Do retinal microvascular abnormalities shed light on the pathophysiology of lacunar stroke?

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Doctor of Philosophy
University of Edinburgh
2010
Declaration

I declare that this thesis was composed by me and that the work contained therein is my own, except where explicitly stated otherwise in the text.

The work within this thesis has not been submitted for any other degree or professional qualification.

Fergus Neil Doubal
Edinburgh, 2010
Publications directly related to results presented in this thesis

**FN Doubal, MS Dennis JM Wardlaw.** Characteristics of patients with minor ischaemic strokes and negative MRI: a cross-sectional study. *J Neurol Neurosurg Psychiatry* 2010 Jun 27th [e-pub ahead of print]


**FN Doubal, TJ MacGillivray, N Patton, B Dhillon, MS Dennis, JM Wardlaw.** Fractal analysis of retinal vessels suggests that a distinct vasculopathy causes lacunar stroke *Neurology* 2010;74:1102-7.

**FN Doubal, AMJ MacLullich, KJ Ferguson, MS Dennis, JM Wardlaw.** Enlarged perivascular spaces on MRI support a distinct lacunar stroke arteriopathy. *Stroke* 2010;41:450-4.
Publications arising from the patients recruited from this thesis but where the results are not directly reported in this thesis

GM Potter, **FN Doubal**, C Jackson, C Sudlow, M Dennis, J Wardlaw. Associations of clinical stroke misclassification (‘clinical-imaging dissociation’) in acute ischemic stroke. *Cerebrovascular Diseases* 2010;29:395-402


Manuscripts submitted and in preparation

GM Potter, **FN Doubal**, C Jackson, MS Dennis, C Sudlow, JM Wardlaw. White matter lesions identified by magnetic resonance imaging are not related to ipsilateral carotid artery stenosis so are unlikely to be embolic. Submitted to *Neurology*.


JM Wardlaw, F Chappell, **FN Doubal**, E Eadie, K Shuler, V Cvoro. Intracranial large artery stenosis is not associated with lacunar stroke.

**FN Doubal**, A Rumley, GDO Lowe, MS Dennis, JM Wardlaw. Coagulation, fibrinolysis and inflammation in ischaemic stroke subtypes.
Acknowledgements

I am extremely grateful to the patients (and their supportive families) who took part in this study, willingly returned for investigations, provided encouragement and showed considerable humbling interest in this work and the results.

This project was no means a single handed effort and succeeded because of the enthusiastic input from many collaborators and advisers. I thank my clinical and radiological colleagues who consistently referred suitable patients and the radiographers at the SFC Brain Imaging Centre who booked and performed all MRI brain imaging. The Wellcome Trust Clinical Research Facility, Western General Hospital housed the retinal camera. Professor Bal Dhillon provided ophthalmological expertise and training for retinal photograph assessment. Professor Martin Dennis, who had initially inspired me to undertake a PhD, provided practical clinical help combined with wise academic counsel.

I am particularly indebted to Dr Tom MacGillivray who developed the software used in the assessment of retinal features. I hope he enjoyed our discussions as much as I did – he was a patient teacher of complex physics. I was fortunate to supervise two enthusiastic and tireless Dutch medical students - Petra Hokke assisted with the systematic review of retinal microvascular abnormalities in stroke and helped develop vessel width measurement techniques and Rosemarie de Haan assisted with retinal geometrical measurements. I was also aided considerably by two medical students from the University of Edinburgh, Susan Stevenson and Fergal Marlborough who helped with the systematic reviews of endothelial dysfunction in lacunar stroke.
I would also like to thank the statisticians Francesca Chappell, Cat Graham and Steff Lewis for their helpful advice.

The Division of Clinical Neurosciences, Western General Hospital, Edinburgh was a fantastic academic environment in which to study and this project benefited immensely from the comments of fellow research fellows, scientists and professors who challenged and encouraged in equal measure. I am grateful to the Wellcome Trust who funded my clinical research training fellowship.

My main thanks are to Professor Joanna Wardlaw who has given her time generously, been a super source of support and provided expert guidance. She has taught me the value of multi-disciplinary research and of proper planning and implementation of ideas. She has given much wise advice and is a continuing source of inspiration.

Final thanks go to my family - my wife Emma for her love and patience (living with a thesis writer is not easy) and my beautiful daughters Eleanor and Poppy.
Abstract

**Background.** Lacunar strokes account for 25% of all ischaemic stroke but the exact nature of the causative cerebral small vessel abnormality remains unknown. Pathological studies are technically difficult and brain imaging cannot adequately characterise the cerebral small vessels. The retinal blood vessels are of similar size and physiology to the cerebral small vessels and may act as a surrogate marker for these cerebral small vessels. We therefore investigated retinal microvascular abnormalities in lacunar stroke.

**Methods.** We performed a systematic review of retinal microvascular abnormalities in lacunar stroke to clarify associations and identify where further research was required. We then established a cohort of patients presenting with lacunar stroke with cortical stroke controls to investigate differences in retinal microvascular abnormalities between stroke subtypes. All patients had MRI brain at presentation and digital retinal photography of both eyes. We investigated the prevalence of retinopathy (hard and soft exudates or haemorrhages/microaneurysms), focal arteriolar narrowing and arteriovenous nicking. We developed, validated and used novel semi-automated techniques for measuring retinal arteriolar and venular widths, retinal arteriolar geometry (branching co-efficients (change in arteriolar cross sectional area across a bifurcation) and branching angles) and fractal dimensions (reflecting branching complexity) of the vasculature. We also assessed MRI parameters in lacunar stroke. We used multivariable analysis to correct for baseline imbalances in vascular risk factors.
**Results.** From the systematic review we demonstrated that retinal microvascular abnormalities are associated with incident and prevalent stroke but that in general, strokes were inadequately characterised and there were no data regarding retinal microvascular abnormalities in ischaemic stroke subtypes. We recruited 253 patients, 129 lacunar strokes and 124 cortical strokes, mean age 68 years. We found no difference in the prevalence of retinopathy, arteriovenous nicking, focal arteriolar narrowing or arteriolar widths between lacunar and cortical stroke subtypes. We found that venules were wider in lacunar stroke. We found no differences in arteriolar branching co-efficients or arteriolar branching angles between lacunar and cortical strokes but found that deep white matter white matter hyperintensities on MRI were associated with increased branching co-efficients and periventricular white matter hyperintensities associated with decreased branching co-efficients. We found that the fractal dimension of the vascular tree was decreased in lacunar stroke. Furthermore we found that enlarged perivascular spaces on MRI are associated with lacunar stroke and white matter disease.

**Conclusions.** We have clearly demonstrated that retinal microvascular abnormalities differ between lacunar and cortical stroke suggesting that a distinct small vessel vasculopathy may cause lacunar stroke. We have also identified MR markers of lacunar stroke. These results suggest that venular disease (a hitherto under-researched area) may play a role in the pathophysiology of lacunar stroke. Retinal microvascular abnormalities can act as markers for cerebral small vessel disease. We plan collaborative analyses with colleagues who have performed similar studies to further assess retinal abnormalities in lacunar stroke.
List of Abbreviations

ACA – Anterior Cerebral Artery
AF – Atrial Fibrillation
AVN – Arterio-venous nicking (or nipping)
ARIC – Atherosclerosis Risk in Communities (study)
AVR – Arterio-venous Ratio
Ausdiab – Australian Diabetes (study)
BC – Branching Coefficient
BDES – Beaver Dam Eye Study
BMES – Blue Mountain Eye Study
CADASIL – Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and leukoencephalopathy
CHS – Cardiovascular Health Study
CI – Confidence Interval
CRAE – Central Retinal Artery Equivalent
CRP – C Reactive Protein
CRVE – Central Retinal Vein Equivalent
CT – Computed Tomography
Dbox – Monofractal dimension (measured with box counting method)
DWI – Diffusion Weighted Imaging
ECG - Electrocardiogram
ECST – European Carotid Surgery Trial
EPVS – Enlarged Perivascular Spaces
FAN – Focal Arteriolar Narrowing
FLAIR – Fluid Attenuated Axial Inversion Recovery
GRE – Gradient Echo
HR – Hazards Ratio
ICAM – Intracellular Adhesion Molecule
IHD – Ischaemic Heart Disease
IL-6 – Interleukin 6
LACS – Lacunar Stroke Syndrome
LVH – Left Ventricular Hypertrophy
MCA – Middle Cerebral Artery
MRI – Magnetic Resonance Imaging
NASCET – North American Symptomatic Carotid Endarterectomy Trial
NHS – National Health Service
NIHSS – National Institutes for Heath Stroke Scale
OCSP – Oxfordshire Community Stroke Project
OR – Odds Ratio
OXVASC – Oxfordshire Vascular (study)
PACS – Partial Anterior Circulation Stroke
PAI – Plasminogen Activator Inhibitor
PCA – Posterior Cerebral Artery
POCS – Posterior Circulation Stroke
PVD – Peripheral Vascular Disease
RAO – Retinal Arteriolar Occlusion
RR – Relative Risk
RS – Rotterdam Study
RVO – Retinal Vein Occlusion
SD – Standard Deviation
sRR – Summary Risk Ratio
TACS – Total Anterior Circulation Stroke
TIA – Transient Ischaemic Attack
TOAST – Trial of Org 10172 in Acute Stroke Treatment
TPA – Tissue Plasminogen Activator
UK – United Kingdom
US – United States
vWF – von Willebrand’s Factor
WGH – Western General Hospital, Edinburgh, UK
WHO – World Health Organisation
WMH – White Matter Hyperintensities
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Chapter 1. Introduction and aims of thesis. Aetiology of lacunar stroke and homology between retinal and cerebral microvasculature.

1.1 Synopsis of thesis

Lacunar strokes account for 25% of ischaemic stroke but the nature of the underlying small vessel vasculopathy is uncertain. This thesis studies retinal vessel abnormalities as surrogate markers of cerebral vessel disease to gain novel insights into this vasculopathy aiming to guide further work into the causes and eventual management of lacunar stroke.

1.2 Importance of stroke

Stroke is common. The World Health Organisation estimates that 15 million stroke and 5 million stroke deaths occur annually worldwide (WHO Collaborative Group 2004). Stroke is the second most common cause of death worldwide behind ischaemic heart disease (Murray and Lopez 1997b). In the United States of America it is predicted that as many as 800,000 subjects will suffer a stroke in 2010 (Lloyd-Jones et al. 2010). Furthermore, stroke is the leading neurological cause of disability in adults (Murray and Lopez 1997a). The incidence of stroke increases with age and by 2030, assuming that the population continues to age as at present, stroke is predicted to become the 4th leading cause of disability in Westernized nations (Lopez et al. 2006). The burden of stroke is also significant and increasing in low and middle income countries (Strong et al. 2007). Stroke therefore carries a significant worldwide economic burden (Dewey et al. 2001).
1.3 Stroke subtypes

Stroke is defined by the World Health Organisation as a clinical syndrome of sudden onset of focal cerebral deficit lasting more than 24 hours or leading to death with a presumed vascular cause (Aho et al. 1980). Approximately 80% of strokes are ischaemic, caused by occlusion or disease in one or more arteries interrupting blood supply to the brain, 15% are caused by intracerebral haemorrhage and 5% are caused by subarachnoid haemorrhage (Warlow et al. 2003). Ischaemic strokes are further sub classified: 50% are caused by large artery atheroma, 20% by cardio-embolism and 25% by small vessel disease (lacunar stroke) The remaining 5% are caused by rare diseases (Sudlow and Warlow 1997).

There are different methods of subdividing ischaemic stroke. Primarily these are based upon clinical findings coupled with, in the first instance, appropriately timed brain imaging and refined in some cases by the results of further investigations.

1.3.1 The OCSP classification

The Oxfordshire Community Stroke Project classification (OCSP) was developed in epidemiological studies and divides stroke primarily on clinical findings according to the likely arterial territories involved. The four categories are total anterior circulation stroke (TACS); partial anterior circulation stroke (PACS); lacunar stroke (LACS) and posterior circulation stroke (POCS) (Bamford et al. 1991). The different subdivisions reflect the size of the underlying infarct and carry different prognoses. The OCSP clinical classification can be performed without investigations and is therefore applicable in most clinical situations.

1.3.2 The TOAST classification

The TOAST criteria for subdivision of ischaemic stroke were derived from a clinical trial and ascribe the most likely aetiological cause for the stroke using the results of investigations such as brain imaging and imaging to identify potential embolic
sources (Adams, Jr. et al. 1993). The TOAST categories are large-artery atherosclerosis (embolus or thrombosis), cardio-embolism (high-risk or medium-risk), small-vessel occlusion (lacune), stroke of other determined cause, stroke of undetermined cause, two or more causes identified, negative evaluation or incomplete evaluation. The TOAST classification certainly uses more information in assigning stroke subtype than the OCSP classification but suffers from using risk factors to assign categories so may bias epidemiological studies (eg the presence of hypertension and diabetes increases the chances of a patient having a lacunar stroke). It may be difficult to always assign a putative aetiology for stroke in many cases either because there are competing causes (eg concurrent carotid stenosis and atrial fibrillation) or because no causes are found due to incomplete or even despite intensive investigation. The proportion of patients in this “stroke of undetermined cause” category varies widely between studies which has led to questioning of the validity of this classification scheme (Landau and Nassief 2005).

1.3.3 Definition of lacunar stroke

Irrespective of which classification system is used, lacunar stroke accounts for approximately 25% of ischaemic stroke yet there has been widespread confusion and variation in the terminology used to define lacunar stroke and in distinguishing the clinical lacunar stroke syndrome from its associated features seen on brain imaging (Wardlaw 2008). A lacunar stroke can be defined as a “stroke with symptoms and signs characteristic of a small deep cerebral infarct” (Bamford et al. 1991). This distinct constellation of neurological deficits, characteristic of a small deep infarct, reflects lesions located where fibres are tightly packed and where relatively small lesions can cause disproportionately large clinical deficits. This can be further refined as a lacunar ischaemic stroke if appropriately timed brain imaging excludes a haemorrhage or other non vascular cause. The brain imaging may show the recent infarct, further strengthening the classification of a lacunar stroke although even within radiological definitions of lacunar infarct there is a startling lack of homogeneity with disagreement regarding the location and size (as well has how to
differentiate old lacunes from other abnormalities such as enlarged perivascular spaces) (Potter and Wardlaw 2010).

The suggested size of lacunes (3-20mm) was derived from the original pathological studies by Fisher (Fisher 1965) and subsequent studies have either adopted this upper size limit of 20mm (Longstreth, Jr. *et al.* 1998) or used a revised upper limit of 15 mm as suggested by the TOAST criteria (Adams, Jr. *et al.* 1993). There is consternation however that this lower limit of 15mm may misclassify infarcts larger than 15mm which are still nonetheless caused by small vessel disease and hence should be lacunar infarcts (Kang *et al.* 2003a). This upper size limit is an arbitrary figure and studies have shown that lesions greater than this may have a small vessel aetiology and also that smaller lesions may have a cardio-embolic or large artery atheromatous cause (Cho *et al.* 2007). It is likely that there is a continuum of decreasing proportions of lesions being caused by small vessel disease as the size of the subcortical lesion increases (Bang *et al.* 2007). The corollary is that as the lesion size increases the proportion caused by cardio-embolism and large artery atheroma increases and large striato-capsular infarcts behave in a similar fashion to cortical infarcts (Boiten and Lodder 1992).

The term “lacune” was a purely descriptive term, attributed to Dechambre, originally referring to the small holes seen deep in the brain on pathological specimens (Dechambre A 1838). This terminology did not imply causation and lacunes were initially subdivided into 3 categories : Type I small areas of cerebral infarction, Type II – cystic scars suggestive of old small haemorrhages and Type III – enlarged perivascular spaces not associated with infarction (Poirier and Derouesne 1985). The term lacune should be reserved for pathologically or radiologically observed holes (Gouw *et al.* 2008; Ikram *et al.* 2006b) but the cause and significance of these “holes” is uncertain. It is not clear what proportion of these holes were associated with clinically significant neurological deficits and indeed lacunes are found in patients without a clinical history of stroke (Wardlaw 2008).
Several studies have used serial MRI scans to identify and assess predictors of incident “lacunar infarcts” (Ikram et al. 2006b; Longstreth, Jr. et al. 1998) which do not have clinical correlates. Again, the significance of these new holes in the brain is uncertain and any associations found using this “stroke” outcome may not extrapolate to understanding the cause of lacunar ischaemic stroke. Furthermore, not all lacunar infarcts, even those associated with lacunar stroke symptoms will cavitate and it can be difficult to distinguish these old non-cavitating infarcts from white matter hyperintensities which do not have focal clinical correlates (Potter et al. 2010). Thus studies which assess the number of radiological lacunes of presumed ischaemic origin without clinical correlates may miss true lacunar strokes where the lesion has not cavitated (or even where there was no lesion present) or counting lacunar infarcts which were not strokes. At best this will lead to a mis-estimation of the true incidence of lacunar stroke and at worst will lead to spurious associations with lacunar stroke. Recent population based studies have, however used the term “infarct-like lesions” which reflects abnormalities visible on MRI without implying causation (Scher et al. 2009). Careful use of terminology so that “lacune” represents an observed hole in the brain and “lacunar infarct” represents a hole in the brain with appropriately timed symptoms suggestive of a stroke is recommended.

1.4 The lacunar hypothesis

Although lacunae were initially described over 170 years ago it is only within the last half century that we have begun to understand their importance and aetiology. The meticulous pathological dissections by CM Fisher in the 1950s and 60s prompted further work leading to the “lacunar hypothesis” (Bamford and Warlow 1988). This suggested that firstly deep small cerebral infarctions were a distinct subset of ischaemic stroke which could be identified clinically by certain patterns of neurological deficit and secondly that these infarcts were caused by disease of lenticulostriate perforating arteries leading to ischaemia in the distribution of the artery involved. Although these infarcts were small (3-20 mm diameter) they can cause severe clinical deficits as they tend occur deep in the brain where there are high concentrations of nerve fibres coursing from the cortex to the spinal cord.
1.4.1 Lacunar stroke syndromes

There are five classical lacunar stroke syndromes which represent a constellation of symptoms which are usually caused by small deep infarcts (Bamford et al. 1991): pure motor stroke, pure sensory stroke, sensorimotor stroke (although in practice distinguishing between these is difficult as many patients with motor stroke will have sensory symptoms which are often short lived), clumsy hand dysarthria syndrome and ataxic hemiparetic syndrome. These 5 categories encompass most cases of lacunar strokes but there is also a large number of atypical syndromes caused by lacunar infarcts which typically account for 7% of all lacunar strokes (Arboix et al. 2006).

1.5 Clinical significance of lacunar stroke

From the UK population based OCSP, the one year case fatality rate for lacunar strokes (10%) was lower than that for TACS (60%) or PACS (20%) (Bamford et al. 1991). This one year case fatality rate was confirmed in the Italian population based L’Aquila study which found a one year case fatality rate post lacunar stroke of 13%. During this first year of follow up post index stroke, the rate of recurrent stroke was lower for lacunar than non-lacunar stroke (3% v 5%) but from the second year the rate of recurrent stroke were similar (Sacco et al. 2006). Lacunar stroke has a low immediate (30 day) case fatality rate of 2% compared with 12% for atherothrombotic and 14% for cardio-embolic stroke (de Jong et al. 2003). Lacunar strokes have similar long term case fatality rates to large artery stroke as a Dutch study using the TOAST criteria with a median follow up time of 15 years in patients who had survived for at least 30 days found that the case fatality rate for lacunar strokes was 76% and large artery atheroma 79% although patients with cardio-embolic stroke had a significantly higher rate of 87% (Staals et al. 2008).
Mortality rates are a blunt tool with which to assess the clinical significance of stroke. Disability is of paramount importance to patients, carers and health service providers. In a follow up study of 196 patients with lacunar stroke, 32% were disabled (modified Rankin score 0-2) at 16 months and 4% had died (Micheli et al. 2008). In addition to its effect on mortality, lacunar stroke is also associated with other clinical markers of cerebral small vessel disease such as cognitive decline and depression as well as radiological markers of small vessel disease such as white matter hyperintensities and microhaemorrhages (Wardlaw et al. 2006). A pathological study found that in 72 brains of patients without evidence of Alzheimer’s Disease or cortical infarction, increased number of lacunes identified in the basal ganglia was associated with decreased cognitive function (Gold et al. 2005). This finding, albeit using “lacunes” identified on MRI, was replicated in the recent LADIS study which found that in a cross sectional study, the number of lacunes present in non-disabled patients was associated with decreased cognition (Benisty et al. 2009). Decreased cognitive performance is common post lacunar stroke with a reported incidence at three months post stroke of 52% (Mok et al. 2004) and at one month of 75% (Rasquin et al. 2007). Neither of these studies however used a non-lacunar stroke control group so it is not clear if this cognitive impairment is due to lacunar stroke or having had any stroke. The Nun Study reported in 1997 that pathological evidence of lacunar infarcts increased the chance of neurofibrillary tangles resulting in dementia (Snowdon et al. 1997) but this study did not correlate post mortem lacunes with life time stroke symptoms. One study which did compare post stroke cognitive function between lacunar and non-lacunar subtypes found no differences between subtypes but was underpowered for this analysis (Srikanth et al. 2004).

1.6 Aetiology of lacunar stroke

Despite having had the better part of two centuries to study the causes of lacunar stroke, the exact nature of the underlying small vessel vasculopathy is not known. Most cortical (or non lacunar) ischaemic strokes are caused by cardio-embolism or large artery atherothromboembolism. There is evidence that these mechanisms can certainly cause a proportion of lacunar stroke: small numbers (6%) of emboli have
been proven in monkey studies to enter the deep penetrating arteries (Macdonald et al. 1995), and studies have linked lacunar stroke with cardio-embolic and larger artery atherothromboembolic phenomena (Cho et al. 2007), but attributing aetiology to stroke is often an imprecise art and whilst cardioembolism and large artery atherothromboembolism can cause lacunar infarcts they are considered to account for only 10-15% of lacunar infarcts (Wardlaw 2005). Clearly there must be an alternative vasculopathy that perhaps may account for up to 80-85% of lacunar strokes.

1.6.1 Why is the aetiology of lacunar stroke poorly understood?

Lacunar stroke has a low immediate (30 day) case fatality rate of 2% compared with 12% for atherothrombotic and 14% for cardio-embolic stroke (de Jong et al. 2003). This, coupled with recent declining autopsy rates over the last 40 years (Burton and Underwood 2007) has resulted in a paucity of brain specimens with well characterised clinically apparent lacunar infarctions available for analysis. In addition to this, further difficulties are caused by the small size of lacunes and it is technically difficult and time consuming to dissect and analyse these lesions and their vascular supply.

Although the field is rapidly progressing and with a powerful 7 tesla MRI scanner the lenticulostriate arterioles can be visualised (Kang et al. 2009), current brain imaging techniques do not have adequate resolution to characterise the cerebral small vessel abnormalities. There have been no suitable animal models of lacunar stroke developed although perhaps the spontaneously hypertensive stroke prone rat may provide useful information (Bailey et al. 2009).

1.6.2 Lacunar stroke is difficult to diagnose

Clinical studies demonstrate that lacunar stroke is difficult to diagnose (Lindgren et al. 2000) without appropriately timed brain imaging – using clinical criteria alone
will result in approximately a 20% misclassification of lacunar as cortical stroke and vice versa (Mead et al. 2000). This misclassification will have diluted the findings of studies which have assessed risk factors and the epidemiology of lacunar ischaemic stroke (Jackson and Sudlow 2005). The 20% mismatch between clinical syndrome and actual lesion site may be explained by the close proximity of the lacune to part of the cortex and may explain the perceived association of some lacunar strokes with potential embolic sources (see below). DWI imaging at the time of the stroke can accurately distinguish stroke subtypes but other imaging, such as CT and T2, T1 or FLAIR MRI, are too non-specific (if the patient has several holes already, one cannot distinguish the new from old infarcts).

1.7 There may exist two different subtypes of lacunar stroke

In addition to the difficulties of identifying and distinguishing lacunar from non-lacunar stroke, the study of lacunar stroke causation has been further confounded by the gradual realisation that there may be at least two different types of lacunar stroke (Boiten et al. 1993; Markus 2008). Type I consists of an isolated lacunar infarct and type II when there is a lacunar infarct with concurrent white matter disease in the form of other silent lacunar infarcts or leukoaraiosis on CT or white matter hyperintensities on MR. The original hypothesis was generated by an observed increased prevalence in hypertension in Type II compared to Type I (Boiten et al. 1993) and follow up data from a Dutch cohort of patients who were recruited between 1987 and 1992 when CT was used to diagnose and subtype lacunar stroke. Patients with type I “isolated” lacunar stroke had a lower 15 year mortality than type II “multiple silent lesion” lacunar strokes (Staals et al. 2008). Risk factor analysis from a separate group confirms that patients with Type II lacunar infarcts have a higher prevalence of hypertension and lower prevalence of hypercholesterolaemia and concomitant ischemic heart disease than those with “isolated” Type I (Khan et al. 2007). Studies of serum markers of endothelial activation (or function) have demonstrated different levels of activation in Type I and Type II lacunar stroke (Hassan et al. 2003b; Knottnerus et al. 2010). The two subtypes may have differing
aetiologies with atherosclerosis causing Type I “isolated” lacunar stroke and a diffuse arteriopathy causing type II lacunar stroke. Interestingly, in the original pathology papers, Fisher intimated that the larger lacunes might have been caused by atheroma and the smaller ones by a diffuse arteriopathy (Fisher 1982).

1.8 Pathological studies of the small vessel abnormality

Much of our knowledge arises from the important and painstaking pathological studies of lacunar stroke, lacunes and the small arteries by C Miller Fisher in the middle of the 20th century. Fisher initially described the pathological appearance of 50 lacunes. He found that in 40 of these 50 lacunes (mostly asymptomatic) there was narrowing and in some cases occlusion of the perforating artery by segmental disorganisation and thickening of the vessel wall. This process was later termed lipohyalinosis (reflecting the fact that the lipid containing macrophages within it stained readily for fat). This process tended to occur in the smaller arteries (40 – 200 microns in diameter). Lipohyalinosis is characterised in the acute phase by fibrinoid necrosis and later by arterial wall disorganisation and collagenous sclerosis and is associated with areas of focal dilatation (see figure 1.1). Fisher found that in patients with symptomatic lacunes the lacunes tended to be larger with larger diseased arteries (200-800 microns in diameter). The disease causing the lacune in these slightly larger arteries was microatheroma with plaques of foam cells (lipid laden macrophages) with or without overlying thrombosis. Fisher’s results offer an intriguing insight into the pathology behind lacunar stroke but there are caveats. Many of the subjects had severe hypertension (a condition less prevalent today) and not all of the lacunes were correlated with lacunar stroke syndromes and in those that were, the lesions were of varying age. Furthermore many of the lacunes studied came from one patient, further decreasing the generalisability of these findings.
Subsequent pathological studies, which have also tended to include small number of patients, have confirmed the presence of intimal thickening, narrowing and hyalinisation in association with lacunar strokes (Arboix et al. 1996; Challa et al. 1990; Ogata 1999). A further study of 70 brains with evidence of small vessel disease found that the main pathological finding was of concentric hyaline wall thickening. The authors did not find significant evidence of fibrinoid necrosis (but these were not patients with clinical lacunar stroke) (Lammie et al. 1997).

It is not clear whether the described hyalinosis and thickening of the vessel wall (especially the intima) is the cause or a reaction to a previous insult. The following discussion reviews the evidence for and against both conventional and novel lacunar stroke aetiologies.
1.9 Possible causes of lacunar stroke.

Lacunar stroke is associated with other manifestations of cerebral small vessel disease such as white matter lesions and microhaemorrhages (Wardlaw et al. 2006). Both lacunar stroke and WML are conventionally considered to be “ischaemic” but the cause of the ischaemia is debated. Although traditional ischaemic stroke mechanisms such as embolism from the heart or carotid atheroma can cause lacunar stroke, they are not common and would not necessarily explain the small vessel pathological changes (originally described as segmental arteriolar disorganisation and latterly as lipohyalinosis or fibrinoid necrosis) described previously. Intracranial microatheroma might explain the small vessel appearance and was initially described by Fisher but the evidence that the vessel changes are associated with other evidence of atheroma is limited. There is increasing evidence that endothelial dysfunction is associated with small vessel disease (Markus 2008) and this could explain the small vessel changes. Again the underlying cause of the endothelial dysfunction is unclear but thickening of the arterioles and capillaries (and possibly even veins) could result in a loss of cerebral autoregulation but again it is unclear how failure of autoregulation might create ischaemic damage. What causes the endothelial malfunction in the first place is uncertain – there is increasing evidence that progression of neurological deficit in lacunar stroke are associated with increased serum markers of inflammation (Castellanos et al. 2002) which may damage the endothelium with consequent vessel wall and brain changes. But could the endothelial dysfunction be the primary problem and release inflammatory markers into peripheral blood? Alternative hypotheses for which there is also increasing hard evidence include altered permeability of the blood brain barrier which could lead to microvessel wall thickening and perivascular damage (Wardlaw et al. 2009). This could arise from endothelial malfunction from inflammation.
1.9.1 Does embolism cause lacunar strokes?

In the original clinicopathological studies by Fisher he suggested that in the absence of atheroma and with the normal appearance of the perforating arteries, embolism may cause lacunes (Fisher 1969). Emboli may arise from the heart or from atheroma in the aorta or the extra or intra-cranial arteries. Animal studies in rats have demonstrated that small numbers of particles can enter and lodge in the lenticulostriate arteries post non-occlusive thrombus in the carotid artery (Futrell et al. 1989), a finding replicated in monkeys (Macdonald et al. 1995). Extrapolating this to humans is difficult however as the rat brain differs in several key respects, notably in the much smaller proportion of white matter compared to human brain. Additionally, in putative animals of lacunar stroke, most emboli caused cortical rather then lacunar stroke (Bailey et al. 2009). Proving that emboli can occur does not prove that they are the usual cause of lacunar stroke.

Clinical researchers have used two main strategies to investigate the association between emboli and lacunar infarct. The first approach demonstrates the co-existence of embolic sources in patients with lacunar stroke (from which determining cause and effect is difficult) and the second assumes - probably erroneously - that if multiple diffusion weighted imaging positive lesions are present on MRI at the time of the lacunar stroke the most likely aetiology is embolism.

Emboli may arise from a stenosed internal carotid artery: Tejada et al found that ipsilateral stenosis was more common than contralateral stenosis in 135 patients with an isolated lacunar infarct on CT (Tejada et al. 2003). Patients with lacunar stroke and an associated carotid stenosis had less severe stenosis than patients with non-lacunar stroke yet still benefitted from carotid endarterectomy (although the benefits were not as large as for non-lacunar strokes) in the North American Carotid Endarterectomy Trial (Inzitari et al. 2000). The European Carotid Surgery Trial was not powered to look at the beneficial effects of endarterectomy in stroke subtypes although patients in this selected group had less severe stenosis than non-lacunar strokes (Boiten et al. 1996). Interestingly the results from the NASCET and ECST
trial have been used to argue both for (benefit with endarterectomy) and against (lacunar stroke associated with less severe stenosis) embolism causing lacunar stroke. One study of 259 patients showed conclusively that the distribution of severe stenosis was the same in the ipsilateral and contralateral internal carotid arteries in first ever lacunar stroke suggesting that the stenosis may be an innocent bystander. Most previous studies either did not compare symptomatic and asymptomatic stenoses or had referral bias – eg those in the NASCET and ECST trials all had a significant carotid artery stenosis as one of the inclusion criteria (Mead et al. 2002). It has tentatively been suggested that emboli may arise from the aortic arch but the only relevant study compared 62 lacunar stroke patients with non-stroke controls and is therefore unable to attribute the presence of aortic arch atheroma either to lacunar stroke or stroke in general (Kazui et al. 2000).

A small study looked at 27 patients with lacunar infarction and atrial fibrillation and compared them with patients with lacunar infarction in sinus rhythm and concluded that cardio-embolism can cause lacunar infarction (Jung et al. 2001). Small vessel disease can occur in the presence of atrial fibrillation illustrating the difficulties and false precision often associated with assigning stroke aetiologies.

Up to a third of patients with lacunar stroke will have concurrent acute DWI lesions either in the subcortex or cortex (Chowdhury et al. 2004; Norrving 2008). These have been interpreted as indicating proximal embolism in some studies which have subsequently investigated these patients seeking possible sources of embolism. One such study (although the authors do not state whether the concurrent lesions were lacunar or not) found that in 43% of lacunar stroke patient with multiple DWI lesions there was another identifiable stroke aetiology other than small vessel disease but this is also true of other stroke subtypes. This finding was repeated in a separate study of 73 patients with lacunar stroke where the presence of multiple lesions (not necessarily subcortical) was associated with non small vessel disease aetiologies (Wessels et al. 2005). A study which did present details about where these concurrent lesions were located (mostly subcortical) suggested that the presence of these lesions was associated with an identifiable source of embolism (Ay et al. 2008).
There are limitations to these studies in that whilst atrial fibrillation or mural thrombus is obviously a major source of embolism, the proportions of patients with embolic sources was perhaps artificially inflated by the inclusions of soft embolic sources eg it is not clear what embolic risk mild aortic stenosis or LVH confer. The results from other studies are inconsistent in that multiple DWI lesions in lacunar stroke were not associated with sources of embolism (Chowdhury et al. 2004) nor were microemboli detected with trans cranial Doppler in patients with lacunar stroke as they were with cortical stroke patients (Kaposzta et al. 1999).

In summary, embolic phenomena can and may cause a small proportion of lacunar stroke (although the presence of multiple infarcts does not necessarily imply embolism but rather a mechanism acting at different locations simultaneously (Moustafa et al. 2010)).

### 1.9.2 Does atheroma cause lacunar strokes?

Since the initial descriptions by Fisher of microatheroma causing disease in the larger small cerebral arteries there has been little further evidence for this, reflecting the paucity of pathological studies. It is conceivable that macroatheroma may cross the origin of the lenticulostriate arteries thus blocking the smaller vessel, however it is more difficult to demonstrate atheroma within the small vessel.

Intracranial stenosis (thought to be more prevalent in Asian populations) has been shown to be associated with a subset of lacunar stroke but it is not clear whether these strokes were caused by emboli or lenticulostriate ostial occlusion or should be taken as evidence for atheroma (Bang et al. 2004). Carotid intima medial thickness can be used as a marker for atheroma but has been found to be increased in non lacunar stroke compared to lacunar stroke (Cupini et al. 2002; Fulton et al. 2006).
1.9.3 Risk factor analysis

It was initially thought that lacunar stroke was associated with diabetes and hypertension and indeed the TOAST classification used these risk factors to diagnose lacunar stroke. A systematic review and meta-analysis however challenged this, finding that diabetes was not associated with lacunar stroke subtype and that hypertension was only weakly associated with lacunar compared with non-lacunar stroke subtype (Relative Risk 1.11 95% CI 1.04 to 1.19) when risk factor free definitions of lacunar stroke were used (Jackson and Sudlow 2005). Reflecting the embolic and atheromatous aetiology behind non-lacunar stroke, this review found that atrial fibrillation and carotid stenosis were associated with non-lacunar rather than lacunar stroke.

1.9.4 Animal models of lacunar stroke

A recent comprehensive systematic review of potential animal models of lacunar stroke found that most animal models either produced lacunar size infarcts in the cortex or that the small artery disease causing lacunar stroke was not adequately modelled (Bailey et al. 2009). An exception to this was the spontaneously hypertensive stroke prone rat which begins to develop hypertension at about 6 weeks which is established at 12 weeks. In the brain, changes suggestive of blood barrier breakdown with perivascular oedema related white matter lesions appear before subsequent arteriolar damage and clinically apparent strokes. The arterioles have not been well characterised but older rats (16-24 weeks) show fibrinoid necrosis, lipohyalinosis and occasional thrombotic occlusion superimposed on a disrupted arteriolar wall as a late secondary phenomenon but long after the brain damage has occurred. If the same changes occur in humans this might explain why Fisher found the occasional occluded arteriole on a background of small vessel brain disease.
1.9.5 Does increased blood brain barrier permeability cause lacunar stroke?

There is mounting evidence apart from the SHSRP model of lacunar stroke that increased blood brain barrier permeability may cause lacunar stroke. The blood brain barrier is a selectively permeable interface that regulates entry and egress of substances between plasma and the brain (Abbott 2000; Abbott et al. 2006). This is controlled by the cerebral vascular endothelium which has tight junctions between cells and this is maintained by glial cells (astrocytes) which together with the neurons and endothelial cells form the neurovascular unit. Blood brain barrier permeability (measured with a variety of techniques) increases with normal ageing and is associated with cognitive impairment and white matter disease (Farrall and Wardlaw 2007; Starr et al. 2003). Lacunar stroke is associated with white matter disease and cerebral microhaemorrhages (Cordonnier et al. 2007; Wardlaw et al. 2006). Fisher demonstrated findings compatible with increased blood brain barrier leak (areas of focal haemorrhage through the disorganised vessel wall) and recent pathological findings have demonstrated perivascular oedema (with no vessel occlusion) in association with lacunes (Lammie et al. 1998; Ma and Olsson 1997).

Permeability MRI, where cerebral accumulation of contrast is measured over 30 minutes provides direct evidence that increased blood brain barrier may cause lacunar stroke. In 97 patients, 51 of whom had lacunar stroke and 46 cortical stroke, patients with lacunar stroke had increased accumulation of contrast representing generalised increased blood brain barrier permeability compared to cortical stroke (Wardlaw et al. 2009) confirming the results of a previous pilot study (Wardlaw et al. 2008). Increased blood brain barrier permeability may cause lacunar stroke and endothelial dysfunction has been implicated as a cause of this increased permeability (Markus 2008).
1.11 Endothelial dysfunction in lacunar stroke

Endothelial dysfunction is a nebulous term that lacks an exact definition but has been interpreted and assessed in a myriad of different methods. Endothelial dysfunction, perhaps more accurately termed endothelial activation and most commonly investigated as a precursor to atherosclerosis, can be assessed by measuring serum biomarkers or the dynamic endothelium dependent performance of blood vessels (Landmesser et al. 2004). The functions of the endothelium are wide ranging but encompass maintaining vascular tone, haemostasis and the expression of adhesion molecules which influence inflammatory pathways (Knottnerus et al. 2009). Endothelial activation represents a change from a quiescent state of the endothelium to one which involves the host response (Deanfield et al. 2007) which can lead to raised serum biomarkers of endothelial dysfunction.

1.12 Serum biomarkers of endothelial dysfunction in lacunar stroke: a systematic review.

1.12.1 Introduction

Endothelial dysfunction may lead to lacunar stroke yet the published literature produces conflicting results and there is a wide range of serum markers that have been measured. We performed a systematic review to ascertain whether serum markers of endothelial dysfunction are associated with lacunar stroke. We were interested primarily in whether serum markers were raised in lacunar ischaemic stroke compared to ischaemic non-lacunar stroke. From an initial search of the literature we chose the following markers to represent endothelial dysfunction: tissue plasminogen activator(t-PA), von Willebrand’s Factor (vWF), Intracellular Cell Adhesion Molecule (ICAM), Vascular Cell Adhesion Molecule (VCAM), plasminogen activator inhibitor (PAI), P & E selectin, fibrinogen, C-reactive protein (CRP), homocysteine, Interleukin-6 (IL-6) and D-dimer.
1.12.2 Methods

We searched Medline (from January 1966) and Embase (from January 1980) on 5th August 2009 for studies which assessed serum markers of endothelial function in lacunar stroke with a control group consisting of ideally non-lacunar stroke but including non-stroke control groups (see appendix 1 for the search strategy). We checked references in review and primary papers and hand-searched the journal *Stroke*.

We included papers in humans which were presented in full and in a language we were able to easily translate. We excluded those which dealt with leukaraoisis or white matter disease solely. Two reviewers (FD, Fergal Marlborough) reviewed all titles and extracted data. A third reviewer (Joanna Wardlaw) adjudicated in any disagreements.

1.12.3 Results

The initial search generated 1190 titles/abstracts from which we reviewed the full text of 40 papers. 8 papers were rejected as we were unable to translate these papers and a further 15 were not relevant after full text review leaving 17 papers that were included in this review. Of these 17 papers, most papers used dual control groups of both non-lacunar stroke and non-stroke controls. Ten papers compared lacunar stroke with non-lacunar stroke (Ageno et al. 2002; Kataoka et al. 2000; Khan et al. 2008; Kilpatrick et al. 1993; Kozuka et al. 2002; Licata et al. 2009; Nakase et al. 2008; Ohira et al. 2006; Parnetti et al. 2004; Takano et al. 1992). 13 papers compared lacunar stroke patients with non-stroke controls (Ageno et al. 2002; Hassan et al. 2003a; Hassan et al. 2003b; Iso et al. 2004; Khan et al. 2008; Kozuka et al. 2002; Licata et al. 2009; Lindgren et al. 1996; Ohira et al. 2006; Parnetti et al. 2004; Salobir et al. 2002; Salobir and Sabovic 2004; Takano et al. 1992).
In general, 14 papers mentioned which stroke classification system was used to delineate lacunar stroke and 15 papers stipulated which method of brain scanning was used (CT or MR). 14 papers provided a definition for lacunar stroke.

In general serum markers of endothelial dysfunction were higher in lacunar stroke patients compared to non-stroke controls although there was heterogeneity between these results and between different serum biomarkers. These results are detailed in the table below.
Table 1.1 showing serum markers of endothelial function in lacunar versus non stroke controls.

<table>
<thead>
<tr>
<th>Study</th>
<th>Lacunar group N(age)</th>
<th>Non-stroke Controls N(age)</th>
<th>Endothelial marker</th>
<th>Lacunar marker level</th>
<th>Control marker level</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ageno 2003</td>
<td>31 (75.4)</td>
<td>63</td>
<td>D-dimer</td>
<td>0.7±0.1g/mL</td>
<td>0.5 ±0.1g/mL</td>
<td>ns</td>
</tr>
<tr>
<td>Hassan 2003</td>
<td>47 (62)</td>
<td>50 (66)</td>
<td>ICAM-1</td>
<td>454.23ng/mL</td>
<td>341.90ng/mL</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Hassan 2004</td>
<td>172 (66±10)</td>
<td>172 (66±10)</td>
<td>Homocyst</td>
<td>13.61 µmol/L</td>
<td>12.01µmol/L</td>
<td>P=0.04</td>
</tr>
<tr>
<td>Iso 2004</td>
<td>75 (64.9)</td>
<td>225 (65)</td>
<td>Homocyst</td>
<td>9.2 µmol/L</td>
<td>8.9 µmol/L</td>
<td>ns</td>
</tr>
<tr>
<td>Kataoka 2000</td>
<td>58 (66±12)</td>
<td>32 (66±12)</td>
<td>Fibrinogen</td>
<td>306 mg/dL</td>
<td>300 mg/dL</td>
<td>ns</td>
</tr>
<tr>
<td>Khan 2008</td>
<td>144 (65)</td>
<td>179 (65)</td>
<td>Homocyst</td>
<td>13.6 µmol/L</td>
<td>11.8 µmol/L</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Kozuka 2002</td>
<td>27 (69.0)</td>
<td>86 (60.0)</td>
<td>vWF</td>
<td>165%</td>
<td>132.5%</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E selectin</td>
<td>41.2ng/mL</td>
<td>24.6 ng/mL</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P selectin</td>
<td>53.9 ng/mL</td>
<td>38.4 ng/mL</td>
<td>P=0.038</td>
</tr>
<tr>
<td>Licata 2009</td>
<td>46 (66.5)</td>
<td>123 (69)</td>
<td>vWF</td>
<td>6 ng/mL</td>
<td>4 ng/mL</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IL-6</td>
<td>4 pg/mL</td>
<td>9 pg/mL</td>
<td>ns</td>
</tr>
<tr>
<td>Lindgren 1996</td>
<td>33 (69)</td>
<td>77 (66)</td>
<td>tPA antigen</td>
<td>11µg/L</td>
<td>6µg/L</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PAI antigen</td>
<td>22µg/L</td>
<td>8µg/L</td>
<td>ns</td>
</tr>
<tr>
<td>Ohira 2006</td>
<td>105 (56.2)</td>
<td>13957 (54)</td>
<td>vWF</td>
<td>136%</td>
<td>117%</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>fibrinogen</td>
<td>326mg/dL</td>
<td>302 mg/dL</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Parnetti 2004</td>
<td>50</td>
<td>152 (69±13)</td>
<td>Homocyst</td>
<td>13.9 µmol/L</td>
<td>8.1 µmol/L</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Salobir 2002*</td>
<td>16(37)</td>
<td>47 (38)</td>
<td>tPA antigen</td>
<td>6.9ng/mL</td>
<td>5.3ng/mL</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tPA activity</td>
<td>0.52 IU/mL</td>
<td>0.54 IU/mL</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PAI antigen</td>
<td>10.6ng/mL</td>
<td>8.8ng/mL</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PAI activity</td>
<td>11.7 IU/mL</td>
<td>8.5 IU/mL</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fibrinogen</td>
<td>2.6±0.4 g/L</td>
<td>2.3±0.4 g/L</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D-dimer</td>
<td>57 ng/L</td>
<td>59 ng/L</td>
<td>ns</td>
</tr>
<tr>
<td>Salobir 2003*</td>
<td>16 (42)</td>
<td>52 (41)</td>
<td>tPA</td>
<td>0.5 IU/mL</td>
<td>0.5 IU/mL</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PAI-1</td>
<td>9.5 IU/mL</td>
<td>6.7 IU/mL</td>
<td>ns</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Fibrinogen</td>
<td>2.4g/L</td>
<td>2.3 g/L.</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D-dimer</td>
<td>57ng/mL</td>
<td>59ng/mL</td>
<td>ns</td>
</tr>
<tr>
<td>Salobir 2004*</td>
<td>16 (42)</td>
<td>47 (41)</td>
<td>IL-6</td>
<td>1.6pg/mL</td>
<td>1.4pg/mL</td>
<td>ns</td>
</tr>
<tr>
<td>Takano 1992</td>
<td>23 (65)</td>
<td>20 (61)</td>
<td>D-dimer</td>
<td>115±15ng/mL</td>
<td>82±9 ng/mL</td>
<td>ns</td>
</tr>
</tbody>
</table>

The numbers in bold represent the significantly higher values. ICAM = intra cellular adhesion molecule, vWF = von Willebrand’s Factor, IL-6 = inter-leukin-6, tPA = tissue plasminogen antigen, PAI-1 = plasminogen activator inhibitor. The Salobir studies marked with * may represent duplicate publication. Ns = non significant.
In lacunar versus non-lacunar stroke 7 different biomarkers were compared in 8 studies. In general there were higher levels of serum biomarkers in non-lacunar stroke compared with lacunar stroke. These results are presented in table 1.2.

*Table 1.2 showing serum markers of endothelial function in lacunar versus non-lacunar stroke controls. Please see footnote.*

<table>
<thead>
<tr>
<th>Study</th>
<th>Lacunar group N(age)</th>
<th>Non-lacunar group N(age)</th>
<th>Endothelial marker level</th>
<th>Lacunar marker level</th>
<th>Non-lacunar marker level</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ageno 2002</td>
<td>31</td>
<td>34</td>
<td>D-dimer 0.7±0.1 µg/mL</td>
<td>1.3±0.2µg/mL</td>
<td>P=0.01</td>
<td></td>
</tr>
<tr>
<td>Kataoka 2000</td>
<td>58 (68±13)</td>
<td>41 (66±14)</td>
<td>Fibrinogen 306 µg/ml</td>
<td>412 µg/ml</td>
<td>P&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Khan 2008</td>
<td>144 (65.4)</td>
<td>40 (65.4)</td>
<td>Homocyst 13.6 µmol/L</td>
<td>11.6µmol/L</td>
<td>P&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Kozuka 2002</td>
<td>27 (69.0)</td>
<td>16 (65.0)</td>
<td>vWF 165%</td>
<td>176.5%</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P selectin 41.2 ng/ml</td>
<td>48.9 ng/ml</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E selectin 53.9 ng/ml</td>
<td>44.3 ng/ml</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Licata 2009</td>
<td>46 (66.5)</td>
<td>50 (75)</td>
<td>IL-6 4 pg/mL</td>
<td>8 pg/mL</td>
<td>P=0.003</td>
<td></td>
</tr>
<tr>
<td>Ohira 2006</td>
<td>105 (56.2)</td>
<td>326 (57.3)</td>
<td>vWF 136%</td>
<td>132%</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>fibrinogen 326 mg/dL</td>
<td>321 mg/dL</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Parnetti 2004</td>
<td>50</td>
<td>43</td>
<td>Homocyst 13.9 µmol/L</td>
<td>17.8 µmol/L</td>
<td>P=0.046</td>
<td></td>
</tr>
<tr>
<td>Takano 1992*</td>
<td>23 (65)</td>
<td>10 (64.9)</td>
<td>D-dimer 115±15 ng/mL</td>
<td>171±29 ng/mL</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

Footnote for table 1.2. The numbers in bold represent the significantly higher values. vWF = vonWillebrand’s Factor, IL-6 = inter-leukin-6, PAI-1 = plasminogen activator inhibitor. Ns = non significant. *Data are mean and standard deviation apart for Takano where data are mean and standard error.
1.12.4 Conclusions

It is likely that the higher levels of most serum biomarkers in non-lacunar stroke compared with lacunar stroke reflects the higher degree of vascular damage in especially cardio-embolic but also large artery atherothromboembolic stroke compared to the relatively small volumes of the parenchymal lesion present in lacunar stroke. The results from this systematic review illustrate the importance of choosing the correct control group as lacunar strokes have higher levels of serum markers of endothelial dysfunction when compared to non-stroke controls (it is likely that having had a stroke of any subtype will raise serum markers) but lower levels of serum endothelial when compared to non-lacunar stroke control groups. There is a relative paucity of studies comparing serum markers of endothelial dysfunction in ischaemic stroke subtypes and significant heterogeneity amongst those published. The endothelial dysfunction evident may be subsequent to having had any stroke or represent the consequences of exposure to common risk factors for stroke such as hypertension, diabetes or cigarette smoking.

1.13 Dynamic measures of endothelial dysfunction in lacunar stroke: a systematic review.

1.13.1 Introduction

Endothelial function can be measured by assessing the endothelial dependent (and primarily nitric oxide dependent) vascular response to an insult (Tousoulis et al. 2005). This can be measured peripherally (by measuring the flow mediated dilatation to cuff inflation in a peripheral artery such as the brachial artery) or in the cerebral circulation by assessing blood flow response to vascular insults such as induced hypercapnia, infusion of acetazolamide or L-arginine. We therefore systematically assessed published studies which tested dynamic endothelial function in patients with lacunar stroke in the cerebral or peripheral circulation.
1.13.2 Methods

We searched Medline and Embase (from Jan 1995) on 15th February 2008 using Ovid and a carefully designed search strategy (see appendix 2) for studies which assessed dynamic endothelial function in patients with lacunar stroke versus cortical ischaemic stroke or non-stroke controls. We checked references in review and primary papers and hand-searched the journal Stroke. Two reviewers extracted data (FD, Susan Stevenson); a third resolved disagreements (Joanna Wardlaw). We were able to combine individual study results by calculating standardised mean difference in vascular reactivity, +/- 95% confidence intervals (SMD, 95%CI) between groups.

1.13.3 Results

The initial search generated 1135 title/abstracts from which we obtained the full text for 27 papers. Three papers were excluded as we were unable to translate them (Russian and Slovakian) and a further nine papers failed to meet the inclusion criteria.

We therefore identified 15 relevant publications (885 patients) reporting on vascular reactivity in the cerebral or peripheral arterial circulations. Twelve papers assessed endothelial function in the cerebral circulation (Chamorro et al. 1996; Cupini et al. 2001; de Leeuw et al. 2003; Gur et al. 2007; Hund-Georgiadis et al. 2003; Immink et al. 2005; Maeda et al. 1993; Mochizuki et al. 1997; Molina et al. 1999; Panczel et al. 1999; Pretnar-Oblak et al. 2006c; Pretnar-Oblak et al. 2006b). Four papers assessed brachial artery flow-mediated dilatation, expressed as the percentage change in arterial diameter (Chen et al. 2006; Pretnar-Oblak et al. 2006c; Pretnar-Oblak et al. 2006a; Pretnar-Oblak et al. 2006b).

Several techniques were used to assess cerebral endothelial function, including the vascular response to hypercapnia, infusion of acetazolamide or L-arginine, expressing the response as a percentage increase in mean arterial blood velocity in the MCA or basilar artery. Change in blood oxygen level dependent signal during
Cerebrovascular reactivity (n=534) was reduced in lacunar stroke patients compared with age-matched normal (SMD -0.94, 95% CI, -1.17, -0.70, P<0.00001), but not age + risk factor-matched controls (SMD 0.08, 95% CI -0.36, 0.53) or cortical strokes (SMD -0.29, 95% CI -0.69, 0.11); forearm flow mediated dilatation (n=312) was reduced compared with age-matched normal controls (SMD -1.16, -1.52, -0.80) and age + risk factor-matched controls (SMD -0.65, 95% CI-1.10, -0.19), but not cortical strokes (SMD -0.23, 95% CI -0.55, 0.08). See figure 1.2.

Figure 1.2. Forest plot showing standardised mean difference in cerebrovascular reactivity in patients with lacunar ischaemic stroke vs. age-matched, age+risk factor matched and cortical ischaemic stroke controls and in patients with lacunar ischaemic stroke with vs. without multiple silent lacunar infarcts. Squares represent the ratio of the lacunar response divided by the control response with the solid black lines representing the 95% CI. The diamond represents the summary result.
From this systematic review we have shown that lacunar stroke patients have impaired endothelial function compared with normal controls but this is not so clear-cut when compared with patients with vascular risk factors or cortical stroke. Again these results reiterate the importance of careful control group selection and suggest that endothelial dysfunction may simply be a consequence of exposure to vascular risk factors. We would recommend further, much larger studies with cortical stroke controls to exclude the possibility that modest endothelial dysfunction is associated specifically with lacunar stroke.

1.13.4 Conclusions.
1.14 Alternative methods for studying lacunar stroke

Despite many efforts over the last 50 years and despite a multifaceted approach to understanding the aetiology of lacunar stroke, little progress has been made (Davis and Donnan 2004). Differences in risk factor analysis, survival data and complications as well as associations with other manifestations of cerebral small vessel disease suggest firstly that lacunar stroke may have a distinct aetiology compared to larger artery and cardio-embolic stroke and secondly that lacunar strokes may not be isolated phenomena and may be one of the cerebral manifestations of a systemic vasculopathy, an idea gaining credence (Thompson and Hakim 2009). The study of vascular beds other than those in the brain e.g. the retina may shed light on the pathophysiology of lacunar stroke.

Fig 1.4 Digital Retinal photograph of a fundus with no macroscopic abnormalities

1.14.1 Retinal and cerebral vessels: comparative anatomy

The retinal arterioles and cerebral arterioles causing small vessel disease share many characteristics. They are embryologically similar as they both arise from the third
branchial arch of the developing foetus at the same developmental stage. They are of similar size: central retinal arteries have been estimated to be approximately 150 microns in diameter with subsequent branches smaller than this (Ikram et al. 2006b) and the cerebral small arterioles range from 50 – 800 microns in diameter (Fisher 1982). Although retinal collaterals have been described as a response to severe sclerotic hypertensive retinopathy (Ball and Henkind 1967), the arterioles tend to be end arterioles with few anastomotic connections. Similarly the lenticulostriate arterioles are end arteries with again few anastomotic connections. Both retinal and cerebral arterioles have tight junctions between endothelial cells which are attached to a basement membrane with surrounding smooth muscle cells (Patton et al. 2005).

At a capillary level the retinal capillaries are slightly smaller (5-6 microns) than their cerebral counterparts and both are lined with a single layer of endothelial cells with tight junctional intercellular complexes which are composed of proteins including claudins, junctional adhesion molecules and occludins (Patton et al. 2005). There is a surrounding basement membrane in both into which pericytes are embedded. These mesenchymal cells provide structural integrity to the capillary (much like the smooth muscle does to the arteriole) and have contractile properties. There is a higher density of pericytes in the retina compared to the cerebrum (Frank et al. 1990). Both retinal and cerebral capillaries are surrounded by the perivascular end feet (Abbott et al. 2006) of astrocytes.

1.14.2 Retinal and cerebral vessels: comparative physiology

The retinal and cerebral microcirculations both have a barrier function which regulates the transport and exchange of particles from the blood to the retina/brain (Bernacki et al. 2008; Kaur et al. 2008). This barrier has a physical (the presence of tight junctions between the endothelial cells) and a chemical component with transport proteins regulating the transport of cells, proteins and molecules (Patton et al. 2005). The surrounding astrocytes play a role in the maintenance of this barrier (Abbott et al. 2006).
1.14.3 Retinal and cerebral vessels: comparative pathology

In an unselected autopsy series in India of 43 patients who did not have clinical evidence of atherosclerosis, the authors report arteriosclerosis in the retinal arteries consisting of fibrosis and hyalanization with thickening of the vessel wall and subsequent narrowing of the lumen. These changes were mirrored in the small vessels of the brain (Patwardhan et al. 1970) and are confirmed in another autopsy series which correlated retinal arteriosclerosis (fibrinoid degeneration and hyalofibrinoid thickening of the arterioles) with cerebral arteriosclerosis (Goto et al. 1975).

1.14.4 Retinal photography

The retinal vasculature can be directly visualised and although direct ophthalmoscopy by a human operator with a fundoscope is prone to error the retina can be easily photographed (Patton et al. 2006b). Initial retinal cameras were analogue and there was a delay until the film was developed and the quality of the photograph known. Recently the development of digital cameras has revolutionised the quality of retinal photographs as the image quality can be assessed immediately and if poor, discarded and repeated.

Fig 1.5 Digital Retinal camera. The subject is the author of this thesis. Note the subject needs to maintain head posture and keep eyes still.
The training for retinal photographers is straightforward and retinal photography is feasible in large population based studies with relatively low rates of ungradeable quality photographs (Ikram et al. 2006a; Wong et al. 2001). Even in a technically demanding cohort of patients with acute stroke the rate of ungradeable photographs was only approximately 10% (Lindley et al. 2009).

1.14.5 Techniques for assessing retinal vessels

There are many different techniques for assessing static retinal photographs ranging from quantitative assessment of focal retinal changes such as retinopathy to semi automated assessment of quantitative parameters such as vessel widths to fully automated assessment such as fractal analysis. I will concentrate on these different methods of retinal analysis in the appropriate chapters of this thesis. There are retinal imaging techniques that can capture dynamic changes in the retinal microvasculature ie record vessel widths changes over a short period of time (Liew et al. 2008c) but these are not dealt with in this thesis.

1.14.6 Associations between retinal microvascular abnormalities and systemic disease in humans

There are well established associations between retinal microvascular abnormalities (especially decreased arteriolar width, focal arteriolar narrowing and arteriovenous nicking) and hypertension (DellaCroce and Vitale 2008; Wong and Mitchell 2007). Retinal microvascular abnormalities have been used in the clinical assessment of patients with hypertension to establish end-organ damage and hence give an indication to duration of hypertension both in the routine assessment of hypertension (Wong and Mitchell 2004) and in the acute phase of malignant hypertension. A recent systematic review assessed whether routine fundoscopy was useful in the assessment of a patients with hypertension and concluded that fundoscopy did not confer additional benefit over measuring the blood pressure (van den Born et al.)
It was not clear if this referred to direct fundoscopy by a clinician or retinal photography, important as direct fundoscopy has a low inter and intra rater reliability for the detection and characterisation of retinal lesions (Dimmitt et al. 1989). Retinal microvascular abnormalities can predict future risk of both hypertension and other cardiovascular disease (Wong and McIntosh 2005b) although the clinical significance of this above measurement and correction of modifiable traditional risk factors is uncertain.

Diabetic retinopathy secondary to thickening of the basement membrane with subsequent increased permeability of the blood retinal barrier (Bernacki et al. 2008) is a common complication of diabetes reflecting glycaemic control and duration of disease and can be an independent predictor of future cardiovascular disease (Liew and Wang 2007).

In this thesis I will present details of the associations between retinal microvascular abnormalities and stroke. Retinal microvascular abnormalities have also been linked with clinical features of small vessel disease in other vascular beds including impaired cognition (Ding et al. 2008; Patton et al. 2007; Wong et al. 2002b), the presence of cerebral white matter lesions on MRI (Qiu et al. 2009; Wong et al. 2002a) and renal disease (Ikram et al. 2008).

1.14.7 Applications for retinal photography

There are three main applications for retinal photography. The first is as a diagnostic aid to guide immediate therapy such as screening for diabetic retinopathy or identifying retinal artery or venule occlusion (Patton et al. 2006b). The second application is as a predictor of future disease whether ocular or systemic. Retinal features have been identified as independent predictors of future cardiovascular events (Wong 2004) although the additional clinical benefit over measuring conventional risk factors is uncertain. Thirdly and of most relevance to this thesis the retinal vessels may act as a marker to aid understanding of the pathophysiology of cerebral vascular disease.
1.15 Aims and objectives of this thesis

The aim of this thesis is to use retinal microvascular abnormalities as a surrogate marker of cerebral small vessel disease in combination with detailed MR brain imaging to gain insights into the hitherto unknown aetiology of lacunar stroke. The main hypothesis is that retinal microvascular abnormalities will be more prevalent in lacunar stroke patients compared with cortical stroke patients.

The specific questions I will address are:

1. To what extent are published retinal microvascular abnormalities associated with stroke and ischaemic subtypes?
2. Can MRI changes in lacunar stroke provide more information regarding the underlying aetiology of lacunar stroke?
3. Does the prevalence of retinopathy (thought to reflect blood-retinal barrier breakdown) differ between lacunar and non-lacunar stroke?
4. Do retinal vessel widths differ in lacunar and non-lacunar stroke?
5. Does retinal vessel geometry differ in lacunar and non-lacunar stroke?
6. Does fractal analysis of the retinal vasculature differ between lacunar and non-lacunar strokes?

To do this, this thesis addresses these questions from several angles: a systematic review of retinal vascular and retinopathic changes in patients with stroke and those who subsequently develop stroke, new data collection of a cohort of very carefully characterised (clinically and radiographically) patients with retinal imaging to examine retinopathic and retinal-vascular associations with lacunar and cortical stroke, detailed analysis of retinal vascular branching patterns including development of new software to assess branching co-efficients, angles and fractal dimensions of the vascular tree and a detailed analysis of associations between retinal and brain imaging features with particular reference to small vessel versus large vessel changes.
The study was part of a large programme of work addressing different aspects of potential mechanisms of vascular disease causing lacunar stroke and cerebral small vessel disease. As such this thesis focuses on the retinal findings but benefited from the substantial pilot work, additional funding and ongoing assessments of other factors (blood markers, carotid and intracranial doppler imaging, detailed brain imaging, blood brain barrier permeability imaging, experimental work and other systematic reviews) that made up the different components of this programme.

Finally the retinal component was designed to complement a contemporaneous study of retinal features in stroke being conducted in Australia and Singapore (Principal Investigator Prof Richard Lindley, University of Sydney) which was anticipated to have a larger sample size but less detailed brain imaging and hence more noisy stroke phenotyping compared with the present study and we ultimately intend to merge the two databases to perform a large individual patient based meta analysis.
Chapter 2. Edinburgh Mild Stroke Study: aims, design and methodology.

2.1 Aims of chapter

In this chapter I describe the background, aims and methods used in the Mild Stroke Study.

2.2 Overview of the Edinburgh Mild Stroke Study

The Mild Stroke Study aimed to elucidate the aetiology of lacunar stroke by comparing retinal, blood markers, large artery disease, risk factors and brain MRI based parameters between patients with lacunar stroke and a control group of patients with cortical stroke. The Mild Stroke Study was part of a large programme of work to address gaps in knowledge concerning mechanisms of lacunar stroke. In addition to the diagnostic MR and retinal imaging, we collected serum biomarkers of coagulation, fibrinolysis and inflammation, performed transcranial doppler studies and a subset of the patients were studied with blood brain barrier permeability contrast enhanced MRI scans. This thesis focuses on the retinal and brain findings.

2.3 Contributors to the Mild Stroke Study

Prof Joanna Wardlaw developed the hypothesis for causes of lacunar stroke which was then tested as part of an extended programme of work which benefitted from several different sources of funding. Prof Wardlaw was my PhD supervisor and all work that I completed on the Mild Stroke Study was under her direct supervision. A component of this programme was to examine retinal features in lacunar stroke which is the focus of this thesis. I, with support from my supervisors and sponsor Peter Sandercock, obtained funding from the Wellcome Trust for my clinical research training fellowship which also funded purchase of the retinal camera. The fellowship application and subsequent development of study protocols benefitted
considerably from previous grants and pilot work in this area by Prof Wardlaw with patient recruitment by research fellows Drs Vera Cvoro, Debashish Chowdhury and Kristin Haga. I liaised with the Ethics Committee regarding modifications to the original approval which had been submitted by Prof Wardlaw. I adapted the main study data collection forms and designed the retinal data collection forms, recruited all patients (other than 8 which were recruited by another research fellow), developed the database (with input from David Perry, head of IT in the Division of Clinical Neurosciences), entered the data and performed all of the statistical analysis (with input and advice from statisticians Cat Graham, Francesca Chappell and Steff Lewis). I co-ordinated the booking of most of the MRI scans all of which were performed by the radiographers in the SFC Brain Imaging Research Centre for Scotland (SBIRC) which is part of the Scottish Imaging Network, A Platform for Scientific Excellence (SINAPSE) collaboration.

I took or directly supervised the taking of all retinal photographs to ensure adequate quality in the Wellcome Trust Clinical Research Facility, Western General Hospital, Edinburgh. I bought and established the retinal camera in the Wellcome Trust Research Facility and trained other photographers with close collaboration with our colleagues in Australia (Prof Richard Lindley) with whom we intend to pool data. I graded all retinal photographs for the presence of retinopathy, arteriovenous nicking and focal arteriolar narrowing. Prof Bal Dhillon, ophthalmologist, graded any questionable lesions. Dr Tom MacGillivray (Senior Image Analyst), in conjunction with me, developed the custom written software with which the retinal photographs were analysed for retinal vessel widths. I graded all retinal images for vessel widths using the semi-automated software. Rosemarie den Haan (a medical student from the University of Amsterdam) helped us develop the software for assessing retinal geometry and subsequent grading of the retinal photographs. Dr MacGillivray processed the retinal photographs to assess their fractal properties. Prof Martin Dennis supervised the clinical aspect of the study and provided help with clinical sub-typing of stroke. University of Edinburgh medical students Susan Stevenson and Fergal Marlborough helped collect data for the systematic reviews on endothelial dysfunction in lacunar stroke. University of Amsterdam medical student Petra
Hokke helped collect data for the systematic review of retinal microvascular abnormalities in stroke and helped pilot early versions of the retinal widths measuring software. Prof Wardlaw obtained the funding for the overarching main study which included funding for the brain scans, all computational analysis and for statistical advice. Prof Wardlaw coded all of the brain scans and supervised the whole project.

2.4 Design of the Mild Stroke Study

The Mild Stroke Study was a prospective observational cross-sectional study.

2.5 Ethical approval

The Mild Stroke Study received ethical approval from the Lothian Regional Ethics Committee and all patients provided written informed consent (ethics committee reference number LREC/2002/8/64). There were several subsequent amendments to address minor study modifications and to keep abreast of regulatory changes. See appendices 3 and 4 for details of the Patient Information Leaflet and the Consent Form.

2.6 Setting

The Mild Stroke Study recruited patients who presented with symptoms suggestive of mild ischaemic stroke (defined as lacunar or mild cortical stroke) to the Western General Hospital in Edinburgh, UK. The Western General Hospital is a tertiary university teaching hospital with a specialist acute stroke service (16 bedded stroke unit, 22 bedded rehabilitation ward) and 24 hour stroke thrombolysis program. The population that the hospital serves is a largely urban population of about 250,000.

During the recruitment period of the Mild Stroke Study patients who had symptoms suggestive of a stroke would have been either referred by their general practitioner (family physician) to the thrice weekly neurovascular clinic or to the medical
receiving unit if their needs were such that in-patient admission might be warranted. Patients who called for an emergency ambulance would either have been taken to the acute receiving unit of the Western General Hospital or the Accident and Emergency Department of the other main hospital in Edinburgh, the Royal Infirmary of Edinburgh.

2.7 Time course of study

Pilot work began in 2003 to test the mechanisms of patient recruitment, the brain imaging methods, the collection of demographic and other patient variables and the retinal photography. The patients described in this thesis were recruited from April 2005 to January 2008.

2.8 Inclusion criteria

We recruited patients at presentation to the hospital with definite lacunar or mild cortical ischaemic stroke as defined in the Oxfordshire Community Stroke Project (Bamford et al. 1991) and diagnosed by an experienced stroke physician. We included patients presenting up to three months after the onset of their symptoms and used diagnostic MRI to diagnose the recent stroke lesion so as to have the best chances of being able to accurately subtype ischaemic stroke. All patients were able to give informed written consent and understand the written patient information sheet (see appendices 3 and 4) which was explained in detail by me to each patient and their relatives/carers if present and relevant.

We defined a lacunar stroke syndrome as one causing one of the 5 classical lacunar syndromes: pure motor stroke involving either weakness of the face and arm, arm and leg or all three, pure hemi sensory stroke involving either sensory loss of the face and arm, arm and leg or all three, sensorimotor stroke as weakness and sensory loss in the face and arm, arm and leg or all three, ataxic hemiparesis or clumsy hand dysarthria syndrome. We defined a mild cortical stroke syndrome as; weakness or sensory loss affecting one limb (or just face) or dysphasia/neglect/inattention or
homonymous hemianopia or any motor weakness and dysphasia/neglect/inattention, dysphasia/neglect/inattention and homonymous hemianopia equivalent to a partial anterior circulation stroke in the OCSP classification (Bamford et al. 1991).

We used a control group of patients with cortical ischemic stroke to control for having a stroke, risk factor profiles, and secondary stroke prevention medications (as opposed to normal age-matched controls, which would not have controlled for any of these factors). The vascular risk factor profiles of patients with lacunar and cortical stroke are similar (Jackson and Sudlow 2005) other than for carotid stenosis and atrial fibrillation. We used mild cortical stroke patients and not those with severe cortical strokes as retinal photography is impractical in patients with severe stroke (Lindley et al. 2009) and the mechanisms behind severe cortical stroke are represented in patients with mild cortical stroke.

2.9 Exclusion criteria

We excluded patients presenting with symptoms or brain imaging findings that were inconsistent with a diagnosis of definite stroke. We excluded patients with contraindications to MR e.g. cardiac pacemakers, metal implants elsewhere in the body and screened with orbital X-rays patients with previous metal fragment injuries to the eyes. We excluded patients who were unable to lie flat or who did not fit into the scanner as they were too wide. We excluded patients whose subsequent MR scan showed a haemorrhage causing their stroke syndrome or in whom the MR scan showed an alternative pathology causing their symptoms e.g. tumour. We performed permeability MRI in some patients which involved injecting intra-venous gadolinium contrast and therefore we excluded patients with impaired renal function (estimated glomerular filtration rate <50 ml/min) to avoid nephrogenic systemic fibrosis. We excluded patients who had complex lesions on MRI scanning such as gliosis secondary to trauma prohibiting reliable sub-typing into lacunar or cortical stroke. We excluded patients who were medically unstable (as lying flat for a MRI for 20 minutes might have exacerbated their condition and compliance with retinal photography is reduced in medically unstable patients).
2.10 Methods of patient recruitment

I identified potential patients in several different ways. All stroke physicians and neurologists who were seeing acute stroke patients in the Western General Hospital were aware of the study and contacted me directly by phone or via letter if they thought a patient was suitable patient. I reviewed on a daily basis the stroke unit and acute receiving wards and all neurovascular clinic lists were reviewed searching for suitable patients. Prof Wardlaw identified potentially suitable patients by screening CT/MR and carotid doppler requests. During the pilot phases of the trial patients were recruited by Drs Vera Cvoro and Debashish Chowdhury. For patients described in this thesis, Dr Kristin Haga recruited the first 8 patients in the study. I recruited all subsequent 245 patients.

2.11 Clinical assessment and data collection

I clinically assessed all potential patients at presentation to the hospital with stroke and collected data on a specifically designed and piloted data collection sheet (see appendix 5). I performed a detailed history and clinical examination to ascertain current symptoms, past medical history and current neurological and cardiovascular impairments. I collected data on the date and time of current symptoms, the date that the patient was clinically assessed and if the symptoms were persisting or if they had resolved how long they had lasted. I recorded the side of the body which was affected (if appropriate). I recorded whether there was weakness and if so whether the face, arm or leg was affected. I recorded whether there was sensory loss and if so whether the face, arm or leg was affected. I recorded the presence of the following: dysphasia, neglect, visual disturbance, posterior circulation symptoms. I recorded whether the patient was left or right handed and assessed NIHSS score both at the time of the clinical assessment and as there were some delays to when patients were seen after their stroke, I also recorded estimated worst NIHSS. I recorded gender of the participant and their date of birth and age at stroke onset.
I recorded presence of hypertension (either previous history of physician/general practitioner diagnosed hypertension or patient on treatment for hypertension), diabetes (either previous history of physician/general practitioner diagnosed Type I or Type II diabetes or on treatment for diabetes – oral hypoglycaemic agents or insulin) and if it was diagnosed at the time of presentation, peripheral vascular disease (either surgeon/physician/general practitioner diagnosed history of or current symptoms of intermittent claudication), ischaemic heart disease subdivided into previous angina, myocardial infarct, coronary angioplasty, coronary artery bypass grafting (either physician/general practitioner diagnosed history of angina or myocardial infarct or previous coronary angioplasty or coronary artery bypass grafting), Transient Ischaemic Attack (TIA) (either physician or GP diagnosed history of), stroke (either physician/GP diagnosed history of) for which I collected details of side of stroke, type of stroke (haemorrhagic/infarct) and location (lacunar/ischaemic) if known. I also recorded if there was a history of atrial fibrillation (physician or general practitioner diagnosed history of), hyperlipidaemia (physician/general practitioner diagnosed history of), left ventricular systolic dysfunction (physician/GP diagnosed or echocardiographic evidence of) and structural heart disease (physician/GP diagnosed history of significant valve lesions, known patent foramen ovale).

I collected data on cigarette/tobacco smoking status (defined as current or ex smoker less than 12 months, ex smoker greater than 12 months or never), current alcohol use measured in unit per week and whether the participant previously drank excessive amount of alcohol and whether the patient was independent in activities of daily living prior to the stroke. I recorded the presence of a family history of stroke in a first degree relative. I recorded a single blood pressure at presentation in either arm, normally in the sitting position unless the patient was unwell and recumbent.

We obtained electrocardiograms on all participants and recorded whether the participant was in sinus rhythm or the presence of atrial fibrillation and the presence of left ventricular hypertrophy on ECG. All patients had carotid arteries assessed and we recorded which method (doppler ultrasound in the majority or magnetic
resonance angiography in a few) and the percentage internal carotid artery stenosis measured using the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria. If a participant had an echocardiogram we recorded any relevant abnormalities that might have been a potential source of embolism. We recorded random serum glucose and total serum cholesterol taken at the time of initial assessment and measured as part of the normal clinical stroke protocol in the local laboratories in the Western General Hospital.

We recorded the use of the following medications or medication types at the time of the stroke: aspirin, dipyridamole, warfarin, diuretic, angiotensin converting enzyme (ACE) inhibitor/angiotensin II receptor blocker (ARB), beta blocker, other antihypertensive medication, oral hypoglycaemic agents, insulin, statin or other cholesterol lowering medication.

2.12 MRI procedures

Patients had diagnostic MR brain imaging on a research dedicated 1.5T MR brain scanner (GE Signa LX EchoSpeed scanner, Milwaukee, WI). The sequences were optimized for diagnostic imaging in stroke after extensive testing. Regular quality assurance tests of all imaging methods were performed. The sequences obtained were T1, T2, Gradient Echo, Diffusion Weighted (DWI) and Fluid Attenuated Axial Inversion Recovery (FLAIR). The table below show technical data for each sequence. Sets of axial DW-echo planar images (sensitization levels b=0 and 1000 s/mm$^2$) were collected with diffusion gradients applied sequentially along 6 non-collinear directions (5 acquisitions including baseline T2WI-EP image and 6 diffusion-weighted EP images per slice position) with 5mm slice thickness, 1mm slice gap, 128 x 128 image matrix and 24 x 24 field of view (FOV). Other MR parameters are shown in the table below. The total scan time was approximately 20 minutes.
Table 2.1. Technical details of MR sequences performed

<table>
<thead>
<tr>
<th>Sequence</th>
<th>TE</th>
<th>TR</th>
<th>Flip angle</th>
<th>Slice thickness</th>
<th>Slice gap</th>
<th>Matrix</th>
<th>FOV</th>
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<td>1.5mm</td>
<td></td>
<td>256x256</td>
<td>24x24</td>
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<tr>
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<td>1.5mm</td>
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<td>1mm</td>
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</tr>
</tbody>
</table>

2.13 Retinal photography

During the pilot phase of this study patients either had their retinal photographs taken on a conventional 45 degree non mydriatic retinal camera (Topcon, Topcon Medical Instruments Inc.) or a widefield Optos P200, a 200 degree scanning laser ophthalmic camera (Optos plc). We found that the Optos widefield camera did not produce images of suitable quality for automated assessment or identification of subtle signs of retinopathy so used a conventional retinal camera for the remainder of the study.
Fig 2.1 Example of an Optos 200 degree (on the top) and an example of a Canon DRGi 45 degree retinal photograph (below) illustrating superior image quality of the Canon DRGi.

The first 8 patients described in this thesis had their photographs taken at the Princess Alexandra Eye Pavilion, Edinburgh by trained medical photographers using a Topcon non mydriatic digital retinal camera. The remaining patients were photographed by me in the Wellcome Trust Clinical Research Facility in the Western
General Hospital with a Canon CR-DGi non-mydriatic 45 degree field of view retinal camera (Canon USA Inc.) with a Canon 20D body. We purchased and established this retinal camera specifically for this study. We were influenced in the choice of this camera by expert advice from our collaborators in Australia (under the instruction of Profs Paul Mitchell and Richard Lindley and A/Prof Jie Jin Wang) who were undertaking a similar study of retinal changes in acute stroke as we were keen to ensure that retinal images were taken with identical cameras and software as we plan to merge study databases to increase power and generalizability.

Images were saved on a dedicated Dell PC which was continuously connected to the retinal camera. All images were captured and manipulated with Digital Healthcare Optomize software (Digital Healthcare, Cambridge, UK). Images were anonymised and stored on a secure server which was backed up daily, on the password protected hard drive of the retinal camera dedicated PC and on a removable hard drive which was kept in a locked filing cabinet in a different building.

After piloting use of the camera without eye drops we decided to use pupil dilatation. We used 1% tropicamide eye drops (2 drops per eye) with a 15 minute wait to dilate the pupil if necessary and if there were no contra-indications (history of glaucoma or if the patient declined eye drops). We screened all patients for glaucoma before administering tropicamide and provided information to the patient about who to contact if they experienced any eye discomfort in the few hours after eye drop administration in the form of a leaflet. If the patient was a car driver and had returned to driving after their stroke we asked the patient not to drive a car for 4 hours after administration of the eye drops. I took photographs of six fields per eye – centred on the optic disc, centred on the macular, lateral to the macula, nasal to the optic disc, upper arcade and lower arcade of each eye for each patient. If I was unable to take all fields I recorded the reason why.
2.14 MRI assessment

All MRI scans for each patient were read by a single experienced consultant neuroradiologist (Joanna Wardlaw) using a data collection sheet designed specifically for this study (see appendix 6). We collected the date and time of and sequences performed. We recorded the presence of a clinically relevant acute lesion to the recent stroke symptoms and whether this was present on DWI and/or FLAIR/T2 weighted sequences. We recorded which side of the brain the lesion was present.

We defined a lacunar infarct as a small round lesion less than 20 mm in diameter in a location typical for and suggestive of infarction secondary to disease in a small perforating artery – normally one of the lenticulostriate branches of the middle cerebral arteries. Lacunar infarcts were differentiated from white matter lesions by their pattern, shape and presence of lesions on different MRI sequences – lacunar infarcts tend to be larger, more likely to be unilateral and single lesions and are more likely to be present on DWI images than white matter lesions. Lacunar infarcts are distinguished from enlarged perivascular spaces (EPVS) as they do not follow characteristic linear patterns that EPVS do either on adjacent slices in the basal ganglia or on single slices in the centrum semiovale. If the lesion was a lacunar infarct we recorded the location from the following categories; internal capsule, internal border zone, centrum semiovale, thalamus, lentiform nucleus, brainstem and the size of the lesion (in millimetres) and whether it extended to the cortical margin.

If the lesion was cortical we classified its location as one of the following; small temporal, small parietal, basal ganglia, subcortical, anterior half of the peripheral Middle cerebral artery territory, posterior half of the peripheral MCA, anterior anterior cerebral artery, posterior ACA, anterior posterior cerebral artery, posterior PCA, anterior border zone, posterior border zone. We recorded the presence and number of old stroke lesions and recorded for each lesion the side (left/right), location (lacunar/cortical/posterior circulation) and type (infarct/haemorrhage).
We counted the number of cerebral microhaemorrhages present (defined as small punctuate round smooth lesions with signal drop out representing haemosiderin deposition on GRE/T2* sequences). We assessed periventricular and deep white matter hyperintensities with the Fazekas scale (none, mild, moderate, severe) for the left and right hemispheres separately and with an overall score for both hemispheres combined (Fazekas et al. 1987). We also rated white matter lesions with the De Leeuw method which counts the number of lesions of certain diameters (and thus enabling an estimate of white matter lesion volume)(de Leeuw et al. 1998). The diameter strata are <3mm, 3-10mm and >10mm for the left and right hemispheres and overall for both hemispheres combined. We rated enlarged perivascular spaces on a previously published scale (MacLullich et al. 2004) from 0-3 (see table) in both the basal ganglia and centrum semiovale for both the left and right hemispheres separately and then combined into an overall score. In the scale EPVS were defined as punctuate round high intensity signal changes on consecutive T2 slices in the basal ganglia and characteristic linear shaped hyperintensities in the centrum semiovale. This scale was developed within the department and counts the number of EPVS in the slice with the largest number of EPVS before categorizing the number as detailed below. This has limitations as we only used one grader and have not yet analysed inter-rater reliability. Furthermore there may be misclassification when the number of EPVS is on the boundaries of the categories eg around 10 or 20. We coded deep and superficial cerebral atrophy as none, mild, moderate and severe compared to a standard age matched template (Farrell et al. 2008).

Table 2.2. EPVS scoring scheme

<table>
<thead>
<tr>
<th>EPVS score</th>
<th>Number of EPVS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1-10</td>
</tr>
<tr>
<td>2</td>
<td>11-20</td>
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<tr>
<td>3</td>
<td>21-40</td>
</tr>
<tr>
<td>4</td>
<td>&gt;40</td>
</tr>
</tbody>
</table>
2.15 Retinal photograph assessment

All retinal photographs were assessed by me blinded to the patients’ clinical details. Photographs were identified with study identification numbers. I was trained under the supervision of Prof Bal Dhillon and the Lothian Diabetic Retinopathy Screening Service to assess retinal photographs for retinopathy and cross checked with standard retinopathy examples from Prof Paul Mitchell in Sydney. I also performed site visits to Singapore and Australia to ensure conformity with our sister retinal stroke study. We collaborated extensively to develop appropriate retinal assessment tools. Details of how retinal photographs were graded for specific features will be presented in detail in the separate results chapters.

2.16 Sub-typing of ischaemic stroke

For each patient I assigned a clinical classification of lacunar or cortical stroke with previously defined criteria based upon the Oxfordshire Community Stroke Project classification (Bamford et al. 1991). Cases were discussed, and stroke diagnosed and sub-typed at a weekly multi disciplinary stroke meeting with consultant stroke physicians, neurologists and neuroradiologists which helped to ensure sub-typing accuracy. Where the clinical differed from the radiological following discussion the imaging classification was used. A neuroradiologist coded diagnostic MRI scans blind to clinical details to assign an imaging classification of cortical or lacunar and the two clinical classification and the imaging classification were combined. A recent infarct was defined as a hyperintense area on diffusion imaging (with corresponding reduced signal on the apparent diffusion coefficient image) with or without increased signal on fluid-attenuated inversion recovery or T2-weighted imaging in a distribution compatible with an arterial territory. Lacunar infarcts were in the cerebral hemispheric white matter, basal ganglia, or brainstem and <2 cm diameter if recent (lesions >2 cm were classed as striatocapsular/cortical because they have large artery disease causes). If there was a discrepancy between the two classifications then we used the imaging classification to assign a final classification.
as this is a more reliable method than using clinical assessment alone in assigning stroke subtype (Mead et al. 2000).

2.17 Database details

Data regarding demographics, clinical details, diagnostic MRI, retinal and Transcranial Doppler findings were manually recorded by me onto specially designed data collection forms which were then entered by me into the specifically designed database. The database was designed to be expandable to include additional data (eg on follow up information) and to match with data derived from other sources such as information from blood brain barrier imaging or blood biomarker results.

The Mild Stroke Study had a bespoke database management system written (by David Perry) to help manage the secure collation, storage, data keying (punching) and encoding of its data. This was written using Microsoft SQL Server 2000 and a web based front end. To use the system, the user first provided their username and password. Each patient had up to 5 data collection forms that were completed by hand and then keyed into the database. The system was tasklist driven which helped manage the workflow to ensure that all data was collected for all registered patients. As data were entered into the system, they were validated and responses were encoded as per the Data Dictionary. This ensured that similar responses were always recorded with the same values; eg M for Male, F for Female. Changes to the data are automatically audited by use of data triggers applied to the SQL database tables. This allowed us to know who changed what and when. The data was stored on centrally managed servers that were protected against data loss.

The database was specifically designed to allow future collaboration and combination of data with collaborators in Australia (Lindley and Multi-Centre Retinal Stroke Study Collaborative Group 2008). Data from the database were
retrieved with Excel. The database had several internal validity data checks and I checked a random sample of data entries to ensure adequate data quality and keying.

### 2.18 Statistical analysis

I performed all analyses using Minitab statistical software (Version 14; Minitab Inc). Further details are given in the specific results chapters.
Chapter 3: Retinal microvascular abnormalities and stroke – a systematic review.

3.1 Aims of chapter

In this chapter I present the results of the systematic review and meta-analysis of the associations between retinal microvascular abnormalities and incident and prevalent stroke and between ischaemic stroke subtypes. Please note that the formatting differs in this chapter due to the large number of references cited.

3.2 Introduction

As discussed in chapter 1, the exact aetiology of lacunar stroke is unclear (Wardlaw 2005). Possible causes include atherothromboembolism, intracranial large artery stenosis, intrinsic microvascular atheroma, or endothelial dysfunction manifesting either through ischaemia or failure of the blood brain barrier (Wardlaw et al. 2003).

The cerebral small vessels that cause lacunar stroke share many characteristics with retinal vessels (Patton et al. 2005), as they are developmentally related during embryogenesis and of similar diameter. Retinal vessels are visibly affected by known risk factors for stroke such as diabetes and hypertension (Wong 2004). Therefore, determining any association between retinal microvascular abnormalities and stroke may improve understanding of the nature of the cerebral small vessel changes underlying lacunar ischaemic stroke and aid cerebrovascular and cardiovascular risk profiling.

Several studies have examined a possible link between stroke and the retina, including large epidemiological studies in community dwelling populations and small detailed studies of patients with specific diseases attending tertiary hospitals.
The presence and strength of any reported associations between retinal vascular abnormalities and stroke vary considerably possibly reflecting the differing populations, methodologies, definitions of disease and abnormalities sought.

We therefore performed a systematic review to clarify associations between a range of retinal microvascular abnormalities and any stroke and with specific subtypes of both stroke and ischaemic stroke. We also determined where more information was needed from new studies.

3.3 Methods

3.3.1 Search strategy

We searched Ovid Medline (from 1st Jan 1950) and Embase (from 1st Jan 1980) to 5th October 2007 for papers investigating associations between retinal microvascular abnormalities and stroke (defined as stroke/transient ischaemic attack/asymptomatic brain infarct identified on imaging). A full list of the search terms used for Medline can be found in appendix 8. The electronic search was supplemented by the authors’ personal files, hand searching the bibliographies of papers selected from the electronic search, checking references of review articles and by surveillance of key journals from April 2005 to October 2007 – (Neurology, Lancet, Brain, Ophthalmology, Stroke, British Journal of Ophthalmology, British Medical Journal and Retina).

I conducted the search, reviewed all titles and extracted data from all of the papers. A second reviewer (Petra Hokke, a Dutch medical student whom I was supervising on a 6 month research placement) independently reviewed all titles and extracted data from all of the papers. A third reviewer (Joanna Wardlaw) assessed a sample of papers for inclusion, extracted data and adjudicated in any disagreements.
3.3.2 Inclusion criteria for papers

We included studies published in full which assessed the association between retinal microvascular abnormalities and stroke (as defined above) in adult humans.

3.3.3 Exclusion criteria for papers

We excluded studies published only as abstracts or presented in conferences without full subsequent publication, review papers, papers dealing with specific single gene related disorders such as CADASIL, papers studying only macular degeneration and duplicate publications. We excluded papers dealing with retinal microvascular abnormalities and only cerebral white matter disease (leukoaraiosis) seen on brain imaging.

3.3.4 Definitions of stroke and retinal outcomes

Stroke is defined by the World Health Organisation as a focal sudden neurological deficit lasting greater than 24 hours of presumed vascular aetiology with brain imaging excluding other causes (Hatano 1976). For the purposes of this review we defined “stroke” as either a clinically diagnosed stroke or transient ischaemic attack (with or without cerebral imaging) or a cerebral infarct identified on brain imaging without definite associated clinical features being documented. We defined “incident stroke” as strokes which occurred after the patient/subject had been enrolled in the study and “prevalent stroke” as strokes which had preceded patient enrolment in the study.

“Retinal microvascular abnormalities” included retinopathy (microaneurysms, soft and hard exudates, haemorrhages), retinal arteriolar and venular width measurements (including arterio-venous ratios - AVR), focal arteriolar narrowing (FAN), arterio-venous nicking (AVN)), retinal artery (RAO) or vein occlusion (RVO), retinal artery emboli and alterations in retinal vascular geometry (angles or vessel tortuosity).
3.3.5 Paper assessment

We collected data on a form designed and piloted specifically for this review. We recorded study population details (age, selection criteria, co-morbidities), study design and assessed definitions of stroke and retinal outcomes.

We assigned the reference standard for retinal assessment as high quality retinal photographs of both eyes (preferably multi field) and blinded assessment of the retinal appearance with a standardised and externally validated tool. We collected data on which retinal features were measured, methods of retinal assessment, use of mydriatic drugs, number of fields imaged per eye and which eyes were photographed, type of camera and field of view of the camera.

The reference standard for stroke diagnosis was assessment of the patient by a stroke specialist at the time of the stroke with appropriate cerebral imaging to differentiate haemorrhagic from ischaemic stroke and stroke mimic and, if ischaemic, to identify whether lacunar or non-lacunar ischaemic stroke. We considered the following methods of stroke diagnosis to be less reliable: patient questionnaires, retrospective case note analysis, death certificates and review of centrally held health records. We assessed studies for the method of subject selection, the presence of a suitable control group, sub-typing of stroke and blinding of assessment of images/patients. We extracted data on numbers with stroke or transient ischaemic attack (TIA) and different retinal features in as much detail as possible, any calculated odds ratios, relative risks or hazards ratios and whether these were unadjusted or adjusted for shared risk factors such as hypertension or diabetes. When counting total numbers of patients and stroke events that contributed to each analysis we were careful to count patients from studies contributing more than one paper only once to avoid unnecessary bias. We did not have the resources to contact authors of studies to ask regarding missing data or individual patient data.
3.3.6 Statistical analysis

We summarised the numbers of subjects in the different population samples, with or without various retinal abnormalities and stroke. Stroke outcomes were rare (incidence less than 10%) and odds ratios, hazards ratios and relative risk were considered to be equivalent. We converted all ratios to summary risk ratios and prepared Forrest Plots using Revman (Review Manager Software Version 5, Cochrane, Oxford, UK) and Excel (version 2003; Microsoft Inc, Redmond, Wash.). We used a random effects generic inverse variance method to plot summary risk ratios and 95% confidence intervals (CI) using a logarithmic scale from the adjusted ratios and 95% CI. We used adjusted ratios in preference to unadjusted to best account for differences in risk factors between populations. We tested for associations between stroke and pre-defined retinal microvascular abnormalities and then separately examined associations in important predefined subgroups such as incident or prevalent stroke, ischaemic versus haemorrhagic stroke and ischaemic stroke subtypes. We tested for heterogeneity between study results with the Chi square test for heterogeneity with an alpha level for significance set at $p=0.05$. When heterogeneity was detected we investigated the methodology and definitions of variables used in the heterogeneous studies to identify possible causes.

3.4 Results

3.4.1 Included studies

The initial search produced 4461 titles and abstracts, subsequent screening of which yielded 52 potentially relevant papers describing probable retinal microvascular abnormalities and stroke for which we assessed the full texts. Of the 52, 15 were excluded (13 were not relevant, and 2 were in Japanese and not easily translated), leaving 37 papers which met the inclusion criteria. Figure 2.1 shows the selection process. Table 3.1 documents the main findings of the included papers.
Figure 3.1. Flow diagram representing selection for papers included in systematic review.

Medline and Embase search yielded 4461 titles and abstracts

Screening of the 4461 titles yielded 52 potentially relevant titles for which we obtained the full text paper

37 paper included in review

37 papers from 23 different study populations

4409 titles/abstracts excluded as not relevant

13 papers excluded as not relevant upon full text reading (looked at retinal vessel function or non-stroke outcomes)
2 were exclude as not able to translate language (Japanese).
Table 3.1 Descriptions of included studies arranged by stroke outcome. Ausdiab – the Australian diabetes, obesity and lifestyle study, CHS – cardiovascular health study, BMES – blue mountains eye study, ARIC – atherosclerosis risk in communities study, WESDR – Wisconsin epidemiological study of diabetic retinopathy, BDES – Beaver Dam eye study, ROTT – the Rotterdam study, RVO – retinal vein occlusion, RAO – retinal arteriolar occlusion, AVR – arteriovenous ratio, AVN – arterio-venous nicking, FAN – focal arteriolar narrowing, inc = increased, dec = decreased, art = arteriolar, ven = venular. ? = not given in paper and unable to calculate from data. Link? = presence of significant association between retinal feature and stroke outcome (continued overleaf).

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<th>Origin</th>
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<th>Stroke outcome</th>
<th>Link</th>
<th>Stroke (n)</th>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inc ven caliber</td>
<td>Incident stroke/TIA</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dec art caliber</td>
<td>Incident stroke/TIA</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Ikram Brain 2006b</td>
<td>ROTT</td>
<td>490</td>
<td>Inc ven caliber</td>
<td>Incident MRI infarct</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Klein WESDR 1999b</td>
<td>WESDR</td>
<td>2366</td>
<td>Retinopathy</td>
<td>Incident death with stroke</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Hirai 2007</td>
<td>BDES</td>
<td>4284</td>
<td>Retinopathy</td>
<td>Incident death with stroke</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Klein BDES 1999a</td>
<td>BDES</td>
<td>4856</td>
<td>Retinal emboli</td>
<td>Incident death with stroke</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Wang 2006</td>
<td>BMES/ BDES</td>
<td>8580</td>
<td>Retinal emboli</td>
<td>Incident death with stroke</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Witt 2006</td>
<td>BDES</td>
<td>684</td>
<td>Dec art caliber</td>
<td>Incident death with stroke</td>
<td>No</td>
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<td>Klein 2003</td>
<td>BDES</td>
<td>4926</td>
<td>Tortuosity</td>
<td>Incident death with stroke</td>
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<td>No</td>
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<tr>
<td>Cugati 2007</td>
<td>BDES/ BMES</td>
<td>8580</td>
<td>RVO</td>
<td>Incident death with stroke</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Tsaloumas 2000</td>
<td></td>
<td>95</td>
<td>RVO</td>
<td>Incident death with stroke</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

57
3.4.2 Description of included studies

Of the 37 papers, 21 originated from large population-based cohort studies (table 3.2 and fig 3.1) (Cheung et al. 2007c; Cooper et al. 2006; Cugati et al. 2007; Hirai et al. 2007; Ikram et al. 2006a; Ikram et al. 2006b; Klein et al. 2004; Klein et al. 2003; Klein et al. 1999b; Klein et al. 1999a; Klein et al. 2000; Longstreth, Jr. et al. 2007; Mitchell et al. 1996; Mitchell et al. 2005; Wang et al. 2006; Witt et al. 2006; Wong et al. 2006; Wong et al. 2005a; Wong et al. 2006; Wong et al. 2001; Wong et al. 2002a). Of the remaining 16 papers, 14 originated from hospital based studies (Abu El-Asrar et al. 2001; Bruno et al. 1995; Hayreh et al. 2001; Howard and Russell 1987; Ikeda et al. 1994; Inoue et al. 1996; Kim et al. 2002; Kobayashi et al. 1997; Kwa et al. 2002; Kwon et al. 2007; Luijckx et al. 1998; Petitti and Bhatt 1995; Tsaloumas et al. 2000; Ueda et al. 2002), one from within a trial of blood pressure reduction (Cohen et al. 2003) and one from a World Health Organization (WHO) study of follow up in diabetic patients (Fuller et al. 2001). The papers were from the US, UK, Australia, Japan, Korea, the Netherlands, Saudi Arabia and Greece, Switzerland, Poland, Germany, Croatia, Hong Kong and Cuba.
Table 3.2  Background information of population based studies contributing to the review

<table>
<thead>
<tr>
<th>Study</th>
<th>Acronym</th>
<th>Size</th>
<th>Age (yrs)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherosclerosis Risk in Communities Study</td>
<td>ARIC</td>
<td>15,792</td>
<td>45-64</td>
<td>Longitudinal US population based study of cardiovascular disease</td>
</tr>
<tr>
<td>Beaver Dam Eye Study</td>
<td>BDES</td>
<td>4,926</td>
<td>43-84</td>
<td>Longitudinal US population based study of eye disease and medical health</td>
</tr>
<tr>
<td>Cardiovascular Health Study</td>
<td>CHS</td>
<td>5,201</td>
<td>&gt;65</td>
<td>Longitudinal US population based study of coronary heart disease and stroke</td>
</tr>
<tr>
<td>Rotterdam Study</td>
<td>RS</td>
<td>7,983</td>
<td>&gt;55</td>
<td>Longitudinal Dutch population based study of chronic diseases</td>
</tr>
<tr>
<td>Blue Mountains Eye Study</td>
<td>BMES</td>
<td>3,654</td>
<td>&gt;49</td>
<td>Longitudinal Australian population based study of vision, common eye diseases and health outcomes</td>
</tr>
<tr>
<td>Wisconsin Epidemiologic Study of Diabetic Retinopathy</td>
<td>WESDR</td>
<td>996</td>
<td>all</td>
<td>Longitudinal US study of patients receiving treatment for type I diabetes</td>
</tr>
<tr>
<td>Ausdiab Study</td>
<td>-</td>
<td>11,247</td>
<td>&gt;25</td>
<td>Cross sectional Australian population based survey</td>
</tr>
</tbody>
</table>

The 37 papers include 62,975 subjects. The mean age of the patients in the 31/37 papers which provided this information was 62.5 years (SD 7.7). Amongst the 62,975 subjects, 1,900 had a clinically evident stroke (total of incident and prevalent stroke) and 993 had infarction seen on brain imaging with no stated clinical correlate. We were unable to extract the number of strokes from 6/37 papers as the raw numbers were not given and any odds ratios presented were already corrected for certain confounders (Hirai et al. 2007; Kim et al. 2002; Klein et al. 2000; Kwa et al. 2002; Mitchell et al. 1996; Ueda et al. 2002). The number of strokes per paper varied from 8-496 (Abu El-Asrar et al. 2001; Longstreth, Jr. et al. 2007).
Figure 3.2 Number of papers each cohort study contributed to the review (ARIC – Atherosclerosis Risk in Communities Study, BDES – Beaver Dam Eye Study, CHS – Cardiovascular Health Study, BMES – Blue Mountains Eye Study, ROTT – The Rotterdam Study, WESDR – Wisconsin Epidemiological Study of Diabetic Retinopathy, AUSDIAB – The Australian Diabetes, Obesity and Lifestyle Study - BDES/BMES relates to papers which combined these study populations.) The numbers on top of each column represent the number of subjects in each cohort study.

3.4.2 Ascertainment of stroke

Kwon et al. 2007; Luijckx et al. 1998; Mitchell et al. 1996; Petitti and Bhatt 1995; Ueda et al. 2002; Wong et al. 2003; Wong et al. 2005a) (total population 21,950; 501 (2.2%) had prior stroke) and one paper examined both prevalent and incident stroke (Longstreth, Jr. et al. 2007).


The methods of stroke identification varied widely. Two papers used the reference standard of specialist examination at the time of stroke (Bruno et al. 1995; Luijckx et al. 1998); twelve papers used clinical assessment but not necessarily at the time of the stroke by study investigators (Abu El-Asrar et al. 2001; Cohen et al. 2003; Fuller et al. 2001; Hayreh et al. 2001; Kim et al. 2002;

3.4.3 Ascertainment of retinal microvascular abnormalities


22/37 papers used retinal abnormality classifications that had been validated either internally or externally (Cheung et al. 2007c; Cooper et al. 2006; Cugati et al. 2007; Hirai et al. 2007; Ikram et al. 2006a; Ikram et al. 2006b; Klein et al. 2004; Klein et al. 2003; Klein et al. 1999b; Klein et al. 1999a; Klein et al. 2000; Kwon et al. 2007; Longstreth, Jr. et al. 2007; Mitchell et al. 1996; Mitchell et al. 2005; Wang et al. 2006; Witt et al. 2006; Wong et al. 2003; Wong et al. 2005a;
Wong et al. 2006; Wong et al. 2001; Wong et al. 2002a). Of the 27 papers which used retinal photography the number of fields used and the angle of the field of view varied: 4 papers gave no information regarding which retinal fields were taken (Bruno et al. 1995; Kobayashi et al. 1997; Kwon et al. 2007; Luijckx et al. 1998), 15 took one field centred on the optic disc and macula (Cooper et al. 2006; Cugati et al. 2007; Hirai et al. 2007; Ikram et al. 2006a; Ikram et al. 2006b; Klein et al. 2003; Klein et al. 1999a; Klein et al. 2000; Kwa et al. 2002; Longstreth, Jr. et al. 2007; Witt et al. 2006; Wong et al. 2003; Wong et al. 2006; Wong et al. 2001; Wong et al. 2002a) and 8 took more than one field (Cheung et al. 2007c; Cohen et al. 2003; Klein et al. 2004; Klein et al. 1999b; Mitchell et al. 1996; Mitchell et al. 2005; Wang et al. 2006; Wong et al. 2005a). 8/27 papers gave no information regarding the angle of the field of view used (Bruno et al. 1995; Cohen et al. 2003; Klein et al. 2004; Klein et al. 1999b; Kobayashi et al. 1997; Kwon et al. 2007; Luijckx et al. 1998; Wong et al. 2005a), 2 papers used 20° cameras (Ikram et al. 2006a; Ikram et al. 2006b), 8 papers used 30° cameras (Cugati et al. 2007; Hirai et al. 2007; Klein et al. 2003; Klein et al. 1999a; Mitchell et al. 1996; Mitchell et al. 2005; Wang et al. 2006; Witt et al. 2006), 8 papers used 45° cameras (Cheung et al. 2007c; Cooper et al. 2006; Klein et al. 2000; Longstreth, Jr. et al. 2007; Wong et al. 2003; Wong et al. 2006; Wong et al. 2001; Wong et al. 2002a) and one paper used a 50° camera (Kwa et al. 2002).

The retinal features that were assessed varied greatly, the most common being retinopathy (19/37 papers) defined as any of the following – microaneurysms, haemorrhages, or exudates (Abu El-Asrar et al. 2001; Cheung et al. 2007c; Cohen et al. 2003; Cooper et al. 2006; Fuller et al. 2001; Hirai et al. 2007; Ikeda et al. 1994; Inoue et al. 1996; Kim et al. 2002; Klein et al. 2004; Klein et al. 1999b; Kwon et al. 2007; Longstreth, Jr. et al. 2007; Mitchell et al. 2005; Petitti and Bhatt 1995; Wong et al. 2003; Wong et al. 2005a; Wong et al. 2001; Wong et al. 2002a). Two papers from the same population based study divided retinopathy into its constituent parts and presented separate associations for these constituent parts (Cooper et al. 2006; Longstreth, Jr. et al. 2007). Other papers used retinal artery occlusion/emboli (6/37 papers) (Bruno et al. 1995; Howard...
3.4.4 Association between retinal microvascular abnormalities and stroke

We were able to extract data and combine risk ratios for 24 papers (total study population 39,376, 1696 stroke (figs 3.2 and 3.3).

3.4.5 Retinopathy and stroke

Retinopathy was associated with incident stroke; amongst 8 papers providing data (total population 25,354; 1,019 strokes) (Cheung et al. 2007c; Fuller et al. 2001; Hirai et al. 2007; Klein et al. 2004; Klein et al. 1999b; Mitchell et al. 2005; Wong et al. 2001; Wong et al. 2002a), the summary Risk Ratio (sRR) for incident stroke in the presence versus absence of retinopathy was 2.1 (95% CI 1.7-2.6; fig. 3.2) with no significant heterogeneity between papers (chi square p=0.46).

Retinopathy was associated with prevalent stroke; amongst 7 papers providing data (total population 8,083; 979 strokes) (Abu El-Asrar et al. 2001; Cooper et al.
2006; Kwon et al. 2007; Longstreth, Jr. et al. 2007; Petitti and Bhatt 1995; Wong et al. 2003; Wong et al. 2005a), the sRR for prevalent stroke in the presence versus absence of retinopathy, was 2.45 (95% CI 1.4-4.3; fig. 3.3) although with significant heterogeneity between papers (chi squared p=0.003).

3.4.6 Arteriolar and venular widths and stroke

Decreased arteriolar width was not associated with incident stroke; amongst 3 papers providing data (total population 9262; 557 strokes) (Ikram et al. 2006a; Ikram et al. 2006b; Wong et al. 2006), decreasing arteriolar width was not associated with incident stroke – sRR 0.9 (95% CI 0.8 to 1.1; fig. 3.2) with no significant heterogeneity between papers (chi squared p=0.67)

Increased venular width was associated with incident stroke; amongst the same three papers providing data for this analysis (total population 9262; 557 strokes) (Ikram et al. 2006a; Ikram et al. 2006b; Wong et al. 2006), venular widening was associated with incident stroke - sRR 1.4 (95% CI 1.1 to 1.7; fig. 3.2) with no significant heterogeneity between papers (chi squared p=0.36). No papers assessed arteriolar or venular widths and prevalent stroke.

Five papers investigated the association between the arteriovenous ratio and incident stroke (Ikram et al. 2006a; Ikram et al. 2006b; Klein et al. 2004; Mitchell et al. 2005; Wong et al. 2001) (total population 21,717;715 strokes) and found that decreased AVR was not associated with incident stroke with a sRR 1.4 (0.9 to 2.0) but with significant clinical heterogeneity between result (p<0.001).

Three papers investigated the association between decreased AVR and prevalent stroke (Cooper et al. 2006; Longstreth, Jr. et al. 2007; Wong et al. 2003), (total population 3734; strokes 679) and found that decreased AVR was associated with prevalent stroke with a sRR of 1.2 (1.1 to 1.3).
Fig 3.3. Summary risk ratios (sRR) showing associations of incident stroke and different microvascular abnormalities. The size of the square denotes weight attributed to each paper and the horizontal lines represent 95% CI. The diamond represents sRR with the width representing the 95% CI. Arrowhead denotes that the upper CI is not marked on the chart. Right hand column indicates key adjustments. BP = blood pressure, DM = Diabetes Mellitus. *denotes studies which used only radiological diagnosis of infarct. BMES = Blue Mountains Eye Study.

### Retinopathy

<table>
<thead>
<tr>
<th>Study</th>
<th>Risk Ratio (95% CI)</th>
<th>Adjusted for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wong⁴ 2001</td>
<td>2.6 (1.6-4.3)</td>
<td>Age Sex BP DM</td>
</tr>
<tr>
<td>Wong⁵ 2002</td>
<td>4.9 (2.0-11.9)</td>
<td>Age Sex BP DM</td>
</tr>
<tr>
<td>Mitchell¹¹ 2005</td>
<td>2.4 (1.1-5.4)</td>
<td>Age Sex BP DM</td>
</tr>
<tr>
<td>Klein²⁰ WESDR 1999</td>
<td>1.9 (1.0-3.4)</td>
<td>Age Sex BP DM</td>
</tr>
<tr>
<td>Klein²⁰ 2004</td>
<td>1.6 (1.1-2.3)</td>
<td>DM</td>
</tr>
<tr>
<td>Hirai¹⁹ 2007</td>
<td>2.3 (0.7-7.4)</td>
<td>Age Sex BP DM</td>
</tr>
<tr>
<td>Fuller⁴⁴ 2001</td>
<td>2.1 (1.4-3.2)</td>
<td>DM</td>
</tr>
<tr>
<td>Cheung⁴⁶ 2007</td>
<td>2.3 (1.1-4.9)</td>
<td>Age Sex BP DM</td>
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### Arteriolar Narrowing

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<tr>
<td>Wong⁶ 2006</td>
<td>1.1 (0.5-2.2)</td>
<td>Age Sex BP DM</td>
</tr>
<tr>
<td>Ikram²⁶ Neurol. 2006</td>
<td>0.9 (0.8-1.2)</td>
<td>Age Sex</td>
</tr>
<tr>
<td>Ikram²⁷ Brain 2006&quot;</td>
<td>0.8 (0.5-1.2)</td>
<td>Age Sex BP DM</td>
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### Venular Widening

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<tr>
<td>Wong⁷ 2006</td>
<td>2.2 (1.1-4.3)</td>
<td>Age Sex BP DM</td>
</tr>
<tr>
<td>Ikram²⁶ Neurol. 2006</td>
<td>1.3 (1.0-1.6)</td>
<td>Age Sex</td>
</tr>
<tr>
<td>Ikram²⁷ Brain 2006&quot;</td>
<td>1.2 (0.7-2.2)</td>
<td>Age Sex BP DM</td>
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</table>

### Decreased AVR

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</tr>
</thead>
<tbody>
<tr>
<td>Klein²² 2004</td>
<td>0.8 (0.6-1.1)</td>
<td>DM</td>
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<td>Wong⁴ 2001</td>
<td>2.3 (1.2-4.3)</td>
<td>Age Sex BP DM</td>
</tr>
<tr>
<td>Mitchell¹¹ 2005</td>
<td>1.7 (0.8-4.0)</td>
<td>Age Sex BP DM</td>
</tr>
<tr>
<td>Klein²⁰ Neurol. 2006</td>
<td>1.6 (1.3-2.1)</td>
<td>Age Sex</td>
</tr>
<tr>
<td>Ikram²⁷ Brain 2006&quot;</td>
<td>1.3 (0.8-2.0)</td>
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### Retinal Artery Occlusion

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</thead>
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<td>Wang¹² (BMES) 2006</td>
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<td>Age Sex BP</td>
</tr>
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<td>Klein²³ 2003</td>
<td>2.4 (1.1-5.0)</td>
<td>Age Sex BP</td>
</tr>
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<td>Bruno⁴⁹ 1995</td>
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### Retinal Vein Occlusion

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<td>Tsaloumas⁴¹ 2000</td>
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</tr>
<tr>
<td>Cugati²⁸ 2007</td>
<td>0.9 (0.4-2.1)</td>
<td>Age Sex BP DM</td>
</tr>
</tbody>
</table>

### Adjusted for

- Age Sex BP DM
- Age Sex
- DM
- Nil

---

66
Six papers assessed the relationship between AVR and incident stroke and we could extract data from 5 papers (21,717 subjects, 748 strokes) (Ikram et al. 2006a; Ikram et al. 2006b; Klein et al. 2004; Mitchell et al. 2005; Wong et al. 2001). Decreased AVR was not associated with incident stroke (sRR 1.4, 95% CI 0.9-2.0; fig 3.2) although there was significant heterogeneity between these papers (chi squared p=0.002). Three further papers found a significant association between decreased AVR and prevalent stroke (5451 subjects, 812 strokes) (Cooper et al. 2006; Longstreth, Jr. et al. 2007; Wong et al. 2003) with sRR 1.2 (95% CI 1.1-1.3; fig. 3.3) with no significant heterogeneity between papers (chi squared p=0.47) but another paper from which we could not extract data reported no association between AVR and prevalent stroke (Klein et al. 2000).

3.4.7 Retinal arteriolar emboli or arteriolar occlusion and stroke

Six papers assessed retinal emboli and incident stroke (Bruno et al. 1995; Howard and Russell 1987; Klein et al. 2003; Klein et al. 1999a; Ueda et al. 2002; Wang et al. 2006) from which we could extract data from 4 papers (Bruno et al. 1995; Klein et al. 2003; Klein et al. 1999a; Wang et al. 2006) however Klein 1999 (Klein et al. 1999a) presents 5 year follow up data from the BDES and Klein 2003 (Klein et al. 2003) presents 10 year follow up from the same patients and thus when combining studies we excluded Klein 1999. Furthermore Wang 2006 (Wang et al. 2006) presented a pooled analysis of data from the BDES and the BMES. As the data from the BDES is included in Klein 2003 we present in this analysis the data presented in Wang 2006 from the BMES (to avoid double counting) therefore a total of 8720 subjects and 266 strokes gave a sRR of 2.9 (95% CI 1.6-5.1; fig. 1) with no significant heterogeneity between papers (chi squared p=0.21). One paper assessed retinal artery occlusion and prevalent stroke but we were unable to extract data from this paper (Ueda et al. 2002).
**Fig 3.3** Summary risk ratios (sRR) showing associations of prevalent stroke and different microvascular abnormalities. The size of the square denotes weight attributed to each paper and the horizontal lines represent 95% CI. The diamond represents sRR with the width representing the 95% CI. Arrowhead denotes that the upper CI is not marked on the chart. Right hand column indicates key adjustments. Risk ratios for Abu El Asrar calculated directly from raw data. BP = blood pressure, DM = Diabetes Mellitus. *denotes studies which used only radiological diagnosis of infarct. BMES = Blue Mountains Eye Study

<table>
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<th>Retinopathy</th>
<th>Risk Ratio (95% CI)</th>
<th>Adjusted for</th>
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<tr>
<td>Wong* 2003</td>
<td>2.0 (1.1-3.6)</td>
<td>Age Sex BP DM</td>
</tr>
<tr>
<td>Wong* 2005</td>
<td>4.2 (1.8-9.7)</td>
<td>Age Sex BP DM</td>
</tr>
<tr>
<td>Pettiti 1995</td>
<td>4.0 (1.0-14.5)</td>
<td>Age Sex BP DM</td>
</tr>
<tr>
<td>Longstreth* 2006*</td>
<td>0.9 (0.6-1.4)</td>
<td>Age Sex BP DM</td>
</tr>
<tr>
<td>Kwon* 2006*</td>
<td>3.0 (1.2-7.5)</td>
<td>Nil</td>
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<tr>
<td>Cooper* 2005*</td>
<td>3.0 (1.3-6.7)</td>
<td>Age Sex BP DM</td>
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<td>Abu El Asrar* 2002</td>
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<td>Wong* 2003</td>
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<td>Age Sex BP DM</td>
</tr>
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<td>Cooper* 2005*</td>
<td>1.7 (0.9-3.2)</td>
<td>Age Sex BP DM</td>
</tr>
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<td>sRR Decreased AVR</td>
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<td></td>
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<table>
<thead>
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<th>Retinal Vein Occlusion</th>
<th>Risk Ratio (95% CI)</th>
<th>Adjusted for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitchell 1996</td>
<td>3.8 (1.9-7.6)</td>
<td>Age</td>
</tr>
</tbody>
</table>
3.4.8 Retinal vein occlusion and stroke

Two papers that investigated the association between RVO and incident stroke (9168 subjects; 359 strokes) found no association - sRR 0.9 (95% CI 0.4-2.0; fig. 3.2) (Cugati et al. 2007; Tsaloumas et al. 2000) with no significant heterogeneity between papers (chi squared p=0.37). A further two papers studied RVO and prevalent stroke (4744 subjects, 43 strokes) - the one paper (3654 subjects) from which we could extract data found RVO was associated with prevalent strokes (sRR 3.8, 95% CI 1.9-7.6; fig. 3.3) (Mitchell et al. 1996).

3.4.9 Retinal microvascular abnormalities in ischaemic versus haemorrhagic stroke

No papers directly compared and reported differences between retinal microvascular abnormalities in haemorrhagic versus ischaemic stroke.

3.4.10 Retinal microvascular abnormalities and large artery versus small artery stroke

Only one pilot study (Luijckx et al. 1998) directly compared retinal microvascular abnormalities in ischaemic stroke subtypes. The diagnosis of stroke and sub-typing was based on clinical and radiological features at the time of the stroke. This small study (59 patients in total) found that there were similarly high levels of mild retinal arteriosclerosis (defined as altered central arteriolar light reflex) in lacunar stroke (prevalence 92%) and cortical stroke (prevalence 80%).
3.4.11 Retinal microvascular abnormalities and transient ischaemic attacks

No papers solely examined retinal disease and TIA. Two papers that included TIAs in their stroke outcomes did not present separate data for TIA (Abu El-Asrar et al. 2001; Mitchell et al. 2005).

3.5 Discussion

This review has demonstrated an association between retinal microvascular abnormalities and any stroke despite large variations in study design, stroke outcomes used and a lack of detail in diagnosing both the presence and subtype of stroke. It has shown that the presence of retinopathy is associated with incident stroke and prevalent stroke. Furthermore, incident stroke was associated with retinal artery embolism, venular widening and decreased AVR but not with arteriolar narrowing. There were no data on arteriolar narrowing or venular widening and prevalent stroke and decreased AVR was not associated with prevalent stroke. Thus there were some inconsistencies in results, which combined with the heterogeneity between studies for some analyses, means that the data on different types of retinal microvascular abnormality and stroke should be viewed cautiously. No published studies have adequately compared retinal microvascular abnormalities in haemorrhagic versus ischaemic stroke or between different ischaemic stroke subtypes therefore we are unable to answer with this systematic review whether retinal microvascular abnormalities may shed light on the pathophysiology of small vessel disease.

Others have reviewed the associations between retinal microvascular abnormalities and cerebrovascular disease (Baker et al. 2008; Sharrett 2007; Wong and McIntosh 2005a) but these have been largely narrative reviews based upon results of large epidemiological studies. They did not employ a systematic search strategy or
combine result in meta-analysis. This is the first systematic review and meta-
analysis of retinal changes in stroke.

Population–based studies contributed the majority of papers and therefore the total
number of subjects was large (62,975 subjects) but in healthy and relatively young
populations (mean age 62.5 years, younger than the mean age of stroke onset in the
UK of 72 years) stroke is rare making it difficult to show differences between
exposure groups. The rareness of strokes is reflected in wide 95% CI (Wong et al.
2002a), the heterogeneity between studies for some associations and the occasional
need to combine cohorts to demonstrate associations in some primary publications
(Wang et al. 2006) indicating that the data on specific retinal features and stroke
should be viewed with caution. Although the general association between any retinal
microvascular abnormalities and stroke may be robust, the details of the associations
between different retinal microvascular features and stroke are inconsistent and may
be less reliable. Case control studies can be used when the outcome of interest is rare
but studies in this review used the general population as controls (Hayreh et al.
2001; Howard and Russell 1987; Tsaloumas et al. 2000) or MRI scans of patients
who self presented to hospital for general health checks (Kobayashi et al. 1997).
We were unable to perform any sub analysis by age group but it is likely that older
populations and populations where all patients have had a stroke will have more
retinal abnormalities thereby increasing the chance of finding associations between
retinal changes and stroke subtypes.

A significant limitation of the literature is that each population base study has
contributed several papers to this review where data from individual patients is
published more than once. This can lead to duplication of publication and it can be
difficult from the information presented to differentiate between patient cohorts
within studies. Although we tried to minimize this we cannot therefore exclude the
possibility that data may have been double counted. We did perform a sensitivity
analysis using only one paper from each study and found that the size or direction of
any of the results presented in Figs 2.2 and 2.3 did not change.
The overall results of the review are promising in that for most analyses, the heterogeneity between studies is low. However there are inconsistencies. For example, it is odd that decreased AVR should be associated with prevalent stroke but not incident stroke. This may be due to the significant heterogeneity in the results from the incident stroke papers (perhaps resulting from Klein 2004 (Klein et al. 2004) using patients with Type I diabetes as their cohort who were younger than the patients in the other studies) possibly causing a type II statistical error for the association between decreased AVR and incident stroke or alternatively recall bias affecting the detection of prevalent stroke. Although there were a similar number of strokes, there were many more subjects in the analysis of incident stroke than of prevalent stroke and AVR. Similarly there is significant heterogeneity between the studies for retinopathy and prevalent stroke but not retinopathy and incident stroke – this may reflect the wide variety of methods used to identify and diagnose prevalent stroke as the number of strokes in the two analyses were similar. Some heterogeneity in the association between retinopathy and prevalent stroke may have resulted from Longstreth (Longstreth, Jr. et al. 2007) investigating associations with MRI defined infarcts in the Cardiovascular Health Study which involved an older age group (>65 years) than the others in the subgroup investigating retinopathy and prevalent stroke. Although we have not demonstrated much heterogeneity it should be noted that with small numbers of outcomes the power to detect heterogeneity is low and our calculations may have missed a small degree of heterogeneity.

Stroke is difficult to diagnose. Ideally the patient should be assessed by a suitably trained stroke expert at the time of the stroke and the diagnosis made with clinical features and appropriately timed brain imaging. Many of the incident stroke studies were population based and it is almost impossible for study investigators to assess every possible stroke. Accordingly, the diagnosis of stroke was made at either regular review when patients were asked if they had had a stroke in the preceding follow-up period and medical records reviewed, or patients were asked to report possible strokes to the investigators. Alternatively discharge summaries or even death certificates were used to diagnose stroke. Although these techniques allow for the assessment of large numbers of subjects they come at the price of reduced
accuracy (Leibson et al. 1994; Leppala et al. 1999; Piriyawat et al. 2002) as discharge summaries and death certificates are often completed by inexperienced junior medical staff. To ascertain whether a patient had a history of stroke, many studies asked the patient and searched medical records. Although prone to recall bias, this approach is deemed acceptable for assessing prevalent stroke epidemiologically, but may be inadequate for detailed studies of pathophysiology. We have grouped together studies investigating certain retinal features and stroke outcomes but there were varying methods of both retinal assessment and more pertinently stroke assessment. We have tried to minimize this variation but it is a limitation of the results of this review that this variation exists.

For this review we used a clearly specified wide definition of stroke to encompass the range of definitions used in the papers. Whilst the vast majority of papers did not use the reference standard for stroke diagnosis (indeed very few did) we considered that there was sufficient clinical homogeneity to permit grouping of the outcome of “stroke” and clearly discuss the limitation that this imposes on our conclusions. In fact, we highlight the heterogeneity as well as the lack of clarity and reliability of the diagnosis of “stroke” used in these papers as one of the main problems affecting this area of research and a major area for methodological improvement in future studies. If we had excluded those which did not conform to the gold standard there would have been virtually no data, and the exercise would have been unhelpful in flagging the fact that better characterisation of the cerebral disorder is needed to match the very detailed classification and characterisation of the retinal one. We note the heterogeneity present in these studies and urge caution in the over-interpretation of the pooled estimates.

In order to assess for small study bias we plotted a Funnel plot (Fig 3.4) of the logarithm of the effect size and standard error of the effect size and found that there was a paucity of small negative studies. Furthermore the larger studies tended to have smaller effect sizes detailing the association between retinal microvascular abnormalities and stroke. One of the possible causes of this small study bias is
publication bias and it is likely that publication bias has affected the results of this review.

*Figure 3.4 Funnel plot of standard error of log effect size versus log effect size showing small study bias and a paucity of negative small studies.*

Stroke is a heterogeneous disorder. The two main pathophysiological divisions are haemorrhagic and ischaemic stroke and ischaemic stroke is further sub-typed into lacunar (or small vessel disease) and cortical (large artery atheroma and cardiac embolism subtypes). Furthermore, whilst stroke subtypes share many vascular risk factors, their underlying pathophysiology vary reflecting different disease processes. Any study aiming to understand the pathophysiology of stroke, or even to assess novel risk factors for stroke, should sub divide stroke into ischaemic and haemorrhage and preferably further subtype ischaemic stroke. This requires careful clinical assessment and appropriate brain imaging. In the ARIC study, strokes were sub-typed according to their likely cause based on clinical and imaging criteria where available – haemorrhagic or ischaemic with ischaemic strokes further sub-typed into the important subdivisions of thrombotic or embolic stroke but not lacunar versus
cortical. However, due to the small number of strokes (and especially haemorrhagic strokes) (Wong et al. 2001; Wong et al. 2002a) direct comparisons between stroke subtypes were not reported. The Rotterdam Study investigated cerebral infarction (but brain imaging was only available for 68% of patients(Ikram et al. 2006a)). Other papers sub-typed stroke but did not compare retinal microvascular abnormalities between the different stroke subtypes but only between any stroke and non-stroke controls (Bruno et al. 1995; Petitti and Bhatt 1995). One study that did compare retinal microvascular abnormalities in lacunar and cortical stroke directly and had adequate diagnosis and sub-typing (Luijckx et al. 1998), was underpowered (n=60), and used subjective measures of retinal arteriolar sclerosis (altered central light reflex).

MRI used appropriately is a powerful tool for diagnosing and sub-typing stroke but in the absence of clinical data about neurological deficits, the abnormal areas seen on imaging are difficult to interpret, especially as they are common in older populations (Vernooij et al. 2007). Lacunar infarcts can have considerable overlap with white matter lesions. We excluded studies dealing solely with white matter lesions but some of the included studies may have misclassified white matter lesions as lacunes and vice versa thus introducing a small bias. Without detailed diffusion weighted MR imaging, or careful CT assessment at the time of any symptoms, it is not clear which, if any, lacunes may have represented previous clinically evident stroke. As such, stroke remains a clinical diagnosis and associations between retinal features and lacunes seen on MRI may be less reliable in aiding to understand the pathophysiology of stroke subtypes.

A few earlier studies used direct ophthalmoscopy to record retinal microvascular abnormalities. Apart from this, the quality of retinal assessment used in most papers was high. Almost all of the later studies and certainly all of the large population-based cohort studies used retinal photography with grading by trained analysts using internally and externally validated techniques blinded to other clinical information.
The strength of the associations between retinal microvascular abnormalities and stroke is encouraging, especially given the consistency between studies. This suggests that retinal examination offers an excellent way to study non-invasively the effects of common vascular risk factors on small vessels and possibly for gaining better understanding of the pathophysiological processes involved in cerebral small vessel disease. The lack of studies which directly compare retinal microvascular abnormalities and stroke ischaemic subtypes is therefore disappointing and further studies carefully characterising stroke subtype with comprehensive assessment at the time of the stroke are required if retinal microvascular abnormalities are to shed light on the pathophysiology of lacunar stroke. The paucity of strokes, lack of detailed stroke assessment and heterogeneity between studies for some associations mean that the data on individual retinal features and stroke should be viewed with caution and underpins the need for more large, stroke-focussed, robust studies.

The results from this review highlighting the lack of knowledge about retinal appearances and ischaemic stroke subtypes provide justification for the Edinburgh Mild Stroke Study which, amongst other factors, investigated retinal microvascular abnormalities in ischaemic stroke subtype. Details of this study are presented subsequently.
Chapter 4. Baseline demographics and MRI based results from the Mild Stroke Study.

4.1 Aims of chapter

This chapter details the baseline characteristics of the study population.

4.2 Introduction

4.1.1 Diagnosis of stroke and rate of negative MRI

Accurate diagnosis and sub-typing of ischaemic stroke is essential to investigate the pathophysiology of stroke sub-types. There is however no gold standard method for diagnosing stroke. The World Health Organisation (WHO) defines stroke as “rapidly developing clinical signs of focal (at times global) disturbance of cerebral function, lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin” (Aho et al. 1980). In the Mild Stroke Study we used the reference standard of stroke as a stroke diagnosis based upon the expert opinion of an experienced stroke physician with a MRI scan including DWI sequences at the time of presentation to hospital with subsequent confirmation by panel discussion in possession of all available data (the panel consisted of at least a stroke physician, a vascular neurologist and a neuroradiologist). We felt that in the absence of a gold standard this was the most robust definition available. We did not however test the reliability of this consensus meeting in diagnosing stroke and it is conceivable that as the personnel in the panel were not fixed there may have been some inter meeting variation in stroke diagnosis. The diagnosis of ischaemic stroke was confirmed by either a MRI scan showing an acute stroke ischaemic lesion or a scan not showing an alternative explanation for the presenting symptoms eg haemorrhage, brain tumour or demyelination.
DWI imaging to identify brain ischaemia is a relatively recent development (Moseley et al. 1990) but it is widely used in the acute setting and by some centres as part of a MR sequence with perfusion scanning to identify tissue at risk of infarction and to guide thrombolysis (Albers 2001). MRI (including DWI) is recommended in UK guidelines as the brain imaging of choice in mild stroke (Department of Health et al. 2007; Scottish Intercollegiate Guidelines Network 2008). MRI involving DWI is more sensitive and specific at diagnosing acute ischaemic stroke than both conventional MRI (without DWI) and CT (Chalela et al. 2007; Fiebach et al. 2002; Lansberg et al. 2000a; Lansberg et al. 2000b; Lutsep et al. 1997; Mullins et al. 2002; Urbach et al. 2000). Abnormal hyperintense DWI signals can persist for at least several months post stroke (Eastwood et al. 2003; Geijer et al. 2001; Schulz et al. 2007) but can produce false negative results both very early and late after stroke onset (Ay et al. 1999a; Oppenheim et al. 2000). False negative DWI lesions occur especially in patients presenting with minor stroke (Schulz et al. 2004; Sylaja et al. 2008) but most studies investigating rates of negative DWI and associated features have included both strokes and TIAs in their cohorts (Schulz et al. 2004; Sylaja et al. 2008) using presence of stroke as an explanatory variable for the presence of a negative scan. We therefore investigated the rate and associated features of negative DWI in patients presenting with mild stroke as one of our preliminary analyses.

Fig. 4.1  DWI showing increased signal right semiovale lacunar infarct (image A) and left hyperintense cortical infarct (image B).
4.2.2 Subtyping of ischaemic stroke

We sub-typed stroke based on radiological and clinical grounds into lacunar or cortical stroke. Where the two differed we sub-typed based on the radiological findings in preference to the clinical symptoms as cortical strokes can be misdiagnosed clinically as lacunar syndromes and vice versa (Mead et al. 2000; Seifert et al. 2005). Furthermore where there is discrepancy between the classifications, patients tend to behave like, and have similar prognosis to the type of lesion seen on imaging, ie a patient with a cortical syndrome and a lacunar ischaemic stroke lesion will have the prognosis of a lacunar stroke patient. Accurate sub-typing is important both clinically for guiding patient management and indicating prognosis but also within research to ensure that any sub-type specific treatments are patient relevant and to ensure accurate epidemiological trials. It is therefore important to fully understand which features may lead to mismatch between clinical and radiological sub-typing. We investigated in this cohort how many patients had their clinical stroke sub-type altered by imaging and which features might predict this and present these results in this chapter, hypothesising that the location and size of the infarct may lead to clinical-radiological mismatch.

4.2.3 Statistical methods

All statistical analyses were performed in Excel (Microsoft Inc,CA, US) and Minitab (version 14, Minitab Inc, PA, USA). In comparing baseline differences between ischaemic stroke sub-types and where the data were normally distributed we used a 2 sample t test (age), where the data was not normally distributed (eg NIH, time to scan) we compared the two groups with a Mann-whitney U test and finally when the variable had a binary nature we used differences in proportions. Significance levels were set at p=0.05 and where possible we reported both the size and direction of the effect rather than just a p value. We used multivariable binary logistic regression to assess predictors of both negative DWI/MRI and the presence of clinico-radiological mismatch.
4.3 Results

4.3.1 Mild Stroke Study recruitment

The Mild Stroke Study recruited patients presenting to the acute stroke services (which covers the inpatient stroke unit, out patient neurovascular clinics and the acute medical receiving unit) of the Western General Hospital (WGH) in Edinburgh, United Kingdom between April 2005 and January 2008. From internal audit data (not published) the WGH saw approximately 550 patients a year with possible (diagnosis of stroke one of many possible diagnoses but not the most likely), probable (diagnosis of stroke is the most likely amongst a list of possible diagnoses) or definite stroke (almost no uncertainty that stroke is the most likely diagnosis).

We recruited 253 patients. Thirty four patients did not have retinal photographs of whom 20 declined further participation in the study after having had the initial MRI scan, we were unable to contact 10 patients to arrange the retinal photography (despite multiple telephone calls and letters inviting the patients to return) and there were technical difficulties photographing 4 patients. The exact numbers of patients included in each analysis are detailed in the relevant result chapters.

4.3.2 Characteristics of study participants

Of the 253 patients, the mean age was 68.1 years with Standard Deviation (SD) 11.6. There were 165 males (65%) and the median National Institute for Health Stroke Scale (NIHSS) was 2 (interquartile range 2-3). We found that age was normally distributed and therefore used parametric tests to assess associations with age. NIH and time to imaging were not normally distributed and therefore we used non-parametric tests. All other variables were tested with differences in proportions. These baseline characteristics are detailed in table 4.1
Table 4.1. Baseline characteristics of the study group. SD – standard deviation, NIHSS – National Institute for Health Stroke Scale, IQR – Inter quartile range, AF – atrial fibrillation, TIA – Transient Ischaemic Attack, IHD – Ischaemic Heart Disease, ACE – angiotensin converting enzyme, ARB – angiotensin receptor blocker,

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>253</td>
</tr>
<tr>
<td>Mean age (SD) years</td>
<td>68.1 (11.6)</td>
</tr>
<tr>
<td>Median NIHSS (IQR range)</td>
<td>2 (2-3)</td>
</tr>
<tr>
<td>Male (n) (%)</td>
<td>165 (65%)</td>
</tr>
<tr>
<td>AF</td>
<td>22 (9%)</td>
</tr>
<tr>
<td>Carotid Stenosis &gt;50%</td>
<td>19 (8%)</td>
</tr>
<tr>
<td>History TIA</td>
<td>32 (13%)</td>
</tr>
<tr>
<td>History Stroke</td>
<td>23 (9%)</td>
</tr>
<tr>
<td>History IHD</td>
<td>51 (20%)</td>
</tr>
<tr>
<td>History Hypertension</td>
<td>154 (61%)</td>
</tr>
<tr>
<td>History Diabetes Mellitus</td>
<td>36 (14%)</td>
</tr>
<tr>
<td>History Peripheral Vascular Disease</td>
<td>11 (4%)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>77 (30%)</td>
</tr>
<tr>
<td>Antiplatelet at time of stroke</td>
<td>132 (52%)</td>
</tr>
<tr>
<td>Statin at time of stroke</td>
<td>92 (36%)</td>
</tr>
<tr>
<td>ACE Inh/ARB at time of stroke</td>
<td>68 (27%)</td>
</tr>
<tr>
<td>Diuretic</td>
<td>68 (27%)</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>62 (24%)</td>
</tr>
<tr>
<td>Other antihypertensive</td>
<td>48 (19%)</td>
</tr>
<tr>
<td>Median alcohol use per week (units)</td>
<td>1 (0-10)</td>
</tr>
<tr>
<td>Median time from stroke onset to scan</td>
<td>12 (IQR 5-27) days</td>
</tr>
</tbody>
</table>
### 4.3.3 Lacunar –Cortical Ischaemic Stroke Baseline Characteristics

Patient characteristics by final ischaemic stroke subtype are detailed in table 4.2.

**Table 4.2 Patient characteristics by stroke sub-type.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lacunar Stroke</th>
<th>Cortical Stroke</th>
<th>Difference and significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>129</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>Mean age (SD) years</td>
<td>66.3 (11.6)</td>
<td>70.0 (11.5)</td>
<td><strong>3.6 (0.8, 6.5) p=0.01</strong></td>
</tr>
<tr>
<td>Median NIHSS (IQR range)</td>
<td>3</td>
<td>2</td>
<td><strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td>Male (n) (%)</td>
<td>77 (60%)</td>
<td>88 (70%)</td>
<td>10% (-1%, 22%) P=0.06</td>
</tr>
<tr>
<td>AF</td>
<td>6 (4.6%)</td>
<td>16 (12.90%)</td>
<td><strong>8% (1, 15) p=0.02</strong></td>
</tr>
<tr>
<td>Carotid Stenosis &gt;50% (NASCET)</td>
<td>5 (3.88%)</td>
<td>14 (11.29%)</td>
<td><strong>7% (1, 13) p=0.02</strong></td>
</tr>
<tr>
<td>History TIA</td>
<td>19 (14.96%)</td>
<td>13 (10.48%)</td>
<td>4% (-4, 12) p=0.29</td>
</tr>
<tr>
<td>History prev Stroke</td>
<td>9 (6.98%)</td>
<td>14 (11.38%)</td>
<td>4% (-3, 12) p=0.23</td>
</tr>
<tr>
<td>History IHD</td>
<td>17 (13.18%)</td>
<td>34 (27.42%)</td>
<td><strong>14% (4, 24) p=0.004</strong></td>
</tr>
<tr>
<td>History Hypertension</td>
<td>84 (65.32%)</td>
<td>70 (56.59%)</td>
<td>9% (-3, 20) p=0.15</td>
</tr>
<tr>
<td>History Diabetes Mellitus</td>
<td>22 (17.05%)</td>
<td>14 (11.29%)</td>
<td>6% (-3, 14) p=0.19</td>
</tr>
<tr>
<td>History Peripheral Vascular Disease</td>
<td>5 (3.88%)</td>
<td>6 (4.84%)</td>
<td>1% (-4, 6) p=0.71</td>
</tr>
<tr>
<td>Current smoker</td>
<td>50 (39%)</td>
<td>27 (22%)</td>
<td><strong>17% (6, 28) p=0.003</strong></td>
</tr>
<tr>
<td>Antiplatelet at time of stroke</td>
<td>63 (48.84%)</td>
<td>69 (55.65%)</td>
<td>7% (-5, 19) p=0.28</td>
</tr>
<tr>
<td>Statin at time of stroke</td>
<td>45 (34%)</td>
<td>47 (37%)</td>
<td>3% (-8, 15) p=0.69</td>
</tr>
<tr>
<td>ACE Inh/ARB at time of stroke</td>
<td>35 (27.13%)</td>
<td>33 (26.61%)</td>
<td><strong>1% (-10, 11) p=0.93</strong></td>
</tr>
<tr>
<td>Diuretic</td>
<td>34 (26.32%)</td>
<td>34 (27.42%)</td>
<td><strong>1% (-10, 12) p=0.85</strong></td>
</tr>
<tr>
<td>Other antihypertensive</td>
<td>29 (22.48%)</td>
<td>19 (15.32%)</td>
<td><strong>7% (-2, 17) p=0.14</strong></td>
</tr>
<tr>
<td>Median alcohol use per week (units)</td>
<td>1</td>
<td>1</td>
<td>P=0.77</td>
</tr>
<tr>
<td>Beta Blocker at time of stroke</td>
<td>20 (15.50%)</td>
<td>42 (33.87%)</td>
<td><strong>18% (8, 29) p=0.001</strong></td>
</tr>
</tbody>
</table>
The cortical patients were significantly older with a lower NIHSS score and rate of currently smoking, higher rates of AF, carotid stenosis >50% NASCET, Ischaemic Heart Disease, and Beta blocker use than lacunar patients. Of the other features, although there were baseline differences, none of these reached conventional levels of significance (p=0.05).

4.3.4 Rates of positive and negative DWI/FLAIR/T2

DWI showed an acute relevant stroke lesion in 165/253 (65%) patients leaving 88 (35%) patients with no relevant hyperintense DWI lesion. For the total study population the median time between onset of symptoms and scan was 12 days (IQR 5-27). Table 4.3 shows the characteristics of the two groups. With univariable regression the group with positive DWI had a higher NIHSS, shorter time between stroke and scan and were more likely to be male. There were small differences that did not reach statistical significance in age, percentage with lacunar stroke, proportion with persisting weakness and proportion with motor weakness between the group with positive DWI and the group with negative DWI.
**Table 4.3** Characteristics of patients with positive and negative DWI scans with univariable analyses.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Positive DWI</th>
<th>Negative DWI</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>165</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>68.8 (11.5)</td>
<td>66.81 (12.0)</td>
<td>2.0 yrs (-1.1, 5.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.20</td>
</tr>
<tr>
<td>Lacunar stroke</td>
<td>49.09</td>
<td>54.55</td>
<td>5.4% (-7.4, 18.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.41</td>
</tr>
<tr>
<td>Median NIHSS</td>
<td>2 (2-3)</td>
<td>2 (1-3)</td>
<td><strong>P=0.007</strong></td>
</tr>
<tr>
<td>Time to imaging (days)</td>
<td>9.5 (3.5-20.6)</td>
<td>21.5 (10.5-35.0)</td>
<td>9.5 days (5.9, 13.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>p&lt;0.0001</strong></td>
</tr>
<tr>
<td>Symptoms persist</td>
<td>87.42</td>
<td>80.23</td>
<td>7.1% (-2.7, 17.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.15</td>
</tr>
<tr>
<td>Motor symptoms</td>
<td>88.34</td>
<td>82.95</td>
<td>5.4% (-3.8, 14.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.26</td>
</tr>
<tr>
<td>Male gender</td>
<td>71.52</td>
<td>54.02</td>
<td>17.4% (5.0, 30.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>p=0.006</strong></td>
</tr>
</tbody>
</table>
With multivariable binary logistic regression using presence of a DWI lesion as the dependent variable and correcting for all of the explanatory variables in the table above, male sex, increased NIHSS score and decreased time to scan were all independently associated with increased likelihood of a positive DWI lesion. These results are detailed in Table 4.4.

Table 4.4 Multivariable predictors of positive DWI scans. All OR are corrected for the presence of the other explanatory variable in the table

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per year increase)</td>
<td>1.02</td>
<td>1.00, 1.05</td>
<td>0.071</td>
</tr>
<tr>
<td>Male sex</td>
<td>2.2</td>
<td>1.22, 3.96</td>
<td>0.008</td>
</tr>
<tr>
<td>Lacunar sub-type</td>
<td>0.77</td>
<td>0.43, 1.40</td>
<td>0.400</td>
</tr>
<tr>
<td>NIHSS</td>
<td>1.47</td>
<td>1.11, 1.95</td>
<td>0.007</td>
</tr>
<tr>
<td>Presence of persisting symptoms</td>
<td>1.48</td>
<td>0.71, 3.10</td>
<td>0.300</td>
</tr>
<tr>
<td>Presence of motor weakness</td>
<td>1.14</td>
<td>0.49, 2.66</td>
<td>0.764</td>
</tr>
<tr>
<td>Time from stroke onset to scan (per day)</td>
<td>0.98</td>
<td>0.97, 0.99</td>
<td>0.006</td>
</tr>
</tbody>
</table>
There were 88/253 patients without an acute DWI lesion visible however 21 of these 88 patients had acute lesions present on T2/FLAIR sequences meaning that there were 67/253 patients who had no evidence of a recent stroke lesion on any sequence of MRI. With univariable analysis, having a positive MRI scan (ie DWI/FLAIR or T2) for the acute lesion was associated with increasing age, decreased time between stroke and brain imaging, the presence of persisting symptoms, and male gender (see Table 4.5).

*Table 4.5. Characteristics of patients with positive and negative MRI scans (T2, FLAIR and DWI sequences) and univariable associations.*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Positive Scan</th>
<th>Negative Scan</th>
<th>p value for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>186</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Age (per year increase)</td>
<td>69.3 (11.2)</td>
<td>64.64 (12.2)</td>
<td>p=0.007</td>
</tr>
<tr>
<td>Lacunar stroke n (%)</td>
<td>91 pts</td>
<td>38 pts</td>
<td>p=0.271</td>
</tr>
<tr>
<td>Median NIH</td>
<td>2 (2-3)</td>
<td>2 (2-3)</td>
<td>p=0.120</td>
</tr>
<tr>
<td>Time to imaging (days)</td>
<td>11 (4-23)</td>
<td>20 (10-32)</td>
<td>p=0.003</td>
</tr>
<tr>
<td>Symptoms persisting</td>
<td>87.22%</td>
<td>78.46%</td>
<td>p=0.123</td>
</tr>
<tr>
<td>Motor symptoms</td>
<td>85.33%</td>
<td>89.55%</td>
<td>p=0.354</td>
</tr>
<tr>
<td>Male gender</td>
<td>70.27%</td>
<td>52.24%</td>
<td>p=0.010</td>
</tr>
</tbody>
</table>
With multivariable binary logistic regression with presence of a positive scan as the dependent variable and correcting for all the explanatory variables present in table 4.6, increasing age, male sex and increased NIH were all associated independently with the presence of a positive MRI scan. Time to imaging, the presence of a motor weakness and time from stroke onset to imaging were not associated with the presence of a positive scan.

**Table 4.6. Multivariable predictors of positive MRI scans. All OR are corrected for the presence of the other explanatory variable in the table**

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per year increase)</td>
<td>1.04</td>
<td>1.01, 1.07</td>
<td>0.002</td>
</tr>
<tr>
<td>Male sex</td>
<td>2.10</td>
<td>1.13, 3.90</td>
<td>0.018</td>
</tr>
<tr>
<td>Lacunar sub-type</td>
<td>0.80</td>
<td>0.43, 1.51</td>
<td>0.496</td>
</tr>
<tr>
<td>NIHSS</td>
<td>1.50</td>
<td>1.11, 2.03</td>
<td>0.009</td>
</tr>
<tr>
<td>Presence of persisting symptoms</td>
<td>1.64</td>
<td>0.75, 3.57</td>
<td>0.211</td>
</tr>
<tr>
<td>Presence of motor weakness</td>
<td>0.44</td>
<td>0.16, 1.23</td>
<td>0.118</td>
</tr>
<tr>
<td>Time from stroke onset to scan</td>
<td>0.99</td>
<td>0.98, 1.01</td>
<td>0.241</td>
</tr>
</tbody>
</table>
4.3.5 Sub-typing misclassification of ischaemic stroke subtypes

Of the 253 patients, 126 presented with a clinical diagnosis of lacunar stroke syndrome and 127 with a clinical diagnosis of cortical stroke syndrome. Overall 41/254 (16.1%) patients had their initial clinical classification changed by imaging eg patient presenting with symptoms suggestive of a lacunar stroke with a cortical infarct on imaging and vice versa. Of the 126 with a lacunar stroke syndrome, 20 had their sub-typing changed to cortical ischaemic stroke and of the 127 with a cortical stroke syndrome, 21 had their classification changed to lacunar ischaemic syndrome.

Table 4.7 Univariable predictors of presence of classification change.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Classification Change Present</th>
<th>No Classification Change Present</th>
<th>P value for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>41</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td>Mean Age (yrs)</td>
<td>70.7 (SD 10.3)</td>
<td>67.6 (SD 11.8)</td>
<td>p=0.09</td>
</tr>
<tr>
<td>Median NIHSS</td>
<td>3</td>
<td>2</td>
<td>P=0.19*</td>
</tr>
<tr>
<td>Median Time to assessment (days)</td>
<td>9</td>
<td>14</td>
<td>P=0.08*</td>
</tr>
<tr>
<td>Motor weakness</td>
<td>40 (98%)</td>
<td>177 (84%)</td>
<td>p=0.023**</td>
</tr>
<tr>
<td>Lacunar Stroke subtype</td>
<td>21 (16%)</td>
<td>20 (16%)</td>
<td>p=0.97</td>
</tr>
</tbody>
</table>

* Mann-Whitney U Test
** Fishers exact test
Table 4.8 Multivariable predictors of the presence of a classification change

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per year increase)</td>
<td>1.03</td>
<td>1.00 to 1.07</td>
<td>0.04</td>
</tr>
<tr>
<td>NIHSS</td>
<td>1.19</td>
<td>0.93 to 1.53</td>
<td>0.16</td>
</tr>
<tr>
<td>Time to assessment</td>
<td>0.99</td>
<td>0.98 to 1.01</td>
<td>0.52</td>
</tr>
<tr>
<td>Motor weakness</td>
<td>6.54</td>
<td>0.84 to 50.97</td>
<td>0.07</td>
</tr>
</tbody>
</table>

With multivariable regression, only increasing age was independently associated with clinical-radiological mismatch. In lacunar strokes we further investigated whether the location of the infarct led to increased clinical–radiological mismatch. There were 129 patients with a final classification of lacunar ischaemic stroke. Of the 129 patients, 92 had acute stroke lesions seen on MRI scan. Of the 92 patients, 72/92 had presented with a clinical lacunar stroke classification but 20/92 had presented with symptoms suggestive of a cortical stroke. The lacunar infarct seen on MRI extended to the cortical margin in 8/20 patients who had presented with a cortical syndrome but had a final diagnosis of lacunar stroke (clinical radiological mismatch) and the lacunar infarct extended to the cortical margin in 10/72 patients who presented with lacunar syndrome and a final classification of lacunar stroke. Patients with a final clinico-radiological classification of lacunar stroke who presented initially with a cortical syndrome were more likely to have lesions extending to the cortical margin than patients with a final diagnosis of lacunar stroke who had presented with a lacunar stroke syndrome (Chi square statistic 6.7, p=0.009) but the small number of outcomes only permits univariable analysis.

In a similar analysis, patients with a final classification of lacunar stroke who presented initially with a cortical classification were more likely to have lesions in the centrum semiovale than patients with a final diagnosis of lacunar stroke who had presented with a lacunar stroke syndrome (Chi square statistic 4.4, p=0.036).
Furthermore, amongst the 92 patients with an acute lacunar infarct on MRI, lesion size was associated with clinical radiological classification mismatch. Patient with clinical radiological mismatch had a mean lesion size of 11.25 mm (SD 3.86mm) and patients with no clinical radiological mismatch had a mean lesion size of 9.1 mm (SD 3.5mm). The difference is 2.1 mm (95% CI 0.2-4.2) p=0.03 (Students t test). Presence of a clinical history of stroke (Mismatch history of stroke prevalence 7.3%, no mismatch history of stroke prevalence 9.4%, difference = 2.1%, (95% CI -6.7% to 11.1%) p=0.63) or the presence of old infarcts on MRI scan (mismatch prevalence of old stroke lesions 34.1%, no mismatch prevalence of old stroke lesions 37.7%, difference = 3.5%, (95% CI -12.3% to 19.5%), p=0.66) were not associated with the presence of mismatch with patients presenting with a final classification of lacunar stroke.

4.4 Discussion

4.4.1 Baseline characteristics and comparison with other cohorts.

The patients in this cohort had a mean age of 68 (SD 11) years, median NIHSS 3 and 65% were male. The mean age of stroke in our cohort is lower than the mean age at stroke onset in the UK which reflects firstly the recruitment of patients with minor stroke and secondly we recruited patients who were able to participate in a research study (that involved MRI scanning and retinal photography) and recruitment bias will have selected a younger more able and willing to participate cohort. The average age for hospital based cohorts investigating risk factors for stroke sub-types however is also lower than the mean age of stroke onset in the UK of 72 years at 66 years (Jackson and Sudlow 2005) which is similar to our cohort.

How do these results compare with other studies? The patients in this study had a considerable number of risk factors for and burden of pre-existing vascular disease and it is useful to compare these rates with other studies from the United Kingdom. OXVASC was a community based study of 91 000 participants cared for by study
general practitioners in Oxfordshire, UK investigating incident major vascular events in any arterial territory (mainly stroke/TIA, coronary and peripheral artery disease) (Rothwell et al. 2005). Within the OXVASC population the researchers piloted a scheme for rapid access to a neurovascular clinic for patients requiring out patient assessment and care for possible stroke/TIA (Rothwell et al. 2007). The main focus of this study was to assess whether a complex intervention of rapid access and instigation of early secondary prevention would influence outcome but the baseline characteristics of patients presenting with stroke/TIA to the outpatients clinic in this population based study are similar to the Mild Stroke Study with a prevalence of hypertension of 59% (MSS 61%), diabetes mellitus 12% (MSS 14%), peripheral vascular disease 8% (MSS 5%), prior antiplatelet use 46% (MSS 52%) and prior statin use 32% (MSS 36%). The authors do not present mean age or data on IHD combined as we do. Other studies have presented similar proportions of patients with hypertension, diabetes and IHD to the MSS (Sandercock et al. 1989). The population based south London Registry has reported vascular risk factors similar to the MSS for patients presenting with first ischaemic stroke (despite representing an ethnically diverse population compared with the MSS where 2 out of 254 patients were of South Asian extraction rather then Caucasian) with prevalences of hypertension 66%, diabetes mellitus 20%, AF 15% and current smoking 32% (Heuschmann et al. 2008). Although these studies represent slightly different cohorts it is encouraging that they report similar prevalences of risk factors to the MSS study which increases the generalisability and likely reproducibility of the results from the Mild Stroke Study.

4.4.2 Baseline characteristics by ischaemic stroke subtype

The patients in the cohort were evenly split between lacunar and cortical stroke. The lacunar patients were significantly younger, with a higher NIHSS and with a lower prevalence of Ischaemic Heart Disease, Atrial Fibrillation and symptomatic carotid stenosis>50% NASCET than the patients with cortical stroke. This is consistent with a large artery disease and cardio embolic causes for cortical stroke. The prevalences of hypertension, diabetes, peripheral vascular disease and previous stroke and TIA
did not differ significantly between the two groups. Prior antiplatelet use, prior statin use, prior ACE Inhibitor/Angiotensin II receptor antagonists use, prior diuretic and other antihypertensive use did not differ between the two groups. Beta blocker use in the cortical stroke group was significantly higher than in the lacunar stroke group reflecting the higher prevalence of ischaemic heart disease in the cortical group.

How do these risk factor differences compare with other populations? It is difficult to generalise from other studies of risk factors for ischaemic stroke sub-types as different studies used different classification systems which produced varying results. Some studies used the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria (Adams, Jr. et al. 1993) which was developed for use in a multicentre clinical trial (Grau et al. 2001; Saposnik et al. 2004). This classification assigns stroke sub-types based on presumptive causation from the clinical findings, risk factor status and brain, heart and vessel imaging results of; large artery atherosclerosis, cardio-embolic, lacunar, undetermined aetiology and other determined aetiology (effectively the rarer causes of stroke). The main flaw with this classification system is that it uses risk factor status to assign sub-type classification e.g. a history of diabetes or hypertension supports the diagnosis of lacunar stroke. Unsurprisingly use of this definition leads to classification bias and these studies report associations between ischaemic stroke sub-types and risk factors that are biased. Other studies have used a classification of ischaemic stroke based upon the clinical features of the stroke syndrome (often the Oxfordshire Community Stroke Project classification (Bamford et al. 1991) altered by brain imaging results that refine the clinical classification to define the location of the infarct. These methods are not biased by risk factor status and infer less about the cause of the stroke than the TOAST method. Other studies have used a purely imaging based method of assessing stroke sub-type with no clinical correlates to the infarcts seen on imaging (Moulin et al. 2000) but this approach is limited as it is not certain what these “silent” holes/infarcts in the brain represent.

A recent systematic review (Jackson and Sudlow 2005) found 28 studies which investigated risk factor profiles in lacunar versus non lacunar stroke. This review
reports a variation in associations between risk factors and ischaemic stroke sub-type which may be in part be due to the classification system used. The studies which used a risk factor free classification system found that the presence of diabetes was not associated with lacunar stroke sub-type (pooled relative risk 0.95; 95% CI 0.83 to 1.09) and that the presence of hypertension was weakly associated with lacunar stroke sub-type (pooled RR 1.11; 95% CI, 1.04 to 1.19). In both cases the pooled relative risk was higher when stroke was sub-typed with risk factor dependent classification systems. These results are consistent with the results from the MSS in not showing a strong association between diabetes and hypertension and lacunar stroke. In keeping with the results of the MSS this review also reports that atrial fibrillation is more common in non-lacunar stroke than lacunar stroke - RR for association with lacunar stroke 0.51; 95% CI 0.42 to 0.62 and that ipsilateral carotid stenosis is also more common in non-lacunar stroke (RR 0.35; 95% CI 0.03 to 0.25) both consistent with atherothromboembolic causes for cortical stroke.

In our cohort we also found that patients with a lacunar stroke had a higher NIHSS score than patients with cortical stroke. Conventionally, patients with lacunar stroke tend to have milder symptoms and lower NIHSS scores than patients with non-lacunar stroke (Adams, Jr. et al. 1999), but we should emphasize that in this study we excluded severe cortical stroke and that the NIHSS (which was originally designed as a research tool to assess differences in intervention groups) may have been influenced by ischaemic stroke classification eg a patient with a lacunar stroke and a weak arm and leg will have a higher NIHSS score than a patient with a cortical stroke and a monoparesis.

The baseline characteristics of this cohort are similar to the characteristics of previously published cohorts.

4.4.3 Rates of negative DWI and associated features

We found that in patients presenting with mild stroke the rate of false negative DWI scans was 35%. Decreased severity of stroke (NIHSS), increased time between
stroke and scan and female gender were associated with the presence of a negative DWI scan (independently of age, presence of persisting symptoms, presence of motor weakness and stroke sub-type). The rate of negative MRI scan (patients with no infarct visible on T2/FLAIR or DWI) was lower at 26% and with multivariable analysis younger age, decreased stroke severity and female gender were associated with a negative MRI scan (independently of time between stroke and scan, presence of persisting symptoms and presence of motor weakness).

This study has several strengths: it is to the best of our knowledge the largest study to investigate the rate and associations of negative DWI solely in minor stroke patients (previous studies included TIA patients), it was a prospective study and all patients had a definite diagnosis of stroke made by an experienced stroke physician with MRI at the time of presentation and the diagnosis of stroke made by a panel of experts. The study was representative of patients who present to the hospital with minor strokes as the clinical deficit is often minimal and therefore there is often a delay between onset of symptoms and the patients seeking clinical attention. We used a dedicated research scanner with standardised sequences and protocols to ensure conformity of scanning and all the scans were coded by an experienced neuroradiologist using a tool based upon a previously validated grading scheme (Wardlaw and Sellar 1994). All patients were carefully characterised and we were able to investigate associations adjusted for key possible confounders.

There are however some weaknesses in the study. Despite aiming to limit it by only including patients where a senior physician felt that there had been a definite stroke and discussing cases at a weekly multidisciplinary meeting to ensure consensus opinion it is inevitable that there will have been a small number of patients in the negative DWI group who did not have a stroke. Other studies have confirmed a diagnosis of stroke in patients with initially negative DWI scans with abnormalities on subsequent follow up scan (Oppenheim et al. 2000) but this approach misses patients who never have a relevant lesion. This is a cross sectional study and as such we can only report associations without implying causality. The presence of DWI lesion in longitudinal studies post stroke demonstrates high inter individual
variability (Schulz et al. 2007) and therefore we are unable to make any comments regarding how long DWI lesions will remain positive on a patient to patient basis other than to assess the median differences between the positive and negative DWI groups. It has been reported that asymptomatic DWI lesions appear after ischaemic stroke (Kang et al. 2003b) and it is possible that the DWI lesions we saw did not correspond to the presenting symptoms but a subsequent event. We sought to minimise this by only reporting a DWI as positive if the lesion was in an arterial territory relevant to the presenting symptoms and using the most recent symptoms that the patients reported to sub-type stroke and estimate time between symptom onset and scan (although most patients only had one episode of symptoms).

The patients for this study were selected according to willingness to take part in a research study and also for suitability for undergoing MRI procedures. This will have inevitably led to selection bias but previous research from this centre has shown that as many as 85% of patients admitted with acute stroke are able and willing to undergo MRI in the acute setting (Hand et al. 2005). Patients who decline MRI scans tend to be older with more medical problems but it is reassuring to see that the mean age of our patients (68.1 years) was similar to hospital based estimates of mean age of stroke onset (65-70) (Jackson and Sudlow 2005).

How do our results compare with other studies? No other studies have investigated the rate and associations with false negative DWI scans in patients presenting solely with minor stroke although several have studied TIA and minor stroke together (Schulz et al. 2004; Sylaja et al. 2008). It is possible to draw simple comparisons between our cohort and certain univariable analyses from these papers but the more useful multivariable analyses were performed for the total study population of patients presenting with TIA and stroke combined. Our rate of negative DWI in patients presenting to hospital with minor stroke was 35%. This is much higher than previous quoted rates of 5.8% in patients scanned within 48 hours of stroke onset where the negative scans were associated with scanning within 24 hours of stroke onset (Oppenheim et al. 2000) and 6% when DWI MRI was used in the emergency room setting (Mullins et al. 2002) although in a similar emergency room setting the
rate of negative DWI in patients with a final diagnosis of stroke was 17% (Chalela et al. 2007).

If, however, we compare our results to other patient cohorts presenting with less severe stroke symptoms and imaged at presentation in the subacute setting the results are remarkably similar. A recent study investigated DWI MRI at presentation in patients presenting with TIA or minor stroke to an outpatients clinic at least 2 weeks after the onset of symptoms (Schulz et al. 2003). This study recruited 101 patients of whom 51 had had a minor stroke. The rate of negative DWI was 43% and the median time to scan 21 days (IQR17-28). With univariable analysis they found that there was no effect of time to scanning on delay (although time was dichotomized to 2-4 weeks versus >4 weeks perhaps losing statistical power to detect small differences and the sample size was small at 51) but that persistent symptoms and signs predicted a positive DWI. A further study from the same authors which recruited patients in the same time period as the first study (although it is not clear whether they are different patients) with TIA or minor stroke at presentation to an outpatients study where the median time to scan was 17 days (IQR10-23) (Schulz et al. 2004). The study recruited 300 patients of whom 164 were minor strokes. Amongst the minor strokes the rate of negative DWI was 30% and with univariable analysis persistence of symptoms/signs were still present (uncorrected for age, NIHSS score and other vascular risk factors). It is impossible to extract data from the multivariable regression correcting for age, sex and diagnosis. A further study from a different group assessing patients presenting with recent TIA/minor stroke found that all of the patients with negative DWI and stroke had lacunar or brainstem infarcts. Despite stating that 26 patients with stroke had false negative scans the study does not however report how many of the 401 patients had TIA or stroke and without a denominator it is therefore impossible to determine the rate of negative DWI scans in minor stroke (Sylaja et al. 2008).

Decreased NIHSS in our study was associated with both negative DWI and MRI scans for relevant ischaemia. This finding is replicated elsewhere (Chalela et al. 2007; Schulz et al. 2004) – it seems logical that small infarcts are less likely to be
picked up on imaging than larger infarcts. Furthermore, although infarct size has been shown in a small longitudinal study not to predict persistence of DWI lesions (Schulz et al. 2007) it is possible that small infarcts may resolve quicker than larger infarcts and then not be seen if there is a delay to imaging.

Increased time to imaging was associated with negative DWI scans although not negative MRI scans. This confirms other findings (Schulz et al. 2004) and is likely to reflect the fact that DWI lesions which reflect areas of impaired water diffusion largely secondary to ischaemia, subsequent cell swelling and then death (Gass et al. 2004) can resolve after time. Although the DWI may have returned to normal there are often persisting abnormalities seen on T2 and FLAIR weighted images and we have demonstrated that T2/FLAIR identifies more infarcts in patients presenting subacutely with minor stroke.

We found that female gender was associated with both negative DWI and negative MRI scans. Other studies have reported similar rates (Schulz et al. 2004) in male and females and the reason for this discrepancy is not clear. Perhaps other studies did not have large enough sample sizes to demonstrate this small effect of perhaps the result was due to residual confounding due to baseline differences in age and vascular risk factors and stroke severity not fully corrected for by our regression model. Despite aiming not to include stroke mimics it is notable that migraine and functional disease may be associated with female gender and this may have biased our sample.

In keeping with some studies (Schulz et al. 2004) we did not find an association between lacunar/cortical sub-types and negative DWI although another study stated that all of the patients with stroke and negative DWI had either brainstem or lacunar infarcts (Sylaja et al. 2008).

We have shown that in a population presenting with minor stroke there is a high rate of negative DWI which is associated with younger age, decreased stroke severity, increased time to scan and female gender. This is of importance to both clinicians
and researchers in that the results will guide clinicians as to when to expect a negative DWI in a patient with minor stroke and should inform them not to exclude the diagnosis in the presence of a negative scan. We included patients with negative DWI if the panel consensus was such that the diagnosis remained a stroke we felt that this was the most robust method for diagnosing stroke. It is a potential limitation of this thesis that inevitably, and despite our best efforts, some patients may have been included who had not had a stroke. Many studies of stroke only include patients in which there is DWI evidence of ischaemia however this approach will exclude a significant proportion of patients presenting with minor stroke leading to significant selection bias. It is this population (along with TIA patients) without significant residual neurological deficit and high risk of early recurrent stroke (Giles and Rothwell 2007) that research should be concentrating on to identify and treat risk factors for subsequent disabling stroke.

4.4.4 Subtyping of ischaemic stroke

We sub-typed stroke using the OCSP classification refined by the results of appropriate MRI imaging to produce a final classification. This method produces a sub-typing of stroke that conveys both information regarding outcome and likely pathophysiology. As mentioned in the previous section there are other classification schemes largely based on the TOAST criteria but these systems have 2 main flaws; firstly the sub-typing is risk factor dependent leading to classification bias in risk factor analysis and secondly in practice there are a large number of strokes which fall into the undetermined category (estimates range from 8-41% of strokes (Jackson and Sudlow 2005) which will lead to loss of data and heterogeneity between different studies. A significant advantage of using clinical features and brain imaging to assign stroke sub-type is that the large proportion of undetermined stroke is avoided as almost every stroke can be sub-typed. There are however problems associated with this as diagnosing stroke and assigning a clinical sub-type can be difficult (Allder et al. 1999) and there is a significant proportion of patients in whom there is a mismatch between the clinical and the radiological sub-type eg a patient presenting...
with a lacunar syndrome has a cortical lesion on their scan and vice versa (Mead et al. 2000).

We found that 41/254 (16%) of patients had their clinical classification changed by the imaging classification and that with multivariable analysis increasing age was independently associated with the presence of mismatch but that clinical classification, NIHSS, time to assessment and presence of motor weakness were not associated with the presence of mismatch. We further investigated the 129 patients with a final diagnosis of lacunar stroke of whom 21 had a presented with cortical syndromes and found that both increased size of the lacunar infarct on MRI and if it extended to the cortical margin were associated with mismatch.

How does this compare to other studies? There have been no other large (>100 patients) studies that have used MRI to accurately identify the recent infarct in all patients. A large Dutch cohort of patients presenting with either first ever deep (lacunar) or territorial (cortical) acute infarcts was investigated with CT at presentation (Lodder et al. 1994). Of 350 patients, 42 (12%) had clinical-radiological mismatch. They further investigated which features may predict the presence of mismatch and found that in patients with deep (lacunar) infarct on scan the patients with mismatch (i.e. who had presented with a syndrome suggestive of a territorial infarct) were more likely to have increased severity of stroke, a cardioembolic source, concurrent leukoaraoisis and asymptomatic infarcts. In patients with a territorial (cortical) infarct on scan the patients who had mismatch (i.e. who had presented with a syndrome suggestive of a deep infarcts) were more likely to have had a cardio-embolic source for their stroke or to have presented with a pure motor stroke. Other studies have reported that in a cohort of 441 patients with a final diagnosis of cortical stroke and 265 patients with a final diagnosis of lacunar stroke mismatch was present in 13% and that neither side of brain or time from scan to brain imaging (dichotomized to less than or greater than 48 hours) predicted the presence of mismatch (Mead et al. 2000).
No other studies have investigate whether the size of the lesion or lesion location (particularly whether the lacune adjoins the cortical margin) predict the presence of mismatch. We found that in patients with final diagnosis of lacunar infarction those patients with mismatch (i.e. who had presented with a cortical syndrome) had larger infarcts than those with no mismatch. This finding does not have a direct correlate in other studies but in a study of patients presenting with subcortical infarcts who were intensively characterised with MRI at presentation and angiography it was found that increased size of the subcortical infarction was associated with an increased chance of finding a non small vessel disease cause for the stroke such as cardio-embolism or large artery disease (Bang et al. 2007). It should be noted however that the mean size of subcortical infarct of the small vessel disease group was 15mm (SD 7 mm) and in the cardio-embolism/large artery group of subcortical infarctions was 20 (SD 11mm). In our study with a maximum cut off of 20 mm for lacunar infarction half of this second group would have been classified as cortical infarctions.

The finding that lacunar infarctions extending to the cortical margin are associated with mismatch is novel. Although lacunar infarcts are often small they often cause a neurological deficit disproportionate to their size largely due to the fact they occur in areas of the brain where the neurons are tightly packed. If a lacunar infarct is situated more peripherally then the nerve fibres will be less tightly packed and a similarly sized but more peripherally located infarct may produce a smaller neurological deficit for example affect a single limb which would lead to a cortical syndrome. We did not find an association between time to assessment and misclassification in keeping with previous studies albeit over a shorter time period (Mead et al. 2000). We had included this variable as we felt that with increased time to assessment and with mild stroke with possible full recovery at the time of assessment there might be recall bias in terms of patients recounting what their maximal deficit had been. This did not appear to have been the case. Previous studies using CT found that the presence of asymptomatic infarcts was associated with the presence of mismatch (Lodder et al. 1994). We did not find that this is the case probably because MRI scans read by an experienced neuroradiologist will permit assessment of old and recent infarcts with greater accuracy than CT scanning.
Ascribing an aetiology to all ischaemic strokes is difficult as the pathophysiology of stroke is complex. Not all lacunar infarcts are caused by small vessel disease either based on clinical/risk factor analysis (Ay et al. 1999b; Baumgartner et al. 2003; Micheli et al. 2008; Seifert et al. 2005) or with pathological studies which have shown small lacune like lesions involving deep cortical layers and arcuate fibres without small vessel disease (De Reuck et al. 2004). We have however shown that a substantial proportion of patients are misclassified using purely clinical methods and that the location of the infarct can affect this misclassification. Judicious use of neuroimaging, preferably MR can lessen the prevalence of clinical radiological mismatch in a population and future studies investigating differences between ischaemic stroke sub-types should be aware of and ensure that they carefully subtype stroke to avoid misclassification bias.

4.5 Chapter summary

This chapter has demonstrated that the patients in the Mild Stroke Study are similar to other patients in hospital based studies and have similar risk factor profiles. We have described rates of clinical-radiological dissociation in sub-typing of minor ischaemic stroke (16%) and demonstrated features which will predict mismatch, largely whether lacunar infarcts extend to the cortical margin, the size of the lacunar infarcts and increasing age of the patient. Finally we have demonstrated that up to 35% of DWI scans in patients presenting sub-acuteley with mild stroke are negative and that this is independently associated with decreased stroke severity, younger age, increased time between onset of stroke symptoms and female gender which will have important implications both for clinicians treating and scientists researching minor stroke.
Chapter 5: Retinopathy in Ischaemic Stroke Subtypes

5.1 Aims of chapter

In this chapter we test the hypothesis that lacunar stroke is caused by increased blood brain barrier permeability by comparing the prevalence of retinopathy (a marker of blood retinal barrier breakdown) in lacunar versus cortical stroke subtypes.

5.2 Introduction

As detailed in previous chapters the exact aetiology of lacunar stroke remains unknown (Wardlaw 2005). There is mounting evidence that disordered small vessel endothelium or blood-brain barrier dysfunction may contribute to the aetiology of cerebral small vessel disease (Rosenberg 2009; Topakian et al. 2008; Wardlaw et al. 2008).

The blood-retinal barrier is analogous to the blood-brain barrier (Patton et al. 2005). Animals studies suggest that in ageing rats with behavioural signs of cognitive dysfunction (one of the clinical manifestations of cerebral small vessel disease) there is evidence of blood retinal barrier breakdown compared to cognitively intact younger rats (Chan-Ling et al. 2007). We have shown in Chapter 3 of this thesis that large population studies demonstrate associations between retinopathy (defined as the presence of hard or soft exudates, haemorrhage or microaneurysms) and previous stroke as well as future stroke risk (Doubal et al. 2009). Retinopathy is associated with increased permeability of the blood-retinal barrier (Krogsaa et al. 1981) and we therefore hypothesized that there would be higher rates of retinopathy in patients with acute ischaemic lacunar stroke compared to acute ischaemic cortical stroke controls where the mechanism is largely atherothromboembolic if lacunar strokes are caused by increased blood brain barrier permeability.
5.3 Methods

We recruited patients prospectively with acute clinical lacunar or mild cortical ischaemic stroke. The exact details of patient recruitment for this study are given in Chapter 2 of this thesis but in brief we included patients who presented with mild cortical or lacunar ischaemic stroke.

All patients were examined by an experienced stroke physician and classified initially into lacunar or cortical stroke clinical syndromes according to the Oxfordshire Community Stroke Project classification (Bamford et al. 1991). Patients had diagnostic cerebral MRI (including diffusion-weighted imaging, DWI and GRE) at presentation to identify the site of the recent infarct and exclude haemorrhage.

All patients had six field retinal photography (centred on the disc, macula, lateral macula, nasal to the disc, upper arcade and lower arcade) of the left and right eyes, with 1% tropicamide eye drops where possible, using a Canon CR-DGi digital retinal camera (Canon USA Inc.).

5.3.1 MRI Image analysis

All MRI scans were coded by an experienced neuroradiologist (JW) for the presence, location and size of the recent symptomatic infarct and any old infarcts or haemorrhages.

5.3.2 Retinal image analysis

I graded each retinal photograph masked to clinical details for retinopathy (defined as haemorrhage, microaneurysms or hard or soft exudates see figures 5.1, 5.2, 5.3 and 5.4 for examples) as absent, questionably present or present according to an established, validated proforma (Mitchell et al. 2005). Retinopathy was deemed present if any of the multiple retinal photographs for each patient showed a
retinopathic lesion (although in practice it was rare for there to be isolated retinopathic lesions). I was trained under the supervision of Professor Bal Dhillon (BD) with test images and with ophthalmologists performing regular diabetic screening. All lesions identified were checked by a consultant ophthalmologist (BD). If an image was missing or I was unable to grade an image I recorded this and the reason why. All images were graded on a standard flatscreen monitor under consistent lighting conditions. The intra-rater repeatability Kappa score for the presence versus absence of retinopathy (for 30 randomly chosen cases in this study performed 1 month apart) was 0.84 (excellent). As I was the only rater for this study I was unable to perform inter-rater reliability scores. This technique was based upon a retinopathy grading scale (microaneurysms and haemorrhages) with a published inter-rater Kappa score of 0.9 (Yu et al. 1998).

5.3.3 Statistical analysis

I used 2 sample t-tests, Fishers Exact Test and Chi Square tests for association to investigate baseline characteristics between the lacunar and cortical groups and associations with retinopathy and multiple binary logistic regression to assess multivariable effects of explanatory variables including assessing for significant interactions. All analyses were performed with Minitab software (version 14, Minitab Inc, PA, USA). Limited sample size calculation based on existing literature on retinal findings in stroke suggested that 197 patients would be needed to detect a difference of prevalence in retinopathy of 10% with 80% power at the 0.05 significance level.
Fig. 5.1 Enlarged section of retinal photograph showing retinal haemorrhages (arrow)

Fig. 5.2 Retinal photograph showing multiple soft exudates (white cotton wool spots)
Fig. 5.3 Enlarged section of retinal photograph showing two dot like red microaneurysms (lower right quadrant of photograph)

Fig. 5.4 Enlarged section of retinal photograph showing yellow/white hard exudates.
5.4 Results

We recruited 220 patients of whom 6 were excluded with photographs of inadequate quality to assess retinopathy (due to cataract, poor compliance or inadequate dilatation) leaving 214 patients for analysis. The mean age was 68.4 years (SD 11.6 years), 62% were male, and the median NIHSS was 2. There were 109 patients with acute cortical stroke and 105 with acute lacunar stroke. Acute stroke lesions were seen on MRI (DWI and/or T2/FLAIR based on signal characteristics and lack of focal atrophy) in 159/214 (74%) patients of whom 144/214 (67%) of the cohort had DWI positive lesions (the rate reflecting the small nature of the lesions and occasional delays to scanning). Consistent with previous studies (Mead et al. 2000) 38/214 (18%) patients had their stroke subtype classification changed by MRI findings from cortical to lacunar or vice versa. 73/214 patients had old infarcts on imaging – in 16 the old infarct was of a different subtype to the acute lesion and 4 had both old cortical and lacunar infarcts.

The baseline characteristics of the lacunar and cortical groups are shown in Table 5.1. The cortical patients were older than the lacunar stroke patients (mean 70.6 years v 66.3 years, 2 sample t test estimate of difference 4.25 95% CI 1.15-7.34 yrs p=0.007) with higher NIHSS (median 3 v 2 Mann-Whitney U test p<0.01) and in keeping with cortical strokes being caused by large artery disease and cardio-embolism had higher rates of atrial fibrillation (14% v 4% p=0.009) and ischaemic heart disease (29% v 13% p=0.004). There were no significant differences between the two groups in gender or rates of hypertension, diabetes or PVD.
Table 5.1. Baseline characteristics of lacunar and cortical stroke subtypes.

Statistical test used – Mean age (2 sample t test), median NIHSS (Mann-Whitney U test), PVD and AF (Fishers Exact Test), all others Chi square test for association.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lacunar Stroke</th>
<th>Cortical Stroke</th>
<th>P value for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>105</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>Age years (SD)</td>
<td>66.3 (11.6)</td>
<td>70.6 (11.4)</td>
<td>0.007</td>
</tr>
<tr>
<td>Male n (%)</td>
<td>60 (57%)</td>
<td>74 (68%)</td>
<td>0.10</td>
</tr>
<tr>
<td>Median NIHSS</td>
<td>3</td>
<td>2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Atrial Fibrillation n (%)</td>
<td>4 (4%)</td>
<td>15 (14%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Past Medical History of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes n (%)</td>
<td>18 (17%)</td>
<td>11 (10%)</td>
<td>0.13</td>
</tr>
<tr>
<td>Ischaemic heart disease n (%)</td>
<td>14 (13%)</td>
<td>32 (29%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Peripheral vascular disease n (%)</td>
<td>5 (5%)</td>
<td>5 (5%)</td>
<td>0.95</td>
</tr>
<tr>
<td>Hypertension n (%)</td>
<td>59 (56%)</td>
<td>62 (66%)</td>
<td>0.14</td>
</tr>
<tr>
<td>TIA n (%)</td>
<td>16 (15%)</td>
<td>13 (12%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Stroke n (%)</td>
<td>9 (9%)</td>
<td>12 (11%)</td>
<td>0.55</td>
</tr>
<tr>
<td>Old infarct on MRI n (%)</td>
<td>35 (33%)</td>
<td>38 (35%)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Of 214 patients, 40 patients (18.7%) had retinopathy present: 19/105 patients (18%) with lacunar stroke and 21/109 patients (19%) with cortical stroke had retinopathy (Chi squared statistic 0.48, p=0.8) See figure 5.4. There was no difference between the rate of retinopathy in stroke subtypes. Table 5.2 shows individual components of retinopathy by stroke subtype. There were no significant differences between individual components of retinopathy and stroke subtype. (Figure 5.5)
Figure 5.4 Bar chart showing proportions of patients with retinopathy by stroke subtype

Table 5.2. Number of patients with individual components of retinopathy by stroke subtype. There are no significant differences in proportions with retinopathy features between stroke subtypes.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lacunar Stroke</th>
<th>Cortical Stroke</th>
<th>% Difference (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hard Exudates (%)</td>
<td>4 (4%)</td>
<td>8 (7%)</td>
<td>3.5% (-2.6%, 9.6%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Soft Exudates (%)</td>
<td>6 (6%)</td>
<td>5 (5%)</td>
<td>1.1% (-4.8%, 7.0%)</td>
<td>0.71</td>
</tr>
<tr>
<td>Haemorrhage/microaneurysm (%)</td>
<td>14 (13%)</td>
<td>12 (11%)</td>
<td>2.3% (-6.4%, 11.0%)</td>
<td>0.60</td>
</tr>
</tbody>
</table>
With univariable analysis the presence of diabetes was associated with retinopathy (Chi square statistic 4.8, p=0.028) but not age, NIHSS, hypertension, IHD, PVD, previous TIA/Stroke or AF. After correcting for mild baseline differences in age, hypertension and diabetes with multivariable binary logistic regression, there was no association between ischaemic stroke subtype and presence of retinopathy (OR 0.76 – 95% CI 0.37-1.57 p=0.46) see Table 5.3. The only variable independently associated with the presence of retinopathy after correction for age, stroke subtype and hypertension was diabetes (OR 3.02 -95% CI 1.24-7.34 p= 0.01).

To examine possible effect modifiers, we assessed all two way interactions (between age, hypertension, diabetes and stroke subtype) and sequentially removed those interactions contributing least to the model where their effect was non significant. After this process the only significant interaction that remained was between hypertension and age in predicting retinopathy (OR for a combined age multiplied by
presence of hypertension explanatory variable of 0.94 95% CI 0.88 -1.00 p=0.04) when modelled with age, hypertension, diabetes and stroke subtype, but this did not significantly alter the associations shown in Table 5.3.

Table 5.3. Multivariable analysis of associations with retinopathy for all 214 patients. All OR are corrected for the other variables in the table.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Multivariable OR for association with retinopathy</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacunar stroke subtype</td>
<td>0.76</td>
<td>0.37-1.57</td>
<td>0.46</td>
</tr>
<tr>
<td>Age</td>
<td>0.98</td>
<td>0.95-1.01</td>
<td>0.25</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.82</td>
<td>0.80-1.75</td>
<td>0.61</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.02</td>
<td>1.24-7.34</td>
<td>0.01</td>
</tr>
</tbody>
</table>

To ensure accurate ischaemic stroke classification, we performed a subgroup analysis excluding patients who had an old lesion of a different type to the acute stroke classification e.g. a patient presenting with an acute lacunar stroke who had an old cortical lesion. Multivariable regression correcting for age, diabetes and hypertension in these 194 patients did not change the size, direction and significance of the results in Table 5.3.

5.5 Discussion

We have not demonstrated an association between ischaemic stroke subtype and the presence of any retinopathy. In this study the only variable which was associated with retinopathy on multivariable analyses was diabetes.
The strengths of the study are that stroke was diagnosed by an expert at the time of the stroke, all patients had diagnostic MRI at presentation to permit accurate diagnosis and subtyping, I used a pre-specified clinical and imaging based hierarchy to classify stroke subtype and our sample size exceeded our original calculated estimate that would be required to detect a 10% difference between stroke subtypes. We can therefore be reasonably confident that we have not missed an important difference in retinopathy prevalence simply through inadequate study design. Furthermore all patients had retinal photography performed at the time of the stroke and no previous studies have compared retinopathy in ischaemic stroke subtypes.

The limitations of this study are that the cross-sectional design means that we can only report on associations between retinopathy and stroke without implying causality or delineating temporal associations. There were modest imbalances in baseline variables which may not have been completely corrected with multivariable analyses. Although I recruited beyond the number indicated in our sample size calculation which, although based upon the best figures available, may not have been accurate, more patients would be needed to show differences in overall prevalence of less than 10%, or in specific subtypes of retinopathy. Whilst we sought to minimize any misclassification of retinopathy by having an experienced ophthalmologist reviewing all questionable retinopathy lesions it is nonetheless a weakness of this study that I was the sole rater for retinopathy. Ideally there would have been another rater with which to compare results but this was not feasible with the funding available for this study.

Fluorescein angiography may have demonstrated subclinical differences in microvascular perfusion between stroke subtypes, however is more invasive (with a significant risk of anaphylaxis) than colour retinal photography, and therefore would have restricted recruitment, would not have been deemed ethical and therefore not feasible in this study. The study sample size was perhaps limited by exclusion of patients with severe strokes (equivalent to total anterior circulation strokes) and the need for patients to have a definite diagnosis of stroke rather than a possible diagnosis of stroke. However retinal photography would have been difficult in severely unwell patients (the subject needs to sit up, hold their head still and follow
commands) and the atherothromboembolic disease mechanisms present in severe cortical strokes will be represented in the included milder cortical strokes.

We subtyped acute ischaemic stroke into lacunar and cortical stroke using the OCSP classification modified by the results of the brain MRI, and therefore unbiased by using risk factors to classify stroke (eg as in the TOAST classification) (Jackson and Sudlow 2005). This study focused on patients with acute clinically proven strokes, however some patients had old lesions on their MRI scans that may or may not have represented clinical strokes (most appeared not to – we did not ascertain a past history of clinically-evident stroke in 65/73 of patients with old infarcts). We repeated the analysis excluding the few patients who had an old lesion type that differed from the acute ischaemic type and this sensitivity analysis did not change the main results shown in Table 5.3. It is a limitation of this study that we included 20 patients who had an old infarct on brain imaging that may have represented a different ischaemic stroke phenotype to the acute event ie acute lacunar stroke with an old cortical infarct. It is not however clear that these old infarct appearing lesions were in fact strokes (indeed the vast majority were not clearly associated with clinical deficits) and therefore probable that this may not have diluted the results significantly. Ideally we would have recruited “pure” lacunar and cortical phenotypes without old infarcts in brain imaging but this would have severely limited recruitment and hence generalisability. It is also reassuring that the sensitivity analysis performed excluding the patients with a different old stroke phenotype did not change the results significantly.

We demonstrated a prevalence of retinopathy of 19% in patients with mild ischaemic stroke and mean age 67 yrs. This is slightly higher than population-based prevalences of 7.0% (ARIC) (Wong et al. 2001) and 8.3% (CHS) (Wong et al. 2003) probably reflecting the fact that the patients in the present study had higher rates of vascular disease than community-dwelling healthy subjects. The prevalence in the present study was slightly lower than in diabetic patients with ischaemic stroke of 33% (ARIC) (Cheung et al. 2007c), but reassuringly similar to that in patients in CHS who had a history of ischaemic stroke, 19% of whom had retinopathy (Wong et al. 2003).
How does our study relate to previous information on retinopathy and stroke? In the large population-based Atherosclerosis Risk in Communities (ARIC) study, the presence of any retinopathy predicted future ischaemic stroke during 3.5 years follow up with a relative risk (RR) of 2.58 (95% CI 1.59-4.20) corrected for diabetes, hypertension, age and other vascular risk factors (Wong et al. 2001). A similar finding is reported for diabetic subjects with a longer follow up from the same study (Cheung et al. 2007c). Furthermore, baseline retinopathy in the Blue Mountains Eye Study was reported as being significantly associated with future risk of stroke and TIA with a RR of 1.7 (95% CI 1.0-2.8) (Mitchell et al. 2005). Although demonstrating a link between retinopathy and future stroke in general, none of these studies reported or compared associations with different subtypes of ischaemic stroke. The evidence for an association between retinopathy and history of stroke is less compelling (perhaps reflecting the inherent difficulties in obtaining accurate medical histories and differing methods of defining prevalent stroke) – the Cardiovascular Health Study (CHS) reported that retinopathy was associated with a clinical history of stroke (OR 2.0 95% CI 1.1-3.6)(Wong et al. 2003) and MRI defined cerebral infarction of unspecified subtype (OR 1.18 95% CI 1.05-1.34) (Longstreth, Jr. et al. 2007). At the time of initial publication there were no other studies which had investigated retinopathy in ischaemic stroke subtypes.

Our collaborators, The Multi Centre Retinal Stroke Study have since published details of associations between retinal microvascular abnormalities and stroke subtypes (Baker et al. 2010). This paper investigated retinal changes in deep intracranial haemorrhage (n=51) compared with both lacunar ischaemic (n=93) and non-lacunar ischaemic stroke (n=486) and not between ischaemic stroke subtypes. It is possible to calculate from the result that the prevalence of retinopathy in this cohort was 32% - higher than the rate of 19% that we found. This difference may partly be explained by the higher rate of diabetes (22% compared with 15%). Incidentally, the prevalence of retinopathy for lacunar strokes in the MCRS cohort was 35% and for non-lacunar stroke 33%. The authors do not present statistics regarding these prevalences (where multivariable analysis would be required) but there would not appear to be a difference certainly with univariable analysis.
Has retinopathy been associated with other clinical markers of brain disease? A systematic review found that retinopathy was associated with decreased cognition but that this association was tempered by heterogeneity between studies and variable study quality (Ding et al. 2008). Studies with improved quality published after this review have shown that in the ARIC study, the presence of retinopathy predicted subsequent cognitive decline (Lesage et al. 2009). In a cross sectional study from the BMES, retinopathy in subjects without hypertension (n=48) and after multivariable correction for age, sex, diabetes, smoking, education level, systolic blood pressure and history of cardiovascular disease (total 8 variables with 48 outcomes of interest) was associated with the presence of severe cognitive impairment (mini mental state exam <23) with an odds ratio of 1.7 (95% CI 1.0-3.2, p=0.03) (Liew et al. 2009). This is a borderline result in a subgroup analysis that just reaches statistical significance in a regression model that may have been over-fitted with variables and so should therefore be treated with caution.

Has retinopathy been associated with other brain imaging markers of brain disease? In a well designed genetic Icelandic study of 4,176 subjects, retinopathy lesions were not associated with white matter lesion load on MRI scans (Qiu et al. 2009). This lack of an association between retinopathy and white matter hyperintensities on MRI had been suggested in the Cardiovascular Health Study (Longstreth, Jr. et al. 2007) yet findings from the ARIC study suggest that patients with retinopathy have a higher prevalence of white matter lesions (after adjustment for vascular risk factors (Wong et al. 2002a). These conflicting results demonstrate the heterogeneity apparent between studies.

What do these results mean? The aim of the present study was to investigate whether there were higher rates of retinopathy in patients with lacunar stroke as this might reflect blood-retinal barrier breakdown. There is growing evidence that cerebral small vessel disease may be associated with dysfunctional blood-brain barrier (Rosenberg 2009; Topakian et al. 2008; Wardlaw et al. 2009) and retinal imaging offers an excellent non-invasive method of studying blood vessels that are similar to cerebral vessels. That the rates of retinopathy do not differ between acute ischaemic
stroke subtypes may be due to several reasons other than our hypothesis being incorrect.

Firstly the simple presence of retinopathy may be too blunt a tool to measure what are likely to be subtle differences between ischaemic stroke subtypes. In diabetic retinopathy the constituent features of retinopathy appear to have a common precursor in that one of the first stages in the development of diabetic retinopathy is subtle breakdown of the blood-retinal barrier (Qaum et al. 2001) (visible on techniques like fluorescein angiography). It is not, however clear what the classic retinopathy changes represent or the final pathways causing them (McLeod 2005; Schmidt 2008; Stanford 2004). In the present study we have looked at any retinopathy (haemorrhage, exudates and microaneurysms) but perhaps only different retinopathic components are associated with blood-retinal barrier leak to varying degrees whilst others reflect other pathological processes. Certainly cotton wool spots are considered to represent focal areas of ischaemia as a late development of the accumulation of axoplasmic constituents (McLeod 2005). Although we have reported the findings for interest and perhaps to generate hypotheses, this study was not powered to identify differences in individual retinopathy features, only the presence of any retinopathy.

Secondly, although very similar, it may be that retinal vessels do not behave exactly like cerebral vessels and the changes are too subtle to be identified without retinal fluorescein angiography. Microvascular changes in the brain and the retina may not move in parallel – it is possible that in these patients (with a low stroke severity) the retinal changes may have not yet developed and perhaps studying patients with more severe stroke may have revealed subtype-specific retinopathic differences. Alternatively, it may be that retinopathy is a marker of vascular risk and is co-associated with stroke but not stroke subtypes, as lacunar and cortical strokes have similar hypertension and diabetes risk profiles (Jackson and Sudlow 2005).

Future research should concentrate on carefully sub-typing stroke and investigating the use of retinopathy as a marker of risk factor effects on end organs in the individual and how this relates to any future stroke risk.
Chapter 6: Retinal vessel width and morphology in ischaemic stroke subtypes.

6.1 Aims of Chapter

In this chapter we describe detailed morphological differences including retinal arteriolar and venular widths, focal arteriolar narrowing and arteriovenous nicking in retinal vessels in ischaemic stroke subtypes aiming to elucidate the underlying vasculopathy in lacunar stroke.

6.2 Introduction

We have demonstrated in chapter 3 of this thesis and others have subsequently confirmed that retinal microvascular abnormalities are associated with stroke: (Baker et al. 2008) retinal vessel widths predict future risk of stroke (McGeechan et al. 2009a) and may be associated with a previous history of stroke (Cooper et al. 2006; Longstreth, Jr. et al. 2007). Arteriovenous nicking (AVN) and focal arteriolar narrowing (FAN) are associated with infarcts on MR brain scanning (Cooper et al. 2006; Longstreth, Jr. et al. 2007). However, although some studies suggest that there are associations between retinal changes and lacunar stroke, there is little information about whether retinal appearances truly differ in ischemic stroke subtypes. This is because these studies either did not subtype ischemic stroke (Ikram et al. 2006a) or only investigated asymptomatic lacunes seen on MRI scanning which are of uncertain relevance to clinical stroke (Ikram et al. 2006b; Wardlaw 2008). Therefore we studied retinal arteriolar and venular widths, focal arteriolar narrowing (FAN) and arteriovenous nicking (AVN) in patients presenting with acute lacunar and cortical ischemic stroke to test the hypothesis that the retinal small vessels would be morphologically different in patients with lacunar stroke compared to patients with large artery cortical atherothromboembolic stroke and that the nature of these abnormalities may shed light on the pathophysiology underlying lacunar stroke.
6.3 Methods

We prospectively recruited consecutive patients with clinical lacunar or mild cortical stroke seen at our hospital stroke service, aiming to recruit all relevant patients as consecutively as possible as detailed in Chapter 3 of this thesis.

All patients had six field retinal photography (centred on the disc, macula, lateral macula, nasal to the disc, upper arcade and lower arcade) of the left and right eyes, with 1% tropicamide eye drops where necessary, using a Canon CR-DGi digital retinal camera (Canon USA Inc.).

6.3.1 MRI Analysis

All MRI scans were coded for the presence, location and size of the recent infarct and any old infarcts or haemorrhages.

6.3.2 Retinal image analysis for vessel widths

All retinal images were analyzed by a grader (FD) who was blinded to clinical and brain imaging features. Each colour retinal image was stored within OptoMize® (Digital Healthcare, Cambridge, UK) software as Tagged Image File Format files. Left and right eye vessel widths are highly correlated (Wong et al. 2004) and we randomly chose one image centred on the optic disc from one eye from each patient. For measurement of Arterio-Venular Ratios (AVR), Central Retinal Arteriolar Equivalent (CRAE) and Central Retinal Venular Equivalent (CRVE), images were analyzed using a custom written validated image analysis program (within MatLab: The MathWorks, Natick, MA).

Processing was performed on the green channel of the colour fundus images as this typically exhibits the greatest contrast between vessel and background. Fundus images are often non-uniformly illuminated and exhibit local luminosity and contrast variability. This may have an adverse affect on the measurement of vessel diameters.
and so each image was corrected by first estimating background illumination using a median filter with a mask of size greater than the expected maximum diameter of vessels (200 pixels x 200 pixels) and then calculating correction coefficients by dividing the maximum grey-level value in the median filtered image by the grey-level value of each pixel in the filtered image. The green channel was then multiplied by the correction coefficients. Finally, contrast stretching was performed whereby the range of gray levels in the corrected image is stretched to make full use of all possible values, i.e. 0 to 255.

After image processing as described above, the software identified the optic disc and drew a circle delineating the disc. After the disc was identified, the position was checked by the grader. The software drew circles at half a disc diameter and a full disc diameter concentrically out from the centre of the disc (Fig 6.1).

*Figure 6.1. Retinal photograph post processing with circles centred on the disc at half disc diameter distance apart superimposed and Zone B where the six largest arteries and venules were measured.*
If the software was unable to locate the disc then the grader marked the centre of the
disc and the outer border and the software fitted a circle based on these 2 reference
points. The grader then identified the six largest arterioles and venules passing
through the circular area between the circle drawn at ½ a disc diameter from the edge
of the optic disc and the circle drawn at 1 disc diameter from the edge of the optic
disc (Zone B shown on figures 6.1 and 6.2) and on each vessel identified 2 points
between which the software tracked, measuring the vessel profile at set intervals
(either 5 pixels or 10 pixels decided by the operator).

*Figure 6.2 showing vessel tracking of a venule in doughnut shaped zone B*

The software measured the vessel profile with microdensitometry and fitted the
profile to a double Gaussian curve (figure 6.3). Each profile was either accepted or
rejected by the grader as being a good fit for the vessel curve (we accepted a r
correlation co-efficient of >0.7). The vessel width was calculated for each profile as
the width of the intensity profiles at half the height of the intensity profile peak (Newsom et al. 1992).

Figure 6.3 Vessel profile: Plot of pixel intensity along a transect across a vessel with superimposed Gaussian Line. The width is taken as the half-height width measure and represented by the dashed line.

We validated this process with Bland Altman plots comparing software performance to best human measurement (with a calliper on images that had been enlarged so that the vessel measured approx 20-30 mm) and found no evidence of systematic bias and a mean difference between human and software measurements for 50 randomly chosen vessels of only 0.006 pixels (95% CI -3.3 to 3.3 pixels). See figure 6.4.
Figure 6.4. Bland Altman plot of difference between human observer derived width measurements (in pixels) and computer derived widths measurements against width of vessel showing mean difference between the two of 0.0066 pixels and most differences falling between +/- 3.3 pixels. There is no systematic bias evident.

We combined arteriolar widths to produce a Central Retinal Arteriolar Equivalent (CRAE) for each eye using the following formula (Patton et al. 2006a) which adjusts Knudston’s formula (Knudtson et al. 2003) to take into account the fact that the branching co-efficient varies with asymmetry of the widths of the daughter vessels. We combined the largest artery with the 6th largest artery to form a summary width in the following formula where \(W_c = \text{summary width}, W_a = 6\text{th largest arteriole width}, W_b = \text{largest arteriole width}\). The values 0.78 and 0.63 represent the regression equation which relates the asymmetry index to the branching co-efficient (Patton et al. 2006a)

\[
W_c = \sqrt{W_a^2 + W_b^2} \left/ \left(0.78 + 0.63 \times \frac{W_a}{W_b}\right)\right.
\]
We then combined the 2nd largest with the 5th largest and the 3rd largest with the 4th largest. This left us with 3 summary widths - we combined the largest with the smallest and then this summary width with the middle value to leave us with a single summary measure (CRAE) after 5 iterations. We performed a similar analysis using the following formula to obtain a summary measure for the venules (CRVE) – where \( W_c = \text{summary width}, \ W_a = \text{6th largest venule width}, \ W_b = \text{largest venule width} \) and 1.22 is the branching co-efficient (for calculating venular summary widths the branching co-efficient is a constant as not affected by asymmetry of the daughter venules) (Patton et al. 2006a)

\[
W_c = \sqrt{W_a^2 + W_b^2 / 1.22}
\]

We calculated AVR for each eye as the ratio of CRAE divided by CRVE.

To convert the pixel measurements obtained from the image to absolute measurements (microns) we assumed that the average disc diameter was 1850 microns (Hubbard et al. 1999) and using our cohort mean disc diameter of 381 pixels multiplied all of our pixels measurements by 4.855 (1850/381) to convert pixel measurements to microns.

In a randomly chosen sample of 20 retinal images the intraclass correlation co-efficients for intra-rater reliability (with assessments performed 1 month apart) were excellent at 0.94 for CRAE, 0.98 for CRVE and 0.91 for AVR. Using a second grader (Tom MacGillivray) the intraclass correlation co-efficients for inter rater reliability were excellent at 0.95 for CRAE, 0.89 for CRVE and 0.90 for AVR.
6.3.3 Retinal image analysis for focal arteriolar narrowing and arteriovenous nicking

Our coding for focal arteriolar narrowing and arteriovenous nicking was based upon that of the ARIC study (Hubbard et al. 1999). A physician (FD) specifically trained in retinal vessel assessment coded the presence (no/questionable/yes) and severity (mild, moderate, severe) of AVN. We defined AVN as a reduced and tapering width of a venule on either side of an arteriole where the arteriole crossed the venule (ignoring rare instances where venules crossed arterioles). We graded AVN mild if the venule was narrowed up to three quarters of its original diameter. We graded
AVN moderate where the venule was narrowed between three quarters and half of its original diameter. We graded AVN severe where the venule was narrowed to less than half of its original diameter.

For FAN we assessed arterioles estimated to be over 50 microns in diameter (approx one third of the diameter of a major venule at the disc margin). FAN was present if there was a focal segmental narrowing of an artery to at least two thirds of the original width with resumption of the original width distal to the area of focal narrowing. FAN was mild/moderate if the sum total length of focal narrowing in a photograph was less than 2 disc diameters and severe if greater than 2 disc diameters. All questionable lesions were graded by an ophthalmologist (BD) with a specialist interest in retinal disease. This method of assessment has intra rater kappa scores of 0.87 for AVN and 0.80 for FAN (Sherry et al. 2002).

*Figure 6.5. Magnified section of original retinal colour photograph illustrating arteriovenous nicking.*
**6.3.4 Statistical analysis**

I compared baseline characteristics between the lacunar and cortical stroke groups with Students t test, Chi square test for association and Fishers exact test. CRAE, CRVE and AVR were normally distributed (figures 6.7, 6.8) and I compared unadjusted CRAE, CRVE and AVR between groups with Students t-test. I performed multiple linear regression analysis with stroke subtype, age and vascular risk factors as explanatory variables and CRAE, CRVE, AVR as the dependent variable. CRAE and CRVE were co-variates and therefore modelled together. I dichotomized FAN and AVN data into present versus absent and used binary logistic regression to assess differences between stroke subtypes correcting for vascular risk factors. I used odds ratios (OR) with 95% confidence intervals (CI) to examine associations between retinal and other features. All analysis was performed with Minitab (Version 14, Minitab Inc, PA, USA). Sample size calculation based on existing literature on retinal findings in stroke suggested that 197 patients would be needed to detect a difference of prevalence of FAN or AVN of 10% with 80% power at the 0.05 significance level between stroke subtypes.
Figure 6.7. Histogram showing distribution of CRVE measured in pixels by 2 pixel measurements indicating normal distribution.

Figure 6.8. Histogram showing distribution of CRAE measured in pixels by 2 pixel measurements indicating normal distribution.
6.4 Results

We recruited 220 patients. Eight were excluded (6 had poor quality photographs, 2 with missing photographs) leaving 212 for analysis of FAN and AVN and 206 with photographic quality permitting analysis of vessel widths. Of the 212 patients there were 105 lacunar strokes and 107 cortical strokes. The mean age was 68.1 (SD 11.5) years and median NIHSS score 2 (interquartile range 2-3) (table 6.1). The lacunar stroke patients were younger with marginally higher stroke severity and lower rates of atrial fibrillation, symptomatic carotid stenosis >50% NASCET and ischemic heart disease than cortical strokes (table 6.1).

Table 6.1 Baseline characteristics of lacunar and cortical stroke subgroups.

Groups are compared with 2 sample t-test (age), Mann Whitney U test (NIHSS) and differences in proportions (all others). SD = Standard Deviation, NIHSS = National Institute for Health Stroke Scale, TIA = Transient Ischemic Attack

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lacunar Stroke</th>
<th>Cortical Stroke</th>
<th>P value for difference between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>105</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>Age years (SD)</td>
<td>66.2 (11.5)</td>
<td>70.0 (11.3)</td>
<td>0.017</td>
</tr>
<tr>
<td>Male gender n (%)</td>
<td>62 (59%)</td>
<td>73 (68%)</td>
<td>0.163</td>
</tr>
<tr>
<td>Median NIHSS</td>
<td>3</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atrial Fibrillation n (%)</td>
<td>4 (4%)</td>
<td>14 (13%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Symptomatic Carotid Stenosis &gt;50% n (%)</td>
<td>4 (4%)</td>
<td>13 (12%)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Past Medical History of:

<table>
<thead>
<tr>
<th></th>
<th>Lacunar Stroke</th>
<th>Cortical Stroke</th>
<th>P value for difference between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes n (%)</td>
<td>19 (18%)</td>
<td>12 (11%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Ischemic heart disease n (%)</td>
<td>14 (13%)</td>
<td>31 (29%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Peripheral vascular disease n (%)</td>
<td>5 (5%)</td>
<td>5 (5%)</td>
<td>0.97</td>
</tr>
<tr>
<td>Hypertension n (%)</td>
<td>59 (56%)</td>
<td>70 (65%)</td>
<td>0.17</td>
</tr>
<tr>
<td>TIA n (%)</td>
<td>17 (16%)</td>
<td>11 (10%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Stroke n (%)</td>
<td>8 (8%)</td>
<td>12 (11%)</td>
<td>0.52</td>
</tr>
</tbody>
</table>
6.4.1 Vessel widths

206 patients contributed to this analysis. For the total study population the mean CRAE was 33.47 pixels (162µ) mean CRVE was 43.87 pixels (212µ) and mean AVR 0.77. CRAE and CRVE were correlated with a Pearson’s correlation coefficient of 0.66. Patients with lacunar stroke had higher CRVE (44.9 SD 5.5 pixels v 42.9 SD 6.4 pixels, p=0.01), lower AVR (0.76 SD 0.1 pixels v 0.78 SD 0.01 pixels, p=0.03) and similar CRAE to patients with cortical stroke (33.2 SD 4.4 pixels v 33.7 SD 4.3 pixels, p=0.4). Table 6.2 shows the adjusted associations with arteriolar and venular widths. Increased CRVE was significantly and independently associated with lacunar stroke subtype, lower age and increased CRAE (after correcting for the presence of diabetes, hypertension and gender). Increased CRAE was significantly and independently associated with female gender and increased CRVE (correcting for age, diabetes, hypertension and stroke subtype). Decreased AVR was significantly and independently associated with lacunar stroke subtype and younger age (correcting for diabetes, hypertension and gender).

I performed a sensitivity analysis excluding patients with a prior history of stroke and found that in 186 patients the results were similar to those presented in table 6.2 although the association between increased venular width and lacunar stroke subtype was strengthened (data not shown).
Table 6.2. Associations between vessel widths and key patient variables on multivariable linear regression showing beta co-efficient and p value for CRAE, CRVE and AVR. All analyses are corrected for the presence of the other variables in the table. CRAE = Central retinal arteriolar equivalent, CRVE = Central Retinal Venular Equivalent, AVR = arteriovenous ratio. Beta = beta coefficient

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>CRAE</th>
<th>CRVE</th>
<th>AVR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta</td>
<td>p value</td>
<td>Beta</td>
</tr>
<tr>
<td>Age</td>
<td>0.03</td>
<td>p=0.12</td>
<td>-0.11</td>
</tr>
<tr>
<td>Male Gender</td>
<td>-1.1</td>
<td>p=0.02*</td>
<td>-0.10</td>
</tr>
<tr>
<td>Lacunar stroke subtype</td>
<td>-0.48</td>
<td>p=0.29</td>
<td>1.33</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.02</td>
<td>p=0.97</td>
<td>-0.29</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.95</td>
<td>p=0.05</td>
<td>0.94</td>
</tr>
<tr>
<td>CRAE</td>
<td>n/a</td>
<td></td>
<td>0.88</td>
</tr>
<tr>
<td>CRVE</td>
<td>0.48</td>
<td>p&lt;0.001*</td>
<td>n/a</td>
</tr>
</tbody>
</table>

*significant p<0.05

6.4.2 Arteriovenous nicking and focal arteriolar narrowing

212 patients contributed to this analysis. AVN was present in 83/107 (78%) patients with cortical stroke and 85/105 (81%) patients with lacunar stroke (difference = 3%, 95% CI -7% to 14% p=0.53). FAN was present in 28/107 (26%) patients with cortical stroke and 37/105 (35%) of patients with lacunar stroke (difference = 9%, 95% CI -3% to 21%, p=0.15), distribution of FAN and AVN by stroke subtype are given in table 6.3. I performed binary logistic regression to adjust for the baseline risk factor and age imbalances between the cortical and lacunar stroke groups with FAN or AVN as the dependent variable (dichotomized to present or absent). Only hypertension independently predicted the presence of AVN; no risk factors were independently associated with FAN (Table 6.4). Excluding patients with a history of prior stroke did not alter these results.
Table 6.3  Numbers of patients with morphological abnormalities by stroke subtype. None of these differences reach statistical significance. FAN = focal arteriolar narrowing, AVN = arteriovenous nicking.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lacunar Stroke (n=105)</th>
<th>Cortical Stroke (n=107)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>20 (19%)</td>
<td>24 (22%)</td>
</tr>
<tr>
<td>Mild</td>
<td>31 (30%)</td>
<td>31 (29%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>29 (28%)</td>
<td>30 (28%)</td>
</tr>
<tr>
<td>Severe</td>
<td>25 (24%)</td>
<td>22 (21%)</td>
</tr>
<tr>
<td>FAN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>68 (65%)</td>
<td>79 (74%)</td>
</tr>
<tr>
<td>Mild</td>
<td>31 (30%)</td>
<td>22 (21%)</td>
</tr>
<tr>
<td>Severe</td>
<td>6 (6%)</td>
<td>6 (6%)</td>
</tr>
</tbody>
</table>

Table 6.4  Multivariable binary logistic regression for associations with presence of FAN and AVN. FAN = focal arteriolar narrowing, AVN = arteriovenous nicking, OR = Odds ratio, CI = Confidence interval

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR predicting presence of AVN (95% CI ) and p value</th>
<th>OR predicting presence of FAN (95% CI ) and p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>0.98 (0.95-1.01) p=0.16</td>
<td>0.99 (0.96-1.02) p=0.52</td>
</tr>
<tr>
<td>Male Gender</td>
<td>0.89 (0.43-1.82) p=0.75</td>
<td>1.13 (0.61-2.10) p=0.70</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.29 (0.45-3.68) p=0.63</td>
<td>0.83 (0.35-1.96) p=0.67</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.05 (1.00-4.19) p=0.049*</td>
<td>1.18 (0.62-2.25) p=0.61</td>
</tr>
<tr>
<td>Lacunar Stroke</td>
<td>1.18 (0.59-2.37) p=0.63</td>
<td>1.55 (0.84-2.84) p=0.16</td>
</tr>
</tbody>
</table>

*significant p<0.05
6.5 Discussion

Retinal microvessel morphology differs between lacunar and cortical ischemic stroke subtypes. Increased retinal venular diameters and decreased AVR are both independently associated with lacunar rather than cortical stroke subtype, supporting the concept of a distinct small vessel vasculopathy in cerebral small vessel disease. We have not demonstrated a strong association between retinal arteriolar diameter, focal arteriolar narrowing or arteriovenous nicking and lacunar stroke subtype. The cross-sectional design means that we can only report on associations so we do not know whether these retinal vessel differences are longstanding and predispose to the lacunar phenotype, or are acquired in later life as part of the lacunar disease. Nor can we tell whether they are causative or associative. The parity of arteriolar parameters (width, AVN and FAN) between stroke subtypes coupled with associations between hypertension and AVN and decreased arteriolar width suggest that arteriolar parameters reflect exposure to vascular risk factors. In response to these risk factors some patients may develop lacunar and others a large artery (cortical) stroke phenotype.

No previous studies have compared quantitative vessel width measurement, AVN or FAN between ischemic stroke subtypes. Previous studies did not subtype stroke and compared retinal arteriolar parameters between subjects with stroke and those without. Those that combined arteriolar and venular diameters into the dimensionless AVR produced conflicting results about associations with both future stroke and history of stroke (Ikram et al. 2006a; Klein et al. 2000; Mitchell et al. 2005; Wong et al. 2006). Changes in AVR were previously thought to reflect only arteriolar narrowing (Hubbard et al. 1999), known to change in response to systemic disease, but it is now known that venular diameter also varies (Ikram et al. 2004). Those studies which analyzed arteriolar and venular widths separately found that the increase in AVR predicting the presence of any stroke (of any subtype) compared with absence of stroke was due to venular widening rather than arteriolar narrowing (Ikram et al. 2006a; Ikram et al. 2006b; Wong et al. 2006).
There are also conflicting reports regarding the associations between AVN and FAN and any stroke (Cooper et al. 2006; Klein et al. 2000; Longstreth, Jr. et al. 2007; Mitchell et al. 2005; Wong et al. 2003). This heterogeneity may reflect inherent methodological difficulties in identifying stroke, i.e. studies which showed an association with stroke tended to use MRI-based definitions of cerebral infarct rather than clinical definitions. However it is uncertain what “holes” in the brain represent (Wardlaw 2008), and case record assessments are of limited accuracy for diagnosing stroke (Piriyawat et al. 2002). We were not able to find any difference in retinopathy between lacunar and large artery stroke as reported in the previous chapter of this thesis despite many previous reports of associations between retinopathy and stroke. The lack of differences in arteriolar changes between stroke subtypes in our study could be because the retinal arteriolar circulation does not accurately reflect cerebrovascular disease, because this studies may not be powered to identify these subtle, fractions of a micron (for CRAE) and subjective (for AVN/FAN), differences. Alternatively retinal features may simply reflect exposure to systemic risk factors and not be specific for small versus large artery disease.

The finding that increased venular diameter and decreased AVR are associated with lacunar compared with cortical ischemic stroke subtype is novel, and may provide useful insights into the microvascular mechanisms of lacunar stroke. Two population-based studies showed that increased venular diameter predicted future risk of any clinical stroke (Ikram et al. 2006a; Wong et al. 2006). One study found larger CRVE in subjects with lacunes seen on MRI (Ikram et al. 2006b), but again silent lacunes are of uncertain clinical relevance (Wardlaw 2008).

Why should venular diameters and AVR differ in lacunar stroke? Increased venular width is associated with plasma markers of inflammation ((De Jong et al. 2007; Klein et al. 2006; Wong et al. 2006), decreased arteriolar oxygen saturation levels (De Jong et al. 2008) and recently with decreased endothelial function measured with forearm plethysmography and decreased flow mediated dilatation in the brachial artery (Nguyen et al. 2010). Plasma markers of endothelial activation and inflammation are elevated in patients with cerebral small vessel disease compared to
age matched controls but raised inflammatory markers might simply reflect any vascular disease (Hassan et al. 2003b). Thus, increased venular diameter could reflect an inflammatory component in patients with lacunar stroke, but clarification of this would require direct comparisons of plasma inflammatory markers between ischemic stroke subtypes.

The strengths of the present study are the subtyping of ischemic stroke and the use of cortical stroke patients to control for potential confounding risk factors and that stroke was diagnosed by an expert with MRI at presentation. We maintained careful blinding of brain and retinal images to each other and to clinical features throughout. We used detailed retinal photos, careful training of retinal graders and validated assessment methods. CRAE and CRVE are correlated, therefore models assessing multivariable associations with either need to correct for the other (Liew et al. 2007a), something which previous studies of retinal arteriolar diameter have not done (Ikram et al. 2006a; Ikram et al. 2004; Ikram et al. 2006b; Wong et al. 2006). In order to minimize confounding of stroke subtyping we performed a sensitivity analysis excluding patients with a history of previous stroke which did not change the results for the total study population. A larger sample size might be needed to show differences in arteriolar diameters, FAN and AVN. We have also used a concrete definition of small vessel disease, ie clinically evident lacunar stroke with best available imaging backup rather than a purely imaging or clinical based definition or one with uncertain relevance even to stroke.

Since the first publication of the results detailed in this chapter (April 2009), our Australian collaborators from the Multicentre Retinal Stroke Study (MCRS) have published the main findings from their large cohort (n=1321) (Lindley et al. 2009). The detailed comparisons between our findings and those of the MCRS study are presented in chapter 10 of this thesis and are not straight forward as although the overall sample size of the MRC study was large there were a number of different stroke subtyping classifications and these subgroup analyses were further stratified by the presence of diabetes. In general, the MCRS found that lacunar stroke was associated with widened venules and narrower arterioles although the strength of the
latter association was weaker than that for venules and the absolute differences were small. Furthermore these associations were not present consistently across the different subtyping methods (and the association with wider venules only present in those patients without diabetes).

The MCRS also reported in some of the subgroup analyses that lacunar stroke was associated with an increased prevalence of focal arteriolar narrowing and arteriovenous nicking although again the odds ratios were not large. These results are consistent with the results presented in this thesis and it is interesting to note that arterioles in lacunar stroke patients were 2 microns smaller than those in non-lacunar stroke patients. We found a similar 2 micron difference but perhaps our study was underpowered to show statistical significance with such a small absolute difference.
Chapter Seven: Retinal vascular geometry in ischaemic stroke subtypes.

7.1 Aims of chapter

In this chapter we assess retinal vascular geometry in ischaemic stroke subtypes studying the hypothesis that patients with lacunar stroke will have altered retinal vascular geometry reflecting small vessel disease compared with patients with cortical stroke.

7.2 Introduction

The exact aetiology of lacunar stroke remains uncertain but possible causes include microatheroma, microemboli, vasospasm or a diffuse arteriopathy possibly related to endothelial dysfunction and altered blood brain barrier permeability (Wardlaw et al. 2003). Lacunar strokes are associated with another manifestation of cerebral small vessel disease, white matter hyperintensities on MRI scans (WMH) (Wiszniewska et al. 2000) which are in turn associated with ageing (Gunning-Dixon and Raz 2000), cognitive impairment and dementia (Sitoh et al. 2004). The exact aetiology of these white matter hyperintensities however remains unknown.

We have demonstrated in chapter 3 of this thesis that retinal vascular abnormalities are associated with stroke but they also predict white matter disease presence and progression (Ikram et al. 2006a; Ikram et al. 2006b; Wong et al. 2002a); and we have previously shown in chapter 6 of this thesis that retinal vessel widths differ between stroke subtypes. Therefore retinal vessel abnormalities may act as markers for cerebral small vessel disease.

In order to fulfil its role of nutrient delivery the retinal arterial tree (a complex and highly developed structure) needs to efficiently deliver blood to tissues with minimal
power losses at bifurcations yet ensuring that blood velocity slows by the time the capillaries are reached to allow delivery of nutrients yet also minimizing total blood volume. The geometry of arterioles affects the efficiency of the arteriolar circulation in achieving these aims (Murray 1926). There are several different measures of retinal vascular geometry. In this chapter we focus on arteriolar branching co-efficients and branching angles.

The branching coefficient of an arteriolar bifurcation measures the change in cross sectional area across a bifurcation i.e. the ratio of the cross sectional area of the parent arteriole and the cross sectional areas of the two summed daughter vessels. In most arteriolar trees, with every bifurcation the total cross sectional area of the vascular tree increases slightly. Taken to extremes, in humans the cross sectional area of the aorta is much smaller than the total summed cross sectional area of the small arterioles. An increased branching co-efficient across a bifurcation represents wider daughter vessels in relation to the parent vessel and a decreased branching coefficient indicates narrower daughters in relation to the parent vessel.

Both increased and decreased branching co-efficients may affect the energy required to deliver blood around the body and hence the efficiency of the circulatory system. A few studies suggest that this theory may hold true in biology; abnormalities in arteriolar branching co-efficient have been associated with cognitive impairment (Patton et al. 2007), peripheral vascular disease (Chapman et al. 2002) and predict death from ischaemic heart disease (IHD) (Witt et al. 2006) although the mechanisms behind these observed changes are not clear and methods used to measure branching co-efficients differ.
Figure 7.1. Examples of inefficient branching co-efficients where blood flow is compromised. With a decreased branching co-efficient (image on the left), blood flow is reduced across the junction resulting in poor blood delivery. With an increased branching co-efficient (image on the right), blood delivery is maximised but in order to maintain such junctions across the arteriolar tree in the human, the blood volume needed would be huge and clearly impractical. Furthermore the design of this junction would not slow down blood flow and one of the functions of the vascular tree is to slow down blood flow over each junction to ensure transfer of nutrients at the capillary level.

Retinal arteriolar branching angles represent the angle subtended by the two daughter vessels. A change in absolute angles or deviation away from a theoretical optimum branching angle may affect the efficiency of the arteriolar tree by altering blood flow across the junction and by altering the ability of the vascular network to distribute blood. A few studies have shown associations between retinal arteriolar branching angles and hypertension (Stanton et al. 1995) and cognitive function (Patton et al. 2007) but not death from stroke (Witt et al. 2006).
We hypothesised that if cerebral small vessel disease is due to an intrinsic small vessel abnormality that might impair the efficiency of nutrient delivery, then patients with cerebral small vessel disease (defined as either lacunar stroke or white matter hyperintensities on MRI) would have altered retinal arteriolar branching co-efficients.
and branching angles compared with patients with large artery atheromatous stroke or patients with fewer or no white matter hyperintensities.

### 7.3 Methods

We recruited patients with lacunar or mild cortical stroke from our university hospital based stroke service as detailed in chapter 2 of this thesis. Patients had diagnostic brain MRI at presentation and six field retinal photography (centred on the disc, macula, lateral macula, nasal to the disc, upper arcade and lower arcade) of the left and right eyes, with 1% tropicamide eye drops where necessary, using a Canon CR-DGi digital retinal camera (Canon USA Inc.).

#### 7.3.1 MRI analysis

MRI scans were coded for the presence, location and size of the recent infarct and any old infarcts or haemorrhages by a neuroradiologist blind to additional clinical details. MRI scans were also coded for deep (lesions not contiguous with the ventricles) and periventricular (lesions contiguous with the ventricles) WMH with the Fazekas scale which rates lesions in both regions from 0-3 (Fazekas et al. 1987). Those whose ages were outside the template range were matched to the nearest age.
Figure 7.3. T2 weighted MRI showing deep (solid arrow) and periventricular (dotted arrow) white matter hyperintensities.

7.3.2 Retinal assessment

For each patient we selected photographs centred on the optic disc for each eye. Images were analysed within custom written Matlab software (The Mathworks Inc, Natick, NA) blind to all clinical and imaging details. Colour images were converted to greyscale and processed to maximize the contrast between vessel and background.

7.3.3 Branching co-efficient assessment

A single trained grader identified the five most proximal measurable arteriolar junctions to the optic disc and used semi-automated computer software to measure the branching co-efficient of each bifurcation. The grader identified the centre point of each bifurcation and placed a cursor on the parent vessel and each daughter vessel. The software then tracked down each vessel from the centre point of each bifurcation (Figure 7.5) fitting a profile of signal intensity at right angles to the longitudinal axis.
of the vessel with a Gaussian curve to determine the width of each vessel (See figure 7.6).

**Figure 7.5. Measurements across a bifurcation. The software tracks down each branch from a central point to obtain width measurements from which to calculate BC. Please note in this image we have used a venule for illustration purposes.**

![Figure 7.5](image1.png)

**Figure 7.6. Vessel profile: Plot of pixel intensity along a transect across a vessel with superimposed Gaussian Line. The width is taken as the half-height width measure and represented by the dashed line.**

![Figure 7.6](image2.png)
Each profile was manually inspected and rejected if the Gaussian line did not fit well (r correlation <0.7). The mean of at least 3 profiles were used to calculate the width of the vessel which was accepted by the grader if the standard deviation of the mean was less that 10% of the vessel width.

We validated this process with Bland Altman plots comparing software performance to best human measurement (with a calliper on enlarged images) and found no evidence of systematic bias and that the mean difference between human and software measurements for 50 randomly chosen vessels was 0.006 pixels (95% CI -3.3 to 3.3 pixels) see figure 6.4 in preceding chapter for further details.

The widths of the parent and daughter vessels were combined to produce the branching co-efficient with the following formula illustrated in Figure 7.3: (Patton et al. 2007)
Figure 7.4. Illustration of branching co-efficient calculation where $BC = \text{branching coefficient}$, $D_1 = \text{width of daughter vessel 1}$, $D_2 = \text{width of daughter vessel 2}$ and $D_0 = \text{width of parent branch}$

$$BC = \frac{D_1^2 + D_2^2}{D_0^2}$$

We aimed to measure the branching co-efficients of the 5 most proximal measurable bifurcations to the optic disk but included patients in whom 3 or more were measurable in our primary analysis. In a prespecified sensitivity analysis, we also analysed patients who had at least 5 branching co-efficient measurements. We avoided assuming that branching co-efficients within each eye were normally distributed by taking the median branching co-efficient for each eye.

The within patient correlation between left and right eyes showed a Pearson’s correlation co-efficient 0.53 for 32 randomly chosen patients and we randomly chose the left or the right eye from a list or randomly generated numbers eye to measure where photographic quality was suitable for both eyes. If one eye had an unsuitable photographic quality we used the other eye. The intra-rater intra class
The correlation co-efficient for a random sample of 10 images with analyses performed 2 weeks apart was 0.82.

Our primary aim was to investigate absolute branching co-efficients but we also assessed deviation from a theoretically calculated optimum branching coefficient of 1.26 (Murray 1926; Patton et al. 2007). The optimum branching co-efficient of 1.26 assumes that the daughter vessels are of equal width but when the daughter vessels have asymmetrical widths the optimum varies between 1.00 and 1.26, according to the asymmetry index which is the ratio of the smaller daughter diameter over the larger daughter diameter (Zamir 1978). We used the corrected (for asymmetry) optimum bifurcation specific branching co-efficient to calculate the deviation for each angle. We took the absolute deviation for each eye and then normalized the data with a square root transformation to permit linear regression.

7.3.4 Arteriolar branching angles

A single trained grader (Rosemarie den Haan) identified the 5 most proximal measurable junctions to the optic disc, selected the centre point of the bifurcation and the parent and daughter vessels. The software tracked down each vessel to a point two parent vessel widths from the bifurcation (where it is suggested from theoretical modelling that turbulent flow after the bifurcation becomes laminar (Tadjfar 2004), and drew a line reflecting the course of the vessel. The branching angle was calculated using the Cosine rule (figure 7.7). Due to the large variation in angles within each eye, we only included patients in the analysis in whom we were able to perform 5 angle measurements to provide a more reliable average than 3 angle measurements. As we could not assume normal distribution of angles within each eye we took the median of the five angles from each eye.
The correlation between angles in the left and right eye was poor (Pearson correlation co-efficient 0.23 for 27 randomly chosen patients) and we therefore measured angles in both eyes where possible to give a median value for each eye. We then took the mean of these two values to give an angle measurement for each patient.

An alternative approach to assessing vascular branching efficiency is to measure deviation from the optimum branching angle (theoretically calculated as 75 degrees) (Murray 1926; Patton et al. 2007) for each bifurcation and we assessed the median deviation from the optimum for each eye. In a random sample of 10 photographs graded 2 weeks apart the intra-rater class correlation coefficient for median angle was excellent at 0.961.
7.3.5 Statistical analysis

All analyses were performed within Minitab (version 14, Minitab Inc, PA, USA). We compared baseline characteristics between the two stroke groups with t-tests, Mann-Whitney U tests and differences in proportions. The branching coefficients and branching angles were normally distributed, as were the deviations from the optimum branching coefficient and angles (after square root transformation) and we therefore performed multivariable linear regression with branching co-efficient and branching angles as the continuous outcomes and vascular risk factors, stroke subtype and WMH as the independent explanatory variables. We set an alpha level for significance of 0.05.

7.4 Results

We recruited 205 patients with mean age 68.0 years (SD11.6). There were 104 lacunar strokes (51%) and 101 cortical strokes (49%) and 135 patients were male (66%). We could not measure at least 3 branching co-efficients in 24 patients (due to poor quality of the photograph, a paucity of bifurcations in the field of view, or local anatomical variations precluding computer measurements of vessel widths). Therefore 181 patients were included in the analysis of branching co-efficients. We were not able to measure 5 branching angles in at least one eye in 61 patients and therefore included 144 patients in the analysis of branching angles. The 24 patients excluded from the branching co-efficient analysis were older (74.5 SD 8.52 years v 67.1 SD 11.7 years) and the 61 patients excluded from the branching angle analysis more often had hypertension (75% v 56%) but did not differ in other respects from the patients in whom these measurements were possible. The baseline characteristics of the 181 patients with at least 3 branching co-efficients are shown in table 7.1.
Table 7.1. Baseline characteristics of 181 patients included with at least 3 measurable branching co-efficients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lacunar stroke</th>
<th>Cortical stroke</th>
<th>P value for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>94</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Mean age (SD) years</td>
<td>65.2 (11.5)</td>
<td>69.2 (11.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Male n (%)</td>
<td>53 (56%)</td>
<td>62 (71%)</td>
<td>0.04</td>
</tr>
<tr>
<td>AF n (%)</td>
<td>4 (4%)</td>
<td>11 (13%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Carotid stenosis&gt;50% n (%)</td>
<td>4 (5%)</td>
<td>10 (12%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Median Deep WMH Fazekas score (IQR)</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
<td>0.97</td>
</tr>
<tr>
<td>Median periventricular WMH Fazekas score (IQR)</td>
<td>1 (1-2)</td>
<td>1 (1-1)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Past medical history of

| Hypertension n (%)                          | 57 (66%)       | 53 (56%)        | 0.21                   |
| Diabetes n (%)                              | 18 (19%)       | 10 (11%)        | 0.15                   |
| Ischaemic Heart Disease n (%)               | 13 (14%)       | 23 (26%)        | 0.03                   |
| Peripheral Vasc. Disease n (%)              | 3 (3%)         | 1 (1%)          | 0.62                   |
| Previous Stroke/TIA n (%)                  | 20 (21%)       | 17 (20%)        | 0.77                   |

AF = atrial fibrillation, WMH = white matter hyperintensity, TIA = Transient Ischaemic Attack, IQR = inter quartile range SD = standard deviation.

7.4.1 Arteriolar branching co-efficients

Of the 181 patients in the branching co-efficient analysis, the mean branching coefficient was 1.44 with a standard deviation of 0.19. There was no difference in mean branching co-efficients between lacunar (1.43, SD 0.17) and cortical stroke (1.44, SD 0.20). On multivariable linear regression (Table 7.2), the presence of IHD and increased periventricular white matter hyperintensities were both significantly and independently associated with an increased branching co-efficients (representing
wider daughter vessels in relation to the parent vessel) and increased deep white matter hyperintensities was significantly and independently associated with a decreased branching co-efficients (representing narrower daughter vessel diameters in relation to the parent vessel). In our pre-specified analysis of patients with 5 branching co-efficients measured in an eye (n=119) the relationships between branching co-efficient and IHD and deep white matter hyperintensities remained but the association between periventricular white matter hyperintensities and branching co-efficient was attenuated and became non-significant (data not shown). When we looked at deviation from the optimum branching co-efficient we found that the results did not change from those in Table 7.2.

Table 7.2. Multivariable adjusted associations with absolute retinal arteriolar branching co-efficient. All values are corrected for the presence of all of the other variables in the table. TIA = transient ischaemic attack, WMH = white matter hyperintensity,

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta co-efficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacunar stroke subtype</td>
<td>-0.001</td>
<td>0.96</td>
</tr>
<tr>
<td>Age</td>
<td>-0.001</td>
<td>0.70</td>
</tr>
<tr>
<td>Deep WMH</td>
<td>-0.076</td>
<td>0.003</td>
</tr>
<tr>
<td>Periventricular WMH</td>
<td>0.072</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Past history of:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.020</td>
<td>0.50</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-0.032</td>
<td>0.38</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>0.155</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td>0.040</td>
<td>0.25</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>0.032</td>
<td>0.73</td>
</tr>
</tbody>
</table>

7.4.2 Arteriolar branching angles

In the 144 patients with 5 angles measured per eye, we found that the mean branching angle was 84.1° with a standard deviation of 7.1°. Median arteriolar
branching angles did not significantly differ between lacunar (mean 85.2° SD 7.3°) and cortical stroke (mean 83.0° SD 7.3°, difference = 2.3 95% CI 0.0 to 4.6 p=0.054). On univariable and also on multivariable analysis, only a history of PVD was associated with increased branching angle (Table 7.3), but note there were very few patients with PVD on which to base this analysis (n=4). Retinal branching angles were not associated with either deep or periventricular white matter hyperintensities. We assessed deviation from an optimum branching angle of 75° but the associations shown in Table 7.3 did not change.

Table 7.3. Multivariable adjusted associations with absolute retinal arteriolar branching angles. All values are corrected for the presence of all of the other variables in the table. TIA = transient ischaemic attack, WMH = white matter hyperintensity.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta co-efficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacunar stroke subtype</td>
<td>2.22</td>
<td>0.07</td>
</tr>
<tr>
<td>Age</td>
<td>-0.03</td>
<td>0.61</td>
</tr>
<tr>
<td>Deep WMH score</td>
<td>1.12</td>
<td>0.34</td>
</tr>
<tr>
<td>Periventricular WMH score</td>
<td>-0.52</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>Past history of:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.27</td>
<td>0.33</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.95</td>
<td>0.57</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>0.51</td>
<td>0.75</td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td>-0.33</td>
<td>0.83</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>9.05</td>
<td>0.006</td>
</tr>
</tbody>
</table>
7.5 Discussion

We have shown that increased retinal arteriolar branching co-efficients are associated with increased periventricular white matter hyperintensities and a history of ischaemic heart disease in patients presenting with mild stroke. Decreased retinal arteriolar branching co-efficient are associated with increased deep white matter hyperintensities but branching co-efficients are not associated with ischaemic stroke subtype. We have not demonstrated significant associations between retinal arteriolar branching angles and ischaemic stroke subtype, white matter hyperintensities or most other vascular risk factors. No previous studies have assessed retinal vascular geometry within ischaemic stroke subtypes or their relationship with white matter hyperintensities.

It is intriguing that deep and periventricular white matter hyperintensities appear to be associated with opposing directions of altered branching co-efficient. A decreased branching co-efficient indicates that the daughter vessels are narrower with respect to the parent vessel and an increased branching co-efficient that the daughters are wider. Pathological studies have indicated that the mechanism of fluid accumulation and subsequent tissue damage in deep and periventricular white matter hyperintensities may differ (Fernando et al. 2006) as deep lesions may have more “ischaemic” causes whilst periventricular changes may be secondary to disruption of the ependymal lining of the ventricles (Fazekas et al. 1998). Deep and periventricular white matter hyperintensities may have slightly different associations with vascular risk factors (de Leeuw et al. 2000). Decreased total cerebral blood flow is associated with the development and worsening of periventricular but not deep white matter hyperintensities (ten Dam et al. 2007). Lower B12 levels in lacunar stroke patients are associated with increased periventricular white matter hyperintensities but not deep white matter hyperintensities (Pieters et al. 2009), a finding replicated independently in a separate cohort (de Lau et al. 2009). Although not definitive, these results suggest that the two types of white matter hyperintensities may have differing causes and we would suggest that deep and
periventricular hyperintensities should, at least for the present, be considered separately in the assessment of white matter disease (Sachdev and Wen 2005).

Nitric oxide levels are associated with altered branching co-efficients (Chapman et al. 2000). Endothelial dysfunction may have an aetiological role in the development of white matter hyperintensities (Markus 2008) so it is possible that disordered nitric oxide production may cause both branching co-efficient changes and white matter disease. Clearly more work is needed to determine whether these observations are true and if so to clarify their association.

We did not find an association between lacunar stroke subtype and retinal arterial branching co-efficient or branching angles. There were limited data on which to base sample size calculations and this study may be underpowered to identify differences between stroke subtypes, particularly when features such as white matter hyperintensities were common in both cortical and lacunar stroke subtypes and may have interacted in the analysis of lacunar versus cortical stroke.

The association between IHD and increased daughter vessel width with respect to the parent vessel is interesting and validates a previous study showing that increased branching co-efficients in a cohort of middle aged adults without vascular disease at baseline predicted death with ischaemic heart disease but not stroke, although the number of stroke deaths was small, n=28 (Witt et al. 2006). The exact mechanisms behind this observation are unclear. It is probably not simply attributable to medication as both our patient groups were taking similar medications and medication is not known to affect certainly retinal vessel widths (Wong et al. 2005b) although the associations with branching co-efficient have not been studied. The true pathophysiological significance of branching coefficients is not known, nor whether these are fixed from birth, alter with age, predispose to or change in the presence of disease. It is therefore difficult to speculate on whether increased branching coefficients might predispose to or be a response to large artery disease. Further studies are needed to examine this interesting finding.
We found no significant associations with branching angles (the apparent association with PVD is based on too few patients so is unlikely to be clinically significant), consistent with some previous studies finding no link between angles and hypertension (Chapman et al. 1997), peripheral vascular disease (Chapman et al. 2002) and death with IHD and stroke (Witt et al. 2006).

The concept of measuring deviation from optimality deserves further comment. In our cohort, the observed mean of 1.43 (SD 0.19) was higher than the optimum theoretically derived branching co-efficient of 1.26. As the majority of our patients had positive deviations from the theoretical optimum, our results did not alter when we assessed deviation from the optimum branching co-efficient rather than absolute values, consistent with other studies (Witt et al. 2006). In study populations where the mean branching co-efficient (or branching angle) is nearer to the theoretical optimum, assessing deviation rather than absolute angles leads to more diverse results (Patton et al. 2007). It is appealing to use the deviation away from the optimum but not all theories attempting to explain biological systems hold true in vivo. Data are too sparse to know whether optimality of branching coefficients and angles differ between arteriolar beds or patient populations and until more is known further studies should assess both absolute and deviation from optimum values.

The strengths of this study include prospective recruitment and careful assessment of all patients at the time of the stroke by an experienced physician with diagnostic MRI scans graded by an experienced neuroradiologist. Assessment of retinal images was blind to clinical and imaging details. We used a specifically written semi-automated software program to assess retinal vessels which minimized human operator variability resulting in excellent intra-rater repeatability scores. We found that angles did not correlate well between left and right eyes so measured both eyes where possible. This poor correlation between left and right eye further questions whether angles have anything to do with systemic disease. Previous studies either did not specify which eye was measured (Patton et al. 2007), looked at one eye only or combined eyes to achieve 5 measurements (Witt et al. 2006). This latter approach assumes that angles do not differ between the eyes. There is no published data on the
strength of the association between left and right eyes. That we did not find a strong association between left and right eyes suggests that retinal arteriolar branching angles may not predispose to or change in response to systemic disease. We would suggest that future studies investigating retinal branching angles stipulate which eye they used and presented data regarding left-right correlation.

Weaknesses should also be acknowledged. Although use of the semi-automated software improved measurement accuracy and repeatability, it limited the number of patients that we were able to include because, unlike a human operator, the semi-automated software is not able to make allowances for anatomically difficult vessels ie those with indistinct edges or where a venule is in close proximity to an arteriole. The sample size may not have been large enough to account for interactions between stroke subtype, WML and other key variables like age. We designed our study using all available data to date on retinal changes and stroke (Doubal et al. 2009). However despite including over 60,000 patients, this literature is limited by lack of precision in stroke diagnosis and subtyping and included few strokes. The theoretical optimum angles and coefficients are just that – theories based on modelling of the circulation as a series of tubes. It is a further limitation that due to a lack of resources we were unable to perform inter-rater reliability testing for retinal arteriolar branching co-efficients and angles which may limit the usefulness of this metric.

Although the concept of using retinal vascular geometrical parameters to reflect cerebral disease is appealing, these techniques are in their infancy and more research in different vascular beds, in response to pharmacological challenges, at different ages, and in the presence of different diseases is required before the real implications of vascular geometry become clear.
Chapter Eight. Fractal analysis of retinal vessels in ischaemic stroke subtypes

8.1 Aims of chapter

In this chapter we describe the how fractal analysis can be applied to the retinal vascular tree and how the fractal properties of the retinal vasculature differ in ischaemic stroke subtypes.

8.2 Introduction

Cerebral small vessel disease causes lacunar stroke, white matter hyperintensities (WMH) on magnetic resonance scanning, cognitive decline, dementia and depression. The exact small vessel abnormalities are uncertain as they are difficult to study. Retinal microvascular abnormalities are associated with white matter disease (Wong et al. 2002a). I have demonstrated earlier in this thesis that retinal microvascular abnormalities are associated with future risk of stroke and having had a stroke and differ in ischaemic stroke subtypes, findings confirmed elsewhere (Lindley et al. 2009; McGeechan et al. 2009a). Therefore, retinal microvascular abnormalities may act as a surrogate marker for cerebral small vessel disease.

Prior research investigating retinal abnormalities in cerebral small vessel disease has focussed on focal retinal abnormalities (eg retinopathy), vessel widths (Lindley et al. 2009) or simple geometrical abnormalities (eg branching angles and branching coefficients) (Witt et al. 2006). The retinal tree is a complex structure and these techniques may not adequately characterise the retinal vascular tree as most of these techniques focus on sampling small sections of the vascular tree. Width measurements, for example are derived from 6 short arteriolar and 6 short venular segments and the geometrical abnormalities presented in chapter 7 of this thesis rely on data from 3 or 5 bifurcations. These techniques therefore do not assess a
considerable proportion of the retinal vascular tree and as a result may have a low
sensitivity for the identification of subtle global changes. An approach which uses a
global measure of the entire visible retinal vascular tree might be more sensitive at
identifying subtle early retinal vascular changes perhaps before macroscopic
abnormalities are present.

“Fractal analysis” is a complex geometrical measure that can be used to characterise
whole structures which branch in a repetitive manner, are self-similar at different
scales, and can be applied to assessment of the retinal vascular tree (Masters 2004).
The fractal dimension measures how a structure fills space or the degree of branching
complexity of a structure. An increased fractal dimension represents increased
branching complexity and a decreased fractal dimension represents decreased
branching complexity. Initially, assessment of retinal vessel fractals involved
laborious and time consuming (up to one hour per eye to process) hand tracing of the
vascular tree to obtain segmented representations of the vascular tree. This
effectively precluded the assessment of large numbers of patients and therefore
hindered the detection of small differences requiring large samples. We have
previously published details of a computer assisted automated technique for the
assessment of fractal dimensions using skeletonised images (MacGillivray et al.
2007). This technique enables a large number of images to be processed much
quicker than previously – 5 minutes rather then 60 minutes per image. Recently,
increased retinal vessel fractal dimensions have been associated with retinopathy in
type 1 diabetic patients aged 12-20 (Cheung et al. 2009) and decreased fractal
dimensions with older age and increasing blood pressure (Liew et al. 2008b).

In this chapter we investigate the associations between fractal properties of the
retinal vessels in patients with clinical and MRI features of lacunar stroke and white
matter disease to test the hypothesis that patients with lacunar stroke (as a distinct
marker of cerebral small vessel disease) will have altered fractal properties of their
retinal vessels compared with a cortical stroke control group (as a distinct marker of
large artery atherothromboembolic disease).
8.3 Methods

These are detailed in chapter 2 of this thesis but in brief we recruited patients presenting with clinical lacunar or minor cortical stroke.

Patients had cerebral Magnetic Resonance Imaging (MRI) at presentation, on a 1.5-T MR scanner (Signa LX; General Electric) with 22 mT m$^{-1}$ maximum strength gradients. All patients had six field retinal photography (centred on the disc, macula, lateral macula, nasal to the disc, upper arcade and lower arcade) of the left and right eyes, with 1% tropicamide eye drops where mydriasis was necessary, using a Canon CR-DGi digital retinal camera (Canon USA Inc.).

8.3.1 MRI analysis

All MRI scans were coded for the presence, location and size of the recent infarct and any old infarcts or haemorrhages and white matter hyperintensities by an experienced neuroradiologist. Scans were coded for deep and periventricular WMH according to the Fazekas scale (Fazekas et al. 1987) from 0-3.

8.3.2 Retinal image analysis

For each eye we chose the image centred on the optic disc. Retinal images were analyzed in Matlab (The Mathworks Inc., USA). Image processing was performed on the green channel of the colour fundus images as this typically exhibits the greatest contrast between vessel and background. All algorithms were performed with custom written software (by Dr Tom MacGillivray) within Matlab (The Mathworks Inc., USA). We combined fractal analysis with an automatic vessel segmentation procedure to speed up analysis. We found that the left and right eye fractal dimensions were correlated (Pearsons co-efficient 0.53), replicating previous results (Masters 1994) and we therefore chose the right eye, where available, for analysis. If the right eye retinal photograph was not suitable we used the left eye.
We converted colour images (figure 8.1, top left image A) to greyscale (figure 8.1, top right image B). We then segmented (traced) the retinal vascular tree (arterioles and venules) using an algorithm previously described (Soares et al. 2006) which denotes each pixel in the retinal image as being vessel or non-vessel to produce segmented images (figure 8.1, bottom left image C). We trained and tested our implementation of this algorithm using a set of 20 retinal images (that were sourced from a separate study of patients aged between 70-72) that had been manually segmented by two human observers (Dr Tom MacGillivray and Adria Rovira). Before fractal analysis, we manually inspected and corrected each computational segmented image removing obvious artefacts such as noise introduced by areas of low contrast in the image and “ring” object caused by dust on the camera face. Less than 5% of images in this cohort required manual correction. We then skeletonised each image (figure 8.1, bottom right image D) using Matlab’s bwmorph algorithm, which is based on iterative deletion of pixels (Lam et al. 1992). When the image is skeletonised information regarding vessel width is lost.
Fig 8.1. Retinal images showing the processing, segmentation and skeletonisation steps. Top left image (A) is the original colour image which is processed to greyscale (top right B) and subsequently segmented to produce the bottom left image (C). Finally, this image is skeletonised to produce the bottom right image (D) upon which fractal analyses are based.

Fractal dimensions can be assessed using a monofractal or a multifractal approach (Stosic and Stosic 2006). We first measured the mono-fractal dimension (Dbox) using the box-counting technique. We covered the skeletonised image with a number of equally sized square boxes and then counted how many of these boxes contained part of the skeletonised retinal vascular tree. We repeated this process with multiple different sized boxes, each time counting how many boxes contained part of the retinal vascular tree and recording the size of the box. We then plotted the logarithm of the number of boxes containing part of the retinal vascular tree against
the logarithm of the size of the box (figure 8.2). The fractal dimension (Dbox) was the slope of the best fit line of these points.

*Figure 8.2. Monofractal analysis. Plot showing Logarithm (ln) N(L) where N is the number of boxes containing part of the skeletonised retinal vascular tree and L is the size of the box against the logarithm of L. Dbox (monofractal dimension) is calculated from the slope of the best fit line*

Previous studies have used monofractal analysis of retinal vessels (Cheung *et al.* 2009; Liew *et al.* 2008b) and it is straightforward to calculate monofractal dimensions. It has however been suggested that multi-fractal techniques are better suited to characterizing complex spatial arrangements such as the retinal arteriolar tree which may represent a composite of many monofractal dimensions (Stosic and Stosic 2006). The retinal tree may possess different fractal dimensions depending on the level of scale (or magnification) used. A multi-fractal approach calculates multiple fractal dimensions from randomly chosen points (in this case 1000 points)
within the skeletonised vascular tree (rather than just one dimension from one run with the mono-fractal approach) and is able to assess the effect of scale on the fractal dimension, ie does magnifying the image change the fractal dimension (or degree of branching complexity).

To calculate the multi-fractal dimensions of the retinal vascular tree we used the generalized sand-box method (Stosic and Stosic 2006). We investigated the effect of scale on the fractal dimension and found that the retinal tree clearly exhibited multi-fractal properties as the fractal dimension changed when different scales were used, ie when the image was magnified the fractal dimension changed. If the retinal vasculature was a simple mono-fractal, the fractal dimension would be constant. We used D0 to denote the multi-fractal dimension.

When a mono-fractal dimension is calculated from a retinal vascular tree there is a confidence interval or uncertainty associated with it that is calculated during the fitting of the straight line to the logarithm graph. We found these uncertainties with multifractal analysis to be much lower (<1%) than the uncertainties from mono-fractal analysis which were in the order of 14%. We assessed both mono-fractal and multi-fractal properties of the retinal vessels to compare methods and to permit comparisons with previous studies which used the mono-fractal approach. Figure 8.3 shows the retinal vascular trees with the highest (left) and lowest (right) multi-fractal dimensions in this study.
Fig 8.3. Segmented vessel maps of two retinal vascular trees. The image on the left (A) was the highest fractal dimension in this study (Multifractal D0 1.736) and the image on the right (B) the lowest fractal dimension (Multifractal D0 1.622). Note these images are segmented vessel maps for illustration purposes. We used the skeletonised images to actually perform the fractal analysis.

The intra-grader reliability estimates for this technique were excellent (in 20 randomly chosen images) with intra-class correlation co-efficients of 0.94 for monofractal Dbox and 0.96 for multifractal D0.

8.3.3 Statistical analysis

All analyses were performed within Minitab (version 15). We assessed differences between the lacunar and cortical groups with t-test, differences in proportions and Mann Whitney U tests. Dbox and D0 were both normally distributed and we therefore used multiple linear regression with Dbox and D0 as the dependent variable with age, stroke subtype, white matter hyperintensity scores as independent variables. We modelled deep and periventricular white matter lesion scores as covariates as they are correlated. There were no immediately applicable data upon which to base sample size calculations for the assessment of fractals in cerebral small vessel disease. The power calculation - based upon our pilot study (MacGillivray et al. 2007) – found that with 80% power at the 5% level of significance to find a difference between groups of a Dbox of 0.01 with a two sample two tailed t test.
which predicted each group should have a minimum of 88 subjects. There were no missing data.

8.4 Results

8.4.1 Patient characteristics

We recruited 183 patients of whom 17 were excluded with poor photographic quality leaving 166 patients for analysis. The mean age was 67.3 years (SD 11.5 years) with a median NIHSS of 2 (IQR 2-3). There were 86 participants with lacunar stroke and 80 with cortical stroke. The baseline characteristics by stroke subtype are detailed in Table 8.1. The lacunar stroke patients were younger with a higher stroke severity than the cortical stroke patients. The prevalence of diabetes, hypertension and white matter hyperintensities did not differ between the groups.

| Table 8.1. Baseline characteristics of participants by ischaemic stroke subtype. |
|----------------------------------------|----------------|----------------|----------------|----------------|
| Feature                               | Lacunar stroke | Cortical Stroke | Difference (lac-cortical) (95% CI) | P value for difference |
| N                                      | 86             | 80             | -                           | -               |
| Age (mean/SD yrs)                     | 65 (11)        | 69 (11)        | -4 yrs (-8 to -1)            | 0.02            |
| Gender (n female)                     | 32 (37%)       | 24 (30%)       | 7% (-7 to 21)                | 0.30            |
| Hypertension n                        | 49 (57%)       | 50 (62%)       | -5% (-20 to 9)               | 0.46            |
| Diabetes n                            | 17 (19%)       | 8 (10%)        | 10% (-1 to 20)               | 0.07            |
| NIHSS (median/IQR)                    | 2 (2-3)        | 2 (1-3)        | -                           | 0.04            |
| DWMH (median/IQR)                     | 1 (1-2)        | 1 (1-2)        | -                           | 0.9             |
| PWMH (median/IQR)                     | 2 (1-2)        | 1 (1-2)        | -                           | 0.4             |
SD – standard deviation, CI – confidence interval, NIHSS – National Institutes for Health Stroke Scale, DWMH – deep white matter hyperintensities, PWMH – periventricular white matter hyperintensities.

### 8.4.2 Fractal dimensions

In this cohort the mean Dbox (monofractal dimension) was 1.42 with SD 0.02, the mean D0 (multifractal dimension) was 1.67 with SD 0.03. With multivariable analysis, decreased Dbox (Table 8.2) and D0 (Table 8.3) (both representing decreased branching complexity) were associated with increasing age and lacunar stroke subtype after correcting for hypertension, diabetes, stroke severity and white matter hyperintensity scores. We did not find associations between Dbox (mono fractal) or D0 (multi fractal) dimensions and the presence of hypertension, diabetes or white matter hypertintensities on either univariable or multivariable analysis.

**Table 8.2. Multivariable predictors of increasing Dbox (mono-fractal dimension) corrected for all listed variables in the table.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per year increase)</td>
<td>-0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lacunar stroke subtype</td>
<td>-0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.002</td>
<td>0.63</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-0.002</td>
<td>0.71</td>
</tr>
<tr>
<td>NIHSS (per point increase)</td>
<td>0.0001</td>
<td>0.52</td>
</tr>
<tr>
<td>DWMH (per point Fazekas increase)</td>
<td>-0.002</td>
<td>0.45</td>
</tr>
<tr>
<td>PVWM (per point Fazekas increase)</td>
<td>0.002</td>
<td>0.46</td>
</tr>
</tbody>
</table>

NIHSS – National Institutes for Health Stroke Scale, DWMH – deep white matter hyperintensities, PWMH – periventricular white matter hyperintensities.
Table 8.3. Multivariable predictors of increasing D0 (multi–fractal dimension) corrected for all listed variables in the table.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta co-efficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lacunar stroke subtype</td>
<td>-0.011</td>
<td>0.002</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.004</td>
<td>0.26</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.002</td>
<td>0.73</td>
</tr>
<tr>
<td>NIHSS</td>
<td>0.0005</td>
<td>0.71</td>
</tr>
<tr>
<td>DWMH (Fazekas score)</td>
<td>-0.002</td>
<td>0.61</td>
</tr>
<tr>
<td>PVWM (Fazekas score)</td>
<td>0.002</td>
<td>0.64</td>
</tr>
</tbody>
</table>

NIHSS – National Institutes for Health Stroke Scale, DWMH – deep white matter hyperintensities, PWVM – periventricular white matter hyperintensities

8.5 Discussion

We have demonstrated in this chapter that decreased fractal dimension (decreased branching complexity) whether measured with either mono or multi-fractal methods is associated with lacunar stroke and increasing age after correcting for other vascular risk factors and WMH. We are able to demonstrate this despite the cortical stroke patients being 4 years older than the lacunar stroke patients which would have tended to mask any associations with lacunar stroke. Furthermore we have demonstrated that it is feasible to perform automated fractal analysis on larger numbers of patients thereby making this tool a feasible tool for assessment of fractal dimensions in large population-based and clinical cohort studies.

This is the first study to investigate retinal fractal properties in the context of cerebral small vessel disease. Therefore there are no previous studies with which direct comparisons can be drawn. Until recently, fractal analysis had only been performed on smaller sample sizes reflecting the laborious nature of hand tracing the vessels. We have previously presented details of our semi-automated approach to fractal
Fractal analysis of the retinal vessels had previously been used with an aim of eventually predicting diabetic retinopathy before macroscopic changes of diabetic retinopathy developed and there have been a number of studies investigating retinal fractals in diabetes but the results from these studies have been inconsistent. An initial small study (n=10) using hand drawn segmentations found that decreased fractal dimension was associated with retinopathy (Avakian et al. 2002) but a subsequent slightly larger and more recent study (n=50) did not replicate this finding, finding no association between fractal dimension and retinopathy (Kunicki et al. 2009). Recent larger studies which use semi-automated techniques of retinal vessel segmentation still produce incongruous results. Two large studies from the same group found that in young (12-18 year old type one diabetic patients) increased fractal dimension was associated with prevalent retinopathy (which was not caused by new vessel formation) (Cheung et al. 2009) but also paradoxically that fractal dimension did not predict incident retinopathy in this same cohort of young diabetic patients (Lim et al. 2009). Others have found that in Type 1 diabetic patients (mean age 57 years) decreased fractal dimension was associated with multivariable analysis with prevalent retinopathy (Grauslund et al. 2010). All of these studies used the same software to assess mono-fractal dimension and the differences between them perhaps either reflect the different ages of participants or that mono-fractal assessment might not be a reliable measure of retinal vascular abnormalities (Anjos et al. 2010; Stosic and Stosic 2006). Further studies are required to assess the associations and determine the clinical implications of retinal fractal analysis.
The strengths of this study are the prospective, accurate and detailed clinical characterisation including MRI assessment of all patients to be certain of stroke subtypes to reduce observer dependency, the use of automated fractal analysis software and the correction with multivariable analysis for vascular risk factors. We used risk factor free definitions of lacunar and cortical stroke to reduce confounding. We were able to assess both monofractal and multifractal measures of the fractal dimension and the automated nature of the work was not labour intensive. We were also able to measure left and right eyes in most of our patients.

We were unable to assess fractal dimension in 10% of patients due to photographic issues but this was similar across both stroke subtypes. Ideally this proportion would have been smaller as we barely made the sample size calculation but we note that this is similar to previous exclusion rates (Cheung et al. 2009) albeit in young patients and our results reflect the difficulties in taking retinal photographs in older patients who have had a stroke. Problems exist with the computerized segmentation: the edge of the optic disk is sometimes wrongly detected as a vessel, incorrect detections result from the underlying choroidal vessels, and some vessel paths are broken. More importantly, very small vessels are frequently missed off the segmentation. All of these issues lead to a reduction in segmentation accuracy and mask the true fractal dimension of the retinal vasculature. Improving segmentation performance by increasing the number of true vessel detections and reducing artefacts would generate a binary object that better reflects the true retinal vascular pattern in the image and thus increase the sensitivity of fractal analysis detecting small changes associated with disease.

In this study we assessed the retinal vascular tree, that is arterioles and venules combined. It is a limitation of the segmentation process that the software cannot differentiate between arteriole and venule and therefore we cannot attribute these changes in branching complexity to either subset of the vascular tree. It is also possible that arterioles and venules behave differently and that assessing both may underestimate changes in one or the other. There has been little work in this area. One study of only 23 normal humans subjects using fluorescein angiography to
delineate the arteries and venules (a technique that is perhaps less reliable than using red free high quality digital photographs as the boundaries of the vessels are less distinct) found no difference between arteriolar and venular tree mono fractal dimensions (Landini et al. 1993) although this study may have been underpowered for this analysis as they did not find an association between fractal dimension and age (over a range of 14-73 yrs) and reflects the time consuming nature of manual vessel segmentation leading to small sample sizes.

It is intriguing that decreased branching complexity is associated with both increasing age and lacunar stroke. It has been suggested previously that many physiological systems become less complex with increasing age (Lipsitz and Goldberger 1992). The results from this study strengthen this theory and suggests a role for fractal analysis in the study of senescence. The exact cause of lacunar stroke is unclear and it has been postulated that lacunar stroke is the focal manifestation of a widespread small vessel vasculopathy perhaps resulting from increased blood brain barrier permeability and possibly associated with endothelial dysfunction. We have demonstrated earlier in this thesis that although the presence of retinopathy (haemorrhages and exudates) does not differ, retinal venules are wider in lacunar compared to cortical stroke which others have confirmed and in addition found narrower arterioles. The presence of these small yet important differences in the retinal vessels point towards a distinct vasculopathy causing lacunar stroke. We did not find an association with white matter hyperintensities and fractal dimension. Although it has been suggested that lacunar stroke and white matter hyperintensities may share a common aetiology (Wardlaw et al. 2003), WMH are common in older people exposed to risk factors like hypertension and diabetes and therefore occur in cortical stroke and lacunar stroke. This study may not have been powered to detect WMH differences between cortical and lacunar stroke patients.

Automated fractal analysis of retinal vessels is an emerging technique that has multiple possible applications. We have used fractals to elucidate underlying pathological mechanisms but future uses might include using the subtle changes visible in the retina as a marker for future development of retinal and cerebral
disease. There is also scope for improving the vessel segmentation which will improve reliability of automated processing of retinal images for fractal analysis and it would be fascinating (although technically difficult) to delineate isolated arteriolar and venular fractal dimensions. We would be delighted to see this work replicated in other populations. Subsequent work should concentrate on clarifying associations between fractal and cognitive function and assessing the predictive power of fractal analysis for future eye or systemic disease.
Chapter 9: Enlarged perivascular spaces on MRI in ischaemic stroke subtypes.

9.1 Aims

Previous chapters have highlighted a possible role for venular disease in lacunar stroke. In chapter 9 we investigate MR markers of cerebral small vessel disease concentrating on enlarged perivascular spaces in ischaemic stroke subtypes to assess the hypothesis that patients with lacunar stroke have higher rates of enlarged perivascular spaces.

9.2 Introduction

Enlarged perivascular spaces (EPVS) or Virchow-Robin spaces are cerebrospinal fluid filled cavities that surround small penetrating cerebral arterioles and venules and correspond with extensions of the subarachnoid space (Braffman et al. 1988). The exact anatomy differs depending on the site and nature of the penetrating vessels but EPVS are contiguous with the perivascular spaces of larger blood vessels in the subarachnoid space (Kwee and Kwee 2007). The exact role of perivascular spaces is not yet clear but tracer studies indicate that there is drainage of interstitial brain fluid down these spaces (Weller and Nicoll 2003) although again the mechanism behind this is unclear (Schley et al. 2006). These may communicate with the lymphatic drainage of the neck and this is reflected by the immunological importance of perivascular spaces – they maintain a stable population of migratory macrophages (Bechmann et al. 2001). EPVS are visible on axial T2-weighted cerebral magnetic resonance imaging as characteristic small high signal areas in the basal ganglia and centrum semiovale that follow the orientation of penetrating arterioles: they appear linear when parallel and dot like when perpendicular to the imaging plane. Although
common and seen at all ages in adulthood, their aetiology and significance are uncertain.

**Figure 9.1.** Axial T2 weighted MRI scans showing multiple punctuate hyperintensities characteristic of EPVS in the basal ganglia on the left and linear hyperintensities characteristic of EPVS in the centrum semiovale on the right.

Although a few EPVS are commonly observed in people at all adult ages, EPVS are associated with ageing (Heier et al. 1989), impaired cognitive function (MacLullich et al. 2004), retinopathy in type I diabetes (Ferguson et al. 2003), hypertension (Hiroki and Miyashita 2001), and are found in and around active lesions in multiple sclerosis (Wuerfel et al. 2008). A possible association with cerebral small vessel disease is suggested by their greater frequency in patients diagnosed as having vascular dementia as opposed to Alzheimer’s disease (Patankar et al. 2005), and their association with white matter hyperintensities (WMH) in patients with lacunar stroke (Rouhl et al. 2008). However, vascular dementia is not per se a marker of small vessel disease and a control group with stroke of a different aetiology (e.g. large artery territorial atherothromboembolic ischaemic stroke) would be required to demonstrate a specific association with lacunar stroke. It is unclear therefore whether EPVS are a marker of small vessel disease or simply represent a generalised brain response to exposure to a range of vascular or other risk factors, or increasing
age. For example, EPVS are present in active MS lesions in association with inflammation, and subside as the inflammation subsides (Wuerfel et al. 2008).

Pathologic studies suggest that EPVS are present at all degrees of WMH severity (Udaka et al. 2002). EPVS defined pathologically may be related to a widespread microangiopathy resulting in WMH which are commonly associated with age related cognitive impairment, (Gunning-Dixon and Raz 2000) vascular dementia (Sitoh et al. 2004) and lacunar stroke (Wiszniewska et al. 2000). The aetiology of EPVS in various brain locations may differ, as distinct associations for basal ganglia and centrum semiovale EPVS have been suggested (Rouhl et al. 2008).

Many lacunar strokes are caused by an intrinsic disease of cerebral small vessels, and cortical strokes are generally caused by large artery atherosclerosis and cardioembolism (Wardlaw 2005). If EPVS are a specific marker of small vessel disease, then they should be more closely associated with lacunar stroke and WMH than with cortical stroke. We investigated firstly whether MRI-defined EPVS (subdivided into basal ganglia and centrum semiovale EPVS) are associated with ischaemic stroke subtype, secondly whether EPVS are associated with WMH, thirdly whether EPVS are associated with any ischaemic stroke, and fourthly whether EPVS are associated with demographic and vascular risk factors.

9.3 Methods

Details of patient recruitment have been given in Chapter 2 but in brief we recruited patients with clinical lacunar or mild cortical stroke at presentation to a teaching university hospital. All patients were examined by an experienced stroke physician with MRI at presentation and classified into lacunar or cortical stroke clinical syndromes according to the Oxfordshire Community Stroke Project classification (Bamford et al. 1991).
Whilst much of the work in this thesis concentrates on phenotypical differences between ischaemic stroke subtypes and no previous studies have investigated this for enlarged perivascular spaces we were also keen to look at associations between the presence of a stroke and non stroke controls addressing the hypothesis that patients with stroke would have more EPVS than subjects without stroke. In order to achieve this we used a control group of patients who had been recruited for a separate study within the same department which had been established to investigate cognition in healthy males. These patients had been scanned in 2002 and were healthy 65-70 year old males who were recruited through primary care. All non-stroke controls were reviewed by a previous research fellow (Alasdair MacLullich) and were free from significant diseases (including diabetes, cancer, heart disease, neurological disease) and were not taking regular medication at the time of the scan. Full details of this cohort have been presented previously (MacLullich et al. 2004).

These non-stroke controls were scanned with a GE prestige scanner (GE Medical, Haifa, Israel) which was a different scanner from that used for the stroke patients. Whilst this was a potential weakness in this study, we felt that this was mitigated as the two groups of patients were imaged in the same centre with the same procedure and techniques. Although the stroke patients were scanned on a 1.5T GE scanner and the controls on a 2T GE scanner the T2 axial sequences were optimised for both scanners, we ran extensive phantom testing to be sure that sequences were optimised and that we could translate for complex computational image analysis between both scanners, and several patient studies were conducted on both scanners (including some of the same subjects) which allowed us to make sure that the change in scanner did not affect structural images. Furthermore, assessing EPVS on optimised T2 images is a robust clinical radiological technique, similar to any other radiological diagnostic visual assessment exercise, and, in contrast to some computational image analysis methods, is not affected by minor between-scanner differences.
9.3.1 Brain image analysis

Images were coded by an experienced neuroradiologist (Joanna Wardlaw) using a standardized classification system for the presence, site and size of the recent infarct (hyperintense on DWI, hypointense on ADC, and possibly also hyperintense on FLAIR and/or T2, hypointense on T1), and also for the presence, site and size of any old infarcts and/or haemorrhages. Scans were coded for deep and periventricular WMH according to the Fazekas scale (Fazekas et al. 1987) from 0-3. Cerebral atrophy was defined as deep (enlargement of the ventricles) or peripheral (enlargement of the gyri) and rated on a subjective scale of 0-3 (absent (0), mild (1), moderate (2), severe (3)) against a reference MR brain template of normal subjects (Farrell et al. 2008).

EPVS were defined as small, sharply delineated structures of cerebrospinal fluid intensity on imaging that followed the orientation of the perforating vessels and ran perpendicular to the brain surface. Therefore they appeared round in axial section (in the basal ganglia) and linear if in longitudinal sections (in the centrum semiovale) and were less than 3 mm wide. They were of high signal on T2 and low signal on T1 and FLAIR sequences. We assessed EPVS in the basal ganglia and centrum semiovale separately as it is possible that due to their different locations and features they may have separate pathophysiologies. EPVS in both the basal ganglia and centrum semiovale were coded with the following scale: (MacLullich et al. 2004) 0 = no EPVS, 1 = <10 EPVS, 2 = 10-20 EPVS, 3 = 30-40 EPVS and 4 = >40 EPVS. The numbers refer to EPVS on one side of the brain; the higher score was used if there was asymmetry between the sides. We summed basal ganglia and centrum semiovale EPVS to form a total EPVS score (0-8).

9.3.2 Statistical Analysis

Total EPVS was normally distributed and analyzed with multiple linear regression. Basal ganglia EPVS and centrum semiovale EPVS were not normally distributed and, to permit binary logistic regression, we dichotomized basal ganglia and centrum
semiovale EPVS into 0 (EPVS scores 0,1) and 1 (EPVS scores 2,3,4). We used t-tests, Mann Whitney U tests and differences in proportions for association to test for differences between the lacunar and cortical groups. We used multiple regression to assess effects of potential explanatory variables in predicting numbers of EPVS, both total EPVS in the acute stroke group and healthy controls combined, and basal ganglia and centrum semiovale EPVS separately within the acute stroke group. We entered both stroke subtype (lacunar versus cortical) and WMH separately into the model as the association between lacunar stroke and WMH is not strong and WMH are found commonly in patients with cortical stroke and normal older people. The alpha value for statistical significance was set at 0.05. All analyses were performed with Minitab software (version 14, Minitab Inc, PA, USA). There were no suitable data upon which to base meaningful study size calculations. There was one patient with missing data regarding MRI analysis and this patient was excluded from all totals given in the paper.

9.4 Results

We recruited 350 patients in total, 253 patients with acute stroke and 97 normal healthy age matched controls (Table 9.1). Of the 253 patients with stroke, the mean age was 68.1 years (SD 11.6 years, range 34-95 years), median NIHSS 2 (interquartile range 2-3), 65% were male and there were 129 patients with lacunar stroke and 124 patients with cortical stroke. Of the 129 lacunar stroke patients, 91 had a recent infarct identified on MRI and of the 124 cortical patients, 95 had a recent infarct identified. No patients had concurrent acute lacunar and cortical infarcts. The mean age of the healthy controls was 66.9 years (SD 1.3 years) and they were all male. Amongst the acute stroke patients, those with cortical stroke were older (70 v 66 years p= 0.01), had less severe strokes (p<0.001) and, in keeping with atherothromboembolic causes for their stroke, had a higher prevalence of atrial fibrillation (p=0.02), carotid stenosis >50% (p=0.02) and ischaemic heart disease (p=0.004) than the lacunar stroke patients. There were no significant differences in sex, race or the prevalence of diabetes, PVD, previous TIA/stroke or hypertension between the stroke subtypes.
### Table 9.1 Baseline characteristics of subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lacunar Stroke n=129</th>
<th>Cortical Stroke n=124</th>
<th>Difference, 95% CI and p value between lacunar and cortical groups</th>
<th>Healthy controls n=97</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (SD) years</td>
<td>66.3 (11.6)</td>
<td>70.0 (11.5)</td>
<td>3.6 (0.8, 6.5) p=0.01</td>
<td>66.9 (1.4)</td>
</tr>
<tr>
<td>Median NIHSS (IQR)</td>
<td>3</td>
<td>2</td>
<td>p&lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>Male (n) (%)</td>
<td>77 (60%)</td>
<td>88 (70%)</td>
<td>10% (-1%, 22%) p=0.06</td>
<td>97 (100%)</td>
</tr>
<tr>
<td>AF</td>
<td>6 (4.6%)</td>
<td>16 (12.90%)</td>
<td>8% (1, 15) p=0.02</td>
<td>0</td>
</tr>
<tr>
<td>Carotid Stenosis &gt;50% (NASCET)</td>
<td>5 (3.88%)</td>
<td>14 (11.29%)</td>
<td>7% (1, 13) p=0.02</td>
<td>0</td>
</tr>
<tr>
<td>History previous TIA</td>
<td>19 (14.96%)</td>
<td>13 (10.48%)</td>
<td>4% (-4, 12) p=0.29</td>
<td>0</td>
</tr>
<tr>
<td>History previous Stroke</td>
<td>9 (6.98%)</td>
<td>14 (11.38%)</td>
<td>4% (-3, 12) p=0.23</td>
<td>0</td>
</tr>
<tr>
<td>History IHD</td>
<td>17 (13.18%)</td>
<td>34 (27.42%)</td>
<td>14% (4, 24) p=0.004</td>
<td>0</td>
</tr>
<tr>
<td>History Hypertension</td>
<td>84 (65.32%)</td>
<td>70 (56.59%)</td>
<td>9% (-3, 20) p=0.15</td>
<td>0</td>
</tr>
<tr>
<td>History Diabetes</td>
<td>22 (17.05%)</td>
<td>14 (11.29%)</td>
<td>6% (-3, 14) p=0.19</td>
<td>0</td>
</tr>
<tr>
<td>History Peripheral Vascular Disease</td>
<td>5 (3.88%)</td>
<td>6 (4.84%)</td>
<td>1% (-4, 6) p=0.71</td>
<td>0</td>
</tr>
<tr>
<td>Median Deep White Matter Fazekas score (IQR)</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
<td>P=0.92</td>
<td>1 (0-1)</td>
</tr>
<tr>
<td>Median Periventricular White Matter Fazekas Score (IQR)</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
<td>P=0.50</td>
<td>1 (0-1)</td>
</tr>
<tr>
<td>Mean Total EPVS Score (SD)</td>
<td>3.81 (1.76)</td>
<td>3.46 (1.72)</td>
<td>0.3 (-0.1, 0.8) p=0.11</td>
<td>1.02 (0.89)</td>
</tr>
</tbody>
</table>
9.4.1 EPVS in ischaemic stroke subtypes

In the 253 patients with acute stroke, total EPVS (basal ganglia and centrum semiovale combined) were independently and significantly associated with lacunar stroke subtype after correction for important confounders including vascular risk factors, age, WMH and deep atrophy, in keeping with our primary hypothesis (Table 9.2). Basal ganglia EPVS were modelled simultaneously with centrum semiovale EPVS to look at separate associations despite being correlated at Spearman’s rank rho 0.47 (p<0.001). Basal ganglia EPVS were associated with lacunar stroke subtype after correcting for other confounders including WMH and deep atrophy (Table 9.3). Centrum semiovale EPVS did not differ between ischaemic stroke subtypes.

*Table 9.2. Multivariable associations with Total EPVS as the dependent variable correcting for all the other explanatory variables in the table in the subgroup of 253 patients with acute stroke*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta Coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>Lacunar stroke subtype (compared with cortical stroke)</td>
<td>0.38</td>
<td>0.04</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.06</td>
<td>0.76</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.067</td>
<td>0.79</td>
</tr>
<tr>
<td>Deep WMH</td>
<td>0.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Periventricular WMH</td>
<td>0.46</td>
<td>0.01</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.01</td>
<td>0.96</td>
</tr>
<tr>
<td>Deep atrophy</td>
<td>-1.4</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Abbreviations
WMH: white matter hyperintensities
Table 9.3 – Univariable and Multivariable associations with basal ganglia EPVS.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariable P value</th>
<th>Univariable OR (95% CI)</th>
<th>Multivariable p value</th>
<th>Multivariable OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt;0.001</td>
<td>1.08 (1.05-1.11)</td>
<td>0.14</td>
<td>1.03 (0.99-1.08)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.11</td>
<td>1.53 (0.91-2.58)</td>
<td>0.46</td>
<td>1.33 (0.63-2.81)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.53</td>
<td>0.79 (0.38-1.65)</td>
<td>0.39</td>
<td>0.65 (0.24-1.74)</td>
</tr>
<tr>
<td>Deep WMH</td>
<td>&lt;0.001</td>
<td>4.47 (2.94-6.80)</td>
<td>0.02</td>
<td>2.03 (1.10-3.74)</td>
</tr>
<tr>
<td>Periventricular WMH</td>
<td>&lt;0.001</td>
<td>4.97 (3.29-7.50)</td>
<td>0.01</td>
<td>2.28 (1.19-4.38)</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.53</td>
<td>1.18 (0.69-2.02)</td>
<td>0.13</td>
<td>1.83 (0.84-3.98)</td>
</tr>
<tr>
<td>Centrum semiovale EPVS</td>
<td>&lt;0.001</td>
<td>7.58 (4.01-14.32)</td>
<td>&lt;0.001</td>
<td>6.34 (2.87-14.0)</td>
</tr>
<tr>
<td>Deep atrophy</td>
<td>&lt;0.001</td>
<td>2.20 (1.60-3.01)</td>
<td>0.58</td>
<td>1.15 (0.70-1.87)</td>
</tr>
<tr>
<td>Lacunar stroke subtype</td>
<td>0.03</td>
<td>1.78 (1.07-2.96)</td>
<td>0.003</td>
<td>3.16 (1.49-6.70)</td>
</tr>
</tbody>
</table>

* = significant p<0.05

Abbreviations

WMH: white matter hyperintensities

9.4.2 EPVS and white matter hyperintensities

In all 350 patients, total EPVS were associated with deep and periventricular WMH after adjusting for age, presence of stroke, hypertension, diabetes and sex (Table 9.4). Furthermore, in the acute stroke group of 253 patients we found that basal ganglia EPVS were associated with deep and periventricular WMH adjusting for lacunar stroke subtype, age and deep atrophy. Centrum semiovale EPVS were not associated with any explanatory variables (after correcting for basal ganglia EPVS - data not shown).
Table 9.4 - Multivariable associations with Total EPVS as the dependent variable correcting for all the other explanatory variables in the table in the total study population of 350 patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta Co-efficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.01</td>
<td>0.35</td>
</tr>
<tr>
<td>Presence of stroke</td>
<td>0.31</td>
<td>0.21</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.08</td>
<td>0.69</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.06</td>
<td>0.81</td>
</tr>
<tr>
<td>Deep WMH</td>
<td>0.60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Periventricular WMH</td>
<td>0.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male gender</td>
<td>-0.16</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Abbreviations
WMH: white matter hyperintensities

9.4.3 EPVS and the presence of stroke

In all 350 patients, EPVS were associated with the presence of any stroke on univariable analysis, but this association was reduced to below statistical significance after adjustment for WMH.
9.4.4 EPVS, vascular risk factors and demographics

In all 350 patients, EPVS were associated with increasing age on univariable analysis but this association was attenuated to below statistical significance after adjustment for WMH. After full adjustment for other variables, only deep and periventricular WMH remaining significantly associated with EPVS.

9.5 Discussion

Our novel findings in this chapter are that total and basal ganglia EPVS are independently and significantly associated with lacunar compared to cortical ischaemic stroke, in keeping with the hypothesis that EPVS and lacunar stroke share a common aetiology. EPVS were also independently and significantly associated with deep and periventricular WMH after correcting for increasing age and vascular risk factors, further emphasizing the specific association of EPVS with small vessel disease.

No previous study has included a control group without stroke to distinguish the relationship between EPVS and any stroke. No previous study has included a control group with a different stroke mechanism to distinguish the relationship between EPVS and ischaemic stroke subtypes. Lacunar stroke may be the focal manifestation of a widespread small vessel angioopathy consequent upon increased blood brain barrier permeability (Wardlaw et al. 2009; Wardlaw et al. 2008) which also leads to the development of WMH (Young et al. 2008). The apparently different associations for basal ganglia and centrum semiovale EPVS might be explained by anatomical differences between the arterioles in the basal ganglia and elsewhere: the former have two layers of surrounding leptomeninges whereas the latter only have one (Pollock et al. 1997).
The exact causes of EPVS are uncertain but the perivascular space is an important conduit for drainage of interstitial fluid to the ventricles (Abbott 2004) and could be affected by various factors including abnormalities at the blood brain interface (Wardlaw et al. 2009). Increased blood brain barrier permeability is associated with EPVS in stroke patients (Wardlaw et al. 2009), in patients with active multiple sclerosis lesions (Wuerfel et al. 2008), and with WMH (Starr et al. 2003). WMH are more common in lacunar than cortical stroke (Wardlaw et al. 2006), again supporting a common small vessel disease pathophysiology. In the case of multiple sclerosis, the appearance of the EPVS followed active inflammation and were associated with a blood brain barrier breakdown; the EPVS resolved as the active inflammation subsided. Endothelial inflammation may be associated with small vessel disease also (Hassan et al. 2003b). It is therefore plausible that EPVS are a manifestation of a cerebral small vessel pathology associated with, and a possible marker for, altered blood brain barrier function (Wuerfel et al. 2008). A possible link with venular pathology in small vessel disease and inflammation has been suggested by previous findings in chapter 6 of this thesis and elsewhere (Lindley et al. 2009) and cerebral venous insufficiency secondary to collagenosis has been implicated in the aetiology of white matter disease (Black et al. 2009; Moody et al. 1995) and it has recently been suggested that research should concentrate on the venular pathophysiology of white matter disease (Andersson 2010).

The strengths of this study are that we directly compared EPVS in ischaemic stroke subtypes and between stroke patients and healthy age matched controls. The sample size was relatively large and thus we were able to investigate several explanatory variables with multivariable regression to correct for confounders. The patients had carefully standardised MRI brain imaging on a dedicated research scanner as part of the study assessment, rather than using either retrospectively recruited patients (Heier et al. 1989) or those who had had clinically indicated MRI (Rouhl et al. 2008). All MRI scans were assessed by one experienced neuroradiologist using a standard EPVS rating scale. Importantly, because we used multi-sequence MRI, we were able to fully characterise WMH and EPVS and simultaneously correct for important
confounding variables, especially WMH and brain atrophy, to ensure that EPVS are not simply caused by parenchymal brain loss.

This study has limitations. The healthy control group did not have basal ganglia and centrum semiovale EPVS characterised separately (though we were able to use a combined total EPVS score to compare groups) and the healthy control group was all male (although sex was not shown to have an independent association with EPVS in the acute stroke group). This is a cross sectional study and therefore can only determine associations not causation, nor the sequence of development of small vessel disease features.

A previous study showed that basal ganglia EPVS were associated on multivariable analysis with periventricular (but not deep) WMH, asymptomatic lacunar infarcts and increasing age in 165 patients with lacunar stroke (Rouhl et al. 2008). Centrum semiovale EPVS were associated on multivariable analysis with diabetes and asymptomatic lacunes. Although this study included patients with large artery stroke (n=41) as well as lacunar stroke, they did not report the EPVS associations for cortical stroke. A retrospective study of 816 patients using data from clinically-indicated brain MRI showed that EPVS were strongly associated with age but not WMH, hypertension or dementia on multiple regression (Heier et al. 1989). The clinically indicated MRI scan, retrospective design, wide age range and a low rate of WMH, may explain the differences with our results. EPVS and WMH were more frequent in patients with vascular dementia than other types of dementia (35 patients with Alzheimer’s, 25 with vascular or fronto-temporal dementia and 35 healthy controls) but this may have been confounded by higher rates of white matter disease in vascular dementia and the small study size (Patankar et al. 2005).

Beyond confirming that EPVS seen on MRI are indeed enlarged spaces around the perforating arterioles, pathologic studies of white matter disease produce conflicting information, and shed little light on their aetiology (Pantoni and Garcia 1997). In 122 patients whose MRI findings were compared with pathology, EPVS were present at all Fazekas grades of deep and periventricular WMH (Udaka et al. 2002). Other
features present in all grades of WMH were myelin pallor and loss, with gliosis and axonal loss in the more severe cases. In the brains of 19 patients >60 years who had died of non-brain disease causes, comparison of MRI and pathologic appearance suggested that arteriosclerosis leads to demyelination, then axonal loss and then subsequent dilatation of perivascular spaces and WMH (van Swieten et al. 1991). However it is difficult to infer a sequence of events from a static picture obtained at post mortem. It is not clear therefore if EPVS precede, follow or appear concurrently with WMH, or if they arise from the same pathologic process or not. The variation in methods for assessing EPVS may account for the discrepancies between studies and points to the need for a robust and reliable classification scheme for EPVS.

EPVS are relatively under-researched but easy to recognise. Amongst older people, they are part of the spectrum of small vessel disease associated with widespread white matter lesions, lacunar stroke and cognitive impairment. We propose that EPVS should be incorporated into white matter rating scales because the associations and significance of EPVS will only be elucidated once EPVS have been studied in more depth. Future research should investigate longitudinal associations between EPVS, WMH, stroke and cognitive function to fully ascertain temporal associations between these common and important abnormalities. More information is needed to provide accurate prognostic indicators and guide development of future therapies for small vessel disease, especially with small vessel disease related to cognitive impairment.
Chapter Ten: Conclusions, implications and suggestions for future research.

10.1 Aims of chapter

In this chapter I summarise the findings of this thesis and compare these results to recently published data from other groups. I will discuss generic strengths and weaknesses of this study before discussing the pathological and clinical implications of these results. Finally I detail ongoing work that has arisen from these results and provide suggestions for further research.

10.2 Summary of findings

We performed a systematic review of retinal microvascular abnormalities in lacunar stroke to clarify associations and identify where further research was required. We then established a cohort of patients presenting with lacunar stroke with cortical stroke controls to investigate retinal microvascular abnormalities in ischaemic stroke subtypes. All patients had MRI brain at presentation and retinal photography of both eyes with a digital retinal camera. We investigated the prevalence of retinopathy (hard and soft exudates or haemorrhages or microaneurysms), retinal arteriolar and venular widths, focal arteriolar narrowing, arteriovenous nicking, retinal arteriolar geometry (branching co-efficients (change in arteriolar cross sectional area across a bifurcation) and branching angles) and fractal dimensions (reflecting branching complexity) of the vasculature. We also assessed MRI parameters in lacunar stroke. We used multivariable analysis to correct for baseline imbalances in vascular risk factors.

From the systematic review we demonstrated that retinal microvascular abnormalities are associated with incident and prevalent stroke but in general stroke was inadequately characterised and there were no data regarding retinal
microvascular abnormalities in ischaemic stroke subtypes. We recruited 253 patients, 129 lacunar stroke and 124 cortical strokes, mean age 68 years. In this cohort of patients presenting with a clinical diagnosis of mild stroke we found that diffusion weighted MRI at presentation was negative for ischaemia in 33% of patients and negative when all sequences were included in 24%. We found no difference in prevalence of retinopathy, arteriovenous nicking, focal arteriolar narrowing or arteriolar widths between lacunar and cortical stroke subtypes. We found that venules were wider in lacunar stroke. We found no differences in arteriolar branching co-efficients or arteriolar branching angles between lacunar and cortical strokes but found that deep white matter hyperintensities on MRI were associated with increased branching co-efficients and periventricular white matter hyperintensities associated with decreased branching co-efficients. We found that fractal dimensions were decreased in lacunar stroke. Furthermore we found that enlarged perivascular spaces on MRI are associated with lacunar stroke and white matter disease.

10.3 Validation by other work

Much of the work presented and previously published from this thesis has been subsequently validated by results from other centres. A recent individual patient based meta-analysis investigated whether retinal arteriolar or venular widths predicted incident stroke (McGeechan et al. 2009a). This systematic review identified 6 population based studies - ARIC, Ausdiab, CHS, BMES, BDES and the Rotterdam study, contacted the principal investigators of each study and merged the data. Their main findings confirmed the results of our systematic review as increased venular width predicted incident stroke but arteriolar width did not predict future stroke. This analysis was limited to studies which assessed arteriolar and venular widths (and did not investigate the predictive nature of other retinal microvascular abnormalities such as retinopathy or retinal arteriolar occlusion) and incident stroke or indeed any associations with prevalent stroke. There will inevitably have been heterogeneity between studies in the definition of stroke and in general stroke was diagnosed by review of medical records (reflecting limitations of
the published literature detailed in chapter 3 of this thesis) but this study included unpublished data from the ARIC and Ausdiab studies.

As detailed previously, the Mild Stroke Study in Edinburgh was designed to complement the similar Multicentre Retinal Stroke (MCRS) study co-ordinated in Sydney, Australia (Lindley and Multi-Centre Retinal Stroke Study Collaborative Group 2008). The MCRS study recruited in three centres – Sydney and Melbourne, Australia and Singapore. The inclusion criteria were slightly different from the Mild Stroke Study – the MCRS recruited patients who were less than 7 days post stroke onset who were admitted to hospital with stroke (including posterior circulation and haemorrhagic stroke). All patients had standard investigations for stroke (plus retinal photographs) which meant that a significant proportion of patients had CT (60%) rather than MRI (40%) which were clinically indicated, to diagnose and subtype ischaemic stroke. To correct for this they performed a series of sensitivity analyses using different methods of subtyping stroke with a hierarchy of classifications. The authors subtyped stroke using lacunar stroke diagnosed with the OCSP classification first, followed by the TOAST method, followed by those with lacunar stroke who had a MRI scan and finally those who had a DWI positive lacunar lesion. There were decreasing numbers of patients in each of these categories. They subsequently repeated all of these analyses in patients without diabetes.

The MCRS study recruited 1321 patients, mean age 66 years and reported that in general, associations between retinal microvascular abnormalities and lacunar stroke subtype strengthened with increasing precision of lacunar stroke diagnosis. Using the OCSP classification in all patients they found no difference in focal arteriolar narrowing, arteriovenous nicking, arteriolar width or venular width between lacunar and non-lacunar stroke. When this analysis was restricted to those patients who had had a MRI, a group perhaps most comparable to those presented in this thesis, (146 patients with lacunar stroke) they found that focal arteriolar narrowing and arteriovenous nicking were more prevalent in lacunar stroke, there was a borderline significant narrowing of arteriolar diameter (OR for the smallest quintile versus the
largest quintile 2.4 (95% CI 1.0 to 5.8) and that retinal venules were not wider in lacunar stroke (OR 1.4 (95% CI 0.9 to 2.3).

When patients with diabetes were excluded from the analysis in the cohort who had had brain MRI (which included 94 patients with lacunar stroke) focal arteriolar narrowing (OR 2.4, 1.1 to 4.5) and arteriovenous nicking (OR 2.4, 1.3 to 4.5) were more prevalent in lacunar stroke, arterioles were narrower (OR 2.1, 1.1 to 4.2) and venules were wider (OR 2.1, 1.1 to 3.9). It is interesting to note however when this analysis was restricted to those with DWI lesions of lacunar stroke (89 patients with lacunar stroke) the association with narrowed arterioles became non-significant. The reported association between narrowed arterioles and lacunar stroke seems inconsistent and perhaps reflect imperfect stroke subtyping as most patients only had CT as their brain imaging which may introduce bias but it would appear that venules are reproducibly wider in lacunar stroke.

Each study independently developed its own protocol for measuring arteriolar widths (although they were based on the same iterative process for combining widths to produce CRAE and CRVE). It is interesting to note that in the MCRS study the absolute mean arteriolar width in lacunar patients was 132 microns and the mean venular width was 214 microns which are similar to our measurements of 161 microns and 208 microns respectively.

Although our study and that of our collaborators were designed to complement each other there were several key methodological and analytical differences between the two studies. All of the patients in the Edinburgh based Mild Stroke Study had diagnostic MRI at presentation and were carefully characterised clinically by a single research fellow using risk factor free definitions. There were slightly more restricted inclusion criteria – we did not include patients with stroke equivalent to total anterior circulation stroke or those with haemorrhage although it is interesting to note that there were only small numbers of patients with haemorrhage in a subset of the MCRS (Baker et al. 2010) – 51 of 630 patients had deep cerebral haemorrhage.
10.4 Variability of retinal features

We have used a cross sectional study design to investigate associations between retinal features and stroke subtypes. Retinal features may vary over short time periods. For certain retinal features such as retinal vessel geometry or fractal analysis it is not clear whether the abnormalities are present at birth, appear at a later stage and precede cerebrovascular disease or are a response to lifetime accumulation of risk factors.

It has been suggested that retinal vessel widths differ at different stage in the cardiac cycle (Chen et al. 1994) but subsequent studies of retinal photographs performed with a digital retinal camera found that the variability in widths from the point in the cardiac cycle was less than the between image variability due to compromised image quality and therefore efforts should be made to ensure maximal image quality rather than co-ordinating photos with the cardiac cycle (Knudtson et al. 2004).

Certain retinal microvascular abnormalities might be present from a young age. A study from Singapore investigated retinal vessel widths in children aged 7-9 years found no relationships between birth markers (weight, length, gestational period, and head circumference) and retinal vessel widths although there were differences in width parameters between children of Chinese, Malay and Indian origin (Cheung et al. 2007b). These finding suggest that retinal vessel width associations develop in response to disease later in life.

If retinal markers can be used as a surrogate marker of disease it is possible that they could be used to assess response to treatment of the associated systemic disease such as hypertension or diabetes. In a small study (n=25) investigating retinal changes before and after treatment for hypertension, both ACE inhibitor and calcium channel treatment were associated with comparable blood pressure falls and a reduction in arteriolar narrowing, widening of arteriolar branching angles and an increase in arteriolar density (Hughes et al. 2008). A study from the same group compared retinal length/diameter ratios (a marker of arteriolar narrowing) in a randomized trial
of antihypertensive regimes finding that treatment with amlodipine was associated with lower ratios than treatment with beta-blockers (Thom et al. 2009). Both of these studies suggest that arteriolar parameters are not fixed and may respond to external factors.

The mechanisms behind these responses are yet to be fully elucidated. In chapter 7 we suggested that endothelial dysfunction (possibly acting via nitric oxide dependent processes) may affect retinal arteriolar geometry. Advanced laser scanning doppler cameras can measure dynamic changes in retinal arterial and capillary blood flow in response to flicker stimuli which under normal circumstances promotes NO dependent vasodilatation. Using laser scanning dopplers, young hypertensive patients had impaired arterial vasodilatation and blood flow increases in response to the flicker light stimulus compared to non-hypertensive controls. Blockage of NO (with N(G)-monomethyl-L-arginine – L-NMA) abolished the dilatation in non-hypertensive controls. Subsequent administration of candesartan (an angiotensin-1 receptor blocker (AT-1) ) restored dilatation in response to the flicker light stimulus (Delles et al. 2004). This suggests a role for NO in the control of retinal blood flow and that blockage of AT-1 receptors may reverse the impaired endothelial function present in hypertensive subjects. No studies have reported associations between the retinal features described in this thesis and response to stimuli.

10.5 Why did we not find a difference in arteriolar widths?

Initial pathological reports suggested that a selection of the key changes apparent in the arterioles are thickened walls with lipohyalinosis and fibrinoid necrosis with associated destruction of the arteriolar wall and finally narrowing and eventual blockage of the arteriolar lumen.(Lammie 2000) We found that arteriolar widths were 2 microns narrower in patients with lacunar stroke, a difference which did not reach statistical significance. There was a paucity of data upon which to base sample size calculations and this study may have been underpowered to detect this small difference. Subsequent publication of results from the Multi-centre retinal
stroke study with a total sample size of 1321 patients of whom 410 had lacunar stroke from our collaborators in Sydney found that there arterioles were 2 microns narrower in lacunar stroke – a similar difference to ours - suggesting that with more power our difference may have reached statistical significance (Lindley et al. 2009). There are inconsistencies however as the MCRS study did not consistently find decreased arteriolar widths in lacunar stroke after multivariable correction for vascular risk factors. Other than our sample being underpowered to look for arteriolar widths differences in lacunar stroke might there be other explanations for the lack of an association between lacunar stroke and decreased retinal arteriolar widths?

Other studies have investigated associations between venular widths and arteriolar widths for the following systemic variables: cognitive function (Liew et al. 2009), incident stroke (Wong et al. 2006), decreased arteriolar saturations and cerebral blood flow (De Jong et al. 2008), MRI evidence of small vessel disease (Ikram et al. 2006b) and serum markers of systemic inflammation (C-reactive protein) (Yim-Lui et al. 2010). All of these studies found associations between increased venular width but not arteriolar width and their variable of choice. It is possible that the retinal venules are more reactive than the retinal arterioles to systemic influences (or their larger size makes any changes easier to identify). The exact role and influences behind alterations in arteriolar and venular widths are imperfectly understood. In general narrower retinal arterioles are associated with hypertension and increased age whilst it is felt that wider venules are associated with younger age (in population based studies of middle aged individuals), hypertension, increased body mass index and increased inflammation (measured with serum markers) (Liew et al. 2008a). It is likely that a genetic influence determines retinal vessel widths (Sun et al. 2009).

Arteriolar narrowing has been demonstrated in cerebral pathological studies yet important information regarding the sequencing of events (which is absent in human pathological studies) is beginning to emerge from animal studies of lacunar stroke (Bailey et al. 2009; Hainsworth and Markus 2008). In the spontaneously hypertensive stroke prone rat the narrowing and subsequent blocking of the lumen of
the arteriole is a late event which follows increased blood brain barrier permeability and parenchymal damage from leaked plasma proteins. It is possible that in this cohort of patients with lacunar stroke the disease process is not far enough advanced for us to be able to identify arteriolar narrowing. In retinal photographs the vessel wall is transparent and what can actually be seen is the red blood cell column in the lumen thereby we are measuring the luminal width rather than the widths of the arterioles. It is possible that even though the arteriolar wall has thickened this has not necessarily resulted in a narrowing of the lumen.

10.6 Why might the retinal venules be wider in lacunar stroke?

The exact reason for this is not clear. There is a paucity of information regarding the cerebral venules in lacunar stroke both from pathological studies (Lammie 2000) or animal models of lacunar stroke (Bailey et al. 2009). Increased retinal venular widths have been associated with decreased oxygen saturations and decreased cerebral blood flow (De Jong et al. 2008), increased serum markers of inflammation (De Jong et al. 2007) and decreased small artery compliance but not large artery compliance (Cheung et al. 2007a). Recent results have found that increased venular width is associated with decreased flow mediated dilatation of the brachial artery (which represent NO mediated endothelial related dilatation) (Nguyen et al. 2010). There is increasing evidence of venular abnormalities in lacunar stroke and altered venular function may underlie lacunar stroke but the venular signs may simply be a sign of the presence of the process that leads to lacunar stroke. At present we would recommend that future research include venular pathology.

10.7 The role of fractals in assessment of the retinal vasculature

When initially presented, the data presented in chapter 8 of this thesis regarding the use of fractals were novel and preliminary. They suggest that patients with lacunar stroke have less complex branching patterns of the retinal vasculature. It is difficult to draw exact pathological correlations from these findings but they reinforce the concept that lacunar stroke is caused by a distinct vasculopathy. Ideally these results
would be replicated elsewhere by another centre. Subsequently our MCRS collaborators have presented data in a subgroup of their cohort study showing that patients with lacunar stroke had increased monofractal dimensions compared to patients with non-lacunar stroke (Cheung et al. 2010), the opposite to our finding of decreased fractal dimensions with lacunar stroke although they also showed that increasing age was associated with decreased fractal dimension. This apparently contradictory result reinforces the nascent nature of retinal fractal associations but it is worthwhile investigating possible causes for this discrepancy.

There are important differences between the two cohorts of patients. Cheung report on a subgroup of 392 patients from a total of 1321 patients recruited for the Multicentre Retina and Stroke Study (Lindley et al. 2009). They used the TOAST criteria to subtype ischaemic stroke. There were 79 patients with lacunar stroke who were compared to patients with non-lacunar stroke. The authors do not detail how many of the non-lacunar stroke were cardio-embolic, large artery, mixed, rare or unknown aetiology. Patients either had a MRI or CT as the brain investigation— but the proportions or reasons for choosing either were not given (the rate of DWI was approximately 40% in the entire MCRS from previous publications ie approximately 30/79 lacunar patients in this sub study). Reliance on CT for the rest will have resulted in noise due to incorrect classification of stroke subtype. All patients presented in this thesis had brain MRI at presentation to subtype stroke and all patients either had a lacunar or cortical stroke using non-risk factor based definitions. Cheung et al report supplementary analysis for patients with MRI but is not clear if these analyses are corrected for age and other vascular risk factors and, given the small number of patients and outcomes it is possible that these regression models are overfitted. These features may explain the differing inter study results.

Is there any evidence from other sources which may indicate which direction fractal dimensions may differ in the presence of cerebral small vessel disease? No other studies have reported on retinal fractal dimensions in stroke but there is evidence of inconsistent associations between retinopathy and fractal dimensions even when the same semi-automated fractal technique is used: Cheung reported that increased
fractal dimension was associated with prevalent retinopathy in young type I diabetic patients (Cheung et al. 2009) but Lim in the same cohort found that fractal dimension did not predict incident retinopathy (Lim et al. 2009). Using the same technique for assessing fractal dimensions, Grauslund found that decreased fractal dimension was associated with prevalent diabetic retinopathy in middle aged adults with type I diabetes (Grauslund et al. 2010). Studies that were performed prior to the recent advent of semi-automated fractal analysis – that were much smaller – again found conflicting results: Avakian found decreased fractal dimension with retinopathy in 5 patients (Avakian et al. 2002) but Kunicki in 35 patients found no relationship between fractal dimension and diabetic retinopathy (Kunicki et al. 2009).

Sng (Sng et al. 2010) investigated retinal fractal dimension and chronic kidney impairment and found that although retinal fractals were lower in those with kidney disease there was evidence for a U shaped relationship between fractal dimension and renal disease. It was suggested that there may be an optimal fractal dimension and that deviation away from this optimum may be associated with the presence of kidney disease. There have been no other fully published papers that support this notion although preliminary data presented in abstract form hints that the presence of retinopathy in non-diabetic patients may be associated with extremes of fractal dimensions (Mitchell et al. 2010). Clearly more work is needed to assess associations and the eventual clinical utility of fractal analysis of the retinal vessels.

10.8 Pathophysiological implications of the work in this thesis

The results in this thesis demonstrate that there are retinal microvascular differences between lacunar and cortical stroke, and perhaps between deep and periventricular white matter hyperintensities on cerebral MRI that are likely to mirror differences in small vessels in the brain. Evidently more work is needed to fully characterise these abnormalities and to ascertain whether they confer clinical risk.
Traditional research into the causes of lacunar stroke has concentrated on the role of the arterioles in an attempt to identify the nature of the underlying arteriopathy. We have demonstrated that venular widths differ between lacunar and cortical stroke which, coupled with the association we demonstrated between enlarged perivascular spaces and lacunar stroke suggests that venular disease may play a prominent role in, or be a major marker for the aetiology of cerebral small vessel disease.

10.9 Clinical implications arising from this thesis

The results in this thesis will not directly alter clinical behaviour although these results reinforce the hypothesis that conventional atherothromboembolic mechanisms do not cause lacunar stroke. If a novel mechanism is responsible for causing lacunar stroke that this subtype of stroke may be treated differently from other subtypes but it would be speculative and is beyond the scope of this thesis to predict how these treatments may differ. There are ongoing clinical trials to assess whether lacunar strokes specifically benefit from conventional secondary stroke prevention strategies (Benavente and Hart 2003). Alternatively many secondary preventative drugs eg statins, ACE inhibitors have other effects such as on the endothelium and this may be effective even if via a different mechanism.

Other than using the retinal vessels as markers of the cerebral vessels to elucidate the underlying pathophysiology are there other applications for assessing the retinal microvasculature? There is current interest in using retinal abnormalities as independent predictors of future and current retinal and systemic disease (McGeechan et al. 2009a). Although retinal micro vessels are perhaps most closely linked to the cerebral small vessels they have been assessed as risk predictors or linked to for a variety of different non-stroke and cognitive clinical features including ischaemic heart disease (McGeechan et al. 2009b), psychological wellbeing (Jensen et al. 2009) and hearing loss (Liew et al. 2007b) to fish consumption (Kaushik et al. 2008). Although the results presented in this thesis are derived from a cross sectional study the techniques developed and tested in this
thesis, notably concerning vessel geometry and fractal analysis will contribute to the future use of novel retinal markers as risk predictors for incident disease.

10.10 Research implications arising from this thesis

These results contribute to the mounting evidence from other disciplines that lacunar stroke is caused by a distinct vasculopathy to other types of stroke. Lacunar stroke is difficult to study and fully elucidating the underlying aetiology will require close collaboration between researchers currently employing different methodologies in such diverse fields as epidemiology, animal models of disease, genetics, advanced brain imaging, retinal imaging, rheology and pathology. Although there has been a recent decline in the rate of autopsies performed in the UK (Bell 2004), post mortem studies in populations where hypertension has been controlled and with careful pre-morbid characterisation of stroke symptoms would be fascinating.

10.11 Mild Stroke Study Cohort follow up

The results presented in this thesis suggest several potential directions for further research. The results presented are cross sectional analyses and we are therefore only able to report associations without implying causation. In the first instance we have designed follow up for these patients clinically collecting data regarding functional outcome and repeat vascular events (stroke and myocardial infarction) and follow up MRI scans and relating these to original presenting clinical and retinal features. Separately we have established a second study where we are investigating cross sectional and longitudinal associations with blood brain barrier permeability. These results will enable us to investigate the predictive power of both retinal vascular abnormalities and blood brain barrier permeability with regard to both lacunar stroke and white matter disease.
10.12 Individual patient data meta-analysis

One of the limitations of our analysis of retinopathy in ischaemic stroke subtypes presented in chapter 4 of this thesis is that the sample size was powered to detect the presence of retinopathy as a combined outcome measure (comprising hard or soft exudates, microaneurysms or haemorrhages). As previously discussed this may have been too blunt a tool as the components of retinopathy may be caused by different factors. The design of this study and the database in particular was such that it would be easy to merge databases with our collaborators in Australia thus permitting individual patient data meta-analyses. We are planning this analysis which will, with its increased power, be able to study the prevalence of the components of retinopathy in ischaemic stroke subtypes.

10.13 Other retinal markers of disease

We have shown associations between certain retinal vascular markers but other investigators have studied other markers such as vessel tortuosity. From the early pathological studies of lacunar stroke it was suggested that arterioles were more tortuous (Fisher 1969) but it is difficult to quantify tortuosity. Previous studies have used the subjective view of an ophthalmologist to ascertain degree of tortuosity against standardised scoring techniques (Kwa et al. 2002) or used semi-automated software to quantify tortuosity (Witt et al. 2006). We are currently developing software to assess retinal arteriolar tortuosity and plan to analyse this parameter in the cohort of patients with mild stroke.

As reported in chapter 9 of this thesis, fractal analysis of the retinal vessels included both the arteriolar and venular trees because segmentation techniques cannot yet reliably distinguish arteriole from venule. We are developing techniques to overcome this difficulty and plan to analyse fractal dimensions of the separate vascular trees.
In our assessment of retinal vascular geometry we had originally concentrated on arteriolar disease as in keeping with other groups we felt the likely pathology was probably arteriolar. The results from our other investigations challenge this assertion and suggest that venular pathology may also be important. Other groups have recently presented abnormalities in venular geometry in preadolescent children (Bayu et al. 2010) and there would be scope to investigate venular geometrical abnormalities in our cohort of stroke patients.

10.14 Associations with serum markers of inflammation, fibrinolysis and coagulation.

In a subset of 125 patients in the Mild Stroke Study (59 patients with cortical stroke and 66 patients with lacunar stroke) we drew serum samples at a median time of 54 days post stroke. These have been analysed in a collaboration with Prof Gordon Lowe in the University of Glasgow for the following serum markers: von-Willebrand factor (vWF), D-dimer, fibrinogen, tissue plasminogen activator antigen(t-PA), tumour necrosis factor alpha (TNF alpha), C-reactive protein (CRP), intracellular adhesion molecule (ICAM) and interleukin-6 (IL-6). We plan to investigate associations between these serum markers with both MRI markers of small vessel disease and retinal vascular abnormalities.

10.15 Summary

We have clearly demonstrated that retinal microvascular abnormalities differ between lacunar and cortical stroke suggesting that a distinct small vessel vasculopathy may cause lacunar stroke. We have also identified MR markers of lacunar stroke. These results suggest that venular disease (a hitherto under-researched area) may play a role in the pathophysiology of lacunar stroke. Retinal microvascular abnormalities can act as markers for cerebral small vessel disease. We plan collaborative analyses with colleagues who have performed similar studies to further assess retinal abnormalities in lacunar stroke and have followed up this cohort to assess longitudinal associations with retinal abnormalities.
Appendix 1. Medline search strategy for systematic review of serum markers of endothelial dysfunction in lacunar stroke.

1. brain ischemia/ or brain infarction/ or brain stem infarctions/ or cerebral infarction/ or hypoxia-ischemia, brain/ or stroke/ 
2. (isch?emi$ adj6 (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or cva or attack$)).tw.
3. ((brain or cerebr$ or cerebell$ or vertebrobasil$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$)).tw.
4. 1 or 2 or 3 
5. (lacun$ or small vessel$ or small infarct$ or microinfarct$ or subcortical lesion$ or subcortical infarct$ or microvascular$ or microcirculation$).tw.
6. 4 and 5 
7. blood-brain barrier/ or endothel$, vascular/ or tunica intima/ or microcirculation/
8. (endotheli$ adj5 (function$ or dysfunction$ or impairment$)).tw.
9. (endogenous tissue plasminogen activator or endogenous tPA).tw
10. thrombosis.tw
11. fibrinogen.tw
12. fibrinolysis.tw
13. homocysteine.tw
14. (ICAM or Intra cellular adhesion molecule).tw
15. (VCAM or Vascular Cell Adhesion Molecule).tw
16. (IL6 or Inter leukin 6).tw
17. (CRP or C reactive protein).tw
18. von Willebrand factor.tw
19. plasminogen activator inhibitor.tw
20. selectin$.tw
21. D-dimer.tw
22. or/7-21
23. 6 and 22
24. limit 23 to humans

1. brain ischemia/ or brain infarction/ or brain stem infarctions/ or cerebral infarction/ or hypoxia-ischemia, brain/ or stroke/
2. (isch?em$ adj6 (stroke$ or apoplect$ or cerebral vasc$ or cerebrovasc$ or cva or attack$)).tw.
3. (brain or cerebr$ or cerebell$ or vertebrobasilar$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation) adj5 (isch?em$ or infarct$ or thrombo$ or embol$ or occlus$ or hypoxi $)).tw.
4. 1 or 2 or 3
5. (lacun$ or small vessel$ or small infarct$ or microinfarct$ or subcortical lesion$ or subcortical infarct$ or microvascular$ or microcirculation$).tw.
6. 4 and 5
7. blood-brain barrier/ or endothel$, vascular/ or tunica intima/ or microcirculation/
8. (endothel$ adj5 (function$ or dysfunction$ or impairment$)).tw.
9. ((vascular or capillary) adj5 endothel$).tw.
10. (endothel$ adj5 (contraction or relaxation)).tw.
11. vascular tone/ or arterial stiffness: mp. [mp=title, original title, abstract, name of substance word, subject heading word]
12. (vascul$ tone or neurovasc$ or coupl$ or arterial stiff$ or vascul$ remodel$ or cerebrovascular reactiv$ or cerebral autoregulation) tw.
13. (Flow mediated adj3 (dilat$ or vasodilat$)).tw.
14. exp Ultrasoundography, Doppler, Transcranial/
15. pulse wave analysis.tw.
16. strain gauge plethysmography.tw.
17. (brachial artery or radial artery or popliteal artery or posterior tibial artery).tw.
18. or/7-17
19. 6 and 18
20. limit 19 to yr='1995 - 2008'
21. limit 19 to humans
22. limit 21 to humans
23. from 22 keep 1-376
24. (strain gauge plethysmography or venous occlusion plethysmography) tw.
25. forearm blood flow.tw.
26. (dorsal hand vein technique or actig technique).tw.
27. stimulated tPA release.tw.
28. or/24-27
29. 18 or 28
Appendix 3. Patient information leaflet for Mild Stroke Study

Patient Information:
The Mild Stroke Study

What is the study about?
We are investigating patients who have had a mild stroke. We are trying to find out why the different types of mild stroke happen. We aim to find links between brain scan and retinal (back of the eye) appearances. This will hopefully mean that in the future we will be able to better understand, prevent and treat patients who have had mild strokes.

Why have I been asked to take part?
You have been asked because you have recently had a mild stroke.

What will the study involve?
If you agree to be in the study we will change the type of brain scan we do from a CT scan to MRI scan. MRI scans give more detailed pictures. We will also take photographs of your retina (the back of the eye) and a blood sample.

For the brain scans you will need to lie flat and still on your back on a table. The table will move into the scanner so that only your head is in the scanner. The rest of your body is outside the scanner.

For the retinal photographs you will need to sit opposite a camera. We may put eye drops into your eye to make the pupil bigger. You will not be able to drive for four hours after the eye drops are put in.

How long will it take?
For the brain scan you will need to lie down in the scanner for twenty to thirty minutes. The retinal photographs will take about ten minutes.
What is the benefit to me?
You will have a more detailed scan than you would normally have. This will help you and the doctors looking after you. You will also be helping future patients who may have a stroke.

Are there any bad points?
All of the tests performed are relatively safe. Occasionally patients feel claustrophobic in the brain scan and we stop the scan if this is the case. Certain patients cannot go into the scanner and we ask all patients about this—for example if the patient has a pacemaker. I will take a small blood sample from you.

How will I travel to the hospital?
If you can have a lift from a relative or friend I can reserve you a parking space in the hospital. If this is not possible then I will arrange a taxi which we will pay for.

What happens if you find something abnormal on the scans?
We write to your General Practitioner anyway to inform them that you are in the study. If we find any other abnormal results we will write another letter to your GP.

What do you do with the scans?
We store the scans and may share anonymous scans with our other researchers.

Can I withdraw at any time?
Yes.

Will being in the trial affect my medical care?
You may get better care as the quality of the scan used is better than normal. But otherwise your quality of care will remain unchanged.

How can I find out the results of the trial?
If you would like to find out we will send you a letter detailing what the results are when the study is finished.

Can I discuss the trial with another doctor?
You can, if you wish, discuss whether or not to have the scans with one of our doctors who is not involved in the research and can give independent advice. His name is Professor Charles Wardlaw, Professor of Neurology, Department of Clinical Neurosciences, Western General Hospital. Please ask if you wish to speak to him or phone 537 1000 to contact him by phone.

If you have any further questions about the study, even after you have left the hospital, you may contact:
Dr. Fergus Dougal—tel number—07789 752200/0131 537 2508
or
Professor Joanna Wardlaw—0131-537-3110

Thank you for taking the time to read this leaflet and considering the study.
Appendix 4. Patient consent form

MAGNETIC RESONANCE IMAGING AND ULTRASOUND STUDIES IN LACUNAR STROKE

PATIENT'S CONSENT FORM

I have read the information leaflet "Magnetic Resonance and Ultrasound Imaging Studies in Lacunar Stroke". I have had time to consider the study and have had all my questions about it answered.

[ ]

I understand that I am free to withdraw at any time from any part of the study, without giving a reason, and without it adversely affecting my future medical care.

[ ]

I agree to take part in the above study.

[ ]

I agree that my anonymous retinal photographs may be shared with other researchers.

[ ]

Signed: .............................................. (patient's signature)
Name: .................................................... (patient's name)
Date: .....................................................

Signed: .............................................. (signature of the investigator)
Name: .................................................... (name of the Investigator)
Date: .....................................................

Please Attach Patient Label

When signed, Please attach this form to patient’s MR request card

Lacunar Stroke Dec 2009
Appendix 5.  Patient data collection sheet.

**Mild Stroke Study Data Collection Form**

(Amended for data punching – if there is a line then answer is numerical and if a box it is a tick – if unticked assume no)

- **Patient Name**: ______________  **DOB**: __/__/  **Sex**: M □ F □
- **CN number**: ______________  **Date of assessment for study**: __/__/___

**PMH of**

- **TIA □**
- **Stroke □ (left □ right □ both □ infarct □ haem □ cortical □ lacunar □)**
- **Ischaemic Heart Disease □ (angina □ MI □ angioplasty □ CABG □)**
- **Peripheral Vascular Disease (or symptoms of) □**
- **Diabetes Mellitus □ (diagnosed at presentation □)**
- **Hypertension □**
- **Atrial Fibr □**
- **Hyperlipidaemia □**
- **LVSD □**
- **Structural Heart Disease □**

**Social and family history**

- **Family history of stroke □**
- **Cigarette smoker □ (ex<12 months □, ex>12 months □, never □)**
- **Alcohol use □ - units per week**: ______  **Previous alcohol excess □**
- **Independent prior to stroke □**

**Details of presenting stroke**

- **Date and time of symptom onset**: __/__/____ - ____
- **Duration of symptoms**: ______ days or persisting
- **Date patient seen by stroke service**: __/__/____
- **Side of body/vision affected**: Left □ Right □

- **Weakness □ (face □, arm □, leg □)**
- **Sensory abnormality □ (face □, arm □, leg □)**
- **Posterior circulation symptoms □**
- **Dysphasia □  Neglect □**
- **Visual field loss □**

- **Right □ Left □ Handed**
- **NIHSS score at assessment**: ______ and estimated worst ______
**Investigations at time of presenting stroke**

<table>
<thead>
<tr>
<th>Total cholesterol</th>
<th>Blood glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECG - sinus</td>
<td>Atrial Fibrillation</td>
</tr>
<tr>
<td>Carotid doppler, MRA - Left ICA stenosis</td>
<td>____%</td>
</tr>
<tr>
<td>Echocardiology abnormality</td>
<td>If so complete free text</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td><strong><strong>/</strong></strong> mmHg</td>
</tr>
</tbody>
</table>

**Medications at time of stroke**

<table>
<thead>
<tr>
<th>Aspirin</th>
<th>Dipyridamole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clopidogrel</td>
<td>Clopidogrel</td>
</tr>
<tr>
<td>Warfarin</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diuretic</th>
<th>ACE inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II R. antagonist</td>
<td>Angiotensin II R. antagonist</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>Beta blocker</td>
</tr>
<tr>
<td>Other antihypertensive</td>
<td>Other antihypertensive</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oral Hypoglycaemic</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statin</td>
<td>Statin</td>
</tr>
<tr>
<td>Other cholesterol medication</td>
<td>Other cholesterol medication</td>
</tr>
</tbody>
</table>

**Clinical Classification** – Lacunar  
**Imaging Classification** – Lacunar  
**Final Clinical and Imaging Classification** – Lacunar  

Free text comments:
## MRI NVSI grading for Mild Stroke Study

Patient CN: ___________ Date/time of MR: ___________

Sequences done (underline): DWI/T2/FLAIR/GRE/T1/OTHER

**Relevant lesion present (age and location):** Yes/No

Sequence on which lesion present: DWI - Yes/No  FLAIR/T2 - Yes/No

Side of brain: **Left/Right**

<table>
<thead>
<tr>
<th>Lacunar (size mm □)</th>
<th>MCA Cortical</th>
<th>Other Cortical</th>
</tr>
</thead>
<tbody>
<tr>
<td>internal capsule □</td>
<td>small temporal □</td>
<td>anterior ACA □</td>
</tr>
<tr>
<td>internal border zone □</td>
<td>small parietal □</td>
<td>posterior ACA □</td>
</tr>
<tr>
<td>centrum semiovale □</td>
<td>basal ganglia □</td>
<td>anterior PCA □</td>
</tr>
<tr>
<td>extends to cortical margin □</td>
<td>sub-cortical □</td>
<td>posterior PCA □</td>
</tr>
<tr>
<td>thalamus □</td>
<td>ant. half periph. MCA □</td>
<td>ant. border zone □</td>
</tr>
<tr>
<td>lentiform □</td>
<td>post. half periph. MCA □</td>
<td>post. border zone □</td>
</tr>
<tr>
<td>brainstem □</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Old lesion(s) present: Yes/No**

<table>
<thead>
<tr>
<th>Left/Right</th>
<th>Side □</th>
<th>Side □</th>
<th>Side □</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lac/Cortical/Post Location □</td>
<td>Location □</td>
<td>Location □</td>
<td></td>
</tr>
<tr>
<td>Infarct/Haemorrhage Type □</td>
<td>Type □</td>
<td>Type □</td>
<td></td>
</tr>
</tbody>
</table>

Number of microhaemorrhages seen □

**White matter rating**

<table>
<thead>
<tr>
<th>Overall</th>
<th>Left</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periventricular lesions - Fazekas 1/2/3 □</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Deep white matter lesions - Fazekas 1/2/3 □</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>De Leeuw n&lt;3mm □</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>De Leeuw n=3-10mm □</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>De Leeuw n&gt;10mm □</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Enlarged perivascular spaces Basal ganglia 0-4 □</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Centrum semiovale 0-4 □</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

**Deep atrophy**

None/Mild/Moderate/Severe

**Superficial atrophy**

None/Mild/Moderate/Severe

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### Appendix 7. Retinal vascular grading form.

#### Mild Stroke Study Retinal Vascular Grading Form

<table>
<thead>
<tr>
<th>Field</th>
<th>Macula</th>
<th>Disc</th>
<th>Lat Mac</th>
<th>Upper Arcade</th>
<th>Lower Arcade</th>
<th>Nasal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ungradable H1</td>
<td>CL</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>PQ</td>
<td>2</td>
<td>PQ</td>
<td>PQ</td>
<td>PQ</td>
<td>PQ</td>
</tr>
<tr>
<td>Missing H2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Focal Art Narrow</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Questionable</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Yes Mild</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>AVN</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Questionable</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Yes Mild</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>No of crossings</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Questionable</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>CG</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Haemorrhage/microaneurysms</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Questionable</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>CG</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Soft Exudates (overall)</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Questionable</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>CG</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
Appendix 8. Medline search strategy for retinal microvascular abnormalities in stroke systematic review.

On stroke/TIA/asymptomatic stroke:
1. exp Ischemic Attack, Transient
2. cerebrovascular disorders/
3. exp brain ischemia/
4. cerebrovascular accident/
5. exp brain infarction/
6. exp hypoxia-ischemia, brain/
7. exp "Intracerebral Embolism and Thrombosis"/
8. exp intracranial hemorrhages/
9. (stroke or cwa$ or isch?em$ attack$ or tia$1 or neurologic$ deficit$).tw.
10. ((brain$ or cerebr$ or cerebell$ or cortical or vertebrobasilar or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or MCA or anterior circulation or posterior circulation or basal ganglia) adj10 (isch?em$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypox$ or vaso$ or obstruction or vasculopathy)).tw.
11. ((lacunar or cortical) adj5 infarct$).tw.
12. ((cerebr$ or cerebell$ or intracerebral or intracran$ or parenchymal or intraventricular or infratentorial or supratentorial or basal ganglia$ or putaminal or putamen or posterior fossa) adj10 (haemorrhage$ or hemorrhage$ or bleed$)).tw.

On retinal abnormalities:
13. exp *Retina/
14. exp *Retinal Vessels/
15. exp *Retinal Artery/
16. exp *Retinal Vein/
17. exp *Retinal Diseases/
18. exp *Retinal Hemorrhage/
19. exp *Retinal Artery Occlusion/
20. exp *Papilloedema
21. (retina$ or retinopath$ or retinal abnormal$).tw

Overall search:
22. or1-12
23. or13-21
24. 22 and 23


Andersson T. What do white matter hyperintensities really represent? Stroke 2010; 41: 574.


Chowdhury D, Wardlaw JM, Dennis MS. Are multiple lacunar infarctions caused by embolic mechanisms? J Neurol Neurosurg Psychiatry 2004; 75: 1416-1420.


Davis SM, Donnan GA. Why lacunar syndromes are different and important. Stroke 2004; 35: 1779-1780.


Ikram MK, De Jong FJ, Van Dijk EJ et al. Retinal vessel diameters and cerebral small vessel disease: the Rotterdam Scan Study. Brain 2006b; 129: 182-188.


Lansberg MG, Norbash AM, Marks MP, Tong DC, Moseley ME, Albers GW. Advantages of adding diffusion-weighted magnetic resonance imaging to conventional magnetic resonance imaging for evaluating acute stroke. Arch Neurol 2000b; 57: 1311-1316.


Mead GE, Lewis SC, Wardlaw JM, Dennis MS, Warlow CP. How well does the Oxfordshire community stroke project classification predict the site and size of the infarct on brain imaging? J Neurol Neurosurg Psychiatry 2000; 68: 558-562.


Norrving B. Lacunar infarcts: no black holes in the brain are benign. Pract Neurol 2008; 8: 222-228.


Poirier J, Derouesne C. [The concept of cerebral lacunae from 1838 to the present]. Rev Neurol (Paris) 1985; 141: 3-17.


Scher AI, Gudmundsson LS, Sigurdsson S et al. Migraine Headache in Middle Age and Late-Life Brain Infarcts. JAMA 2009; 301: 2563-2570.


Schulz UGR, Briley D, Meagher T, Molyneux A, Rothwell PM. Abnormalities on diffusion weighted magnetic resonance imaging performed several weeks after a minor stroke or transient ischaemic attack. J Neurol Neurosurg Psychiatry 2003; 74: 734-738.


Starr JM, Wardlaw J, Ferguson K, MacLullich A, Deary IJ, Marshall I. Increased blood-brain barrier permeability in type II diabetes demonstrated by gadolinium magnetic resonance imaging. J Neurol Neurosurg Psychiatry 2003; 74: 70-76.


Tadjfar M. Branch angle and flow into a symmetric bifurcation. J Biomech Eng 2004; 126: 516-518.


ten Dam VH, van den Heuvel DMJ, deCraen AJM et al. Decline in total cerebral blood flow is linked with increase in periventricular but not deep white matter hyperintensities. Radiology 2007; 243: 198-203.


Wardlaw JM, Lewis SC, Keir SL, Dennis MS, Shenkin S. Cerebral microbleeds are associated with lacunar stroke defined clinically and radiologically, independently of white matter lesions. Stroke 2006; 37: 2633-2636.


