S., Colson, K. E., Condon, J., Connor, M. D., Cooper, L. T., Corriere, M., Cortinovis, M., de Vaccaro, K. C., Couser, W., Cowie, B. C., Criqui, M. H., Cross, M., Dabhadkar, K. C., Dahodwala, N., De, L. D., Degenhardt, L., Delossantos, A., Denenberg, J., Des, J., Dharmaratne, S. D., Dorsey, E. R., Driscoll, T., Duber, H., Ebel, B., Erwin, P. J., Espindola, P., Ezzati, M., Feigin, V., Flaxman, A. D., Forouzanfar, M. H., Fowkes, F. G., Franklin, R., Fransen, M., Freeman, M. K., Gabriel, S. E., Gakidou, E., Gaspari, F., Gillum, R. F., Gonzalez-Medina, D., Halasa, Y. A., Haring, D., Harrison, J. E., Havmoeller, R., Hay, R. J., Hoen, B., Hotez, P. J., Hoy, D., Jacobsen, K. H., James, S. L., Jasrasaria, R., Jayaraman, S., Johns, N., Karthikeyan, G., Kassebaum, N., Keren, A., Khoo, J. P., Knowlton, L. M., Kobusingye, O., Koranteng, A., Krishnamurthi, R., Lipnick, M., Lipshultz, S. E., Ohno, S. L., Mabweijano, J., MacIntyre, M. F., Mallinger, L., March, L., Marks, G. B., Marks, R., Matsumori, A., Matzopoulos, R., Mayosi, B. M., McAnulty, J. H., McDermott, M. M., McGrath, J., Mensah, G. A., Merriman, T. R., Michaud, C., Miller, M., Miller, T. R., Mock, C., Mocumbi, A. O., Mokdad, A. A., Moran, A., Mulholland, K., Nair, M. N., Naldi, L., Narayan, K. M., Nasseri, K., Norman, P., O'Donnell, M., Omer, S. B., Ortblad, K., Osborne, R., Ozgediz, D., Pahari, B., Pandian, J. D., Rivero, A. P., Padilla, R. P., Perez-Ruiz, F., Perico, N., Phillips, D., Pierce, K., Pope, C. A., III, Porrini, E., Pourmalek, F., Raju, M., Ranganathan, D., Rehm, J. T., Rein, D. B., Remuzzi, G., Rivara, F. P., Roberts, T., De Leon, F. R., Rosenfeld, L. C., Rushton, L., Sacco, R. L., Salomon, J. A., Sampson, U., Sanman, E., Schwebel, D. C., Segui-Gomez, M., Shepard, D. S., Singh, D., Singleton, J., Sliwa, K., Smith, E., Steer, A., Taylor, J. A., Thomas, B., Tleyjeh, I. M., Towbin, J. A., Truelsen, T., Undurraga, E. A., Venketasubramanian, N., Vijayakumar, L., Vos, T., Wagner, G. R., Wang, M., Wang, W., Watt, K., Weinstock, M. A., Weintraub, R., Wilkinson, J. D., Woolf, A. D., Wulf, S., Yeh, P. H., Yip, P., Zabetian, A., Zheng, Z. J., Lopez, A. D., & Murray, C. J. 2013, "Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010", *Lancet*, vol. 380, no. 9859, pp. 2095-2128.

Lunetta, K. L. 2008, "Genetic association studies", *Circulation*, vol. 118, no. 1, pp. 96-101.

Ly, J. V., Donnan, G. A., Villemagne, V. L., Zavala, J. A., Ma, H., O'Keefe, G., Gong, S. J., Gunawan, R. M., Saunder, T., Ackerman, U., Tochon-Danguy, H., Churilov, L., Phan, T. G., & Rowe, C. C. 2010, "11C-PIB binding is increased in patients with cerebral amyloid angiopathy-related hemorrhage", *Neurology*, vol. 74, no. 6, pp. 487-493.

Maas, M. B., Nemeth, A. J., Rosenberg, N. F., Kosteva, A. R., Guth, J. C., Liotta, E. M., Prabhakaran, S., & Naidech, A. M. 2013a, "Subarachnoid extension of primary intracerebral hemorrhage is associated with poor outcomes", *Stroke*, vol. 44, no. 3, pp. 653-657.

Maas, M. B., Nemeth, A. J., Rosenberg, N. F., Kosteva, A. R., Prabhakaran, S., & Naidech, A. M. 2013b, "Delayed intraventricular hemorrhage is common and worsens outcomes in intracerebral hemorrhage", *Neurology*, vol. 80, no. 14, pp. 1295-1299.

Manchester, K. L. 1995, "Value of A260/A280 ratios for measurement of purity of nucleic acids", *Biotechniques*, vol. 19, no. 2, pp. 208-210.

Mandybur, T. I. 1975, "The incidence of cerebral amyloid angiopathy in Alzheimer's disease", *Neurology*, vol. 25, no. 2, pp. 120-126.

Mann, D. M., Pickering-Brown, S. M., Takeuchi, A., & Iwatsubo, T. 2001, "Amyloid angiopathy and variability in amyloid beta deposition is determined by mutation position in presenilin-1-linked Alzheimer's disease", *Am.J.Pathol.*, vol. 158, no. 6, pp. 2165-2175.

Marti-Fabregas, J., Delgado-Mederos, R., Marin, R., de la Ossa, N. P., onso de, L. M., Rodriguez-Yanez, M., Sanahuja, J., Purroy, F., De Arce, A. M., Carrera, D., Dinia, L., Guardia-Laguarta, C., & Lleo, A. 2014, "Prognostic value of plasma beta-amyloid levels in patients with acute intracerebral hemorrhage", *Stroke*, vol. 45, no. 2, pp. 413-417.

Martinez-Gonzalez, N. A. & Sudlow, C. L. 2006, "Effects of apolipoprotein E genotype on outcome after ischaemic stroke, intracerebral haemorrhage and subarachnoid haemorrhage", *J.Neurol Neurosurg.Psychiatry*, vol. 77, no. 12, pp. 1329-1335.

Martini, S. R., Flaherty, M. L., Brown, W. M., Haverbusch, M., Comeau, M. E., Sauerbeck, L. R., Kissela, B. M., Deka, R., Kleindorfer, D. O., Moomaw, C. J., Broderick, J. P., Langefeld, C. D., & Woo, D. 2012, "Risk factors for intracerebral hemorrhage differ according to hemorrhage location", *Neurology*, vol. 79, no. 23, pp. 2275-2282.

Massaro, A. R., Sacco, R. L., Mohr, J. P., Foulkes, M. A., Tatemichi, T. K., Price, T. R., Hier, D. B., & Wolf, P. A. 1991, "Clinical discriminators of lobar and deep hemorrhages: the Stroke Data Bank", *Neurology*, vol. 41, no. 12, pp. 1881-1885.

Mastaglia, F. L., Byrnes, M. L., Johnsen, R. D., & Kakulas, B. A. 2003, "Prevalence of cerebral vascular amyloid-beta deposition and stroke in an aging Australian population: a postmortem study", *Journal of Clinical Neuroscience*, vol. 10, no. 2, pp. 186-189.

Masuda, J., Tanaka, K., Ueda, K., & Omae, T. 1988, "Autopsy study of incidence and distribution of cerebral amyloid angiopathy in Hisayama, Japan", *Stroke*, vol. 19, no. 2, pp. 205-210.

Matsukawa, H., Shinoda, M., Fujii, M., Takahashi, O., Yamamoto, D., Murakata, A., & Ishikawa, R. 2012, "Factors associated with lobar vs. non-lobar intracerebral hemorrhage", *Acta Neurol Scand.*, vol. 126, no. 2, pp. 116-121.

Matthews, F. E., Brayne, C., Lowe, J., McKeith, I., Wharton, S. B., & Ince, P. 2009, "Epidemiological pathology of dementia: attributable-risks at death in the Medical Research Council Cognitive Function and Ageing Study", *PLoS Medicine / Public Library of Science*, vol. 6, no. 11, p. e1000180.

McArthur, K., Beagan, M. L. C., Degnan, A., Howarth, R. C., Mitchell, K. A., McQuaige, F. B., Shannon, M. A. C., Stott, D. J., & Quinn, T. J. 2012, "Properties of proxy-derived modified Rankin Scale assessment", *International Journal of Stroke*.

McCarron, M. O., Nicoll, J. A., & Graham, D. I. 1998, "A quartet of Down's syndrome, Alzheimer's disease, cerebral amyloid angiopathy, and cerebral haemorrhage: interacting genetic risk factors", *J.Neurol Neurosurg.Psychiatry*, vol. 65, no. 3, pp. 405-406.

McCarron, M. O., Nicoll, J. A., Stewart, J., Ironside, J. W., Mann, D. M., Love, S., Graham, D. I., & Dewar, D. 1999a, "The apolipoprotein E epsilon2 allele and the pathological features in cerebral amyloid angiopathy-related hemorrhage", *J Neuropathol Exp Neurol*, vol. 58, no. 7, pp. 711-718.

McCarron, M. O., Nicoll, J. A. R., Ironside, J. W., Love, S., Alberts, M. J., & Bone, I. 1999b, "Cerebral Amyloid Angiopathy–Related Hemorrhage: Interaction of Apo e2 with putative clinical risk factors", *Stroke*, vol. 30, no. 8, pp. 1643-1646.

McGowan, E., Pickford, F., Kim, J., Onstead, L., Eriksen, J., Yu, C., Skipper, L., Murphy, M. P., Beard, J., Das, P., Jansen, K., Delucia, M., Lin, W. L., Dolios, G., Wang, R., Eckman, C. B., Dickson, D. W., Hutton, M., Hardy, J., & Golde, T. 2005, "Abeta42 is essential for parenchymal and vascular amyloid deposition in mice", *Neuron*, vol. 47, no. 2, pp. 191-199.

Mclean, S. A. M. Independent review group on the retention of organs at post-mortem. The Scottish Government . 2003. 11-12-2012.
Ref Type: Internet Communication

McNamee, R. 2005, "Regression modelling and other methods to control confounding", *Occupational and Environmental Medicine*, vol. 62, no. 7, pp. 500-506.

Mead, G. E., Wardlaw, J. M. W., Dennis, M., & Lewis, S. C. 2002, "Extensive haemorrhagic transformation of infarct: might it be an important cause of primary intracerebral haemorrhage?", *Age and Ageing*, vol. 31, pp. 429-433.

Medical Research Council 2009, "A network of brain banks fit for the future", *Lancet Neurol*, vol. 8, no. 8, p. 691.

Mendis, S., Thygesen, K., Kuulasmaa, K., Giampaoli, S., Mahonen, M., Ngu Blackett, K., Lisheng, L., & Writing group on behalf of the participating experts of the WHO consultation for revision of WHO definition of myocardial infarction 2010, "World

Health Organization definition of myocardial infarction: 2008–09 revision", *International Journal of Epidemiology*.

Meretoja, A., Strbian, D., Putaala, J., Curtze, S., Haapaniemi, E., Mustanoja, S., Sairanen, T., Satopaa, J., Silvennoinen, H., Niemela, M., Kaste, M., & Tatlisumak, T. 2012, "SMASH-U: a proposal for etiologic classification of intracerebral hemorrhage", *Stroke*, vol. 43, no. 10, pp. 2592-2597.

Millar, J. H., Wardlaw, J. M., & Lammie, G. A. 1999, "Intracerebral haemorrhage and cerebral amyloid angiopathy: CT features with pathological correlation", *Clinical Radiology*, vol. 54, pp. 422-429.

Millar, T., Walker, R., Arango, J. C., Ironside, J. W., Harrison, D. J., MacIntyre, D. J., Blackwood, D., Smith, C., & Bell, J. E. 2007, "Tissue and organ donation for research in forensic pathology: the MRC Sudden Death Brain and Tissue Bank", *Journal of Pathology*, vol. 213, no. 4, pp. 369-375.

Minakawa, T., Takeuchi, S., Sasaki, O., Koizumi, T., Honad, Y., Fujii, Y., Ozawa, T., Ogawa, H., Koike, T., & Tanaka, R. 1995, "Surgical experience with massive lobar haemorrhage caused by cerebral amyloid angiopathy", *Acta Neurochir.(Wien.)*, vol. 132, no. 1-3, pp. 48-52.

Miners, J. S., Kehoe, P., & Love, S. 2011, "Neprilysin protects against cerebral amyloid angiopathy and Abeta-induced degeneration of cerebrovascular smooth muscle cells", *Brain Pathol.*, vol. 21, no. 5, pp. 594-605.

Mittal, M. K. & Lele, A. 2011, "Predictors of poor outcome at hospital discharge following a spontaneous intracerebral hemorrhage", *Int.J.Neurosci.*, vol. 121, no. 5, pp. 267-270.

Miyahara, K., Murata, H., & Abe, H. 2007, "Predictors of intracranial hematoma enlargement in patients undergoing hemodialysis", *Neurol Med Chir (Tokyo)*, vol. 47, no. 2, pp. 47-51.

Monoranu, C. M., Apfelbacher, M., Grunblatt, E., Puppe, B., Alafuzoff, I., Ferrer, I., Al-Saraj, S., Keyvani, K., Schmitt, A., Falkai, P., Schittenhelm, J., Halliday, G., Kril, J., Harper, C., McLean, C., Riederer, P., & Roggendorf, W. 2009, "pH measurement as quality control on human post mortem brain tissue: a study of the BrainNet Europe consortium", *Neuropathol.Appl.Neurobiol.*, vol. 35, no. 3, pp. 329-337.

Moser, D. K., Kimble, L. P., Alberts, M. J., Alonzo, A., Croft, J. B., Dracup, K., Evenson, K. R., Go, A. S., Hand, M. M., Kothari, R. U., Mensah, G. A., Morris, D. L., Pancioli, A. M., Riegel, B., & Zerwic, J. J. 2006, "Reducing Delay in Seeking Treatment by Patients With Acute Coronary Syndrome and Stroke", *Circulation*, vol. 114, no. 2, pp. 168-182.

Muir, K. W., Buchan, A., von Kummer, R., Rother, J., & Baron, J.-C. 2006, "Imaging of acute stroke", *The Lancet Neurology*, vol. 5, pp. 755-768.

Muir, K. W. & Santosh, C. 2005, "Imaging of acute stroke and transient ischaemic attack", *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 76, no. suppl 3, p. iii19-iii28.

Nag, C., Das, K., Ghosh, M., & Khandakar, M. R. 2012, "Prediction of Clinical Outcome in Acute Hemorrhagic Stroke from a Single CT Scan on Admission", *N Am.J.Med Sci.*, vol. 4, no. 10, pp. 463-467.

Nagasawa, S., Handa, H., Okumura, A., Naruo, Y., Moritake, K., & Hayashi, K. 1979, "Mechanical properties of human cerebral arteries. Part 1: Effects of age and vascular smooth muscle activation", *Surg.Neurol*, vol. 12, no. 4, pp. 297-304.

Naito, K. S., Sekijima, Y., & Ikeda, S. 2008, "Cerebral amyloid angiopathy-related hemorrhage in a middle-aged patient with Down's syndrome", *Amyloid*, vol. 15, no. 4, pp. 275-277.

National Institute for Health and Clinical Excellence (NICE) 2010, *Venous thromoboembolism - reducing the risk*.

Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) 2001, "Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales.", *Lancet*, vol. 357, no. 9251, pp. 169-175.

Newcombe, R. 1998, "Two-sided confidence intervals for the single proportion: comparison of seven methods", *Statistics in Medicine*, vol. 17, pp. 857-872.

Ng, T. H., Leung, S. Y., & Wong, M. P. 1991, "Cerebral amyloid angiopathy in Chinese: incidence and significance", *Clinical Neurology & Neurosurgery*, vol. 93, no. 1, pp. 19-23.

Nicoll, J. A., Yamada, M., Frackowiak, J., Mazur-Kolecka, B., & Weller, R. O. 2004, "Cerebral amyloid angiopathy plays a direct role in the pathogenesis of Alzheimer's disease. Pro-CAA position statement. [Review] [61 refs]", *Neurobiology of Aging*, vol. 25, no. 5, pp. 589-597.

Nilsson, O. G., Lindgren, A., Stahl, N., Brandt, L., & Säveland, H. 2000, "Incidence of intracerebral and subarachnoid haemorrhage in southern Sweden", *J.Neurol Neurosurg.Psychiatry*, vol. 69, no. 5, pp. 601-607.

Nilsson, O. G., Lindgren, A., Brandt, L., & Säveland, H. 2002, "Prediction of death in patients with primary intracerebral hemorrhage: a prospective study of a defined population", *Journal of Neurosurgery*, vol. 97, no. 3, pp. 531-536.

Office for National Statistics 2012, *Chapter 2: Population Change*.

Oide, T., Takahashi, H., Yutani, C., Ishihara, T., & Ikeda, S. 2003, "Relationship between lobar intracerebral hemorrhage and leukoencephalopathy associated with cerebral amyloid angiopathy: clinicopathological study of 64 Japanese patients", *Amyloid*, vol. 10, no. 3, pp. 136-143.

Okamoto, Y., Yamamoto, T., Kalaria, R. N., Senzaki, H., Maki, T., Hase, Y., Kitamura, A., Washida, K., Yamada, M., Ito, H., Tomimoto, H., Takahashi, R., & Ihara, M. 2012, "Cerebral hypoperfusion accelerates cerebral amyloid angiopathy and promotes cortical microinfarcts", *Acta Neuropathol*, vol. 123, no. 3, pp. 381-394.

Okazaki, H., Reagan, T. J., & Campbell, R. J. 1979, "Clinicopathologic studies of primary cerebral amyloid angiopathy", *Mayo Clinic Proceedings*, vol. 54, no. 1, pp. 22-31.

Olichney, J. M., Hansen, L. A., Galasko, D., Saitoh, T., Hofstetter, C. R., Katzman, R., & Thal, L. J. 1996, "The apolipoprotein E epsilon 4 allele is associated with increased neuritic plaques and cerebral amyloid angiopathy in Alzheimer's disease and Lewy body variant", *Neurology*, vol. 47, no. 1, pp. 190-196.

Olichney, J. M., Hansen, L. A., Hofstetter, C. R., Grundman, M., Katzman, R., & Thal, L. J. 1995, "Cerebral infarction in Alzheimer's disease is associated with severe amyloid angiopathy and hypertension", *Archives of Neurology*, vol. 52, no. 7, pp. 702-708.

Ooneda, G., Yoshida, Y., Suzuki, K., & Sekiguchi, T. 1973, "Morphogenesis of plasmatic arterionecrosis as the cause of hypertensive intracerebral hemorrhage", *Virchows Arch A Pathol Pathol Anat.*, vol. 361, no. 1, pp. 31-38.

Oppenheim, G. 1909, "Uber "drusige Nekrosen" in der Großhirnrinde", *Neurol Centralbl.*, vol. 28, pp. 410-413.

Oshima, K., Akiyama, H., Tsuchiya, K., Kondo, H., Haga, C., Shimomura, Y., Iseki, E., Uchikado, H., Kato, M., Niizato, K., & Arai, H. 2006, "Relative paucity of tau accumulation in the small areas with abundant Abeta42-positive capillary amyloid angiopathy within a given cortical region in the brain of patients with Alzheimer pathology", *Acta Neuropathol*, vol. 111, no. 6, pp. 510-518.

Paciaroni, M., Agnelli, G., Corea, F., Ageno, W., Alberti, A., Lanari, A., Caso, V., Micheli, S., Bertolani, L., Venti, M., Palmerini, F., Biagini, S., Comi, G., Previdi, P., & Silvestrelli, G. 2008, "Early Hemorrhagic Transformation of Brain Infarction: Rate, Predictive Factors, and Influence on Clinical Outcome", *Stroke*, vol. 39, no. 8, pp. 2249-2256.

Palm, F., Henschke, N., Wolf, J., Zimmer, K., Safer, A., Schroder, R. J., Inselmann, G., Brenke, C., Becher, H., & Grau, A. J. 2013, "Intracerebral haemorrhage in a population-based stroke registry (LuSSt): incidence, aetiology, functional outcome and mortality", *J Neurol*, vol. 260, no. 10, pp. 2541-2550.

Pantelakis, S. 1954, "Un type particulier d'angiopathie sénile du système nerveux central: l'angiopathie congophile. Topographie et fréquence", *Monatsschr Psychiatr Neurol*, vol. 128, pp. 219-256.

Pantoni, L. 2010, "Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges", *Lancet Neurol*, vol. 9, no. 7, pp. 689-701.

Parameswaran, B., Gregson, B., Kuskoor, S. M. P., Mitchell, P., Soh, C., Mitra, D., Gholkar, A., & Mendelow, A. 2003, "Reliability assessment of computerized

tomography scanning measurements in intracerebral hematoma", *Neurosurgical Focus*, vol. 15, no. 4, p. E6.

Parizel, P. M., Makkat, S., Van, M. E., Van Goethem, J. W., van den, H. L., & De Schepper, A. M. 2001, "Intracranial hemorrhage: principles of CT and MRI interpretation", *Eur Radiol.*, vol. 11, no. 9, pp. 1770-1783.

Park, J. H., Seo, S. W., Kim, C., Kim, G. H., Noh, H. J., Kim, S. T., Kwak, K. C., Yoon, U., Lee, J. M., Lee, J. W., Shin, J. S., Kim, C. H., Noh, Y., Cho, H., Kim, H. J., Yoon, C. W., Oh, S. J., Kim, J. S., Choe, Y. S., Lee, K. H., Lee, J. H., Ewers, M., Weiner, M. W., Werring, D. J., & Na, D. L. 2013, "Pathogenesis of cerebral microbleeds: In vivo imaging of amyloid and subcortical ischemic small vessel disease in 226 individuals with cognitive impairment", *Ann.Neurol*, vol. 73, no. 5, pp. 584-593.

Passero, S., Burgalassi, L., D'Andrea, P., & Battistini, N. 1995, "Recurrence of bleeding in patients with primary intracerebral hemorrhage", *Stroke*, vol. 26, no. 7, pp. 1189-1192.

Patel, D. V., Hier, D. B., Thomas, C. M., & Hemmati, M. 1984, "Intracerebral hemorrhage secondary to cerebral amyloid angiopathy", *Radiology*, vol. 151, no. 2, pp. 397-400.

Patel, P., FitzMaurice, E., Nandigam, R. N. K., Auluck, P., Viswanathan, A., Goldstein, J. N., Rosand, J., Greenberg, S. M., & Smith, E. E. 2009, "Association of Subdural Hematoma With Increased Mortality in Lobar Intracerebral Hemorrhage", *Archives of Neurology*, vol. 66, no. 1, pp. 79-84.

Payne, E. M., Fetzer, D. L., MacFall, J. R., Provenzale, J. M., Byrum, C. E., & Krishnan, K. R. R. 2002, "Development of a semi-automated method for quantification of MRI gray and white matter lesions in geriatric subjects", *Psychiatry Research Neuroimaging*, vol. 115, pp. 63-77.

Peck, G., Smeeth, L., Whittaker, J., Casas, J. P., Hingorani, A., & Sharma, P. 2008, "The genetics of primary haemorrhagic stroke, subarachnoid haemorrhage and ruptured intracranial aneurysms in adults", *PLoS One*, vol. 3, no. 11, p. e3691.

Peduzzi, P., Concato, J., Kemper, E., Holford, T. R., & Feinstein, A. R. 1996, "A simulation study of the number of events per variable in logistic regression analysis", *J Clin Epidemiol.*, vol. 49, no. 12, pp. 1373-1379.

Pena, F., Gutierrez-Lerma, A., Quiroz-Baez, R., & Arias, C. 2006, "The role of beta-amyloid protein in synaptic function: implications for Alzheimer's disease therapy", *Curr.Neuropharmacol.*, vol. 4, no. 2, pp. 149-163.

Penke, L., Valdes Hernandez, M. C., Munoz Maniega, S., Gow, A. J., Murray, C., Starr, J. M., Bastin, M. E., Deary, I. J., & Wardlaw, J. M. 2012, "Brain iron deposits are associated with general cognitive ability and cognitive aging", *Neurobiology of Aging*, vol. 33, pp. 510-517.

Pezzini, A., Grassi, M., Paciaroni, M., Zini, A., Silvestrelli, G., Iacoviello, L., Di, C. A., Del, Z. E., Caso, V., Nichelli, P. F., Giossi, A., Volonghi, I., Simone, A. M., Lanari, A.,

Costa, P., Poli, L., Pentore, R., Falzone, F., Gamba, M., Morotti, A., Ciccone, A., Ritelli, M., Guido, D., Colombi, M., De, G. G., Agnelli, G., & Padovani, A. 2013, "Obesity and the risk of intracerebral hemorrhage: the multicenter study on cerebral hemorrhage in Italy", *Stroke*, vol. 44, no. 6, pp. 1584-1589.

Pfeifer, L. A., White, L. R., Ross, G. W., Petrovitch, H., & Launer, L. J. 2002, "Cerebral amyloid angiopathy and cognitive function: the HAAS autopsy study", *Neurology*, vol. 58, no. 11, pp. 1629-1634.

Plagnol, V., Cooper, J. D., Todd, J. A., & Clayton, D. G. 2007, "A method to address differential bias in genotyping in large-scale association studies", *PLoS Genet*, vol. 3, no. 5, p. e74.

Poon, M. T., Fonville, A. F., & Al-Shahi Salman, R. 2013, "Long-term prognosis after intracerebral haemorrhage: systematic review and meta-analysis", *J Neurol Neurosurg.Psychiatry*, vol. 85, pp. 660-667.

Portenoy, R. K., Lipton, R. B., Berger, A. R., Lesser, M. L., & Lantos, G. 1987, "Intracerebral haemorrhage: a model for the prediction of outcome", *J.Neurol Neurosurg.Psychiatry*, vol. 50, no. 8, pp. 976-979.

Potter, G. M., Doubal, F., Jackson, C., Chappell, F., Sudlow, C. L. M., Dennis, M., & Wardlaw, J. M. 2010, "Counting Cavitating Lacunes underestimates the burden of Lacunar Infarction", *Stroke*, vol. 41, pp. 267-272.

Premkumar, D. R., Cohen, D. L., Hedera, P., Friedland, R. P., & Kalaria, R. N. 1996, "Apolipoprotein E-epsilon4 alleles in cerebral amyloid angiopathy and cerebrovascular pathology associated with Alzheimer's disease", *American Journal of Pathology*, vol. 148, no. 6, pp. 2083-2095.

Quinn, T. J., Dawson, J., Walters, M. R., & Lees, K. R. 2009, "Functional outcome measures in contemporary stroke trials", *International Journal of Stroke*, vol. 4, pp. 200-206.

Quinn, T. J., Ray, G., Atula, S., Walters, M. R., Dawson, J., & Lees, K. R. 2008, "Deriving modified Rankin scores from medical case-records", *Stroke*, vol. 39, pp. 3421-3423.

Radberg, J. A., Olsson, J. E., & Radberg, C. T. 1991, "Prognostic parameters in spontaneous intracerebral hematomas with special reference to anticoagulant treatment", *Stroke*, vol. 22, no. 5, pp. 571-576.

Rankin, J. 1957, "Cerebral vascular accidents in patients over the age of 60. II. Prognosis", *Scott.Med J*, vol. 2, no. 5, pp. 200-215.

Rannikmae, K., Kalaria, R. N., Greenberg, S. M., Chui, H. C., Schmitt, F. A., Samarasekera, N., Al-Shahi Salman, R., & Sudlow, C. L. 2013a, "APOE associations with severe CAA-associated vasculopathic changes: collaborative meta-analysis", *J Neurol Neurosurg.Psychiatry*.

Rannikmae, K., Samarasekera, N., Martinez-Gonzalez, N., Annan, R., Al-Shahi Salman, R., & Sudlow, C. 2013b, "Genetic associations of cerebral amyloid angiopathy-systematic review and meta-analysis", *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 84, no. 8, pp. 901-908.

Raposo, N., Viguier, A., Cuvinciuc, V., Calviere, L., Cognard, C., Bonneville, F., & Larrue, V. 2011, "Cortical subarachnoid haemorrhage in the elderly: a recurrent event probably related to cerebral amyloid angiopathy", *Eur.J.Neurol*, vol. 18, no. 4, pp. 597-603.

Rebeck, G. W., Reiter, J. S., Strickland, D. K., & Hyman, B. T. 1993, "Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions", *Neuron*, vol. 11, no. 4, pp. 575-580.

Redfern, M. The Royal Liverpool Children's Inquiry: summary and recommendations. Department of Health . 2001. 12-12-2012.
Ref Type: Internet Communication

Revesz, T., Holton, J. L., Lashley, T., Plant, G., Frangione, B., Rostagno, A., & Ghiso, J. 2009, "Genetics and molecular pathogenesis of sporadic and hereditary cerebral amyloid angiopathies", *Acta Neuropathol.*, vol. 118, no. 1, pp. 115-130.

Richard, E., Carrano, A., Hoozemans, J. J., van, H. J., van Haastert, E. S., Eurelings, L. S., de Vries, H. E., Thal, D. R., Eikelenboom, P., van Gool, W. A., & Rozemuller, A. J. 2010, "Characteristics of dyshoric capillary cerebral amyloid angiopathy", *J Neuropathol Exp Neurol*, vol. 69, no. 11, pp. 1158-1167.

Rinkel, G. J. 2005, "Intracranial aneurysm screening: indications and advice for practice", *Lancet Neurol*, vol. 4, no. 2, pp. 122-128.

Ritter, M. A., Droste, D. W., Hegedus, K., Szepesi, R., Nabavi, D. G., Csiba, L., & Ringelstein, E. B. 2005, "Role of cerebral amyloid angiopathy in intracerebral hemorrhage in hypertensive patients", *Neurology*, vol. 64, no. 7, pp. 1233-1237.

Roberts, I. S., Benamore, R. E., Benbow, E. W., Lee, S. H., Harris, J. N., Jackson, A., Mallett, S., Patankar, T., Peebles, C., Roobottom, C., & Traill, Z. C. 2012, "Post-mortem imaging as an alternative to autopsy in the diagnosis of adult deaths: a validation study", *Lancet*, vol. 379, no. 9811, pp. 136-142.

Rodriguez-Luna, D., Rubiera, M., Ribo, M., Coscojuela, P., Pineiro, S., Pagola, J., Hernandez-Guillamon, M., Ibarra, B., Romero, F., varez-Sabin, J., Montaner, J., & Molina, C. A. 2011, "Ultraearly hematoma growth predicts poor outcome after acute intracerebral hemorrhage", *Neurology*, vol. 77, no. 17, pp. 1599-1604.

Roher, A. E., Lowenson, J. D., Clarke, S., Woods, A. S., Cotter, R. J., Gowing, E., & Ball, M. J. 1993, "beta-Amyloid-(1-42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer disease", *Proc.Natl.Acad.Sci.U.S A*, vol. 90, no. 22, pp. 10836-10840.

Rosenow, F., Hojer, C., Meyer-Lohmann, C., Hilgers, R. D., Muhlhofer, H., Kleindienst, A., Owega, A., Koning, W., & Heiss, W. D. 1997, "Spontaneous intracerebral hemorrhage. Prognostic factors in 896 cases", *Acta Neurol Scand.*, vol. 96, no. 3, pp. 174-182.

Ross Russell, R. 1963, "Observations on intracranial aneurysms", *Brain*, vol. 86, pp. 425-442.

Rost, N. S., Rahman, R. M., Biffi, A., Smith, E. E., Kanakis, A., Fitzpatrick, K., Lima, F., Worrall, B. B., Meschia, J. F., Brown, R. D., Jr., Brott, T. G., Sorensen, A. G., Greenberg, S. M., Furie, K. L., & Rosand, J. 2010, "White matter hyperintensity volume is increased in small vessel stroke subtypes", *Neurology*, vol. 75, no. 19, pp. 1670-1677.

Rost, N. S., Smith, E. E., Chang, Y., Snider, R. W., Chanderraj, R., Schwab, K., FitzMaurice, E., Wendell, L., Goldstein, J. N., Greenberg, S. M., & Rosand, J. 2008, "Prediction of Functional Outcome in Patients With Primary Intracerebral Hemorrhage", *Stroke*, vol. 39, no. 8, pp. 2304-2309.

Ruiz-Sandoval, J. L., Cantu, C., & Barinagarrementeria, F. 1999, "Intracerebral hemorrhage in young people: analysis of risk factors, location, causes, and prognosis", *Stroke*, vol. 30, no. 3, pp. 537-541.

Ruiz-Sandoval, J. L., Chiquete, E., Garate-Carrillo, A., Ochoa-Guzman, A., Arauz, A., Leon-Jimenez, C., Carrillo-Loza, K., Murillo-Bonilla, L. M., Villarreal-Careaga, J., Barinagarrementeria, F., & Cantu-Brito, C. 2011, "Spontaneous intracerebral hemorrhage in Mexico: results from a Multicenter Nationwide Hospital-based Registry on Cerebrovascular Disease (RENAMEVASC)", *Rev Neurol*, vol. 53, no. 12, pp. 705-712.

Ruiz-Sandoval, J. L., Chiquete, E., Romero-Vargas, S., Padilla-Martinez, J. J., & Gonzalez-Cornejo, S. 2007, "Grading scale for prediction of outcome in primary intracerebral hemorrhages", *Stroke*, vol. 38, no. 5, pp. 1641-1644.

Sacco, S., Marini, C., Toni, D., Olivieri, L., & Carolei, A. 2009, "Incidence and 10-year survival of intracerebral hemorrhage in a population-based registry", *Stroke*, vol. 40, no. 2, pp. 394-399.

Sackett, D. L., Haynes, R. B., & Tugwell, P. 1991, *Clinical epidemiology: a basic science for clinical medicine.* Little, Brown & Company, Boston,MA.

Salanti, G., Amountza, G., Ntzani, E. E., & Ioannidis, J. P. 2005, "Hardy-Weinberg equilibrium in genetic association studies: an empirical evaluation of reporting, deviations, and power", *Eur.J.Hum.Genet.*, vol. 13, no. 7, pp. 840-848.

Samarasekera, N., Al-Shahi Salman, R., Huitinga, I., Klioueva, N., McLean, C. A., Kretzschmar, H., Smith, C., & Ironside, J. W. 2013, "Brain banking for neurological disorders", *Lancet Neurol*, vol. 12, no. 11, pp. 1096-1105.

Samarasekera, N., Potter, G. M., & Al-Shahi Salman, R. 2011, "Microbleed Mimics," in *Cerebral Microbleeds: Pathophysiology to Clinical Practice*, Cambridge University Press, Cambridge, pp. 44-48.

Samarasekera, N., Smith, C., & Al-Shahi Salman, R. 2012, "The association between cerebral amyloid angiopathy and intracerebral haemorrhage: systematic review and meta-analysis", *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 83, no. 3, pp. 275-281.

Sampson, J. N. & Zhao, H. 2009, "Genotyping and inflated type I error rate in genome-wide association case/control studies", *BMC Bioinformatics.*, vol. 10, p. 68.

Sanner, M. 2006, "People's attitudes and reactions to organ donation", *Mortality*, vol. 11, no. 2, pp. 133-150.

Sattianayagam, P., Hawkins, P., & Gillmore, J. 2009, "Amyloid and the GI tract", *Expert Rev Gastroenterol.Hepatol.*, vol. 3, no. 6, pp. 615-630.

Schilling, S., DeStefano, A. L., Sachdev, P. S., Choi, S. H., Mather, K. A., DeCarli, C. D., Wen, W., Hogh, P., Raz, N., Au, R., Beiser, A., Wolf, P. A., Romero, J. R., Zhu, Y. C., Lunetta, K. L., Farrer, L., Dufouil, C., Kuller, L. H., Mazoyer, B., Seshadri, S., Tzourio, C., & Debette, S. 2013, "APOE genotype and MRI markers of cerebrovascular disease: systematic review and meta-analysis", *Neurology*, vol. 81, no. 3, pp. 292-300.

Schmitt, F. A., Wetherby, M. M., Wekstein, D. R., Dearth, C. M., & Markesbery, W. R. 2001, "Brain donation in normal aging: procedures, motivations, and donor characteristics from the Biologically Resilient Adults in Neurological Studies (BRAiNS) Project", *Gerontologist*, vol. 41, no. 6, pp. 716-722.

Scholz, W. 1938, "Studien zur Pathologie der Hirngefäβe II. Die drusige Entartung der Hirnarterien und-capillaren (Eine Form seniler Gefässerkrankung)", *Zeitshrift für die gesamte Neurologie und Psychiatrie*, vol. 162, pp. 694-715.

Schrag, M., McAuley, G., Pomakian, J., Jiffry, A., Tung, S., Mueller, C., Vinters, H. V., Haacke, E. M., Holshouser, B., Kido, D., & Kirsch, W. M. 2010, "Correlation of hypointensities in susceptibility-weighted images to tissue histology in dementia patients with cerebral amyloid angiopathy: a postmortem MRI study", *Acta Neuropathol.*, vol. 119, no. 3, pp. 291-302.

Schulz, K. F. & Grimes, D. A. 2002, "Case control studies: research in reverse", *Lancet*, vol. 359, pp. 431-434.

Scolding, N. J., Joseph, F., Kirby, P. A., Mazanti, I., Gray, F., Mikol, J., Ellison, D., Hilton, D. A., Williams, T. L., MacKenzie, J. M., Xuereb, J. H., & Love, S. 2005, "Abeta-related angiitis: primary angiitis of the central nervous system associated with cerebral amyloid angiopathy", *Brain*, vol. 128, no. Pt:3, pp. 3-15.

Seifert, T., Lechner, A., Flooh, E., Schmidt, H., Schmidt, R., & Fazekas, F. 2006, "Lack of association of lobar intracerebral hemorrhage with apolipoprotein E genotype in an unselected population", *Cerebrovasc Dis*, vol. 21, no. 4, pp. 266-270.

Sherrington, R., Rogaev, E., Liang, Y., Rogaeva, E., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K., Tsuda, T., Mar, L., Foncin, J. F., Bruni, A., Montesi, M., Sorbi, S., Rainero, I., Pinessi, L., Nee, L., Chumakov, I., Pollen, D., Brookes, A., Sanseau, P., Polinsky, R., Wasco, W., Da Silva, H., Haines, J., Pericak-Vance, M., Tanzi, R., Roses, A., Fraser, P., Rommens, J., & George-Hyslop, P. H. 1995, "Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease", *Nature*, vol. 375, no. 6534, pp. 754-760.

Sheth, K. N., Cushing, T. A., Wendell, L., Lev, M. H., Romero, J. M., Schwab, K., Smith, E. E., Greenberg, S. M., Rosand, J., & Goldstein, J. N. 2010, "Comparison of hematoma shape and volume estimates in warfarin versus non-warfarin-related intracerebral hemorrhage", *Neurocrit.Care*, vol. 12, no. 1, pp. 30-34.

Shin, H. K., Jones, P. B., Garcia-Alloza, M., Borrelli, L., Greenberg, S. M., Bacskai, B. J., Frosch, M. P., Hyman, B. T., Moskowitz, M. A., & Ayata, C. 2007, "Age-dependent cerebrovascular dysfunction in a transgenic mouse model of cerebral amyloid angiopathy", *Brain*, vol. 130, no. Pt 9, pp. 2310-2319.

Shoamanesh, A., Kwok, C. S., & Benavente, O. 2011, "Cerebral microbleeds: histopathological correlation of neuroimaging", *Cerebrovasc Dis*, vol. 32, no. 6, pp. 528-534.

Shrout, P. E. & Fliess, J. L. 1979, "Intraclass Correlations : Uses in Assessing Rater Reliability", *Psychological Bulletin*, vol. 86, no. 2, pp. 420-428.

Simpkin, A. L., Robertson, L. C., Barber, V. S., & Young, J. D. 2009, "Modifiable factors influencing relatives' decision to offer organ donation: systematic review", *BMJ*, vol. 339, p. b991.

Smith, E. E., Gurol, M. E., Eng, J. A., Engel, C. R., Nguyen, T. N., Rosand, J., & Greenberg, S. M. 2004, "White matter lesions, cognition, and recurrent hemorrhage in lobar intracerebral hemorrhage", *Neurology*, vol. 63, no. 9, pp. 1606-1612.

Smith, E. E., Nandigam, K. R., Chen, Y. W., Jeng, J., Salat, D., Halpin, A., Frosch, M., Wendell, L., Fazen, L., Rosand, J., Viswanathan, A., & Greenberg, S. M. 2010, "MRI markers of small vessel disease in lobar and deep hemispheric intracerebral hemorrhage", *Stroke*, vol. 41, no. 9, pp. 1933-1938.

Snyder, E. M., Nong, Y., Almeida, C. G., Paul, S., Moran, T., Choi, E. Y., Nairn, A. C., Salter, M. W., Lombroso, P. J., Gouras, G. K., & Greengard, P. 2005, "Regulation of NMDA receptor trafficking by amyloid-beta", *Nat Neurosci.*, vol. 8, no. 8, pp. 1051-1058.

Sobel, D. F., Baker, E., Anderson, B., & Kretzschmar, H. 1985, "Cerebral amyloid angiopathy associated with massive intracerebral hemorrhage", *Neuroradiology*, vol. 27, no. 4, pp. 318-321.

Soontornniyomkij, V., Lynch, M. D., Mermash, S., Pomakian, J., Badkoobehi, H., Clare, R., & Vinters, H. V. 2010, "Cerebral microinfarcts associated with severe cerebral beta-amyloid angiopathy", *Brain Pathol*, vol. 20, no. 2, pp. 459-467.

Sprigg, N., Machili, C., Otter, M. E., Wilson, A., & Robinson, T. G. 2009, "A systematic review of delays in seeking medical attention after transient ischaemic attack", *J.Neurol Neurosurg.Psychiatry*, vol. 80, no. 8, pp. 871-875.

Stead, L. G., Jain, A., Bellolio, M. F., Odufuye, A. O., Dhillon, R. K., Manivannan, V., Gilmore, R. M., Rabinstein, A. A., Chandra, R., Serrano, L. A., Yerragondu, N., Palamari, B., & Decker, W. W. 2010, "Effect of anticoagulant and antiplatelet therapy in patients with spontaneous intra-cerebral hemorrhage: Does medication use predict worse outcome?", *Clin.Neurol Neurosurg.*, vol. 112, no. 4, pp. 275-281.

Stehling, C., Wersching, H., Kloska, S. P., Kirchhof, P., Ring, J., Nassenstein, I., Allkemper, T., Knecht, S., Bachmann, R., & Heindel, W. 2008, "Detection of asymptomatic cerebral microbleeds: a comparative study at 1.5 and 3.0 T", *Acad.Radiol.*, vol. 15, no. 7, pp. 895-900.

Stein, M., Luecke, M., Preuss, M., Boeker, D. K., Joedicke, A., & Oertel, M. F. 2010, "Spontaneous Intracerebral Hemorrhage With Ventricular Extension and the Grading of Obstructive Hydrocephalus: The Prediction of Outcome of a Special Life-Threatening Entity", *Neurosurgery*, vol. 67, no. 5.

Steiner, T., Kaste, M., Forsting, M., Mendelow, D., Kwiecinski, H., Szikora, I., Juvela, S., Marchel, A., Chapot, R., Cognard, C., Unterberg, A., & Hacke, W. 2006, "Recommendations for the management of intracranial haemorrhage - part I: spontaneous intracerebral haemorrhage. The European Stroke Initiative Writing Committee and the Writing Committee for the EUSI Executive Committee", *Cerebrovasc Dis*, vol. 22, no. 4, pp. 294-316.

Steiner, T., Petersson, J., Al-Shahi Salman, R., Christensen, H., Cordonnier, C., Csiba, L., Harnof, S., Krieger, D., Mendelow, D., Molina, C., Montaner, J., Overgaard, K., Roine, R. O., Schmutzhard, E., Tatlisumak, T., Toni, D., & Stapf, C. 2011, "European research priorities for intracerebral haemorrhage", *Cerebrovasc Dis*, vol. 32, no. 5, pp. 409-419.

Stevens, M. 1998, "Factors influencing decisions about donation of the brain for research purposes", *Age and Ageing*, vol. 27, pp. 623-629.

Strong, K., Mathers, C., & Bonita, R. 2007, "Preventing stroke: saving lives around the world", *Lancet Neurol*, vol. 6, no. 2, pp. 182-187.

Sudlow, C., Martinez Gonzalez, N. A., Kim, J., & Clark, C. 2006, "Does apolipoprotein E genotype influence the risk of ischemic stroke, intracerebral hemorrhage, or subarachnoid hemorrhage? Systematic review and meta-analyses of 31 studies among 5961 cases and 17,965 controls", *Stroke*, vol. 37, no. 2, pp. 364-370.

Sudlow, C. L. & Warlow, C. P. 1997, "Comparable studies of the incidence of stroke and its pathological types: results from an international collaboration. International Stroke Incidence Collaboration", *Stroke*, vol. 28, no. 3, pp. 491-499.

Sundqvist, N., Garrick, T., & Harding, A. 2010, "Families reflections on the process of brain donation following coronial autopsy", *Cell and Tissue Banking* pp. 1-13.

Tanaka, A., Ueno, Y., Nakayama, Y., Takano, K., & Takebayashi, S. 1999, "Small Chronic Hemorrhages and Ischemic Lesions in Association With Spontaneous Intracerebral Hematomas", *Stroke*, vol. 30, no. 8, pp. 1637-1642.

Tang, Y. J., Wang, S., Zhu, M. W., Sun, Y. L., & Zhao, J. Z. 2013, "Severe pathological manifestation of cerebral amyloid angiopathy correlates with poor outcome from cerebral amyloid angiopathy related intracranial hemorrhage", *Chin Med J (Engl.)*, vol. 126, no. 4, pp. 603-608.

Tanskanen, M., Makela, M., Myllykangas, L., Rastas, S., Sulkava, R., & Paetau, A. 2012, "Intracerebral hemorrhage in the oldest old: a population-based study (vantaa 85+)", *Front Neurol*, vol. 3, p. 103.

Tanskanen, M., Kiuru-Enari, S., Tienari, P., Polvikoski, T., Verkkoniemi, A., Rastas, S., Sulkava, R., & Paetau, A. 2006, "Senile systemic amyloidosis, cerebral amyloid angiopathy, and dementia in a very old Finnish population", *Amyloid*, vol. 13, no. 3, pp. 164-169.

Tatsumi, S., Shinohara, M., & Yamamoto, T. 2008, "Direct comparison of histology of microbleeds with postmortem MR images: a case report", *Cerebrovasc Dis*, vol. 26, no. 2, pp. 142-146.

Tatu, L., Moulin, T., El Mohamad, R., Vuillier, F., Rumbach, L., & Czorny, A. 2000, "Primary intracerebral haemorrhages in the Besancon stroke registry: initial clinical and CT findings, early course and 30 day outcome in 350 patients", *European Journal of Neurology*, vol. 43, no. 4, pp. 209-214.

Telman, G., Hlebtovsky, A., Sprecher, E., Zaaroor, M., & Kouperberg, E. 2010, "Ethnic disparities in first primary intracerebral hemorrhage in northern Israel", *Neuroepidemiology*, vol. 34, no. 4, pp. 208-213.

Thal, D. R., Ghebremedhin, E., Orantes, M., & Wiestler, O. D. 2003, "Vascular pathology in Alzheimer disease: correlation of cerebral amyloid angiopathy and arteriosclerosis/lipohyalinosis with cognitive decline", *J Neuropathol Exp Neurol*, vol. 62, no. 12, pp. 1287-1301.

Thal, D. R., Ghebremedhin, E., Rub, U., Yamaguchi, H., Del, T. K., & Braak, H. 2002a, "Two types of sporadic cerebral amyloid angiopathy", *J.Neuropathol.Exp.Neurol*, vol. 61, no. 3, pp. 282-293.

Thal, D. R., Papassotiropoulos, A., Saido, T. C., Griffin, W. S., Mrak, R. E., Kolsch, H., Del, T. K., Attems, J., & Ghebremedhin, E. 2010, "Capillary cerebral amyloid angiopathy identifies a distinct APOE epsilon4-associated subtype of sporadic Alzheimer's disease", *Acta Neuropathol.*, vol. 120, no. 2, pp. 169-183.

Thal, D. R., Rub, U., Orantes, M., & Braak, H. 2002b, "Phases of A beta-deposition in the human brain and its relevance for the development of AD", *Neurology*, vol. 58, no. 12, pp. 1791-1800.

Thorvaldsen, P., Kuulasmaa, K., Rajakangas, A. M., Rastenyte, D., Sarti, C., & Wilhelmsen, L. 1997, "Stroke Trends in the WHO MONICA Project", *Stroke*, vol. 28, no. 3, pp. 500-506.

Tian, J., Shi, J., Bailey, K., Lendon, C. L., Pickering-Brown, S. M., & Mann, D. M. 2004, "Association between apolipoprotein E e4 allele and arteriosclerosis, cerebral amyloid angiopathy, and cerebral white matter damage in Alzheimer's disease", *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 75, no. 5, pp. 696-699.

Tian, J., Shi, J., Bailey, K., & Mann, D. M. 2003, "Negative association between amyloid plaques and cerebral amyloid angiopathy in Alzheimer's disease", *Neuroscience Letters*, vol. 352, no. 2, pp. 137-140.

Tilling, K. 2001, "Capture-recapture methods: useful or misleading?", *International Journal of Epidemiology*, vol. 30, no. 1, pp. 12-14.

Tomonaga, M. 1981, "Cerebral amyloid angiopathy in the elderly", *Journal of the American Geriatrics Society*, vol. 29, no. 4, pp. 151-157.

Tuhrim, S., Horowitz, D. R., Sacher, M., & Godbold, J. H. 1995, "Validation and comparison of models predicting survival following intracerebral hemorrhage", *Crit Care Med*, vol. 23, no. 5, pp. 950-954.

Tveiten, A., Ljostad, U., Mygland, A., Thomassen, L., Pripp, A. H., & Naess, H. 2012, "Intracerebral hemorrhage in southern Norway--a hospital-based incidence study", *Eur.Neurol*, vol. 67, no. 4, pp. 240-245.

Tzourio, C., Arima, H., Harrap, S., Anderson, C., Godin, O., Woodward, M., Neal, B., Bousser, M. G., Chalmers, J., Cambien, F., & MacMahon, S. 2008, "APOE genotype, ethnicity, and the risk of cerebral hemorrhage", *Neurology*, vol. 70, no. 16, pp. 1322-1328.

van Asch, C. J. J., Luitse, M. J. A., Rinkel, G. J. E., van der Tweel, I., Algra, A., & Klijn, C. J. M. 2010, "Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis", *The Lancet Neurology*, vol. 9, no. 2, pp. 167-176.

van Swieten, J. C., Hijdra, A., Koudstaal, P. J., & van, G. J. 1990, "Grading white matter lesions on CT and MRI: a simple scale", *J Neurol Neurosurg.Psychiatry*, vol. 53, no. 12, pp. 1080-1083.

van Swieten, J. C., Koudstaal, P., Visser, M. C., Schouten, H. J. A., & van Gijn, J. 1988, "Interobserver Agreement for the Assessment of Handicap in Stroke Patients", *Stroke*, vol. 19, pp. 604-607.

Venkatasubramanian, C., Mlynash, M., Finley-Caulfield, A., Eyngorn, I., Kalimuthu, R., Snider, R. W., & Wijman, C. A. 2011, "Natural history of perihematomal edema after intracerebral hemorrhage measured by serial magnetic resonance imaging", *Stroke*, vol. 42, no. 1, pp. 73-80.

Verghese, P. B., Castellano, J. M., & Holtzman, D. M. 2011, "Apolipoprotein E in Alzheimer's disease and other neurological disorders", *Lancet Neurol*, vol. 10, no. 3, pp. 241-252.

Vernooij, M. W., van der, L. A., Ikram, M. A., Wielopolski, P. A., Niessen, W. J., Hofman, A., Krestin, G. P., & Breteler, M. M. 2008, "Prevalence and risk factors of cerebral microbleeds: the Rotterdam Scan Study", *Neurology*, vol. 70, no. 14, pp. 1208-1214.

Vinters, H. V. 1987, "Cerebral amyloid angiopathy. A critical review", *Stroke*, vol. 18, no. 2, pp. 311-324.

Vinters, H. V. & Gilbert, J. J. 1983, "Cerebral amyloid angiopathy: incidence and complications in the aging brain. II. The distribution of amyloid vascular changes", *Stroke*, vol. 14, no. 6, pp. 924-928.

Viswanathan, A. & Greenberg, S. M. 2011, "Cerebral amyloid angiopathy in the elderly", *Ann.Neurol*, vol. 70, no. 6, pp. 871-880.

Viswanathan, A., Rakich, S. M., Engel, C., Snider, R., Rosand, J., Greenberg, S. M., & Smith, E. E. 2006, "Antiplatelet use after intracerebral haemorrhage", *Neurology*, vol. 66, no. 2, pp. 206-209.

Vittinghoff, E. & McCulloch, C. E. 2007, "Relaxing the Rule of Ten Events per Variable in Logistic and Cox Regression", *American Journal of Epidemiology*, vol. 165, no. 6, pp. 710-718.

von Elm E., Altman, D. G., Egger, M., Pocock, S. J., Gotzsche, P. C., & Vandenbroucke, J. P. 2007, "The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies", *Lancet*, vol. 370, no. 9596, pp. 1453-1457.

Vonsattel, J. P., Myers, R. H., Hedley-Whyte, E. T., Ropper, A. H., Bird, E. D., & Richardson, E. P., Jr. 1991, "Cerebral amyloid angiopathy without and with cerebral hemorrhages: a comparative histological study", *Annals of Neurology*, vol. 30, no. 5, pp. 637-649.

Wagle, W. A., Smith, T. W., & Weiner, M. 1984, "Intracerebral hemorrhage caused by cerebral amyloid angiopathy: radiographic-pathologic correlation", *AJNR Am.J.Neuroradiol.*, vol. 5, no. 2, pp. 171-176.

Wakai, S., Kumakura, N., & Nagai, M. 1992, "Lobar intracerebral hemorrhage. A clinical, radiographic, and pathological study of 29 consecutive operated cases with negative angiography", *J Neurosurg.*, vol. 76, no. 2, pp. 231-238.

Wang, Z. 2007, "Two postestimation commands for assessing confounding effects in epidemiological studies", *Stata Journal*, vol. 7, no. 2, pp. 183-196.

Wardlaw, J. M., Bastin, M. E., Valdes Hernandez, M. C., Munoz Maniega, S., Royle, N. A., Morris, Z., Clayden, J. D., Sandeman, E. M., Eadie, E., Murray, C., Starr, J. M., &

Deary, I. J. 2011, "Brain aging, cognition in youth and old age and vascular disease in the Lothian Birth Cohort 1936: rationale, design and methodology of the imaging protocol", *International Journal of Stroke*, vol. 6, pp. 547-559.

Wardlaw, J. M., Farrall, A., Perry, D., von Kummer, R., Mielke, O., Moulin, T., Ciccone, A., Hill, M., & for the Acute Cerebral CT Evaluation of Stroke Study (ACCESS) Study Group. 2007, "Factors influencing the detection of early computed tomography signs of cerebral ischemia. An internet-based, international multiobserver study", *Stroke*, vol. 38, pp. 1250-1256.

Wardlaw, J. M., Ferguson, K., & Graham, C. 2004, "White matter hyperintensities and rating scales – observer reliability varies with lesion load", *Journal of Neurology*, vol. 251, pp. 584-590.

Wardlaw, J. M., Lewis, S. C., Dennis, M., Counsell, C., & McDowall, M. 1998, "Is visible infarction on computed tomography associated with an adverse prognosis in acute ischaemic stroke?", *Stroke*, vol. 29, pp. 1315-1319.

Wardlaw, J. M., Smith, E. E., Biessels, G. J., Cordonnier, C., Fazekas, F., Frayne, R., Lindley, R. I., O'Brien, J. T., Barkhof, F., Benavente, O. R., Black, S. E., Brayne, C., Breteler, M., Chabriat, H., Decarli, C., de Leeuw, F. E., Doubal, F., Duering, M., Fox, N. C., Greenberg, S., Hachinski, V., Kilimann, I., Mok, V., Oostenbrugge, R., Pantoni, L., Speck, O., Stephan, B. C., Teipel, S., Viswanathan, A., Werring, D., Chen, C., Smith, C., van, B. M., Norrving, B., Gorelick, P. B., & Dichgans, M. 2013, "Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration", *Lancet Neurol*, vol. 12, no. 8, pp. 822-838.

Wardlaw, J. M. & Statham, P. F. 2000, "How often is haemosiderin not visible on routine MRI following traumatic intracerebral haemorrhage?", *Neuroradiology*, vol. 42, no. 2, pp. 81-84.

Wardlaw, J. M. W. & Farrall, A. 2004, "Diagnosis of stroke on neuroimaging", *British Medical Journal*, vol. 328, pp. 655-656.

Warlow, C., van Gijn, J., Dennis, M., Wardlaw, J., Bamford, J. M., Hankey, G., Sandercock, P. A., Rinkel, G. J. E., Langhorne, P., Sudlow, C., & Rothwell, P. 2008, "What caused this intracerebral haemorrhage?," in *Stroke Practical Management*, 3rd editiion edn, Blackwell Publishing, pp. 411-456.

Weimar, C., Benemann, J., & Diener, H. C. 2006, "Development and validation of the Essen Intracerebral Haemorrhage Score", *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 77, no. 5, pp. 601-605.

Weimar, C., Benemann, J., Terborg, C., Walter, U., Weber, R., & Diener, H. C. 2011, "Recurrent stroke after lobar and deep intracerebral hemorrhage: a hospital-based cohort study", *Cerebrovasc Dis*, vol. 32, no. 3, pp. 283-288.

Weingarten, K., Zimmerman, R. D., Cahill, P. T., & Deck, M. D. 1991, "Detection of acute intracerebral hemorrhage on MR imaging: ineffectiveness of prolonged interecho interval pulse sequences", *AJNR Am.J.Neuroradiol.*, vol. 12, no. 3, pp. 475-479.

Weir, C. J., Murray, G. D., Adams, F. G., Muir, K. W., Grosset, D. G., & Lees, K. R. 1994, "Poor accuracy of stroke scoring systems for differential clinical diagnosis of intracranial haemorrhage and infarction", *Lancet*, vol. 344, no. 8928, pp. 999-1002.

Weissleder, R., Wittenberg, J., Harisinghani, M., & Chen, J. 2011, "Neurologic Imaging," in *Primer of Diagnostic Imaging*, Elsevier Mosby, Missouri, pp. 363-374.

Weller, R. O., Boche, D., & Nicoll, J. A. 2009, "Microvasculature changes and cerebral amyloid angiopathy in Alzheimer's disease and their potential impact on therapy", *Acta Neuropathol.*, vol. 118, no. 1, pp. 87-102.

Weller, R. O., Djuanda, E., Yow, H. Y., & Carare, R. O. 2009a, "Lymphatic drainage of the brain and the pathophysiology of neurological disease", *Acta Neuropathol.*, vol. 117, no. 1, pp. 1-14.

Weller, R. O., Preston, S. D., Subash, M., & Carare, R. O. 2009b, "Cerebral amyloid angiopathy in the aetiology and immunotherapy of Alzheimer disease", *Alzheimers Res Ther*, vol. 1, no. 2, p. 6.

Weller, R. O., Subash, M., Preston, S. D., Mazanti, I., & Carare, R. O. 2008, "Perivascular drainage of amyloid-beta peptides from the brain and its failure in cerebral amyloid angiopathy and Alzheimer's disease. [Review] [106 refs]", *Brain Pathology*, vol. 18, no. 2, pp. 253-266.

Wells, GA., Shea, B., O'Connell, D., Peterson, J., Welch, V., Losos, M., & Tugwell, P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2002. 19-11-2012.
Ref Type: Internet Communication

Wermer, M., Rinkel, G. J. E., van Rooij, W.-J. J., Witkamp, T. D., Ziedses des Plantes, B. G., & Algra, A. 2002, "Interobserver agreement in the assessment of lobar versus deep location of intracerebral haemoatomas on CT", *Journal of Neuroradiology*, vol. 29, pp. 271-274.

White, P. M., Wardlaw, J. M., & Easton, V. 2000, "Can noninvasive imaging accurately depict intracranial aneurysms? A systematic review", *Radiology*, vol. 217, no. 2, pp. 361-370.

Whiting, P., Rutjes, A. W., Reitsma, J. B., Bossuyt, P. M., & Kleijnen, J. 2003, "The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews", *BMC Med Res Methodol.*, vol. 3, p. 25.

Willinsky, R. A., Taylor, S. M., TerBrugge, K., Farb, R. I., Tomlinson, G., & Montanera, W. 2003, "Neurologic complications of cerebral angiography: prospective analysis of 2,899 procedures and review of the literature", *Radiology*, vol. 227, no. 2, pp. 522-528.

Wilson, J. T. L., Hareendran, A., Grant, M., Baird, T., Schulz, U. G. R., Muir, K. W., & Bone, I. 2002, "Improving the Assessment of Outcomes in Stroke", *Stroke*, vol. 33, no. 9, pp. 2243-2246.

Wisniewski, T. & Frangione, B. 1992, "Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid", *Neurosci.Lett.*, vol. 135, no. 2, pp. 235-238.

Wong, G. K. C., Siu, D. Y. W., Abrigo, J. M., Poon, W. S., Tsang, F. C. P., Zhu, X. L., Yu, S. C. H., & Ahuja, A. T. 2011, "Computed Tomographic Angiography and Venography for Young or Nonhypertensive Patients With Acute Spontaneous Intracerebral Hemorrhage", *Stroke*, vol. 42, no. 1, pp. 211-213.

Woo, D., Sauerbeck, L. R., Kissela, B. M., Khoury, J. C., Szaflarski, J. P., Gebel, J., Shukla, R., Pancioli, A. M., Jauch, E. C., Menon, A. G., Deka, R., Carrozzella, J. A., Moomaw, C. J., Fontaine, R. N., & Broderick, J. P. 2002, "Genetic and environmental risk factors for intracerebral hemorrhage: preliminary results of a population-based study", *Stroke*, vol. 33, no. 5, pp. 1190-1195.

Xu, D., Yang, C., & Wang, L. 2003, "Cerebral amyloid angiopathy in aged Chinese: a clinico-neuropathological study", *Acta Neuropathologica*, vol. 106, no. 1, pp. 89-91.

Yamada, M. 2002, "Risk factors for cerebral amyloid angiopathy in the elderly", *Annals of the New York Academy of Sciences*, vol. 977, pp. 37-44.

Yamada, M., Sodeyama, N., Itoh, Y., Suematsu, N., Otomo, E., Matsushita, M., & Mizusawa, H. 1997, "Association of presenilin-1 polymorphism with cerebral amyloid angiopathy in the elderly", *Stroke*, vol. 28, no. 11, pp. 2219-2221.

Yamada, M., Tsukagoshi, H., Otomo, E., & Hayakawa, M. 1987, "Cerebral amyloid angiopathy in the aged", *Journal of Neurology*, vol. 234, no. 6, pp. 371-376.

Yamada, S., Saiki, M., Satow, T., Fukuda, A., Ito, M., Minami, S., & Miyamoto, S. 2012, "Periventricular and deep white matter leukoaraiosis have a closer association with cerebral microbleeds than age", *Eur.J.Neurol*, vol. 19, no. 1, pp. 98-104.

Yamanouchi, H., Shimada, H., & Kuramoto, K. 1990, "Subtypes and proportions of cerebrovascular disease in an autopsy series in a Japanese geriatric hospital", *Klinische Wochenschrift*, vol. 68, no. 23, pp. 1173-1177.

Yaqub, B. A., Shamena, A. R., Kolawole, T. M., & Patel, P. J. 1991, "Cerebrovascular disease in Saudi Arabia", *Stroke*, vol. 22, no. 9, pp. 1173-1176.

Yen, C. C., Lo, Y. K., Li, J. Y., Lin, Y. T., Lin, C. H., & Gau, Y. Y. 2007, "Recurrent primary intracerebral hemorrhage: a hospital based study", *Acta Neurol Taiwan.*, vol. 16, no. 2, pp. 74-80.

Yeung, R., Ahmad, T., Aviv, R. I., de Tilly, L. N., Fox, A. J., & Symons, S. P. 2009, "Comparison of CTA to DSA in determining the etiology of spontaneous ICH", *Can.J.Neurol Sci.*, vol. 36, no. 2, pp. 176-180.

Yong, W. H., Robert, M. E., Secor, D. L., Kleikamp, T. J., & Vinters, H. V. 1992, "Cerebral hemorrhage with biopsy-proved amyloid angiopathy", *Arch Neurol*, vol. 49, no. 1, pp. 51-58.

Yoshimura, M., Yamanouchi, H., Kuzuhara, S., Mori, H., Sugiura, S., Mizutani, T., Shimada, H., Tomonaga, M., & Toyokura, Y. 1992, "Dementia in cerebral amyloid angiopathy: a clinicopathological study", *J Neurol*, vol. 239, no. 8, pp. 441-450.

Yu, W. H., Wang, W. H., Dong, X. Q., Du, Q., Yang, D. B., Shen, Y. F., Wang, H., Zhang, Z. Y., Zhu, Q., Che, Z. H., Liu, Q. J., Jiang, L., & Du, Y. F. 2014, "Prognostic significance of plasma copeptin detection compared with multiple biomarkers in intracerebral hemorrhage", *Clin Chim.Acta*, vol. 433C, pp. 174-178.

Zahuranec, D. B., Brown, D. L., Lisabeth, L. D., Gonzales, N. R., Longwell, P. J., Eden, S. V., Smith, M. A., Garcia, N. M., & Morgenstern, L. B. 2006, "Differences in intracerebral hemorrhage between Mexican Americans and non-Hispanic whites", *Neurology*, vol. 66, no. 1, pp. 30-34.

Zahuranec, D. B., Brown, D. L., Lisabeth, L. D., Gonzales, N. R., Longwell, P. J., Smith, M. A., Garcia, N. M., & Morgenstern, L. B. 2007, "Early care limitations independently predict mortality after intracerebral hemorrhage", *Neurology*, vol. 68, no. 20, pp. 1651-1657.

Zannis, V. I., Breslow, J. L., Utermann, G., Mahley, R. W., Weisgraber, K. H., Havel, R. J., Goldstein, J. L., Brown, M. S., Schonfeld, G., Hazzard, W. R., & Blum, C. 1982, "Proposed nomenclature of apoE isoproteins, apoE genotypes, and phenotypes", *J.Lipid Res.*, vol. 23, no. 6, pp. 911-914.

Zhan, R. Y., Tong, Y., Shen, J. F., Lang, E., Preul, C., Hempelmann, R. G., Hugo, H. H., Buhl, R., Barth, H., Klinge, H., & Mehdorn, H. M. 2004, "Study of clinical features of amyloid angiopathy hemorrhage and hypertensive intracerebral hemorrhage", *J.Zhejiang Univ Sci.*, vol. 5, no. 10, pp. 1262-1269.

Zheng, H. & Koo, E. H. 2011, "Biology and pathophysiology of the amyloid precursor protein", *Mol.Neurodegener.*, vol. 6, no. 1, p. 27.

Zhu, X. L., Chan, M. S., & Poon, W. S. 1997, "Spontaneous intracranial hemorrhage: which patients need diagnostic cerebral angiography? A prospective study of 206 cases and review of the literature", *Stroke*, vol. 28, no. 7, pp. 1406-1409.

Zhu, Y.-C., Chabriat, H., Godin, O., Dufouil, C., Rosand, J., Greenberg, S. M., Smith, E., Tzourio, C., & Viswanathan, A. 2012, "Distribution of white matter hyperintensity in cerebral haemorrhage and healthy ageing", *Journal of Neurology*, vol. 3, pp. 530-536.

Zia, E., Engstrom, G., Svensson, P. J., Norrving, B., & Pessah-Rasmussen, H. 2009, "Three-year survival and stroke recurrence rates in patients with primary intracerebral hemorrhage", *Stroke*, vol. 40, no. 11, pp. 3567-3573.

Zia, E., Pessah-Rasmussen, H., Khan, F. A., Norrving, B., Janzon, L., Berglund, G., & Engstrom, G. 2006, "Risk factors for primary intracerebral hemorrhage: a population-based nested case-control study", *Cerebrovasc Dis*, vol. 21, no. 1-2, pp. 18-25.

Appendix of study materials

Proformas

1. LATCH proforma
2. LINCHPIN scan reading form (MRI proforma)

Patient information leaflets

3. LATCH patient information leaflet
4. LINCHPIN patient information leaflet
5. LINCHPIN patient information leaflet (adults with incapacity)
6. LINCHPIN patient information leaflet for relatives of people who have died from suspected intracerebral haemorrhage

Consent forms

7. LINCHPIN consent form-general
8. LINCHPIN consent form-MRI
9. LINCHPIN consent form-post-mortem examination
10. LINCHPIN consent form-general (adults with incapacity)
11. LINCHPIN consent form-MRI (adults with incapacity)
12. LINCHPIN consent form-post-mortem examination (adults with incapacity)
13. NHS Lothian post-mortem examination authorisation form

Annual questionnaire

14. LATCH annual questionnaire

Sampling protocol

15. LINCHPIN sampling protocol for brain tissue

Lothian Audit of the Treatment of Cerebral Haemorrhage: Checklist

Patient name: ……Patient ID: ☐☐☐☐

LATCH

Seen by BHT Yes: Leaflet given ☐ Yes: No leaflet given ☐ No: not seen ☐ Leaflet posted ☐

	Done?	Entered?
Scan reviewed at Wednesday stroke imaging meeting	☐	☐
Case reviewed with Rustam	☐	☐

LINCHPIN	DD / M M / YYYY	Done?	Entered?
Verbal consent given	☐☐/☐☐/☐☐☐☐		
Clinical consent	☐☐/☐☐/☐☐☐☐		
IQCODE consent	☐☐/☐☐/☐☐☐☐		
Records consent	☐☐/☐☐/☐☐☐☐		
Blood consent	☐☐/☐☐/☐☐☐☐		
Blood taken?		☐	☐
MRI consent	☐☐/☐☐/☐☐☐☐		
MRI brain requested		☐	☐
MRI completed		☐	☐
Patient & GP informed of MRI result		☐	☐
LP consent		☐	☐
LP completed		☐	☐
LINCHPIN consent letter sent		☐	☐
Newsletter wanted?		☐	☐
Autopsy consent	☐☐/☐☐/☐☐☐☐		
Biopsy consent		☐	☐
Donor card given to patient		☐	☐
PM alert letter & donor card sent to GP		☐	☐
Placement aware of PM consent and has forms?		☐	☐

If the patient dies…

Timescale for funeral: ……

Details of undertaker to be used: ……

Clinical autopsy report received	☐	☐
LINCHPIN autopsy report received	☐	☐
Do NOK want PM results? Yes / No		
Who should be informed (check NOK details)?.....	☐	☐
Inform by letter/phone?	☐	☐
NOK informed of PM result	☐	☐

LATCH ID ☐☐☐☐ **LATCH opt out** : dd/mm/yyyy

Personal Information

Title[i]:	Forename:	Surname:	
Gender: M / F	Date of Birth: dd/mm/yyyy	CHI:	Age:
Religion[ii]:		Ethnicity[iii]:	
Address:			Postcode:
Phone (H):	Phone (Mob):	Email:	

Next of Kin details

Name:			Relationship[iv]:
Address:			Postcode:
Phone (H):	Phone (Mob):	Email:	

GP details

Name:			Practice name:
Address:			Postcode:
Phone (H):	Phone (Mob):	Email:	

[i] Mr, Mrs, Miss, Ms, Dr, Prof, Lord, Lady, Sir,Dame, Reverend
[ii] Bahai, Brahma Kumaris, Buddhism, Chinese, Christianity, Hinduism, Humanism, Islam, Jehovah's witness, Judaism, Mormon, Paganism, Sikhism, Other
[iii] Asian or asian british: Indian, Asian or asian british:Bangladeshi, Asian or asian british: Pakistani, Asian or asian british:Chinese, Asian or asian british: other, Black or black british:African, Black or black british: Caribbean, black or black british: other, white british, white other, mixed ethnic background
[iv] Spouse/Partner/Parent/child/brother/sister/grandparent/grandchild/niece/nephew/stepfather/stepmother/half-brother/half-sister/cousin/friend/ Welfare guardian/legal representative/Other

Lothian Audit of the Treatment of Cerebral Haemorrhage: ICH – notification, general PMHx

Notification

Source	✓ as appropriate
Collaborator	List collaborator names
ISD	
SSCA	
CT search	
TRAK search	
NXR	
Autopsy report	

Is **this patient excluded from LATCH?** Yes ☐ No ☐

If Yes: Reason for exclusion - HTI ☐ Intraventricular haemorrhage ☐ Postcode ☐

Other non-ICH diagnosis ☐ ICH without pathology/imaging ☐ Trauma ☐

Has LINCHPIN been discussed? Yes ☐ No ☐

If No, give reason LINCHPIN not discussed:

No next of kin found ☐ Team did not think it was appropriate to discuss ☐

Consent unlikely to be obtained[v] ☐ Late ascertainment[vi] ☐

Patient had died when ascertained ☐ Patient died before LINCHPIN discussed[vii] ☐

Unable to do PM if consented ☐ Secondary ICH ☐

Consented to any/all of LINCHPIN Yes ☐ No ☐

(Document consent discussion on page 15)

If No, date of withdrawal from LINCHPIN : …….

[v] Clinical team did not advise against discussing LINCHPIN but BHT judged LINCHPIN inadvisable – e.g. patient failed to engage with clinical care.
[vi] Late ascertainment – patient ascertained at least 3 months after date of ICH
[vii] Patient ascertained whilst still alive but died before LINCHPIN discussed

Lothian Audit of the Treatment of Cerebral Haemorrhage: ICH – notification, general PMHx

Past medical history – general

Illness	dd/mm/yy[viii]

[viii] If date & month unknown, insert mid-point of year (01/07/yy), Chronic kidney disease – should be stage 2 or above to be documented as Chronic renal failure

Past medical history – ICH related

1.	**History of ischaemic events?**[ix]	Y	N	IF NO, GO TO QUESTION 2
a.	Angina	Y	N	
b.	Myocardial Infarction	Y	N	
c.	Heart failure	Y	N	
d.	Diabetes mellitus	Y	N	
e.	Hyperlipidaemia	Y	N	
f.	Peripheral vascular disease	Y	N	
g.	Transient ischaemic attack	Y	N	
h.	Ischaemic Stroke	Y	N	
i.	Deep vein thrombosis	Y	N	
j.	Pulmonary embolus	Y	N	
k.	Atrial fibrillation	Y	N	
2	**History of hypertension?**[x]	Y	N	IF YES, YEAR OF DIAGNOSIS ?
3	**History of haemorrhagic events?**[xi]	Y	N	IF NO, GO TO QUESTION 4

[ix] Any ischaemic event listed in 1a-1k, diagnosis made prior to admission with *index* ICH, recorded in GP or hospital notes (or for Diabetes mellitus, or hyperlipidaemia only, where a patient has been prescribed treatment commonly used for this indication) For heart failure, a diagnosis of heart failure made prior to admission or clinical evidence from echocardiography. For atrial fibrillation, a diagnosis made prior to admission with index ICH or a previous ECG showing atrial fibrillation.

[x] Diagnosis made prior to admission with index ICH recorded in GP or hospital notes or where a patient has been prescribed drugs used in the treatment of hypertension.

[xi] Diagnosis made prior to admission with index ICH recorded in GP or hospital notes

3a	Gastrointestinal haemorrhage	Y	N	
3b	Coagulopathy[xii]	Y	N	
4	**History of Migraine?**	Y	N	IF NO, GO TO QUESTION 5
4a	**Type of migraine?** (✓ as appropriate)			COMMENTS
	aura			
	no aura			
	acephalgic			
	Retinal			
	unknown			
5.	**History of Epilepsy?**[xiii]	Y	N	COMMENTS
6	**History of dementia?**	Y	N	IF NO, GO TO QUESTION 6
6a	**Type of dementia?** (✓ as appropriate)			COMMENTS
	Alzheimer's disease			
	vascular			
	Mixed Alzheimer's disease & vascular			
	Lewy-Body disease			
	Frontotemporal			
	Other (no formal dx, suspected by relatives)			
	Not specified (demented but type unknown)			
	Unknown			
6b	Year of dementia diagnosis			
6.	Transient neurological symptoms?[xiv]			
7.	Progressive neurological symptoms[xv]			

[xii] Recurrent nosebleeds, bleeding following dental extraction, easy bruising, liver disease, abnormal premenopausal bleeding, post menopausal bleeding, idiopathic thrombocytopaenia purpura, thrombocytopaenia-other, haemophilia, other haematological disorder (excluding ICH)
[xiii] Diagnosis made prior to admission with index ICH recorded in GP or hospital notes, Epilepsy types : Focal, generalised, unknown

[xiv] confusion, decreased conscious level Sensory, hemiparesis/plegia, myoclonus, dysphasia, hemianopia, other visual disturbance, confusion, ataxia, chorea, tics, dystonia, movement disorder-unspecified, other

[xv] Amnesia, behavioural change, confusion, cranial nerve palsies, decreased consciousness, decreased concentration, dyscalculia, dysgraphia, dyspraxia, dysphasia, encephalopathy, gait disturbance, hallucinations, headaches, mood disturbance, other nerve palsy, seizures, sensory disturbance, visual disturbance, weakness, other

Haemorrhage details

1a.	Is this the first ICH[xvi]?	First	R	N/K
1b	ICH definite / possible[xvii]?			
1c	Single or multiple ICH?			
1d	Symptomatic ICH?			
2.	Date of ICH?	dd/mm/yyyy		
3a.	Time of symptom onset?	hh:mm		
3b.	Is the time stated :	Last seen well	From sleep	Awake at onset
4.	Who is the responsible consultant?			
5a.	Date of hospital assessment	dd/mm/yyyy		
5b.	Time of hospital assessment	hh:mm		
6a	Referral date	dd/mm/yyyy		
6b	Date seen (BHT)	dd/mm/yyyy		
6c	Time seen (BHT)	hh:mm		
7a	Hospital[xviii]			
7b	Inpatient/Outpatient			
7c	Ward			
7d	Date admitted to the stroke unit	dd/mm/yyyy		

[xvi] (F= first, R= recurrent, N/K=not known)
[xvii] Definite ICH if confirmed by imaging/pathology
[xviii] This is either the hospital where BHT first saw the patient or the hospital where the patient was admitted if BHT did not see the patient. If the patient was transferred between hospitals, enter the first hospital the patient was admitted to in 7e and the second hospital in 7a. WGH, RIE, SJH, Liberton, Astley Ainslie, Roodlands

7e	Has this patient been transferred from another hospital[xix]?			
8	Handedness (A= ambidextrous, N/K=not known)	R	L	A N/K
9	**History of presenting bleed**			
10a	Head injury prior to the ICH?	Y	N	
10b	Is the patient pregnant or is she post partum?	Y	N	IF YES: SPECIFY TRIMESTER/POST PARTUM
10c	Date of thrombolysis pre-ICH	dd/mm/yyyy		

DHx on admission

Medication	Dose	Frequency	Date started (mm/yy)	Date stopped (mm/yy)

[xix] WGH, RIE, SJH,

1. Is the participant adopted ?

   Yes ☐ No ☐ Unknown ☐ does not want to answer ☐

2. Are you one of multiple births ?

   Twin – identical ☐ Twin- non identical ☐

   Triplet ☐ Other ☐ Unknown ☐

3. Draw the family tree of first degree relatives* only :

   * - mother, father, sibling, half-sibling, child

4.

Relative*	Alive? Y / N	Age / age @ death	Cause of death	Stroke?		Dementia?	
				Type[xx]	Age	Y/ N/Unknown	Age

[xx] ICH, ischaemic stroke, Unknown

Social details

1a.	Does this person live alone?	Y	N	N/K
2a.	Employment (Unemployed, Full time, Part time, Retired)			
2b	Highest level of education (B=basic, F=further, H=higher)	Basic	Further	Higher
2c	Age left education (years)			
3a	Alcohol intake (units/week)[xxi]			
3b	Smoker?	never	current	ex
3c	Recreational drug use?	Y	N	N/K
3d	Last date drugs taken	dd/mm/yyyy		

PRE MORBID MODIFIED RANKIN SCALE (✓ as appropriate)

Do you have any symptoms? (if No: 0, Yes: 1)

0 – no symptoms at all ☐

Are you able to look after yourself and carry out all usual (previous) activities? (if No: 2)

1 - No significant disability despite symptoms: Able to carry out all usual activities. ☐

Are you able to pay the bills, do the shopping, cleaning etc? (if Yes: 2, No: 3)

2 - Slight disability (i.e. independent for ADL but unable to do all usual activities). ☐

Can you walk without the help of other people? (if Yes: 3, No: 4)

3 - Moderate disability: Requiring some help but able to walk without assistance. ☐

Can you be left alone for at least some hours of the day or do you need constant attention? (if Yes: 4, No: 5)

4 - Moderate to severe disability: Unable to walk without assistance and unable to attend to own bodily needs without assistance. ☐

5 - Severe disability: Bedridden, incontinent and requiring constant nursing car ☐

[xxi] If recording from notes, use most recently documented alcohol history

Examination

Systolic BP on admission		Diastolic BP on admission
Glasgow coma scale		
Eyes[xxii]: 1 2 3 4 UN	Verbal [xxiii]: 1 2 3 4 5 T UN	Motor[xxiv]: 1 2 3 4 5 6 UN
Can talk? Yes / No / NK	Orientated? Yes / No/ NK	Able to lift both arms off bed? Yes / No / NK
Main examination findings : weakness/dysphasia/dysarthria/neglect/field defect and R/L		

Admission results

Hb (g/l)		Na (mmol/l)		Gluc(mmol/l)	
WCC ($\times 10^9$/l)		K (mmol/l)		Chol (mmol/l)	
Plts ($\times 10^9$/ l)		Urea (mmol/l)		TG (mmol/l)	
PT (secs)		Creat (µmol/l)		CRP (mg/l)	
APTT (secs)		Bil (µmol/l)			
Fib (g/l)		Alb (g/l)			
INR		ALP (U/l)			
ESR (mm/h)		ALT (U/l)			

ECG	AF : Y / N / NK LVH[xxv] : Y / N / NK
Urine toxicology	

Blood results - comments :

[xxii] 1 = Does not open eyes, 2 = Opens eyes in response to painful stimuli, 3 = Opens eyes in response to voice, 4 = Opens eyes spontaneously
[xxiii] 1 = No sounds, 2 = Incomprehensible sounds, 3 = Inappropriate words, 4 = Confused, disoriented, 5 = Oriented, converses normally, T= intubated
[xxiv] 1 = No movements, 2 = Extension to stimuli, 3 = Abnormal flexion to stimuli, 4 = Flexion/Withdrawal, 5 = Localizes, 6 = Obeys commands
[xxv] LVH : LVH - R wave in leads V5 or 6 plus S wave in lead V1 >35 mm (BMJ, 2002;324:1264-1267)

1.	**Scan ID**	
2.	**Scan modality**	CT / MR
3.	**Date of scan**	dd/mm/yyyy
4.	**Time of scan**	hh:mm
5.	**ICH classification**	Lobar / deep / cerebellum / brainstem
6.	**ICH location**[xxvi]	
7.	**ICH side**	Left / Right / Left and Right / Midline / unknown
8.	**Intraventricular extension**	Yes / No
9.	**Subarachnoid extension**	Yes / No
10.	**Subdural extension**	Yes / No
11.	**Midline shift (cm)**	
12.	**ICH A dimension (cm)** [xxvii]	
13.	**ICH B dimension (cm)**	
14.	**ICH C dimension (cm)**	
15.	**ICH computerised volume (ml)**	
16.	**Cause of ICH**[xxviii]	

[xxvi] Specify location as frontal / parietal / Temporal / occipital / fronto-parietal / fronto-temporal / parieto-occipital / temporo-parietal/ temporo-occipital/ temporo-parieto-occipital/ basal ganglia/ thalamic/ brainstem

cerebellar / midbrain / pontine / medulla

[xxvii] ABC/2 method. Identify the CT slice with the largest area of haemorrhage on it. A= largest diameter of haemorrhage on CT slice (cm), B= diameter perpendicular to A (cm, measured on the same slice), C= no of slices on which ICH visible × thickness of brain CT slices ($1cm^3$ =1ml). (Kothari et al. *Stroke.* 1996; 27: 1304–1305.)

[xxviii] CLINCH classification: Acquired small vessel disease, cerebral amyloid angiopathy – without a detected genetic mutation, cerebral amyloid angiopathy – with a detected genetic mutation, genetic small artery diseases – CADASIL, genetic small artery diseases – COL4A1 mutation, genetic small artery diseases – familial without an identified mutation, moya-moya phenomenon, vasculitis, reversible cerebral vasoconstriction syndrome, haemorrhagic transformation of an infarct, arterial aneurysm, arteriovenous malformation, dural arteriovenous fistula, cavernous malformation, acute leucoencephalopathy syndromes, intracranial venous thrombosis, malignancy, multiple – evidence of at least 2 of the previously mentioned causes, unknown

Date of auditable event[xxix] : ____ / _____ / _____

A. Appropriate referral made as per audit criteria (✓one option)

cerebellar ICH >2cm diameter ☐ ICH causing brainstem compression ☐

ICH causing hydrocephalus ☐ ICH within 1cm of cortical surface ☐

ICH causing deterioration in GCS from 9-12 to less than or equal to 8 ☐

B. Patient referred when referral inappropriate as per audit criteria (✓one option)

Small deep ICH ☐ Large ICH & prior comorbidities ☐

Lobar ICH without either hydrocephalus or rapid neurological deterioriation ☐

GCS at time of referral[xxx]: E ☐ V ☐ M ☐

Neurosurgical referral made? Yes / No / Unknown

Neurosurgical opinion : (✓as appropriate)

Opinion	Yes	No	Not applicable
Conservative management by other specialty			
Transfer ITU			
Transfer DCN (inc HDU)			
Further imaging			
Randomise STICH2			
Other investigation recommended			

Neurosurgical intervention: (✓as appropriate)

Intervention	Yes	No	Not applicable
Conservative management			
ICP monitor			
Insertion of ventricular drain			
Burr hole/mini-craniotomy and aspiration of clot			
Craniotomy+evacuation of clot +/- craniectomy			
Decompressive craniectomy			
Excision of lesion			

[xxix] Tick one option in A or B. Check up to 72hrs from the time of admission for auditable neurosurgical events

[xxx] E: 1 = Does not open eyes, 2 = Opens eyes in response to painful stimuli, 3 = Opens eyes to voice, 4 = Opens eyes spontaneously
V: 1 = No sounds, 2 = Incomprehensible sounds, 3 = Inappropriate words, 4 = Confused, disoriented, 5 = Oriented,T= intubated
M: 1 = No movements, 2 = Extension to stimuli, 3 = Abnormal flexion to stimuli, 4 = Flexion/Withdrawal, 5 = Localizes, 6 = Obeys commands

Imaging record

Scan	Date of scan	Diagnostic scan	CT/CTA/CTV/MRI /MRA/MRV /MRI&MRA /MRI &MRV/ DSA
1.			
2.			
3.			
4.			
5.			

Date of DNAR:

In-patient treatment (✓ as appropriate)

Treatment	Yes	No	Unknown
Antihypertensives			
Platelets			
FFP			
PCC			
Vitamin K			

Discharge medication

Medication	**Type**	**Dose**	**Freq.**	**Date started (mm/yy)**	**Date stopped (mm/yy)**

Treatment	One	Many	No	Unknown
Antihypertensives				
Antiplatelets				
Anticoagulants				

Treatment change notes:

Place of discharge: Home ☐ Residential home ☐ Nursing home ☐

Lothian Audit of the Treatment of Cerebral Haemorrhage: ICH – Death

Date of death : ____/____/_____ Time of death :

Place of death : hospital ☐ community ☐ (✓ one as appropriate)

Death certificate	Condition
1a	
1b	
1c	
1d	
2	

PM performed : LINCHPIN ☐ Fiscal ☐ No ☐ (✓ as appropriate)

PM exam date : ____/____/_____

Autopsy done by whom? Colin Smith ☐ James Ironside ☐ Toni Torgersen ☐

Ralph BouHaidar ☐

GIVEN BY	
First approach? (BHT vs Clinical team)	
Date of first approach to discuss PM	
Relationship between lead NOK & deceased	
Number of family members involved in PM discussion	
CONSENT PROCESS	
Other Trials : PATCH/STICH 2 (given dates of consent to any other ICH studies)	

LINCHPIN: SCAN READING FORM

P1

Adapted with permission from http://www.sbirc.ed.ac.uk/documents/

SCAN ID: _____ SCAN DATE: _____

LATCH/LINCHPIN ID_____ READ DATE: _____

SCAN TYPE: CT - CONTRAST: ☐ + CONTRAST: ☐

CT FORMAT: _____

CTA ☐ CTV ☐

MR	T1 sag	☐	ADC	☐	T2 sag	☐
	T2 axial	☐	GRE	☐	T1 cor	☐
	FLAIR	☐	SWAN	☐	MRA	☐
	DWI	☐	SWI	☐	MRV	☐

1.	Is this scan for assessment of the notifying event (*e.g.* detection and / or classification of an acute infarct or haematoma)?	Y	N	IF NO, GO TO Q.6
2.	Is there any sign of acute ischaemic change?	Y	N	IF NO, GO TO Q.3
a.	On which side of the brain is the ischaemia?	R	L	TICK R AND L IF BOTH
3.	Is there any acute parenchymal haemorrhage?	Y	N	IF NO, GO TO Q.6
b.	Is the main haemorrhage most likely Haemorrhagic Transformation of an Infarct or Primary Intracerebral Haemorrhage?	HTI	PICH	IF HTI, GO TO Q.6

4. Characterize each separate acute parenchymal haemorrhage starting with the largest / symptomatic/ most important at number 1. Rank haemorrhages in order of importance.

	1	2	3	4	5	6	7	8	9	10
Left / Right	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Anatomic Location code (ICHs)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Anatomic Location Codes for haemorrhages (use multiple codes if necessary)

F	Frontal lobe (not basal ganglia)	T	Temporal lobe
BG	Basal Ganglia (not thalamus)	C	Cerebellum
Th	Thalamus	Mi	Midbrain
P	Parietal lobe	P	Pons
O	Occipital lobe	Me	Medulla

SCAN ID: _____

Adapted with permission from http://www.sbirc.ed.ac.uk/documents/ for use in: LINCHPIN P2

b.	Is there any intraventricular haemorrhage?	Y	N	
c.	Is there any subarachnoid extension?	Y	N	
d.	Is there any subdural extension?	Y	N	
e.	Is there any midline shift?	Y	N	
5.	Characterize the haemorrhage(s) listed in 4a further.			
a.	What is the size of haemorrhage no.1? (A x B x C) method in centimeters (cm)	A	B	C
b.	What is the size of haemorrhage no.2? (A x B x C) method in centimeters (cm)	A	B	C
c.	What is the size of haemorrhage no.3? (A x B x C) method in centimeters (cm)	A	B	C
d.	What is the size of haemorrhage no.4? (A x B x C) method in centimeters (cm)	A	B	C
e.	What is the size of haemorrhage no.5? (A x B x C) method in centimeters (cm)	A	B	C
f.	Is the main haemorrhage lobar or deep? If lobar AND deep, tick both.	L	D	Uncertain / n/a
6.	Are there any chronic infarcts or haemorrhages (which are not microbleeds)?	Y	N	IF NO, GO TO Q.7
a.	Classify the chronic infarcts and haemorrhage (for ICHs use anatomic location codes in 4a)			

	1	2	3	4	5	6	7	8	9	10
Left / Right	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Infarct / Haem	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Location code	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Vascular Territory Location Codes for infarcts

MCA cortical
1 small cortical
2 basal ganglia
3 subcortical
4 ant half periph MCA
5 post half periph MCA
6 whole peripheral MCA
7 whole periph + lat BG
8 whole MCA territory

Other cortical
9 anterior ACA
10 posterior ACA
11 anterior PCA
12 posterior PCA
13 anterior BZ
14 posterior BZ

Lacunar**
15 int & ext capsules/lent nucleus
16 internal border zone
17 centrum semiovale
18 thalamus
19 brainstem
20 anterior (mainly) borderzone
21 posterior (mainly) borderzone

Cerebellum
22 small cortical
23 <1/2 hemisph.
24 >1/2 hemisph.

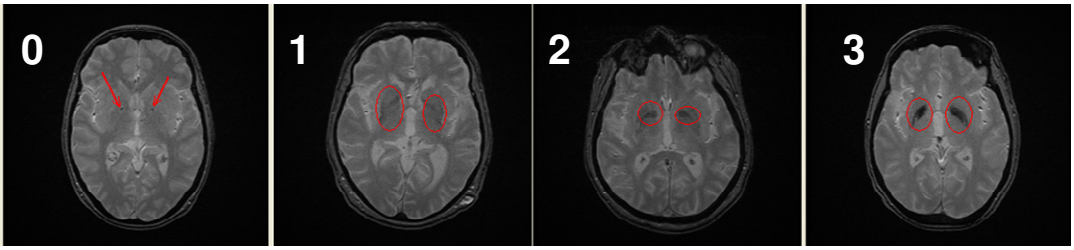
Brainstem
25 small
(i.e. <1/2 medulla)
26 extensive

1. Record in the following order: MCA cortical > other cortical > lacunar > cerebellum > brainstem
2. Defined as a 'CSF-containing cavity' in an appropriate site, measuring 3-20mm in diameter

SCAN ID: _____

Adapted with permission from http://www.sbirc.ed.ac.uk/documents/ for use in: LINCHPIN P3

7.	Is this scan a CT scan?	Y	N	IF YES, STOP.
8.	Rate any BASAL GANGLIA MINERAL DEPOSITS			[GRE most useful]
				Code: ☐



9.	Rate any WHITE MATTER HYPERINTENSITIES Fazekas scale: 0 1 2 3			[FLAIR / T2 most useful]
a.	Periventricular	R	L	Overall ☐
b.	Deep (subcortical white matter)	R	L	Overall ☐
10	Rate any ATROPHY using normative age template 0 = None. 1 = Mild. 2 = Moderate. 3 = Severe			[T2 to match template]
a.	Deep			Overall ☐
b.	Cortical / superficial			Overall ☐
11	Rate any ENLARGED PERIVASCULAR SPACES 0 = None. 1 = < 10. 2 = 11 – 20. 3 = 21 – 40. 4 = > 40.			[T2 best]
a.	Hippocampus	R	L	Overall ☐
b.	Basal ganglia	R	L	Overall ☐
c.	Centrum semiovale	R	L	Overall ☐

SCAN ID: _____

Adapted with permission from http://www.sbirc.ed.ac.uk/documents/ for use in: LINCHPIN P4

12	Quantify MICROBLEEDS	RIGHT		LEFT		Notes:
	Certain or Uncertain	C	U	C	U	
a.	Frontal lobe Cortex / grey-white junction	☐ C	☐ U	☐ C	☐ U	
b.	Frontal lobe Subcortical white matter	☐ C	☐ U	☐ C	☐ U	
c.	Temporal lobe Cortex / grey-white junction	☐ C	☐ U	☐ C	☐ U	
d.	Temporal lobe Subcortical white matter	☐ C	☐ U	☐ C	☐ U	
e.	Parietal lobe Cortex / grey-white junction	☐ C	☐ U	☐ C	☐ U	
f.	Parietal lobe Subcortical white matter	☐ C	☐ U	☐ C	☐ U	
g.	Occipital Cortex / grey-white junction	☐ C	☐ U	☐ C	☐ U	
h.	Occipital lobe Subcortical white matter	☐ C	☐ U	☐ C	☐ U	
	Lobar Totals	C	U	C	U	
i.	Basal ganglia grey matter	☐ C	☐ U	☐ C	☐ U	
j.	Internal & external capsules	☐ C	☐ U	☐ C	☐ U	
k.	Thalamus	☐ C	☐ U	☐ C	☐ U	
	Deep Totals	C	U	C	U	
l.	Cerebellum	☐ C	☐ U	☐ C	☐ U	
m.	Brainstem	☐ C	☐ U	☐ C	☐ U	
	Infratent Totals	C	U	C	U	

LOTHIAN AUDIT OF THE TREATMENT OF CEREBRAL HAEMORRHAGE

(L.A.T.C.H.)

An audit of the care of adults in Lothian who are affected by brain haemorrhage

Patient information leaflet

This leaflet is about a Lothian-wide clinical audit called LATCH, which involves you because you have been diagnosed as having a brain haemorrhage. It is important for you to understand why the audit exists and what it involves.

What is LATCH?

The purpose of LATCH is to monitor the quality of care, treatment and outcomes for adults with brain haemorrhages, in order to improve the care and services that we provide. The audit also monitors how common the condition is, and the problems it causes for those affected.

LATCH does this by trying to find every adult resident in Lothian, who is newly diagnosed with a brain haemorrhage and enrolling them in this confidential audit. We are looking for people who were diagnosed after 1st June 2010.

How does LATCH work?

In the course of your care we collect information about you, your diagnosis, your investigations, your treatment and how you get on. This information is obtained from your general practitioner (GP) and hospital medical records, brain scans, GP,

Information Services Division, General Register Office for Scotland and the NHS central register. The data are held confidentially under secure conditions. You may be invited to take part in associated research projects, and will be informed of them at a later date.

How does LATCH handle information?

The information is gathered and processed by the LATCH team and held securely in an electronic database within the NHS. All names, addresses and other identifying information are removed before the data are analysed. We comply with the Data Protection Act, 1998.

The team includes medical staff, as well as administrative and computing staff with NHS contracts and a duty of confidentiality.

How can I obtain more information?

In this leaflet we have attempted to give a basic description of the audit. For more information, you may contact the number below. You may also seek advice from your hospital consultant or general practitioner.

What if I want my information removed from the audit?

If you wish to opt out of the audit, contact LATCH using the details below.

If you would like further information please contact:

Rosemary Anderson or Dr Rustam Al-Shahi Salman, Bramwell Dott Building, Division of Clinical Neurosciences, Western General Hospital, Edinburgh. EH4 2XU

Telephone: 0131 537 2944 / 07872416010

Lothian study of INtraCerebral Haemorrhage Pathology, Imaging and Neurological outcome (LINCHPIN)

Participant information sheet

We understand that you have had a stroke due to a bleed in the brain. This is also known as a 'brain haemorrhage'. We would like to invite you to take part in our research study on brain haemorrhages.

Before you decide, we want you to understand why the research is being done and what it would involve.

Brain haemorrhage team

We are doctors and nurses who look after and have a special interest in patients with brain haemorrhage. If required, we will provide the doctor looking after you with advice on tests and treatments that may help you. We will provide you with information about brain haemorrhage. We also do research into the causes of brain haemorrhage. This research project may help us to find out what caused your brain haemorrhage.

One of our team will go through this information sheet with you and answer any questions you have. **Part 1** tells you the purpose of this research study and what will happen to you if you take part. **Part 2** gives you more detailed information about the research study.

Part 1

What is the purpose of the study?

About 1 in every 5 strokes are caused by bleeding into the brain. Around 15,000 people in the UK will have a bleed like this every year and the effects can be very serious. At the moment,

we know very little about what causes a bleed and do not have any effective medical treatments for it. This study will use blood samples, brain scans and samples of brain tissue from people who have had a brain haemorrhage to find out more about what causes them. This could lead to specific treatments, which might benefit you personally by preventing another brain haemorrhage. This might also benefit others in the future.

Why have I been asked to take part?

We understand that this is a difficult time for you. We are asking you to take part because you have had a bleed into the brain. We are giving you the opportunity to participate in this study because it may help us to understand why you have had a bleed and this could have implications for your future health or the health of your relatives.

Do I have to take part?

It is up to you. You have the option to participate in as much of the study as you wish. If you agree to take part, we will ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. This will not affect the care you receive.

What will happen to me if I take part?

There are five parts to the research and you can contribute to any or all of them.

1. Assessment by the brain haemorrhage team

As a clinical care team with a special interest in brain haemorrhage we would like to talk to you and/or your family to find out more about what led up to your bleed. We will ask your next of kin to complete a short questionnaire about your memory. We will review your medical records to investigate what may have caused the bleed into your brain.

2. Blood samples

We invite you to provide a blood sample. This will help study genetic and non-genetic influences on bleeding in the brain. The sample will be used in collaboration with researchers overseas, where laws governing the use of data and tissue differ from ours.

3. Brain scan

We would like to offer you the opportunity to have a detailed magnetic resonance imaging (MRI) brain scan, which is not usually performed for people with brain haemorrhage in standard practice. This scan could identify a cause of your brain haemorrhage. The scan will also search for tiny deposits of blood in the brain, called microbleeds, which may indicate that your bleed has been caused by a protein called amyloid. Amyloid might affect the risk of you having another bleed in the future.

This scan will take approximately 30 minutes to complete and does not involve any exposure to x-rays or other forms of radiation. This should happen within one month of the bleed if you are well enough, or if you are well enough when we see you in clinic six months after the bleed.

4. Checking how you get on in the future

We would like to check how you get on either by telephone or in person in approximately six months time and by reviewing your medical records as well as sending your GP a short questionnaire every year.

5. Examination of your brain tissue

The best way of understanding what caused your brain haemorrhage is by examining your brain under the microscope. We are therefore inviting you to donate samples of your brain to our research study when you die. This could also be very helpful in establishing whether the 'microbleeds' identified by the MRI scan do reliably identify the amyloid protein in the brain.

We will provide you with a 'card' to carry identifying you as a member of the LINCHPIN study, so that if you choose to donate tissue, your wishes to do so are known.

If the information in **Part 1** has interested you and you are considering participation, please read the additional information in **Part 2** before making any decision.

Part 2

Will my taking part in the study be kept confidential?

Yes. Your identity is totally confidential and no identifying details will ever be made public.

How will information about me be handled?

The study is run by a team from the Department of Clinical Neurosciences in Edinburgh. The information is processed by this team which includes medical, computing and administrative staff. All members have NHS contracts and a professional duty of confidentiality. The information is held securely in a password protected database. All identifying information is removed before the data are analysed. We comply with the Data Protection Act, 1998.

Which gene is being tested for and why?

Your genes can affect your health in different ways. Firstly, an abnormality in one of your genes may directly cause an illness or secondly, a variation in a gene may make you more likely to have an illness. We are looking at a gene called Apolipoprotein E which falls into the second group. It will not directly cause a bleed into the brain, but may make a bleed more

likely to occur. As far as we know, having different variations of the gene will not directly affect the health of you or your family.

What happens to the results of my brain scan?

The MRI scan will be reported by a doctor who specialises in looking at brain scans. If the scan does reveal a specific cause for your bleed, or any other abnormality which has implications for your health or that of your family, we will inform you and the doctors who are looking after you.

The scan will be kept securely so that it forms part of your medical record and can be seen by the doctors looking after you or if you have to return to hospital in future. The scans will be stored on University computers for analysis by University staff, only identified by study number, not by your name. We may want to come back to re-examine the data after the study is finished, and possibly to share scan data with other researchers to answer relevant research questions. These points are important because sometimes new ideas come out during or after the study and we can then go back and re-examine the study data to see if these new ideas might be true.

What does examining my brain tissue involve?

Brain tissue is essential to establish whether amyloid protein does cause brain haemorrhage, and whether microbleeds on MRI diagnose amyloid. If you choose to donate brain tissue, a doctor trained in tissue examination, called a pathologist, will carry out a post mortem examination restricted to the head only. Small samples of tissue from different areas of your brain will be taken and examined. These would be 1cm by 1cm by 1cm in size. A small sample of the fluid surrounding the brain will also be taken. Afterwards, your brain will be returned to your body. Any funeral arrangements will not be affected by your decision to make a donation to this study.

What are the potential disadvantages?

Apart from the time taken considering your case in detail, there are no disadvantages of clinical examination. A blood test may be uncomfortable. Some people become claustrophobic in the MRI scanner – but if that happened to you, the scan would not have to continue. There is a 1 in 37 chance of finding an abnormality on your MRI scan that is completely incidental to your brain haemorrhage.

What happens to the samples I have donated?

The blood samples and samples of brain tissue will be stored in a secure laboratory in an anonymous form. They may be used in other ethically approved research studies (including genetic research) to benefit human health in the future. Neither your relatives nor the research groups would profit financially from any developments of this kind.

What happens when the research study stops?

Information about you and your samples will be retained indefinitely. Retained tissue may be kept for future medical research and disposed of lawfully when they have served this purpose.

Will my GP be informed about my participation?

Yes. We will inform your GP if you decide to participate in this study.

If I agree now, can I change my mind later?

Yes. Do contact us if you need to discuss anything (see back page). If you change your mind later on, any samples or brain scans done will be retained as part of your medical record but will not be used for research or education.

What if there is a problem?

If you have a concern about any aspect of this study, you should speak to one of the research team who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this via the NHS Complaints Procedure, whose details can be obtained from the hospital.

Can I find out the results of the research?

We would be very happy to send you a yearly newsletter about the results of the research, if you wish. The results of the study will be submitted for publication in relevant professional journals and also made available in a format appropriate to the general public. When the results of our study are published, we will not include any individual information about you that would be identifiable.

Who is funding this research?

The study is funded by the UK Medical Research Council and The Stroke Association.

Who has reviewed the study?

The Multi-Centre Research Ethics Committee for Scotland, Committee A, approved this study.

Thank you for reading this information leaflet.

Independent advice about this study is available from:

Professor M Dennis, Consultant Stroke Physician, Ward 55, Western General Hospital, Edinburgh. EH4 2XU. Telephone: 0131 537 2082.

If you would like further information please contact one of the study team:

Mrs Chris Lerpiniere or Mrs Tracey Millar (research nurses)

Prof. Rustam Al-Shahi Salman (chief investigator)

Dr Neshika Samarasekera (clinical fellow)

Division of Clinical Neurosciences, Bramwell Dott Building, Western General Hospital, Edinburgh. EH4 2XU. Telephone: 0131 537 2944. Mobile: 07872 416 010. Email: Rosemary.Anderson2@nhs.net

This study is funded by:

MRC Medical Research Council and Stroke association

Lothian study of INtraCerebral Haemorrhage Pathology, Imaging and Neurological outcome (LINCHPIN)

Information sheet for relatives of participants without capacity

We understand that your relative has had a stroke due to a bleed in the brain. This is also known as a 'brain haemorrhage'. We would like to invite your relative to take part in our research study on brain haemorrhages.

Before you decide, we want you to understand why the research is being done and what it would involve.

Brain Haemorrhage Team

We are doctors and nurses who look after and have a special interest in patients with brain haemorrhage. If required, we will provide the doctor looking after your relative with advice on tests and treatments that may help them. We will provide you and your relative with information about brain haemorrhage. We also do research into the causes of brain haemorrhage, because more needs to be known about them. This research project may help us to find out what caused your relative to have a brain haemorrhage.

We feel your relative is unable to decide for him/herself whether to take part in this study. We are therefore asking you for your consent on their behalf for them to join the study. We ask you to consider the following information about the study and to let us know whether you wish them to take part.

We ask you to set aside your own views and consider their interests and what you feel would be their wishes and feelings, had they been able to make a decision for themselves. Any advance decisions they may have made and that you are aware of should take precedence.

If you feel unable to decide whether or not to give consent, please say so. You may seek independent advice. We will understand if you do not want this responsibility.

If you feel you cannot decide, it will not affect the care your relative receives.

One of our team will go through this information sheet with you and answer any questions you have. This information is the same as would have been provided to your relative.

Part 1 tells you the purpose of this research study and what will happen to your relative if they take part.

Part 2 gives you more detailed information about the research study.

Part 1

What is the purpose of the study?

About 1 in every 5 strokes are caused by bleeding into the brain. Around 15,000 people in the UK will have a bleed like this every year and the effects can be very serious. At the moment, we know very little about what causes a bleed and do not have any effective medical treatments for it. This study will use blood samples, brain scans and samples of brain tissue from people who have had a brain haemorrhage to find out more about what causes them. This could lead to specific treatments, which might benefit your relative personally by preventing another brain haemorrhage. This might also benefit others in the future.

Why has my relative been asked to take part?

We understand that this is a difficult time for you. We are asking your relative to take part because they have had a bleed into the brain. We are giving your relative the opportunity to participate in this study because it may help us to understand why they have had a bleed and this could have implications for their health in the future.

Does my relative have to take part?

It is up to you. Your relative has the option to participate in as much or as little of the study as you wish. If you agree to them taking part, we will ask you to sign a consent form. You are free to withdraw your relative at any time, without giving a reason. This will not affect the care your relative receives.

What will happen to my relative if they take part?

There are five parts to the research and your relative can contribute to any or all of them.

1. Assessment by the Brain Haemorrhage Team

As a clinical care team with a special interest in brain haemorrhage we would like to talk to you to find out more about what led up to your relative's bleed. We will ask you to complete a short questionnaire about their memory. We will review their medical records to investigate what may have caused the bleed into their brain.

2. Blood samples

We invite you to provide a blood sample. This will help study genetic and non-genetic influences on bleeding in the brain. The sample will be used in collaboration with researchers overseas, where laws governing the use of data and tissue differ from ours.

3. Brain scan

We would like to offer your relative the opportunity to have a detailed magnetic resonance imaging (MRI) brain scan, which is not usually performed for people with brain haemorrhage in standard practice. This scan could identify a cause of their brain haemorrhage. The scan will also search for tiny deposits of blood in the brain, called microbleeds, which may indicate that their bleed has been caused by a protein called amyloid. Amyloid might affect the risk of them having another bleed in the future. This scan will take approximately 30 minutes to complete and does not involve any exposure to x-rays or other forms of radiation.

This should happen within one month of the bleed if they are well enough, or if they are well enough when we see them in clinic six months after the bleed.

4. Checking how they get on in the future

We would like to check how your relative gets on **either** by telephone **or in person**, in approximately six months time and by reviewing their medical records as well as sending their GP a short questionnaire every year.

5. Examination of brain tissue

The best way of understanding what caused your relative's brain haemorrhage is by examining their brain under the microscope. We are therefore inviting them to donate samples of their brain to our research study when they die. This could also be very helpful in establishing whether the 'microbleeds' identified by the MRI scan do reliably identify the amyloid protein in the brain.

We will provide you or your relative with a 'card' to carry identifying them as a member of the LINCHPIN study, so that if they choose to donate tissue, their wishes to do so are known.

If the information in **Part 1** has interested you and you are considering participation, please read the additional information in **Part 2** before making any decision.

Part 2

If my relative takes part in the study, will it be kept confidential?

Yes. Your relative's identity is totally confidential and no identifying details will ever be made public.

How will information about my relative be handled?

The study is run by a team from the Department of Clinical Neurosciences in Edinburgh. The information is processed by this team which includes medical, computing and administrative staff. All members have NHS contracts and a professional duty of confidentiality. The information is held securely in a password protected database. All identifying information is removed before the data are analysed. We comply with the Data Protection Act, 1998.

Which gene is being tested for and why?

Your genes can affect your health in different ways. Firstly, an abnormality in one of your genes may directly cause an illness or secondly, a variation in a gene may make you more likely to have an illness. We are looking at a gene called Apolipoprotein E which falls into the second group. It will not directly cause a bleed into the brain, but may make a bleed more likely to occur. As far as we know, having different variations of the gene will not directly affect the health of your relative or their family.

What happens to the results of the brain scan?

The MRI scan will be reported by a doctor who specialises in looking at brain scans. If the scan does reveal a specific cause for your relative's bleed, or any other abnormality which has implications for their health or that of your family, we will inform the doctors who are looking after your relative.

The scan will be kept securely so that it forms part of your relative's medical record and can be seen by the doctors looking after them or if they have to return to hospital in future. The scans will be stored on University computers for analysis by University staff, only identified by a study number, not by your relative's name. We may want to come back to re-examine the data after the study is finished, and possibly to share scan data with other researchers to answer relevant research questions. These points are important because sometimes new ideas come out during or after the study and we can then go back and re-examine the study data to see if these new ideas might be true.

What does examining brain tissue involve?

Brain tissue is essential to establish whether amyloid protein does cause brain haemorrhage, and whether microbleeds on MRI diagnose amyloid. In the event of your relative dying a doctor trained in tissue examination, called a pathologist, will carry out a post mortem examination restricted to the head only. Small samples of tissue from different areas of your relative's brain will be taken and examined. These would be 1cm by 1cm by 1cm in size. A small sample of the fluid surrounding the brain will also be taken. Afterwards, their brain will be returned to their body. Any funeral arrangements will not be affected by your decision to make a donation to this study.

What are the potential disadvantages?

Apart from the time taken considering their case in detail, there are no disadvantages of clinical examination. A blood test may be uncomfortable. Some people become claustrophobic in the MRI scanner – but if that happened to your relative, the scan would not have to continue. There is a 1 in 37 chance of finding an abnormality on their MRI scan that is completely incidental to their brain haemorrhage.

What happens to the samples I have donated?

The blood samples and samples of brain tissue will be stored in a secure laboratory in an anonymous form. They may be used in other ethically approved research studies (including

genetic research) to benefit human health in the future. Neither relatives nor the research groups would profit financially from any developments of this kind.

What happens when the research study stops?

Information about your relative and their samples will be retained indefinitely. Retained tissue may be kept for future medical research and disposed of lawfully when they have served this purpose.

Will my relative's GP be informed about their participation?

Yes. We will inform their GP if they participate in this study.

If I agree now, can I change my mind later?

Yes. Do contact us if you need to discuss anything. If you change your mind later on, any samples or brain scans done will be retained as part of your relative's medical record but will not be used for research or education.

What if there is a problem?

If you have a concern about any aspect of this study, you should speak to one of the research team who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this via the NHS Complaints Procedure, whose details can be obtained from the hospital.

Can I find out the results of the research?

We would be very happy to send you a yearly newsletter about the results of the research, if you wish. The results of the study will be submitted for publication in relevant professional journals and also made available in a format appropriate to the general public. When the results of our study are published, we will not include any individual information about you or your relative that would be identifiable.

Who is funding this research?

The study is funded by the UK Medical Research Council and The Stroke Association.

Who has reviewed the study?

The study was approved by the Multi-Centre Research Ethics Committee for Scotland, Committee A.

Thank you for reading this information leaflet.

Independent advice about this study is available from:

Professor M Dennis, Consultant Stroke Physician, Ward 55, Western General Hospital, Edinburgh. EH4 2XU. Telephone: 0131 537 2082.

If you would like further information please contact one of the study team:

Mrs Chris Lerpiniere or Mrs Tracey Millar (research nurses)

Prof. Rustam Al-Shahi Salman (chief investigator)

Dr Neshika Samarasekera (clinical fellow)

Division of Clinical Neurosciences, Bramwell Dott Building, Western General Hospital, Edinburgh. EH4 2XU. Telephone: 0131 537 2944. Mobile: 07872 416 010. Email: Rosemary.Anderson2@nhs.net

This study is funded by:

MRC Medical Research Council **and** Stroke association

Lothian study of INtraCerebral Haemorrhage Pathology, Imaging and Neurological outcome (LINCHPIN)

Information sheet for relatives of people who have died from suspected intracerebral haemorrhage

We understand that your friend/relative has died from a suspected stroke due to a bleed in the brain. We also call this a 'brain haemorrhage'. We would like to offer you the opportunity to donate samples of your relative/friend's brain tissue to our research study on brain haemorrhages.

Before you decide, we want you to understand why the research is being done and what it would involve.

Brain haemorrhage team

We are doctors who look after patients with brain haemorrhage. We also do research into the causes of brain haemorrhage.

We ask you to consider the following information about the study and to let us know whether you wish to take part. We ask you to set aside your own views and consider what you feel would have been your friend/relative's wishes and feelings, had they been able to make a decision for themselves. Any advance decisions they may have made and that you are aware of should take precedence.

If you feel unable to decide whether or not to give consent, please say so. You may seek independent advice. We will understand if you do not want this responsibility.

One of our team will go through this information sheet with you and answer any questions you have.

Part 1 tells you the purpose of this research study. **Part 2** gives you more detailed information about the research study.

Part 1

What is the purpose of the study?

About 1 in every 5 strokes are caused by bleeding into the brain. Around 15,000 people in the UK will have a bleed like this every year and the effects can be very serious. At the moment, we know very little about what causes a bleed and do not have any effective medical treatments for it. This study uses samples of brain tissue from people who have had a brain haemorrhage to find out more about what causes them. This could give you a better idea of why your friend/relative had a brain haemorrhage. It might also help others in the future by leading to specific treatments for brain haemorrhage.

Why have you asked for tissue from my friend/relative?

We are asking for a tissue donation from your friend/relative because they may have died as a result of a bleed into the brain. We understand that this is a very difficult time for you. However, we are giving you the opportunity to consent to tissue donation because it may help us to understand why your friend/relative could have had a bleed, and it will help us understand the cause of brain haemorrhage in general.

Does my friend/relative have to donate tissue?

It is up to you. If you consent to tissue donation, we will ask you to sign a consent form. You are free to withdraw your consent at any time, without giving a reason.

What will happen if I provide consent?

1. Assessment by the brain haemorrhage team

We are neurology doctors with a special interest in brain haemorrhage and we will talk to you to find out more about what led up to your friend / relative's bleed. We will review their medical records to look for any factors which may have contributed to the bleed into their brain.

2. Examination of brain tissue

We understand that the Procurator Fiscal has asked for a post mortem examination to be carried out. The post mortem examination is done to try and confirm the cause of death. A doctor trained in tissue examination, called a pathologist, will take and examine small samples of tissue from different areas of your friend/relative's brain.

The post mortem examination is also an opportunity for you to donate samples taken for use in research and if you wish, to donate additional tissue samples from the brain which may help us to understand why your friend/relative had a bleed. We are therefore inviting donation of additional samples of brain tissue for our research.

Any funeral arrangements will not be affected by your decision to make a donation to this study.

If the information in **Part 1** has interested you and you are considering participation, please read the additional information in **Part 2** before making any decision.

Part 2

If my friend/relative donates tissue to the study, will it be kept confidential?

Yes. Your identity and that of your friend / relative is totally confidential and no identifying details will ever be made public.

How will information about my friend / relative be handled?

The study is run by a team from the Department of Clinical Neurosciences in Edinburgh. The information is processed by this team which includes medical, computing and administrative staff. All members have NHS contracts and a professional duty of confidentiality. The information is held securely in a password protected database. All identifying information is removed before the data are analysed. We comply with the Data Protection Act, 1998.

What does examining brain tissue involve?

If you consent to tissue donation, the pathologist would use the routine samples taken at post mortem for research purposes and examine additional tissue samples from different areas of your friend / relative's brain. These samples would be 1cm by 1cm by 1cm in size. A small sample of the fluid surrounding the brain will also be taken. Afterwards, your friend/relative's brain would be returned to their body.

In some people a protein called amyloid is deposited in the brain and this protein may be one cause of brain haemorrhages. Brain tissue is essential to determine whether amyloid is present. The pathologist would look for different causes of brain haemorrhage, one of which would be amyloid.

Any funeral arrangements will not be affected by your decision to make a donation to this study.

Will my decision affect the post mortem examination?

You can be assured that whether you decide to participate in this study or not, this will not affect the care with which the post mortem examination is carried out.

What happens to the results of the post mortem examination?

The post mortem findings will be reported by the pathologist and a report will be sent to your friend/relative's GP. You can discuss the report with the GP. We will also inform you if the post mortem reveals a specific cause for the bleed or any other abnormality which has implications for your family.

What happens to the donated tissue samples?

The samples of brain tissue will be stored in a secure laboratory in an anonymous form. They may be used in other ethically approved research studies (including genetic research) to benefit human health in the future. Neither relatives nor the research groups would profit financially from any developments of this kind.

What happens when the research study stops?

Information about your friend/relative and their samples will be retained indefinitely. Retained tissue may be kept for future medical research and disposed of lawfully when they have served this purpose.

Will my friend/relative's GP be informed about this study?

Yes. We will seek relevant information from the GP records.

If I agree now, can I change my mind later?

Yes. Do contact us if you need to discuss anything (see back page). If you change your mind later on, any samples taken will be retained as part of your friend/relative's medical record but will not be used for research or education.

What if there is a problem?

If you have a concern about any aspect of this study, you should speak to one of the research team who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this via the NHS Complaints Procedure, whose details can be obtained from the hospital.

Can I find out the results of the research?

We could send you a yearly newsletter about the results of the research, if you wish. The results of the study will be submitted for publication in relevant professional journals and also made available in a format appropriate to the general public. When the results of our study are published, we will not include any individual information about you or your relative/friend that would be identifiable.

Who has reviewed the study?

The study was approved by the Multi-Centre Research Ethics Committee for

Scotland, Committee A.

Thank you for reading this information leaflet.

Independent advice about this study is available from:

Professor M Dennis, Consultant Stroke Physician, Ward 55, Western General Hospital, Edinburgh. EH4 2XU

Telephone: 0131 537 2082.

If you would like further information please contact:

Dr Neshika Samarasekera, Division of Clinical Neurosciences, Bramwell Dott Building, Western General Hospital, Edinburgh. EH4 2XU

Telephone: 0131 537 2626 / 07872416010

This study is funded by:

MRC Medical Research Council **and** The STROKE Association

Lothian study of INtraCerebral Haemorrhage Pathology, Imaging and Neurological outcome (LINCHPIN): Consent form

Name of researcher: Prof Rustam Al-Shahi Salman

Please initial box

I have read the information leaflet for the above study and had the opportunity to ask questions. ☐

I understand that my participation is voluntary and I am free to withdraw from the project at any time and my medical care will not be affected. ☐

I give my consent for my general practitioner and consultant to be contacted about the study, for follow up and for my medical records to be examined. ☐

I give my consent to a clinical assessment and a questionnaire to be completed by my next of kin / nearest relative / friend. ☐

I agree to being contacted in about 6 months to see how I am getting on. ☐

I consent to two blood samples being taken; one for genetic analysis and one to be stored for use in future studies of stroke. ☐

I consent to DNA, tissue and anonymised information about me being shared with collaborators within or outside the European Union. ☐

I understand that my doctor and I/my family will be informed if any of the results of the medical tests done as a part of the research have implications for my health or that of my family. ☐

In the event of my death I would like the wishes that I have expressed here to be respected by others. ☐

I would / would not * like to receive information about the results of the research. (*delete as appropriate) ☐

_____	_____	_____
Name of Participant	Date	Participant's signature
_____	_____	_____
Researcher	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from the researcher)	Date	Signature

Lothian study of INtraCerebral Haemorrhage Pathology, Imaging and Neurological outcome (LINCHPIN): Consent form for magnetic resonance imaging (MRI)

Name of researcher: Prof Rustam Al-Shahi Salman

Please initial box

I consent to have a magnetic resonance imaging (MRI) scan of my brain. ☐

I consent to my doctor and I / my family being informed of any scan findings which have implications for my health. ☐

I agree that my brain scans, may be used in other ethically approved research studies in the future as long as all the information including the images is anonymised. ☐

_____	_____	_____
Name of Participant	Date	Participant's signature
_____	_____	_____
Researcher	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from the researcher)	Date	Signature

Lothian study of INtraCerebral Haemorrhage Pathology, Imaging and Neurological outcome (LINCHPIN): Consent form for post mortem examination

Name of researcher: Prof Rustam Al-Shahi Salman

Please initial box

When I die, I consent to a post mortem examination limited to my brain, to look into the cause of my brain haemorrhage. ☐

I consent to tissue samples and fluid taken at the time of a post-mortem examination being retained by the University of Edinburgh and being used for audit, ethically approved research and the education and teaching of healthcare staff. ☐

I consent that the University of Edinburgh may keep indefinitely, any tissues and fluid donated for medical research and dispose of them lawfully when the research is complete or they are no longer usable. ☐

_____	_____ _____	_____
Name of Participant	Date	Participant's signature
_____	_____	_____
Researcher	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from the researcher)	Date	Signature

Lothian study of INtraCerebral Haemorrhage Pathology, Imaging and Neurological outcome (LINCHPIN): Consent form for participants with incapacity

Name of researcher: Prof Rustam Al-Shahi Salman

Please initial box

I have been consulted about my relative's participation in this research project.	☐
I have had the opportunity to ask questions about the study, understand what is involved and give consent on behalf of my relative.	☐
I understand that I am free to withdraw him/her from the study at any time without giving a reason and his/her medical care will not be affected.	☐
I give my consent for his/her general practitioner and consultant to be contacted about the study and for follow up and for his/her medical records to be examined.	☐
I consent to a clinical assessment of my relative and to completing a questionnaire.	☐
I agree to being contacted in 6 months to see how he/she is getting on.	☐
I consent to two blood samples being taken; one for genetic analysis and one to be stored for use in future studies of stroke.	☐
I consent to DNA, tissue and anonymised information about him/her being shared with collaborators within or outside the European Union.	☐
I understand that his/her doctor will be informed if any of the results of the medical tests done as a part of the research have implications for their health or that of their family.	☐
I would / would not * like to receive information about the results of the research. (*delete as appropriate)	☐

_____

Name of Participant

_____	_____	_____
Researcher	Date	Signature
_____	_____	_____
Name of Person providing consent (on behalf of the participant)	Date	Signature

I am (✓ one): ☐ Welfare guardian / legal representative

☐ Nearest relative ⟶ & there is no closer relative: Yes / No [circle one]

Lothian study of INtraCerebral Haemorrhage Pathology, Imaging and Neurological outcome (LINCHPIN): Consent form for participants with incapacity-MRI

Name of researcher: Prof Rustam Al-Shahi Salman

Please initial box

I consent to my relative having a magnetic resonance imaging (MRI) scan of his/her brain. ☐

I consent to my relative's doctor being informed of any scan findings which have implications for his/her health. ☐

I agree that my relative's brain scans, may be used in other ethically approved research studies in the future as long as all the information including the images is anonymised. ☐

_____

Name of Participant

_____	_____	_____
Researcher	Date	Signature

_____	_____	_____
Name of Person providing consent (on behalf of the participant)	Date	Signature

↓

I am (✓ one): ☐ Welfare guardian / legal representative
☐ Nearest relative ⟶ & there is no closer relative: Yes / No [circle one]

Lothian study of INtraCerebral Haemorrhage Pathology, Imaging and Neurological outcome (LINCHPIN): Consent form for participants with incapacity post mortem examination

Name of researcher: Prof Rustam Al-Shahi Salman

I consent to a post mortem examination limited to the brain, to look into the cause of my relative's brain haemorrhage. ☐

I consent to tissue samples and fluid taken at the time of a post-mortem examination being retained by the University of Edinburgh and being used for audit, ethically approved research and the education and teaching of healthcare staff. ☐

I consent that the University of Edinburgh may keep indefinitely, any tissues and fluid donated for medical research and dispose of them lawfully when the research is complete or they are no longer usable. ☐

_____

Name of Participant

_____	_____	_____
Researcher	Date	Signature

_____	_____	_____
Name of Person providing consent (on behalf of the participant)	Date	Signature

I am (✓ one): ☐ Welfare guardian / legal representative
☐ Nearest relative ⟶ & there is no closer relative: Yes / No [circle]

TOP COPY TO BE RETAINED BY NOMINATED REPRESENTATIVE(S)/ NEAREST RELATIVE

Authorisation for the Hospital Post-Mortem Examination on an Adult who left no formal authorisation

Attach patient identification label or addressograph label here.

CHI no. _____

This form is:

- to help you understand what is involved in a hospital post-mortem examination; and
- to provide a record for you and for the hospital about what you want to happen if you decide to authorise a post-mortem examination.

If you wish more information, there are two leaflets. One is short, and gives important general information. The other gives more detailed information. If there is anything you do not understand, or want to know more about, please ask the hospital staff.

Please note: the post-mortem examination usually takes place one or two days after you give your authorisation but (rarely) may take place later the same day.

☐ I am/We are the nominated representative(s) of:

_____

OR

☐ I am the nearest relative of:

_____

(If acting as a nominated representative:) I confirm I am an adult (16 years of age or over).

I/We have no actual knowledge that the person named above was unwilling (a) for a post-mortem examination to be carried out and (b) for organs to be removed, retained or used for any of the purposes of audit, education, training or research which are authorised by virtue of this form.

Section 1A. Authorisation of a full post-mortem examination

☐ I/We authorise the carrying out of a full post-mortem examination on the person named above, which involves internal examination of the body, and the keeping of small tissue samples as blocks and slides, samples of blood and bodily fluids, and may involve taking photographs, X-rays and scans. These will be kept as part of the medical record and may be used for audit, education, training or research.

Section 1B. Authorisation of a limited post-mortem examination

☐ I/We authorise the carrying-out of a limited post-mortem examination on the person named above, which may involve keeping small tissue samples as blocks and slides, samples of blood and bodily fluids and may involve taking photographs, X-rays and scans. These will be kept as part of the medical record and may be used for audit, education, training or research.

Please say what you authorise to be examined:

☐ Head ☐ chest ☐ abdomen

other *(please state what is to be examined)*

Section 2. Authorisation of retention and use of whole organs

There may be benefits in removing whole organs during post-mortem examination and retaining and using those organs afterwards. If so, you will be asked if you are willing to complete this section. Please note: whole organs will only be removed, retained and used under this section on instructions left by the deceased or with your authorisation.

☐ I/We authorise the removal, retention and use of the deceased's organs:

☐ brain ☐ heart ☐ lungs

☐ other – *(please specify):*

for the following purposes, as this may help to better understand the cause of death and the effects of treatment *(please tick as many as you wish):*

☐ audit ☐ education/training ☐ research

☐ I/We understand that blocks and slides may be made from these organs and I/we authorise the keeping of these as part of the medical record so that they can be used for audit, education, training or research.

Section 3. Genetic testing

☐ I/We are willing to allow testing for genetic diseases to be carried out on any material which has been retained under sections 1 or 2.

Section 4. Other requests or wishes

Would you like to make any other requests or set out any other wishes about the post-mortem examination or any retention or future use of tissue or organs? If no, *please tick box.* ☐

If yes, hospital staff should document here any special wishes you have:

Aeros 840786 6/06

To be completed by member of hospital staff who has discussed authorisation

I confirm that:

- ☐ I have asked if the deceased had authorised the matters covered by this form.
- ☐ I have attached a copy of any instructions left by the deceased or a note of any objections he or she is believed to have had.
- ☐ I have offered information to the deceased's nominated representative(s)/nearest relative about the procedures involved and the reasons for the investigations requested. I have offered to explain any procedures and options available in the level of detail that the nominated representative(s)/nearest relative wish and have given any explanations asked for.
- ☐ I have explained that unless the procedures authorised have already taken place, the authorisation given by the nominated representative(s)/nearest relative can be withdrawn at any time, but that withdrawal must be in writing and witnessed by one witness. An amended version of this form would then be passed to the person who would otherwise have undertaken the post-mortem examination.

Written information given:

☐ Basic leaflet ☐ Advanced leaflet ☐ None

(To be completed where whole organs were removed under section 1:)

I have discussed the options for disposal of whole organs which have not been retained under section 2, and have noted the following wishes:

- ☐ the organs should be returned to the body after the examination. I have explained that this may delay the funeral.
- ☐ the hospital should arrange for respectful disposal of the organs.
- ☐ the funeral director should collect and arrange for respectful disposal of the organs.

Signature of member of staff

Signature _____

Name *(block capitals)* _____

_____

Date _____

Job title _____

Telephone contact no. _____

Page no. _____

To be completed by nominated representative(s) / nearest relative

I am the deceased's nominated representative(s)/nearest relative and I am not aware of anyone with a closer relationship who should be asked if there is an objection to post-mortem examination of the deceased. The post-mortem examination has been explained to me and I feel that I have been provided with enough information to give the authorisation set out in this form.

Signature _____

Name *(block capitals)* _____

Date _____

Signature of person witnessing authorisation

(Note: there must be one witness to authorisation. The witness must be 16 years of age or over and is required to witness the content of the form and the signature of the person providing authorisation. A person who is a nominated representative of the deceased adult cannot act as a witness to authorisation by another nominated representative.)

Signature _____

Name *(block capitals)* _____

_____

Job title* _____

Date _____

* If member of hospital staff

University Hospitals Division

<Title> <Initials> <surname of GP>
<Practice name>
<address< <postcode of practice>

Dear <insert GP title and surname>

Re: <Name><Address><Postcode>
Date of birth: <DOB>
CHI: <CHI number>

It is now approximately one year since the NHS Lothian Audit of the Treatment of Cerebral Haemorrhage (LATCH) collected data on <insert forename and surname of person>.

We would be very grateful to you for completing the attached one page questionnaire. There is vital follow-up information that only you will be able to contribute to this NHS Lothian audit.

Please return the attached form with the relevant copies of their GP notes, if applicable, to us in the freepost envelope provided. Do let us know if we can help in any way, or if you would like to discuss this request, or the audit in general.

Thank you

Yours sincerely

Dr Neshika Samarasekera

Clinical fellow in neurology

GMC No. 6076136

Dr Rustam Al-Shahi Salman

Consultant neurologist

GMC No. 4067993

Patient name «PatForename» «PatSurname»

Date of birth «PatDOB»

Patient address «PatAddress» «PatPostCode»

Our ref LATCH Annual questionnaire, LATCH ID <Insert LINCHPIN ID>

Please tick (✓) appropriate box

	Yes	**No**
1. Is «PatTitle» «PatSurname» still alive?	☐	☐
If you answered '**No**', please provide date of death:		
2. Is their address the same (as above)?	☐	☐
If you answered '**No**', please amend the address.	☐	☐
3. I enclose an electronic summary of «PatTitle» «PatSurname»'s comorbidities and prescriptions	☐	☐

4. **Has «PatTitle» «PatSurname» suffered from any of the following in the last year?** If they have, please provide brief information below, and **send relevant copies of their case notes.**

a. Ischaemic/thromobotic event (ischaemic stroke,TIA, DVT, PE,acute coronary syndrome)	**Yes / No**	_____ day/month/year
b. Haemorrhagic event (intracerebral or other intracranial haemorrhage,gastrointestinal bleed, other extracranial bleeding)	**Yes / No**	_____ day/month/year
c. New diagnosis of dementia	**Yes / No**	_____ day/month/year
d. New diagnosis of hypertension	**Yes / No**	_____ day/month/year

5. Please give «PatTitle» «PatSurname»'s last BP recording _____

BP ____ / _____ mmHg day/month/year

6. Which of these best describes <Patient forename & surname>'s current state?

- No symptoms ☐
- Minor symptoms, which do not interfere with her lifestyle ☐
- Some restrictions to her lifestyle, but she looks after herself ☐
- Requiring some help with activities of daily living but able to walk unaided ☐
- Requiring help to walk and attend to bodily needs but doesn't need constant attention ☐
- Severe handicap, totally dependent, requiring attention night and day ☐

Signature _____ Date _____ day/month/year

«GPTitle» «GPSurname»
«GPPracticeName»
«GPAddress»

BLOCK SHEET FOR LINCHPIN RESEARCH CASES

Name …… Date of PM…….....

SD Number……. Brain weight……

UA Number…… Neuropath Number…….

Received……..…
Processed……

Sent to Neuropathologist……..
Neuropathologist……

Region	Left	Right	Immuno	Comments
Frontal Parasagittal				
Frontal Convexity				
Frontal white matter				
Basal Ganglia (Ant.Comm)				
Basal Ganglia (Mamm body)				
Amygdala				
Temporal Superior				
Parietal Parasagittal				
Parietal Convexity				
Hippocampus				
Thalamus				
Occipital				
Occipital white matter				
Midbrain				
Pons				
Medulla				
Vermis				
Cerebellum				
Cervical Spinal Cord				
Thoracic spinal cord				
Lumbar spinal cord				
Caudia equina				
Dura				
Other				
Unspecified				

Author; Chris-Anne McKenzie Date 04/05/2012
SOP Number Version Review Date