Cerebral small vessel disease: mechanistic insights, ethnic differences and prognostic value

Thesis submitted for the degree of D.Phil.

Dr Gary Kui Kai Lau

St Edmund Hall

Nuffield Department of Clinical Neurosciences

University of Oxford

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To Mum, Dad, Linda and Timothy

For their unconditional love, support and encouragement

ABSTRACT

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Small vessel disease (SVD) accounts for approximately 25% of all strokes and 45% of all dementias. Although the small vessels cannot be visualised with conventional neuroimaging, the pathological changes in the cerebral white and deep grey matter secondary to SVD has been adopted as markers of SVD. These are best appreciated with magnetic resonance imaging (MRI) and includes recent small subcortical infarcts, white matter hyperintensity (WMH), lacunes, cerebral microbleeds and enlarged perivascular spaces (PVSs). There are however a number of outstanding questions regarding these surrogate neuroimaging markers of SVD and how these markers may influence clinical management.

First, although a high burden of microbleeds have been associated with an increased risk of intracerebral haemorrhage (ICH) and possibly recurrent ischaemic stroke in patients with TIA or ischaemic stroke, how microbleeds should influence antithrombotic treatment use after TIA or ischaemic stroke remains uncertain. Second, the long-term prognostic implications of enlarged PVSs in patients with TIA or ischaemic stroke have not been studied. Third, although previous studies have shown possible ethnic differences in prevalence of microbleeds, whether there are any ethnic differences in prevalence of other neuroimaging markers of SVD remains unclear. Fourth, although a Total SVD Score was recently proposed to measure the global SVD burden, the prognostic value of this score in patients with TIA or ischaemic stroke has yet to be studied. Fifth, the relationships of long-term premorbid blood pressure with global SVD burden is unknown. Finally, the age and sex specific associations between renal impairment, carotid and cerebral pulsatility with burden of SVD has yet to be studied.

The aim of my thesis was therefore to determine the clinical correlates, ethnic differences and long-term prognostic implications of a range of neuroimaging markers and global burden of SVD. I also aimed to determine the relationships of global SVD burden with long-term mean premorbid blood pressure, renal impairment and carotid pulsatiltiy.

I have collected, collated and analysed clinical and neuroimaging data from two independent cohorts - the Oxford Vascular Study (OXVASC) and The University of Hong Kong (HKU). In particular I worked as one of the Clinical Research Fellows at OXVASC and was involved in regular recruitment, assessment and follow up of study patients. In OXVASC, 1080 predominantly Caucasians with TIA or ischaemic stroke who had a cerebral MRI performed at baseline was recruited during 2004 to 2014. I interpreted all these MRIs, specifically coding the burden of microbleeds, enlarged perivascular spaces and lacunes. I was involved in obtaining funding and developing the HKU cohort, which includes 1003 predominantly Chinese with ischaemic stroke recruited during 2008-2014 who had a cerebral MRI performed at baseline. I saw about 25% of the patients in the cohort and was involved in interpreting all of the MRIs of the cohort. All patients from both cohorts were followed-up regularly and adverse events including recurrent ischaemic stroke and ICH was determined. Presence and burden of periventricular and subcortical WMH, lacunes, microbleeds, basal ganglia and centrum semiovale PVSs was determined for all patients and the global burden of SVD estimated according to the Total SVD Score.

There are several clinically relevant findings in this thesis. First, I have shown that in Caucasians and Chinese with ≥5 microbleeds, withholding antiplatelet drugs during the first year after TIA or ischaemic stroke may be inappropriate, especially early after TIA. However, the risk of ICH is likely to outweigh any benefit thereafter. Second, I have shown that TIA or ischaemic stroke patients with microbleeds on warfarin had an increased risk of subsequent ICH. However, this risk was not different from that of antiplatelet users with microbleeds. Third, I have shown that a high burden of MRI-visible basal ganglia PVSs is independently associated with an increased risk of recurrent ischaemic stroke, but not ICH. However, the prognostic value of MRI-visible centrum semiovale PVSs in the TIA or ischaemic stroke population is limited. Fourth, I demonstrated significant ethnic differences in underlying prevalence and burden of neuroimaging markers of SVD – Chinese had a greater prevalence of microbleeds, lacunes and subcortical WMH, whilst Caucasians had a greater prevalence of periventricular WMH and PVSs. Fifth, I validated the Total SVD Score and showed that the SVD Score is able to predict risk of recurrent ischaemic stroke and ICH in Caucasians and Chinese, but is unable to identify patients at high risk of ICH from those at high risk of recurrent ischaemic stroke. Sixth, I showed that mean premorbid blood pressure, especially diastolic blood pressure measurements taken 10-20 years prior to TIA or ischaemic stroke was most strongly associated with global SVD burden suggesting a latency effect of hypertension on the pathogenesis of SVD. Finally, I demonstrated age-specific associations between renal impairment, internal carotid artery pulsatility index and SVD burden.

DECLARATION

I certify that this thesis entitled “Cerebral small vessel disease: mechanistic insights, ethnic differences and prognostic value” was performed whilst I was a full-time postgraduate student at the University of Oxford.

I declare that this thesis is of my own composition, and the research contained herein is my own original work under the supervision of Professor Peter Rothwell. No portion of this work has been submitted in support of an application for any other degree.

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PUBLICATIONS AND PRESENTATIONS

The work in this thesis has led to the following publications and presentations:

Publications:

Lau KK, Li L, Schulz, U, Simoni M, Chan KH, Ho SL, Cheung RTF, Küker W, Mak HKF, Rothwell PM.Total small vessel disease score and risk of recurrent stroke: validation in 2 large cohorts. Neurology 2017; 88(24);2260-2267

Lau KK, Li L, Lovelock CE, Zamboni G, Chan TT, Chiang MF, Lo KT, Küker W, Mak HK, Rothwell PM. Clinical correlates, ethnic differences and prognostic implications of perivascular spaces in transient ischaemic attack and ischaemic stroke. Stroke 2017; 48(6):1470-1477

Lau KK, Wong YK, Teo KC, Chang RSK, Tse MY, Hoi CP, Chan CY, Chan OL, Cheung RHK, Wong EKM, Kwan JSK, Hui ES, Mak HKF. Long-term prognostic implications of cerebral microbleeds in Chinese with ischaemic stroke. J Am Heart Association 2017 Dec 7;6(12). pii:e007360

Lau KK, Lovelock CE, Li L, Simoni M, Küker W, Mak HKF, Rothwell PM. Antiplatelet treatment after TIA and ischaemic stroke in patients with cerebral microbleeds: time-course and severity of recurrent stroke in Caucasian and Chinese cohorts. Stroke (accepted)

Lau KK, Pego P, Mazzucco S, Li L, Howard DPJ, Küker W, Rothwell PM. Age and sex-specific associations of carotid pulsatility with small vessel disease burden in TIA and ischaemic stroke (submitted)

Lau KK, Li L, Simoni M, Mehta Z, Küker W, Rothwell PM. Long-term premorbid blood pressure and cerebral small vessel disease burden on imaging in TIA and ischaemic stroke: population-based study (submitted)

Liu B, **Lau KK**, Li L, Lovelock CE, Liu M, Küker W, Rothwell PM. Age-specific associations of renal impairment with MRI markers of cerebral small vessel disease in TIA and stroke. Stroke (in press)

Presentations:

Lau KK, Li L, Schulz U, Simoni S, Chan KH, Ho SL, Cheung RTF, Küker W, Mak HKF, Rothwell PM. Total small vessel disease score and risk of recurrent stroke – validation in two large cohorts. Presented at the NIHR Stroke Research Workshop, Cambridge, September 2017

Lau KK, Li L, Schulz U, Simoni S, Chan KH, Ho SL, Cheung RTF, Küker W, Mak HKF, Rothwell PM. Total small vessel disease score and risk of recurrent stroke – validation in two large cohorts. Presented at the European Stroke Organisation Conference, Prague, May 2017

Lau KK, Li L, Lovelock C, Zamboni G, Chan TT, Chiang MF, Lo KT, Küker W, Mak HKF, Rothwell PM. Clinical correlates, ethnic differences and prognostic implications of perivascular spaces in TIA and ischaemic stroke. Presented at the European Stroke Organisation Conference, Prague, May 2017

Lau KK, Li L, Lovelock CE, Simoni M, Schulz U, Zamboni G, Wong YK, Teo KC, Küker W, Mak HKF, Rothwell PM. Prognostic implications of neuroimaging markers of small vessel disease in patients with transient ischemic attack and ischemic stroke. Presented at Thomas Willis Day, Department of Clinical Neurosciences, University of Oxford, April 2017

Lau KK, YK Wong, Teo KC, Li OY, Chan J, Chan S, Cheung R, Wong E, Wong E, Ho SL, Mak HKF, Rothwell PM. Clinical correlates and prognostic implications of cerebral microbleeds detected by susceptibility weighted imaging in Chinese with ischaemic stroke. Presented at the European Stroke Organisation Conference, Glasgow, April 2015.

Lau KK, YK Wong, Teo KC, Li OY, Chan J, Chan S, Cheung R, Wong E, Wong E, Ho SL, Mak HKF, Rothwell PM. Clinical correlates and prognostic implications of cerebral microbleeds detected by susceptibility weighted imaging in Chinese with ischaemic stroke. Presented at the International Symposium of Healthy Aging, Hong Kong, March 2015.

Books:

Gary KK Lau, Sarah T Pendlebury and Peter M Rothwell. Transient Ischemic Attack and Stroke: Diagnosis, Investigation and Management (Second Edition, Cambridge University Press) (in press)

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ABBREVIATIONS

ADC	apparent diffusion coefficient
BP	blood pressure
BG	basal ganglia
CAA	cerebral amyloid angiopathy
CADASIL	cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CCA	common carotid artery
CI	confidence interval
CKD-EPI	Chronic Kidney Disease Epidemiology
CS	centrum semiovale
CT	computed tomography
CVR	cerebrovascular reactivity
DBP	diastolic blood pressure
DKI	diffusion kurtosis imaging
DTI	diffusion tensor imaging
DWI	diffusion weighted imaging
FA	fractional anisotropy
FLAIR	fluid attenuation inversion recovery
GRE	gradient echo
GFR	glomerular filtration rate
HKU	University of Hong Kong
HR	hazard ratio
ICA	internal carotid artery
ICH	intracerebral haemorrhage
IQR	interquartile range
MCA	middle cerebral artery
MD	mean diffusivity
MDRD	Modification of Diet in Renal Disease
MRI	magnetic resonance imaging
NAWM	normal appearing white matter
NNT	number needed to treat
NOAC	non-vitamin K antagonist oral anticoagulant

OR	odds ratio
OXVASC	Oxford Vascular Study
PI	pulsatility index
PVS	perivascular space
SBP	systolic blood pressure
SD	standard deviation
STRIVE	Standards for Reporting Vascular Changes on Neuroimaging
SVD	small vessel disease
SWI	susceptibility weighted imaging
TCD	transcranial Doppler
TIA	transient ischaemic attack
TOAST	Trial of ORG 10172 in Acute Stroke Treatment
T	Tesla
WMH	white matter hyperintensity

Chapter 1

Introduction

1.1 Definition of cerebral small vessel disease

Cerebral small vessel disease (SVD) comprises a syndrome of clinical, cognitive, neuroimaging and neuropathological findings as a result of pathological processes affecting the cerebral microcirculation and predominantly affects the blood supply (small arteries, arterioles, capillaries; <800μm, and mostly <400μm) to tissue of the deep white and grey matter areas of the brain.[1, 2] Various manifestations of cerebral SVD will be briefly introduced below, but the main focus of this thesis will be on the mechanisms, ethnic differences and prognostic implications of a range of neuroimaging markers of cerebral SVD.

Cerebral arterioles can originate superficially, where they branch from arteries from the subarachnoid circulation to form cortico-leptomeningeal arterioles. They can also arise deep within the brain, where they stem directly from large arteries to form deep perforators. These two systems form the small vessel networks of the brain which is essential in maintaining the optimal functioning of some of the most metabolically active nuclei and white matter networks within the brain.[3]

As these small vessels cannot be visualised in vivo or with conventional neuroimaging such as with computed tomography (CT) or with magnetic resonance imaging (MRI), the pathological changes in the cerebral white matter secondary to SVD have been adopted as markers of SVD. Leukoaraiosis (attenuation of the white matter), lacunes and brain atrophy may be visualised on conventional CT. However, conventional structural MRI incorporating T1-weighted, T2-weighted, fluid attenuation inversion recovery (FLAIR) and haemosiderin sensitive sequences, such as T2*-gradient echo (GRE) and susceptibility-weighted imaging (SWI) would be able to best capture the full spectrum of neuroimaging markers of SVD such as recent small subcortical infarcts, white matter hyperintensity (WMH), lacunes, cerebral microbleeds, enlarged perivascular spaces (PVSs) and brain atrophy.

1.2 History of research on small vessel disease

The history of small vessel disease dates back to around the 19th century. Amédée Dechambre (1812-1886), a French physician in Paris, first reported in 1838 in the Gazette Médicale de Paris the post-mortem pathological findings in the subcortical white matter of a patient who recovered from a stroke. He described in this patient, 'a number of small lacunes of variable size and forms, more or less filled with milky fluid…' ('la lacune' in French means a gap or empty space, and in Latin, 'lacuna', a pit or hole). Several years later in 1842, Maxine Durand-Fardel (1816-1899) confirmed Dechambre's fndings and applied the term 'lacunes' to deep, healed, small infarcts and the term 'l'état cribalé' (meaning 'tissue riddled with holes') to describe the dilatation of visible perivascular spaces associated with SVD. In 1894, Alois Alzheimer and Otto Binswanger described an arteriosclerotic form of brain atrophy characterized by 'miliary apoplexies' (suggestive of multiple lacunes) affecting the basal ganglia, internal capsule and white matter of the centrum semi-ovale.

Later on in 1901, Pierre Marie (1853-1940), whilst working at the Hospice for the Elderly near Paris, coined the term 'état lacunaire', where he described the clinical picture of recurrent motor deficits in the elderly, often with partial recovery, accompanied by pseudobulbar palsy, incontinence and small-steppage gait. He correlated these clinical presentations to pathological brain specimens which showed multiple lacunes. In the 1960s, Miller Fisher (1913-2012), assessed serial sections of blood vessels in autopsy specimens of patients with small deep seated infarcts.[4] He noted that the penetrating arteries that supplied the territory of these lacunar infarcts often contained focal enlargements and small haemorrhagic extravasations through the walls of the arteries. At times, subintimal foam cells were noted to obliterate the lumens, and pink-staining fibrinoid material were present within the vessel walls. In some regions, the penetrating arteries were replaced by whorls, tangles and wisps of connective tissue that obliterated the usual vascular layers. Fisher called these processes segmental arterial disorganization, fibrinoid degeneration and lipohyalinosis.[4] Many of these patients with lacunes at autopsy were noted to have underlying hypertension and Fisher subsequently described a number of lacunar syndromes with detailed anatomical and clinicopathological correlations.

With advances in neuroimaging techniques and introduction of the CT scanner in 1972 and MRI scanner in 1977, this has enabled researchers to study the mechanisms, risk factors, clinical correlates and prognostic significance of cerebral small vessel disease *in vivo*. In 1987, Vladimir Hachinski, Paul Potter and Harold Merskey coined the term 'leukoaraiosis' (meaning rarefaction of the white matter) to describe the loss of white matter density due to hypertension, as noted on CT.[5] MRI has also enabled lacunes, white matter lesions and perivascular spaces to be identified and with new MRI techniques, such as haem-sensitive T2*-gradient echo, cerebral microbleeds were first identified and described in 1996.[6-8]

1.3 Burden and clinical manifestations of cerebral small vessel disease

Cerebral SVD resulting in lacunar ischaemic strokes accounts for about 25% of all strokes worldwide and is a common cause of intracerebral haemorrhage (ICH) due to hypertensive or cerebral amyloid angiopathy (CAA).[9] Cerebral SVD is also the most common cause of vascular cognitive impairment, and contributes up to 45% of all dementias.[10] The burden of SVD on public health and cost to society is substantial.[11]

The clinical manifestations of cerebral SVD are broad and include sudden onset focal neurological symptoms or stroke syndromes, subjective cognitive impairment,[12] progressive cognitive decline[13] and depression.[14] However, presentations of SVD can also be subtle and by disrupting white matter tracts connecting important sensory and motor regions, may present with mild and often ignored neurological symptoms and signs,[15] gait and balance disturbances[16] and bladder dysfunction.

1.4 Classification and mechanisms leading to cerebral small vessel disease

An aetiological classification system of cerebral SVD has been proposed by Pantoni, where type 1 refers to cerebral SVD due to arteriosclerosis (age-related and vascular risk factor-related

SVDs); type 2 - sporadic and hereditary CAA; type 3 – inherited or genetic SVDs [e.g. cerebral autosomal dominant arteriopathy with subcortical ischaemic strokes and leukoencephalopathy (CADASIL)]; type 4 – inflammatory and immunological mediated SVD (e.g. granlomatosis with polyangiitis and eosinophilic granulomatosis with polyangiits); type 5 – venous collagenosis and type 6 – other SVDs (e.g. post-radiation angiopathy).[1] The first two types of cerebral SVD are the most common.

1.4.1 Arteriosclerosis and blood brain barrier dysfunction

A number of pathological processes occur in age- and hypertension-related small vessel arteriopathy.

Atherosclerosis may affect distal vessels of diameter 200-800μm in size. Atheromatous microplaques may cause micro-occlusions within proximal perforating arteries (microatheromas), at their origin (junctional atheroma) or in the parent artery of the circle of Willis (mural atheroma).

Lipohyalinosis, originally defined by Fisher as ‘segmental arteriolar wall disorganization’ occurs in small, long, scarcely branching arteries of diameter 40 to 300μm in size.[17, 18] It is due to hypertension resulting in endothelial dysfunction, which causes leakage of plasma proteins through the damaged endothelium leading to thickening of the basal lamina and deposition of fibrohyaline material. There is also proliferation of smooth muscle cells from the tunica media. Perforating vessels arising directly from large vessels, e.g. the lenticulo-striate perforating branches of the middle cerebral artery, the thalamo-perforating branches of the proximal posterior cerebral artery, the perforating branches of the basilar artery and the vessels in the periventricular white matter are frequently affected.

Hyalinosis appears as a light eosinophilic amorphous wall in intracerebral small arteries of hypertensive patients. In contrast, fibrinoid necrosis describes the accumulation of amorphous, proteinaceous material in the tissue matrix with a staining pattern similar to fibrin. It occurs in the walls of arterioles in patients with malignant hypertension, but can also occur in patients with

immune-mediated vascluitides, pre-eclampsia and hyperacute transplant rejection. Fibrohyalinosis refers to perforating arteries with thickened hyalinized wall that contains areas of fibrosis.

Arteriolosclerosis affects arterioles of diameter 5 to 150 μm and causes hyaline thickening of vessel walls and concentric fibrohyalinosis. It occurs in the brain, but is also seen in other organs such as the kidneys and retinas. Arteriolosclerosis is strongly associated with ageing, hypertension and diabetes and is likely the underlying cause of leukoaraiosis in the elderly.[19-21] The decreased cerebral blood flow associated with arteriolosclerosis results in an accumulation of fibrous collagen within in the microvascular walls, including those within the white matter, causing thickening of the microvascular walls and narrowing of the vessel lumen.

Microaneurysms result from destruction of the elastic interna of smooth muscle cells by hyaline connective material. They occur at branching sites in vessels 100 to 300μm in diameter.

The mechanisms leading to these vessel wall changes remains uncertain but has largely been attributed to age and vascular risk factors, in particular hypertension.[20] However, age and vascular risk factors such as hypertension, diabetes mellitus, hyperlipidaemia and smoking appears to be equally common in patients with lacunar stroke and those with cortical atherothrombotic stroke.[22] Furthermore, non-hypertensive cases of SVD without genetic aetiology have also been described,[23] and randomised controlled trials of antihypertensive treatment have been minimally effective in slowing the progression of WMHs.[24, 25] These findings have led to the postulation that alternative mechanisms leading to cerebral SVD may be present.

In particular, recent studies have suggested that the blood brain barrier is likely to play a pivotal role in the pathogenesis and development of cerebral SVD.[2] The blood brain barrier is important in regulating materials that enters and leaves the brain. However, as we age, the cerebrovascular endothelium and hence, the blood brain barrier becomes more permeable.[26] The blood brain barrier also becomes more leaky in patients with acute stroke, cerebral neoplasms or traumatic brain injury. Blood brain barrier dysfunction also occurs in processes such as inflammation[27] and

oxidative stress[27] and increased permeability of the blood brain barrier with diffuse cerebrovascular endothelial failure may lead to leakage of plasma components and inflammatory cells into the vessel wall and perivascular tissue. Smooth muscle cells of arterioles may then be replaced by collagenous tissue, foamy macrophages and inflammatory cells resulting in lipohyalinosis. This subsequently results in thickening and narrowing of the vessel, arterial stiffness and impaired autoregulation, subsequently leading to the SVD changes seen on neuroimaging.[2] Studies have supported the hypothesis of blood brain barrier leakage in the pathogenesis of SVD by showing that the blood brain barrier is more leaky in the white matter in patients with lacunar stroke versus those with atherothromboembolic stroke. In patients with minor stroke, blood brain barrier was also noted to be more leaky in patients with WMH compared with normal appearing white matter (NAWM), and in NAWM close to compared with remote from WMH.[28, 29] Furthermore, BBB leakage was noted to be associated with hypertension and WMH severity and predicted a decline in cognitive function. Novel risk factors, such as the oral microbiota has hence also been implicated in the pathogenesis of SVD via inflammatory pathways leading to blood brain barrier dysfunction.[30, 31]

However, studies that have investigated the relationship of hypertension and other novel risk factors with SVD have a number of short-comings. SVD is a slowly progressive disorder, but the majority of previous studies on risk associations of hypertension and SVD (or other novel risk factors where hypertension has been adjusted for) have been based on single clinic or ambulatory blood pressure (BP) measurements at baseline, known history of hypertension and/or prior use of antihypertensive agents,[32-35] potentially underestimating the effect of BP changes accrued during the many years prior to clinical presentation. Studying this 'latency effect' of long-term premorbid BP on SVD is important, especially as systolic BP increases, and diastolic BP decreases with age.[36] Only relating baseline BPs with SVD without age stratification may therefore potentially undermine significant age-specific associations.

Moreover, although renal impairment has been associated with a number of individual neuroimaging markers of SVD,[37, 38] recent meta-analysis did not find an association between renal impairment with lacunar versus non-lacunar stroke subtypes.[37] Whether the relationships

between renal impairment and SVD may be age-specific has yet to be determined. Similarly, although carotid and cerebral pulsatility, a marker of large artery stiffness have been associated with leukoaraiosis,[39, 40] possibly mediated by transmission of pulsatile flow along the carotid and cerebral circulation, previous studies have also shown that the risk of leukoaraiosis varies with age and sex.[41] Sex differences in carotid anatomy exists,[42] potentially explaining this phenomenon. However, whether there are age and sex-specific associations between carotid pulsatility with global SVD burden has yet to be determined.

1.4.2 Cerebral amyloid angiopathy

Cerebral amyloid angiopathy (CAA) is an organ-specific form of amyloid-ß protein deposition (Aß40) in small and medium-sized arteries, and less commonly capillaries, of the cerebral cortex and leptomeninges.[43] In severe cases, there may be focal fragmentation of the vessel wall, with or without microaneurysmal dilatation and occasionally luminal occlusion.[44, 45]

The majority of CAA cases are sporadic and affect the elderly.[46] However, hereditary forms of CAA also exist, and several genetic mutations have been associated with the development of CAA including those affecting the amyloid-ß protein precursor, presenilin and cystatin C genes.[47] Population-based autopsy studies have shown that CAA is present in 20-40% of elderly without dementia, and up to 60% of those with dementia.[46] Amongst patients with Alzheimer's disease, CAA is present in more than 90% of cases - most cases have a mild form of CAA, but severe forms are found in ~25% of cases.[48, 49] Individuals with polymorphisms affecting the apolipoprotein E (apoE) gene, in particular ε2 and ε4, are more likely to develop CAA as well as CAA-related lobar intracerebral hemorrhage.[46, 50, 51] Those with both ε2 and ε4 alleles are at highest risk of early CAA and ICH as ε4 and ε2 works synergistically by promoting amyloid-ß deposition and inducing structural changes in amyloid laden vessels, allowing them to be prone to rupture.[46 52, 53]

Individuals with CAA often presents with multiple, recurrent lobar hemorrhages.[46] Approximately 15% of patients also present with transient focal neurological episodes (also known as amyloid

spells) in the form of recurrent, stereotyped and short-lasting positive (spreading paresthesias, visual phenomena or limb jerking) or negative symptoms (sudden-onset limb weakness, dysphasia or visual loss).[54] Cognitive impairment is also common as a result of vascular amyloid deposition leading to cortical atrophy.[55] Domains in memory, executive function and processing speech are most frequently affected.[56] In a minority of patients with CAA, especially those with apoE ε4/ε4 polymorphisms, amyloid-ß within the vessel wall may trigger an intense inflammatory reaction and patients may present with neuropsychiatric symptoms, seizures, headache and focal neurological deficits.[57]

Diagnosis of CAA-related ICH is based on the classic and modified Boston criteria (Table 1.1).[58, 59] MRI will frequently show subcortical SVD and demyelination, strictly lobar microbleeds, cortical superficial siderosis and enlarged centrum semiovale PVSs.[60-63]

1.5 Neuroimaging biomarkers of cerebral small vessel disease on conventional MRI

1.5.1 Recent small subcortical infarct

Also commonly referred to as lacunar infarcts, recent small subcortical infarcts that are symptomatic accounts for approximately 25% of all ischaemic strokes. Approximately 50% of recent small subcortical infarcts are visible on CT,[64] whilst at least 70% of recent small subcortical infarcts are visible on diffusion-weighted MRI.[65] Studies have shown that not all recent small subcortical infarcts cavitate into lacunes (small fluid-filled cavities).[66, 67] Some may develop into subcortical WMHs without apparent cavitation, whilst others may even disappear completely on conventional MRI. Therefore, recent consensus have proposed the term ‘recent small subcortical infarct’ rather than lacunar infarct.[11] Recent small subcortical infarcts refers to MRI neuroimaging evidence of infarction in the territory of a perforating arteriole with imaging features or clinical symptoms consistent with a lesion occurring in the recent few weeks. They are characterized by a high signal intensity on diffusion weighted imaging (DWI), reduced signal intensity on apparent diffusion coefficient (ADC) map, increased signal on FLAIR and T2-weighted imaging and reduced signal on T1-weighted MRI. These lesions are round, ovoid or tubular in shape and

usually less than 20mm in diameter in the axial plane, although they may appear slightly larger in the coronal plane.[11] Recent small subcortical infarcts excludes striatocapsular infarcts affecting the basal ganglia and internal capsule that are larger than 20mm and are likely due to middle cerebral artery embolism, occlusion or atheroma occluding several penetrating arteries. However, it should be noted that these striatocapsular infarcts may reduce in size markedly after the acute stage, leaving a residual lacune-like cavity, and hence previous pathological and imaging studies imaging patients later on their illness have associated atherothrombtoic and embolic disease with lacunar infarcts.[2]

1.5.2 White matter hyperintensity of presumed vascular origin

WMHs are usually bilateral, symmetrical confluent areas and appear hyperintense on T2-weighted and FLAIR images and isointense or hypointense on T1-weighted sequences. WMHs are often distributed in the periventricular and subcortical white matter of the cerebral hemispheres (Figure 1.1). However, WMHs may also occur in the subcortical deep grey matter structures, such as the basal ganglia and brainstem, and any non-cortical hyperintensities are often collectively termed 'non-cortical hyperintensties'.[11] WMHs are common in the elderly and are strongly associated with vascular risk factors, covert neurological and cognitive symptoms, gait disturbances and cerebrovascular disease.[12, 13, 15, 16, 68, 69] They are also more prevalent and severe in patients with acute lacunar stroke compared with other stroke subtypes,[70] and are associated with other neuroimaging markers of SVD such as lacunes,[71] enlarged PVSs,[72] cerebral microbleeds[73] and brain atrophy.[74] However, as white matter lesions may also be present in other disorders such as multiple sclerosis and leukodystrophies, the term WMH of presumed vascular origin has been proposed to exclude white matter lesions due to non-vascular causes.[11]

1.5.3 Lacunes of presumed vascular origin

Lacunes of presumed vascular origin are round or ovoid fluid-filled cavities, usually located in the subcortical regions such as the centrum semiovale, basal ganglia, internal capsule, thalamus or

pons (Figure 1.2). They are normally between 3 to 15 mm in diameter, and have a similar signal change as cerebrospinal fluid on MRI. Lesions larger than 15mm are usually due to mechanisms other than SVD. Lacunes are frequently seen in elderly patients who are asymptomatic and have been associated with an increased risk of stroke, gait impairment and dementia.[75-77] They are likely the consequence of small subcortical infarcts, either symptomatic or silent. However, small lacunes may also be secondary to small deep haemorrhages.[11]

1.5.4 Cerebral microbleeds

Cerebral microbleeds are small, perivascular haemosiderin deposits that result from blood leakage from pathologically fragile small vessels affected by hypertensive or cerebral amyloid angiopathy, or secondary to an ischaemic insult.[73, 78, 79] Pathological studies of patients with microbleeds have shown that they occur at the capillary, small artery and arteriolar levels.[80] The underlying cause of cerebral microbleeds are heterogeneous, and histologically, they may correspond to erythrocytes (suggestive of recent haemorrhages), iron-positive siderophages without erythrocytes (suggestive of old haemorrhages) and vasculopathies (fibrinoid necrosis).[81] In patients with CAA, the microbleeds are predominantly lobar in location (Figure 1.3A), but pathological studies have shown that cerebral microbleeds seem to appear independent to the site of amyloid deposition.[80] In contrast, in patients with hypertensive angiopathy, microbleeds are predominantly deep-seated in location (Figure 1.3B).

Cerebral microbleeds can be as small as 50-200μm, but may also be up to 4-5mm in diameter and hence are not readily visible on CT scans. However, haemosiderin-sensitive sequences on MRI, such as T2*-GRE or SWI are able to detect the presence of microbleeds, but consistently overestimates their size by about 1.6-fold due to the 'blooming effect' as a result of the magnetic properties of MRI on the iron-containing haemosiderin.[82, 83] The sensitivity of detecting cerebral microbleeds is approximately 30% higher in SWI compared with T2*-GRE (Figure 1.3).[84] The reported prevalence of microbleeds amongst transient ischaemic attack (TIA) and ischaemic stroke patients ranges from 8% to 68%.[85, 86] MIcrobleeds have also been associated with lacunar stroke and WMH.[73, 87]

Recent systematic reviews of mainly small studies have shown that a high burden of microbleeds is associated with an increased risk of ICH and possibly also of ischaemic stroke.[88-90] Other retrospective studies have reported an increased risk of ICH amongst aspirin users with microbleeds.[91] Whilst aspirin is highly effective in patients with TIA or ischaemic stroke in reducing the early risk of recurrent ischaemic events,[92] and this benefit outweighs the risks of ICH,[93] antiplatelet-related ICH is associated with a high risk of morbidity and mortality on longer-term treatment.[94] However, in TIA or ischaemic stroke patients with microbleeds, current guidelines make no recommendations on the safety of antiplatelet treatments,[95] although there is clinical uncertainty, particularly in those with ≥5 microbleeds.[96] Clinicians therefore face a treatment dilemma,[97] and may err on the side of caution by not prescribing antiplatelets in patients with multiple microbleeds, potentially jeopardising the early benefits of antiplatelet treatment after TIA or ischaemic stroke.[92]

Similarly, warfarin is highly effective in reducing the risk of ischaemic stroke in patients with atrial fibrillation, but is associated with an ICH risk of ~0.5% per year.[98] Compared with antiplatelet users and non-antithrombotic users, an excess of microbleeds have been noted in warfarin users with ICH, suggesting that microbleeds also increase the risk of warfarin-associated ICH.[89] Whilst these findings may have implications on atrial fibrillation management,[99] long-term prognostic data of the risk of recurrent stroke in warfarin users with microbleeds are lacking. Although current guidelines suggest that warfarin for stroke prevention in atrial fibrillation should probably be avoided in patients with lobar ICH and probable or confirmed CAA,[100, 101] they make no recommendations on the safety of warfarin in TIA or ischaemic stroke patients with microbleeds.[95] In these instances, some clinicians may be tempted to avoid anticoagulation and prescribe antiplatelet agents, due to a perceived lower risk of ICH.[102]

These treatment dilemmas might be solved by reliable data on the prognostic implications of microbleeds, but the current evidence-base has a number of shortcomings. First, previous studies were mostly either small or had a short duration of follow-up, and may have been subject to publication bias. Second, meta-analyses have combined cohorts including patients who were

variously on antiplatelets, anticoagulants or no anti-thrombotic drugs. Third, although microbleeds are much more prevalent in Asians than Caucasians, and there is some evidence that they are more significantly associated with ICH in Asians, and possibly more strongly associated with ischaemic stroke in Caucasians, racial differences in prognosis remain uncertain. Fourth, although the balance of risk and benefits from antiplatelet agents in patients with microbleeds who present with a TIA or ischaemic stroke might well vary over time, particularly if the risk of recurrent ischaemic events is highest early whereas the risk of ICH and extracranial bleeding accrue more gradually, the time-course of risk of ICH versus ischaemic stroke has not been addressed in previous studies. Furthermore, whether the risk of ICH in antiplatelet users with microbleeds is indeed lower than that of warfarin users has not been studied.

1.5.5 Perivascular spaces

PVSs are tiny cavities of cerebrospinal fluid that surround arterioles and venules as they course from the subarachnoid space and penetrate the brain parenchyma.[103] They are important drainage conduits for cerebral interstital fluid and if enlarged, can be seen on T2-weighted or T1-weighted brain MRI as they contain increased fluid of similar signal to CSF (MRI-visible enlarged PVSs). Compared with lacunes of presumed vascular origin, visible PVSs are generally less than 3 mm in diameter on MRI and appear as punctate (if perpendicular to the plane of scan) or linear (if longitudinal to the plane of scan) hyperintensities on T2 images. Visible PVSs are most frequently found in the inferior basal ganglia (BG), centrum semi-ovale (CS) and midbrain (Figure 1.4).[11] Although it is normal to have a few visible PVSs on MRI,[104] the presence of many is not. Visible PVSs have been attributed to the pulsatile effects secondary to arterial stiffness and also to the changes in the handling of interstitial fluid, e.g. due to cerebral endothelial failure and secondary to vascular amyloid deposition resulting in fluid and metabolic waste clearance dysfunction.[2, 105]

A high burden of visible BG and CS-PVSs have been associated with increasing age,[106-109] hypertension,[34, 106-108] renal impairment,[110] WMH,[34, 72, 106-108] and lacunes.[34, 72, 106, 108] A high burden of visible BG-PVSs have in addition also been associated with male sex,[108] mean systolic

and diastolic BP,[34, 111] deep or infratentorial cerebral microbleeds,[34, 109] and also stroke due to small vessel occlusion.[72] A high burden of visible BG-PVSs have hence been considered a marker of hypertensive arteriopathy secondary to endothelial dysfunction,[34, 109] and has recently been proposed as one of the four components of the 'Total SVD score' (see section 1.6).[112] In contrast, a high burden of visible CS-PVSs have been associated with lobar microbleeds in healthy adults and those with cognitive impairment.[34, 107] A high burden of visible CS-PVSs have also been noted in patients with CAA.[109, 113] It has therefore been hypothesised that in contrast to visible BG-PVSs, CS-PVSs may be a neuroimaging marker of CAA by representing fluid and metabolic waste clearance dysfunction due to vascular amyloid deposition.[34, 105, 109] Visible PVSs are not merely the consequence of brain atrophy as they are often appreciated in patients with little brain atrophy.[108]

Although visible PVSs have shown potential as an imaging biomarker of hypertensive angiopathy and CAA, the long-term prognostic implications of visible PVSs amongst patients with TIA or ischaemic stroke have yet to be determined. Ethnic differences in visible PVS are also uncertain.

1.5.6 Brain atrophy

Brain atrophy may be classified into general or focal (e.g. medial temporal lobe in patients with Alzheimer's disease), symmetrical or asymmetrical and may only affect certain types of tissues (e.g. white matter). The causes leading to brain atrophy are numerous. Indeed brain atrophy occurs as part of normal ageing. However, the extent and rate varies from individual to individual. In patients with cerebrovascular disease and vascular cognitive impairment, neuropathological processes leading to brain atrophy includes arteriolosclerosis, white matter rarefaction and shrinkage, neuronal loss, cortical thinning and secondary neurodegenerative changes.[114, 115] Presence and severity of SVD has also been associated with brain atrophy.[48, 74] and indeed, brain atrophy has been implicated to mediate the effects of vascular lesison on cognition.[116-118]

1.6 Scoring systems to study the global burden of small vessel disease

A "Total SVD Score" was recently proposed,[35, 112, 119] which incorporates four neuroimaging biomarkers of SVD and aims to capture the overall burden of cerebral SVD. The score not only provides a composite measure to further study the epidemiology and pathogenesis of SVD, but may also serve as a potential surrogate endpoint for clinical trials.[112, 120] In this score, one point is allocated to each of the following: 1) presence of lacunes, 2) presence of cerebral microbleeds, 3) moderate-severe (>10) BG-PVSs and 4) severe periventricular and/or moderate-severe deep WMH.[112] The score has been associated with age, male sex, hypertension, smoking and lacunar stroke subtype in patients with ischaemic stroke.[112] In patients with lacunar infarct, the Total SVD Score has also been associated with increased ambulatory BP[35] and cognitive impairment.[119]

However, although the "Total SVD Score" has also been shown to be associated with cognitive impairment,[121, 122] its long-term prognostic implications for recurrent stroke in patients with TIA or ischaemic stroke have yet to be determined.[112] Whether the Total SVD Score is able to better identify patients on antithrombotic treatment who are at high risk of ICH from those at risk of recurrent ischaemic strokes has not been studied. Furthermore, whilst the Total SVD Score is associated with lacunar stroke subtype,[112] whether the score also predicts risk of recurrent stroke in non-lacunar stroke subtypes is also unknown. In addition, whether refinements to the score (e.g. by incorporating different weightings based on microbleed[90] and WMH burden) may improve its predictive value has not been explored.[112, 120]

1.7 Novel neuroimaging techniques for imaging cerebral small vessel disease

With an increasing interest in understanding the pathogenesis and progression of SVD, a number of new techniques have been developed in the recent years. These techniques have shown importance as a means to identify the earliest changes in brain tissue prior to the development of overt disease on structural imaging.

1.7.1 Measuring structural and functional integrity of white matter tracts or regions

Structural and functional integrity of the white matter tracts or regions can now be imaged using diffusion tensor imaging (DTI) or diffusion kurtosis imaging (DKI). These techniques enables markers such as fractional anisotropy (FA) and mean diffusivity (MD) to be derived (FA decreases and MD increases with damage to the white matter tracts) and provides a quantitative measure of integrity of the white matter tract or region or interest. FA and MD could be measured in regions with noted damage, e.g. areas of WMH on conventional neuroimaging or in NAWM. Recent systematic reviews have demonstrated that hypertension, smoking and physical inactivity are associated with increased microstructural damage in NAWM, although some of the risk associations were attenuated after adjusting for other neuroimaging markers of SVD.[123] Motor symptoms of SVD and cognitive function however, were consistently associated with integrity of NAWM, even after adjusting for other neuroimaging markers of SVD.[123] Prospective studies have also shown that in stroke-free individuals, impaired global white matter integrity on DTI was associated with a subsequent increased stroke risk and cardiovascular death, independent of age, sex, vascular risk factors, WMH volume and presence of lacunes.[124]

Metrics derived from DTI or DKI could also be converted into maps of connectivity to study the strength of white matter connections between different brain networks and also how efficient these networks are. Nodal efficiency is a commonly used marker in studies of connectivity and by studying the path length between a node and other nodes, provides a measurement of the underlying white matter integrity. Shorter path lengths are more efficient and therefore a longer path length would mean that there is a reduction in nodal efficiency. In patients with cognitive impairment of mixed aetiology, a decrease in nodal efficiency has been associated with lacunes and WMH volume.[125] Furthermore, frontal nodal efficiency was shown to mediate the effects of SVD on frontal atrophy and frontal executive dysfunction, whilst temporal and parietal nodal efficiency was shown to mediate the effects of SVD on temporal and parietal atrophy and memory dysfunction.[125] Similarly, a lower global network efficiency has been shown to correlate with a higher cortical amyloid load, WMH volume, brain atrophy and consequently worse performance on processing speed and executive function tests and gait velocity in patients with probable CAA

without dementia.[126] Nevertheless, although structural and functional connectivity provides an attractive measure of microstructural damage secondary to SVD, further prospective studies are required to determine the prognostic value of these measurements and also whether these markers could be utilised in clinical trials.

1.7.2 Cerebrovascular reactivity

Cerebrovascular reactivity (CVR) can now be assessed at a tissue level via MRI and measures the subject's cerebral vasomotor response to a vasoactive stimulus, e.g. hypercapnia through inhalation of carbon dioxide. Studies in this area are however scarce and sample sizes are small. However, recently, in a cross sectional study, an association between falling CVR with carbon dioxide inhalation (i.e. a reduced flow suggesting areas at risk of ischaemia) and increasing WMH burden and enlarged PVSs was noted.[127] Other longitudinal studies have shown that CVR was lower in areas of NAWM that subsequently developed into WMH.[128] Measurment of CVR via a carbon dioxide challenge is currently utlised in multi-centre observational studies (INVESTIGATE@SVDs ISRCTN10514229) and in randomised clinical trials (LACI-1 ISRCTN 12580546 and TREAT@SVDs) to further study the association of CVR with SVD and to determine if this marker can be utilised to test potential treatments of SVD as an intermediate marker of treatment efficacy.

1.7.3 Assessment of blood brain barrier leakage

Although blood brain barrier leakage is increasingly implicated in the pathogenesis of SVD, techniques such as using dynamic contrast-enhanced MRI for measurement of blood brain barrier leakage are technically challenging as the signal changes caused by slow leakage are small, is time-consuming and needs to adjust for a high level of noise, artefact and signal drift. There remains to be numerous pitfalls and limitations of the technique and results need to be interpreted with caution.[29, 129-131] At present, there is also no consensus as to the standard imaging acquisition protocol although an international working group (HARNESS) has recently

been formed to assess the standardization of the blood brain barrier leakage method for studies in SVD.

1.8 Aims of thesis

The aims of my thesis are to determine the following:

1) Ethnic differences in prevalence and clinical correlates of cerebral microbleeds in patients with TIA or ischaemic stroke
2) Long-term prognostic implications of cerebral microbleeds in patients with TIA or ischaemic stroke on antithrombotic treatment
3) Ethnic differences in prevalence and clinical correlates of basal ganglia and centrum semiovale perivascular spaces in patients with TIA or ischaemic stroke
4) Long-term prognostic implications of basal ganglia and centrum semiovale PVSs in patients with TIA or ischaemic stroke
5) To validate the Total SVD Score by determining its prognostic value in prediction of recurrent ischaemic stroke and ICH in patients with TIA or ischaemic stroke
6) To determine whether refinements to the Total SVD Score might improve its prognostic value in prediction of recurrent ischaemic stroke and ICH in patients with TIA or ischaemic stroke
7) To determine the associations of long-term mean premorbid BP with individual neuroimaging markers and global burden of SVD in patients with TIA or ischaemic stroke
8) To determine the relationships between renal impairment and individual neuroimaging markers and global burden of SVD in patients with ischaemic stroke
9) To determine the age- and sex-specific relationships of carotid pulsatility and SVD burden in patients with TIA or ischaemic stroke

Table 1.1 Classic and modified Boston criteria for diagnosis of CAA-related haemorrhage

Definite CAA

Full postmortem examination demonstrating:

- Lobar, cortical or corticosubcortical hemorrhage
- Severe CAA with vasculopathy
- Absence of other diagnostic lesions

Probable CAA with supporting pathology

Clinical data and pathologic tissue (evacuated hematoma or cortical biopsy) demonstrating:

- Lobar, cortical or corticosubcortical hemorrhage
- Some degree of CAA in the specimen
- Absence of other diagnostic factors

Probable CAA

Clinical data and MRI or CT demonstrating:

- Multiple hemorrhages restricted to lobar, cortical, or corticosubcortical regions (cerebellar hemorrhage allowed)
  [or single lobar, cortical, or corticosubcortical hemorrhage, and focal[a] or disseminated[b] superficial siderosis]
- Age ≥55 years
- Absence of other causes of hemorrhage [or superficial siderosis]

Possible CAA

Clinical data and MRI or CT demonstrating:

- Single lobar, cortical, or corticosubcortical hemorrhage [or focal[a] or disseminated[b] superficial siderosis]
- Age ≥55 years
- Absence of other causes of hemorrhage [or superficial siderosis]

Modified criteria are indicated in [ ].
[a]Siderosis restricted to 3 or fewer sulci; [b]Siderosis affecting at least 4 sulci

Figure 1.1 Magnetic resonance imaging (T2-weighted, axial cut, at level of lateral ventricle) showing severe periventricular white matter hyperintensity

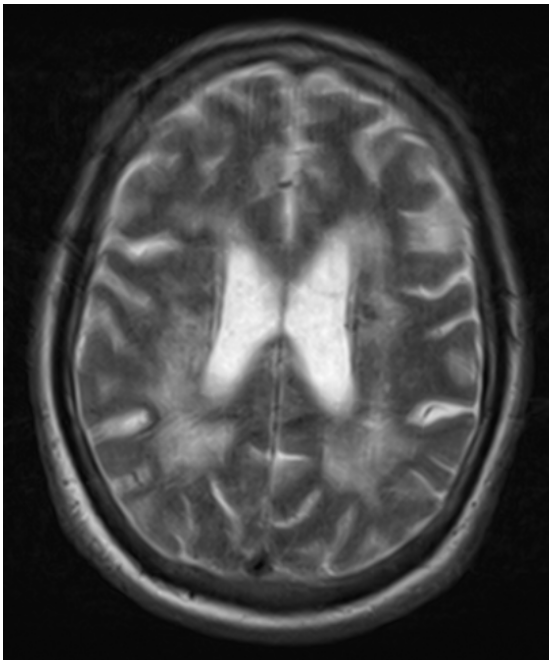


Figure 1.2 Magnetic resonance imaging (T2-weighted, axial cut, at level of basal ganglia and thalamus) showing lacune at left basal ganglia

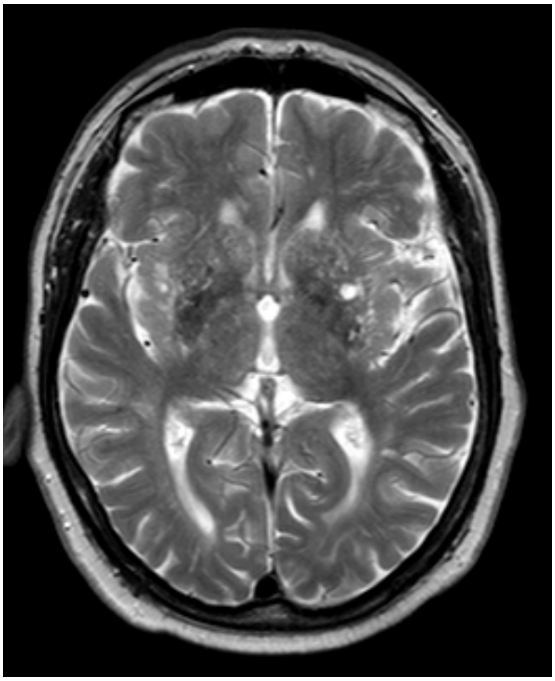
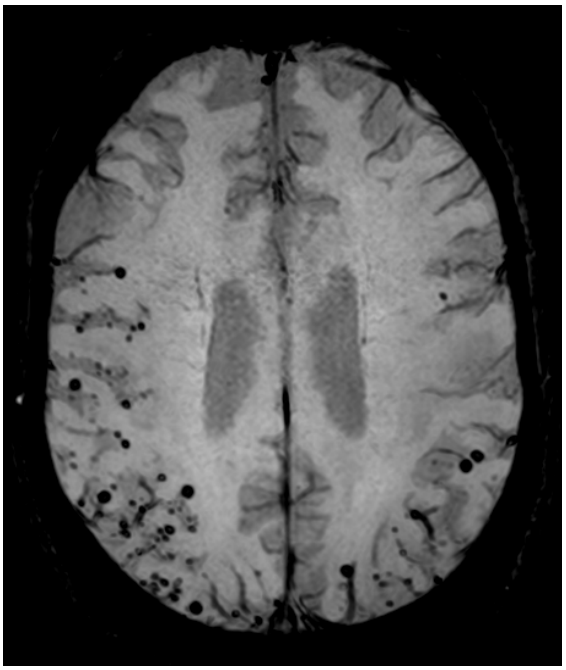


Figure 1.3 Cerebral microbleeds in A) patient with cerebral amyloid angiopathy as detected by susceptibility weighted imaging and B) patient with hypertensive angiopathy detected by T2*-gradient echo on magnetic resonance imaging (axial cut)

A)



B)

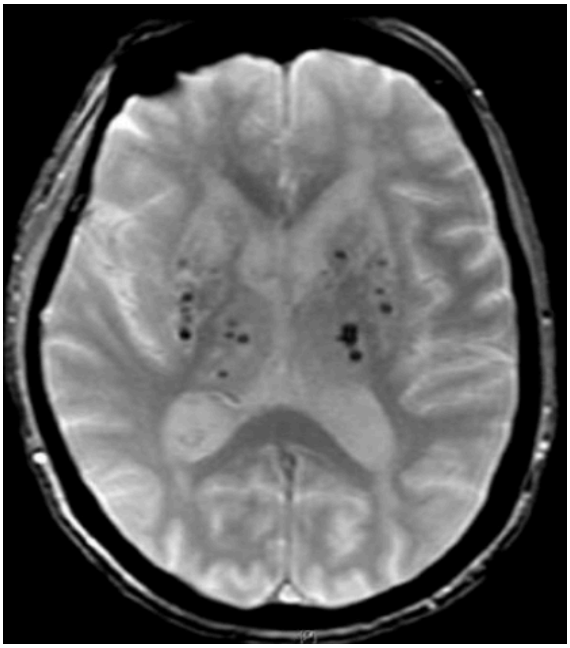
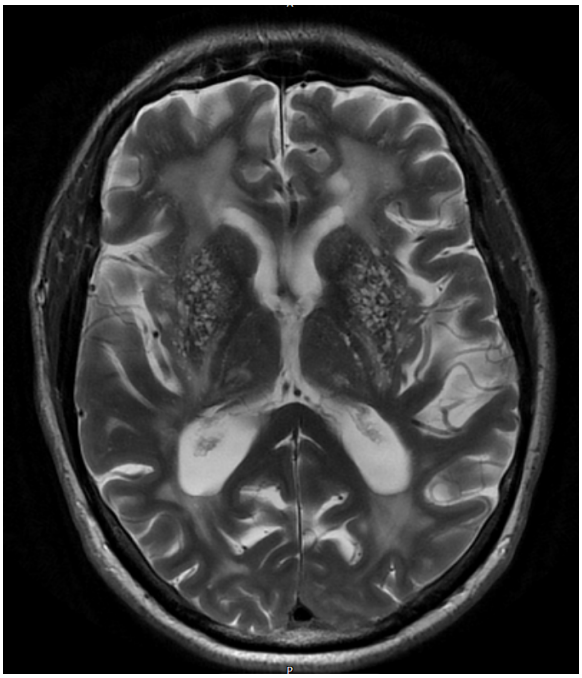
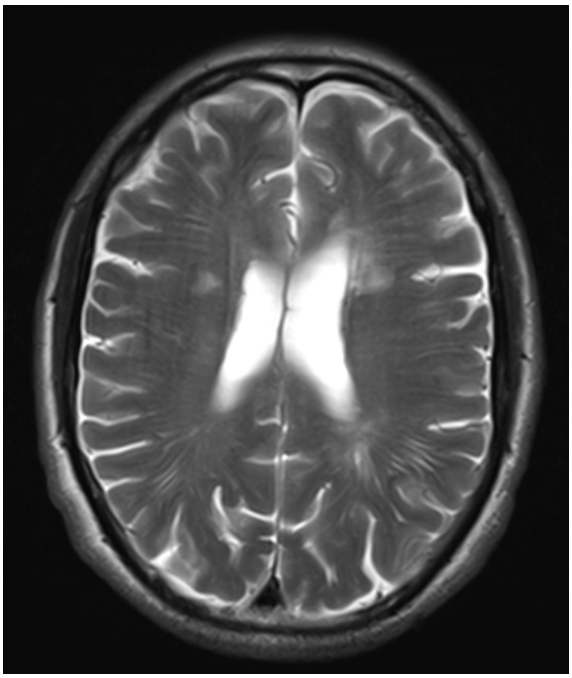


Figure 1.4 A) Severe burden (>40) of visible basal ganglia perivascular spaces in a patient with cerebal autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and B) Frequent (20-40) visible centrum semiovale perivascular spaces in patient with TIA or ischaemic stroke on magnetic resonance imaging (T2-weighted, axial cut)

A)



B)



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Chapter 2

Methods

2.1 Study populations

I prospectively studied 2156 patients with TIA or ischaemic stroke recruited from two study centres - The Oxford Vascular Study (OXVASC), and The University of Hong Kong (HKU). OXVASC is an on-going population-based study of all acute vascular events occurring within a predominantly Caucasian population of 92728 individuals within Oxfordshire, irrespective of age, who are registered with 100 general practitioners in eight general practices of Oxfordshire, UK.[1] The analysis in this thesis includes 1080 consecutive cases of transient ischaemic attack (TIA) or ischaemic stroke recruited from November 1, 2004, to September 30, 2014 who had a cerebral magnetic resonance imaging (MRI) incorporating a haemosiderin-sensitive sequence and was subsequently diagnosed to have a TIA or ischaemic stroke. From April 1, 2002, to March 31, 2010, computed tomography of the brain and carotid Doppler ultrasound were the first-line imaging methods, with MRI and MR angiography done in selected patients when clinically indicated. From April 1, 2010 onwards, brain MRI and MR angiography of the intra- and extracranial vessels became the first-line imaging methods.

A further 1076 consecutive patients who were predominantly Chinese with a diagnosis of acute ischaemic stroke who received a MRI scan incorporating a haemosiderin-sensitive sequence at the HKU MRI Unit was recruited from March 1, 2008, to September 30, 2014.

Demographic data, atherosclerotic risk factors, premorbid antithrombotic use, details of hospitalisation of index event and medications on discharge during face-to-face interview were collected and cross-referenced with primary care and hospital records in both cohorts. Cause of TIA or ischaemic stroke was classified according to the modified Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria.[2] Blood tests for renal function was taken upon ascertainment and glomerular filtration rate was calculated according to the Modification of Diet in Renal Disease (MDRD) Study equation[3] in OXVASC and Chronic Kidney Disease Epidemiology (CKD-EPI) Collaboration equation for Asian populations in HKU.[4]

All patients gave written informed consent, or assent was obtained from a relative of patients who were unable to provide consent. The two studies were approved by the local research ethics committee.

2.2 MRI scanners and parameters

TIA or ischaemic stroke patients recruited from OXVASC were scanned either at 1.5-Tesla (T) (493/630 on an Achieva MRI/Philips Healthcare) or 3-T (388/450 patients on Magnetom Verio/Siemens Healthcare). All 1076 HKU patients were scanned using a 3-T MRI scanner Achieva (Philips Healthcare). Details of all MRI scanners in OXVASC and HKU as well as the scan parameters are provided in Table 2.1. Two neurologists, supervised by two consultant neuro-radiologists interpreted all MRI images.

2.3 Definitions of neuroimaging markers of small vessel disease

Definitions of neuroimaging biomarkers were based on Standards for Reporting Vascular Changes on Neuroimaging (STRIVE).[5]

The severity of white matter disease was determined for each patient according to the Fazekas scale.[6] Subcortical white matter hyperintensity (WMH) was graded as 0 (absent), 1 (puntate foci), 2 (beginning confluence of foci) and 3 (large confluent areas); whilst periventricular WMH was graded as 0 (no WMH except for small triangular foci surrounding the frontal horns), 1 (periventricular hyperintensity surrounding the anterior and posterior horns ± discrete WMHs), 2 (extensive patchy WMHs and their early confluent stages), and 3 (confluent, completely surrounding lateral ventricles).[6]

Lacunes were defined as rounded or ovoid lesions, >3mm and <20mm in diameter, in the basal ganglia (BG), internal capsule, centrum semiovale (CS) or brainstem, of cerebrospinal fluid signal density on T2 and fluid attenuation inversion recovery (FLAIR) and no increased signal on diffused weighted imaging.[5]

Microbleeds were defined as rounded, hypodense foci up to 10mm in size and were differentiated from microbleed mimics based on current guidelines.[7] The location and number of microbleeds were scored according to the Microbleed Anatomical Rating Scale,[8] and microbleed-burden was graded as absent, 1, 2-4, and ≥5.

MRI-visible enlarged perivascular spaces (PVSs) were defined as small, sharply delineated structures of cerebrospinal fluid intensity measuring <3mm in cross-sectional diameter. They should follow the course of perforating vessels (and in very high field MRI scanners, a vessel within a PVS may be visible) and normally appear round if imaged in the axial plane, and longitudinal if cut in the long axis of the PVS. The burden of MRI-visible enlarged PVSs were coded according to a previously validated scale.[9] In patients with an asymmetrical number of visible PVSs, the side with the higher number of visible PVSs was counted.[9] Burden of visible PVSs were then stratified into 3 groups: <11(normal to mild), 11-20 (moderate) and >20 (frequent to severe).[9] Ideally, PVSs may be differentiated from lacunes based on whether a vessel is visible within the PVS, as this may not be feasible in our MRI scanners (1.5 and 3-T), PVSs were differentiated arbitrarily from lacunes based on size (<3mm vs. ≥3mm) with the limitation that this may potentially have misclassified a small proportion of the lesions.

The intra-rater κ for burden for 50 randomly selected scans in OXVASC and HKU was: periventricular WMH burden (Fazekas grade 0, 1, 2, 3) - 0.66 (OXVASC), 0.69 (HKU); subcortical WMH burden (Fazekas grade 0, 1, 2, 3) - 0.75 (OXVASC), 0.71 (HKU); lacunes - 0.85 (OXVASC), 0.82 (HKU); microbleed burden (0, 1, 2-4, ≥5) – 0.88 (OXVASC), 0.81 (HKU) and visible PVS burden (<11, 11-20, >20) – 0.86 (BG), 0.84 (CS) in OXVASC and 0.86 (BG), 0.72 (CS) in HKU.

2.4 Determination of adverse outcomes on follow-up

All patients in OXVASC were followed-up regularly by a research nurse or physician at 1, 3, 6, 12, 24, 60 and 120 months after the index event. Patients recruited from HKU were followed-up by a clinician every 3-6 months, or more frequently if clinically indicated. All patients were assessed for

the following clinical outcomes: 1) recurrent stroke (ischaemic and haemorrhagic), 2) acute coronary events (acute coronary syndrome and sudden cardiac death), 3) major extracranial bleeding and 4) mortality (vascular and non-vascular).

The definition of recurrent stroke required a sudden new neurological deficit fitting the definition of ischaemic stroke or intracerebral haemorrhage, occurring after a period of unequivocal neurological stability and not attributable to cerebral oedema, mass effect, or haemorrhagic transformation of the incident cerebral infarction. Patients with suspected recurrent stroke received a cranial computed tomography or MRI to support the diagnosis. An acute coronary syndrome was defined as non-ST or ST-segment elevation myocardial infarction based on current guidelines,[10, 11] and sudden cardiac death was defined as a sudden pulseless condition, presumed to be due to a cardiac arrhythmia, in a previously stable individual without a non-cardiac cause of cardiac arrest. Severity of extracranial bleeding were classified according to the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial,[12] where major bleeding episodes was defined as substantially disabling bleeding, intraocular bleeding leading to the loss of vision, or bleeding necessitating the transfusion of at least 2 units of blood. Major bleeding was classified as life-threatening if the bleeding episode was fatal or led to a reduction in the haemoglobin level of at least 5 grams per deciliter, resulted in hypotension requiring the use of intravenous inotropic agents, if it necessitated a surgical intervention, if it was a symptomatic intracranial haemorrhage, or it if necessitated the transfusion of 4 or more units of blood. Minor bleeding episodes included other haemorrhages that led to the interruption of the antithrombotic medications.[12] Vascular death was defined as death due to lethal cardiac arrhythmias, acute coronary syndrome, congestive heart failure, fatal stroke, pulmonary embolism, aortic dissection or unexplained sudden death.

Where needed, details of clinical outcomes were supplemented by electronic or paper medical records from primary care practices, hospitals as well as the Deaths General Register Office.

Table 2.1 MRI sequence parameters of the OXVASC and HKU cohort

MR parameters	HKU Achieva, Philips Healthcare	OXVASC scanner 1 Magnetom Verio, Siemens Healthcare	OXVASC scanner 2 Discovery MR750, GE Healthcare	OXVASC scanner 3 Achieva, Philips Healthcare	OXVASC scanner 4 Signa HDxt, GE Healthcare
Patients scanned	1076	388	62	493	137
Field strength (T)	3	3	3	1.5	1.5
T1W TR/TE/TI (ms)	2000/20/800	2000/1.94/880	-	701/16	-
T2W TR/TE (ms)	2377/80	6000/96	5800/94	5061/100	3760/100
FLAIR TR/TE/TI (ms) (3D)	4800/282/1650	9000/88/2500	9600/130/2350	11000/140/2800	8080/112/2200
Diffusion TR/TE (ms)	2874/46	5300/91	6000/84	2891/73	6100/71
GRE / SWI TR/TE (ms) (3D)	SWI 28/23	GRE 504/15	GRE 500/20	GRE 694/23	GRE 560/25
Pixel bandwidth (Hz)	218.5 (T1W) 350.7 (T2W) 144.7 (FLAIR) 40.2 (Diffusion) 455.7 (SWI)	240 (T1W) 220 (T2W) 202 (FLAIR) 1374 (Diffusion) 200 (GRE)	- 50 (T2W) 41.7 (FLAIR) 250 (Diffusion) 31.3 (GRE)	87.4 (T1W) 88.5 (T2W) 375 (FLAIR) 25.3 (Diffusion) 109.3 (GRE)	- 47.6 (T2W) 31.3 (FLAIR) - 75 (GRE)
Matrix	308x207 (T1W) 308x235 (T2W) 228x227 (FLAIR) 112x87 (Diffusion) 256x224 (SWI)	256x256 (T1W) 320x320 (T2W) 192x192 (FLAIR) 130x130 (Diffusion) 320x256 (GRE)	- 512 (T2W) 384x224 (FLAIR) 128x128 (Diffusion) 288x224 (GRE)	118x214 (T1W) 356x193 (T2W) 236x159 (FLAIR) 97x84 (Diffusion) 256x163 (GRE)	416x256 (T2W) 256x224 (FLAIR) 128x128 (Diffusion) 288x192 (GRE)
No. of slices	25 (T1W) 25 (T2W) 30 (FLAIR) 25 (Diffusion) 25 (SWI)	208 (T1W) 25 (T2W) 50 (FLAIR) 25 (Diffusion) 25 (GRE)	25	25 (T1W) 25 (T2W) 28 (FLAIR) 25 (Diffusion) 22 (GRE)	25
Slice thickness (mm)	5 (T1W) 5 (T2W) 5 (FLAIR coronal) 2.5 (FLAIR axial) 5 (Diffusion) 5 (SWI)	1 (T1W) 5 (T2W) 3 (FLAIR) 5 (Diffusion) 5 (GRE)	5	5	5
Inter-slice gap (mm)	0.5 (T1W) 0.5(T2W) 0.5 (FLAIR coronal) 0 (FLAIR axial) 0.5 (Diffusion) 0.5 (SWI)	0 (T1W) 1 (T2W) 0 (FLAIR coronal) 1 (Diffusion) 1 (GRE)	1	1	1
Voxel size (mm^3)	0.75x0.95x5.0 (T1W) 0.75x0.76x5.0 (T2W) 1.10x1.10x1.12 (FLAIR) 2.05x2.64x5.0 (Diffusion) 0.90x0.90x2.00 (SWI)	1.0x1.0x1.0 (T1W) 0.8x0.8x5.0 (T2W) 1.0x1.0x3.0 (FLAIR) 1.8x1.8x5.0 (Diffusion) 0.9x0.8x5.0 (GRE)	-	0.53x0.53x5.0 (T1W) 0.65x0.65x5.0 (T2W) 0.82x0.81x5.0 (FLAIR) 1.74x1.73x5.0 (Diffusion) 0.90x0.90x5.0 (GRE)	-

2.5 References

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Chapter 3

Antiplatelet treatment after TIA and ischaemic stroke in patients with cerebral microbleeds: time-course and severity of recurrent stroke in Caucasians and Chinese cohorts

3.1 Chapter outline

In patients with transient ischaemic attack (TIA) and ischaemic stroke, microbleed burden predicts intracerebral haemorrhage (ICH), and possibly ischaemic stroke, but implications for antiplatelet treatment are uncertain. Previous cohort studies have been small, had insuffient follow-up to assess the time-course of risks, have not stratified risks by antithrombotic use and have not reported extracranial bleeds or functional outcome of ICH versus ischaemic stroke. Guidelines do not comment on appropriate antiplatelet treatment, and it is uncertain whether recommendations should differ in Asian versus Caucasian populations.

Therefore in this Chapter, I determined the risks, time-course and outcome of ICH, extracranial bleeds, and recurrent ischaemic events in two independent prospective cohorts with TIA and ischaemic stroke [Oxford Vascular Study (OXVASC), mainly Caucasian and University of Hong Kong (HKU), mainly Chinese], stratified by microbleed-burden (0 vs. 1, 2-4 and ≥5) and adjusting for age, sex and vascular risk factors. I also obtained pooled risk associations by performing a meta-analysis with previous cohorts.

I found that microbleeds were more frequent in the Chinese cohort (450/1003 vs. 158/1080; $p<0.0001$), but risk associations were similar during 7433 patient-years of follow-up. Among 1811 patients on antiplatelet drugs, risk of major extracranial bleeds was unrelated to microbleed-burden ($p_{trend}=0.87$), but the 5-year risk of ICH was steeply related ($p_{trend}<0.0001$), with 73% (11/15) of ICH in 7.7% (140/1811) of patients with ≥5 microbleeds. However, risk of ischaemic stroke also increased with microbleed-burden ($p_{trend}=0.013$), such that ischaemic stroke and coronary events exceeded ICH and major extracranial bleeds during the first-year even amongst patients with ≥5 microbleeds (11.7% vs. 3.7%). However, this ratio changed over time (time-course $p_{interaction}=0.034$), with risk of haemorrhage matching that of ischaemic events after one-year (11.0% vs. 10.5%). Moreover, whereas the association between microbleed-burden and risk of ischaemic stroke was due mainly to non-disabling events ($p_{trend}=0.007$), the association with ICH was accounted for ($p_{trend}<0.0001$) by disabling or fatal events (e.g. ≥5 microbleeds: 82% disabling or fatal ICH vs. 40% ischaemic stroke; $p=0.035$). Pooled analysis (16025 patient-years) with 14 smaller studies yielded risk associations for ICH that were consistent with our cohorts, but

heterogeneous estimates for risk of ischaemic stroke were noted in particular amongst patients with >1 microbleeds (p_{het}=0.0003). However, heterogeneity was accounted for by two TIA-only cohorts with short follow-up that exaggerated (p_{het}=0.004) the longer-term association.

I concluded that in Caucasian and Chinese patients with ≥5 microbleeds, withholding antiplatelet drugs during the first-year after TIA or ischaemic stroke may be inappropriate. However, the risk of ICH may outweigh any benefit thereafter.

3.2 Introduction

Cerebral microbleeds are small, perivascular haemosiderin deposits that result from blood leakage from pathologically fragile small vessels affected by hypertensive or cerebral amyloid angiopathy, or occur secondary to an ischaemic insult.[1-3] They can be detected using haemosiderin-sensitive sequences on magnetic resonance imaging (MRI), and hence serve as a biomarker of underlying small vessel disease (SVD) burden.[4] The reported prevalence of microbleeds amongst transient ischaemic attack (TIA) and ischaemic stroke patients ranges from 8% to 68%.[5, 6]

Recent systematic reviews of mainly small studies have shown that a high burden of microbleeds is associated with an increased risk of intracerebral haemorrhage (ICH) and possibly also of ischaemic stroke.[7-9] Other retrospective studies have reported an increased risk of ICH amongst aspirin users with microbleeds.[10] Whilst aspirin is highly effective in patients with TIA and ischaemic stroke in reducing the early risk of recurrent ischaemic events,[11] and this benefit outweighs the risks of ICH,[12] antiplatelet-related ICH is associated with a high risk of morbidity and mortality on longer-term treatment.[13] However, in TIA and ischaemic stroke patients with microbleeds, current guidelines make no recommendations on the safety of antiplatelet treatments,[14] although there is clinical uncertainty, particularly in those with ≥5 microbleeds.[15] Clinicians therefore face a treatment dilemma,[16] and may err on the side of caution by not prescribing antiplatelets in patients with multiple microbleeds, potentially jeopardising the early benefits of antiplatelet treatment after TIA or ischaemic stroke.[17]

The treatment dilemma might be solved by reliable data on the prognostic implications of microbleeds, but the current evidence-base has a number of shortcomings.[7-9] First, previous studies were mostly either small or had a short duration of follow-up, and may have been subject to publication bias.[18] Second, meta-analyses have combined cohorts including patients who were variously on antiplatelets, anticoagulants or no antithrombotic drugs.[8, 9] Third, although microbleeds are much more prevalent in Asians than Caucasians, and there is some evidence that they are more significantly associated with ICH in Asians, and possibly more strongly associated with ischaemic stroke in Caucasians,[8] racial differences in prognosis remain

uncertain. Fourth, although the balance of risk and benefits from antiplatelet agents in patients with microbleeds who present with a TIA or ischaemic stroke might well vary over time, particularly if the risk of recurrent ischaemic events is highest early whereas the risk of ICH and extracranial bleeding accrue more gradually, the time-course of risk of ICH versus ischaemic stroke has not been addressed in previous studies. The particularly high early risk after TIA versus ischaemic stroke might also be relevant here.[19]

In order to improve the reliability of previous estimates of risk of recurrent stroke from meta-analyses of small studies and to address these unanswered questions, I analysed data from The Oxford Vascular Study (OXVASC) and The University of Hong Kong (HKU) that consisted of more than 2000 Caucasians and Chinese with TIA and ischaemic stroke, with similar policies of routine long-term antiplatelet treatment irrespective of microbleed burden. I hypothesise that in TIA/ischaemic stroke patients, the long-term absolute risk of recurrent ischaemic stroke is greater than the risk of ICH in patients with <5 microbleeds and hence antiplatelet agents should be prescribed unless contraindicated. However, in patients with ≥5 microbleeds, I postulate that the long-term absolute risk of ICH may be similar to that of ischaemic events. In view of the possible benefit of antiplatelet agents in preventing against early recurrent ischaemic events in patients with ≥5 microbleeds, and also the likely higher risk of disability and mortality of ICH compared with ischaemic stroke, I postulate that in TIA/ischaemic stroke patients with ≥5 microbleeds, whilst antiplatelet agents may potentially be beneficial in the short-term, the potential risks of intra and extracranial haemorrhages associated with antiplatelet-use may outweigh its benefits in the long-run.

3.3 Methods

3.3.1 Study populations

1080 consecutive cases with TIA or ischaemic stroke who were predominantly Caucasians and 1076 consecutive cases with ischaemic stroke who were predominantly Chinese were recruited from OXVASC and HKU (see section 2.1). Baseline data was collected as described in section 2.1. All patients received a cerebral MRI incorporating a haemosiderin sensitive sequence. Microbleeds were detected using T2*-weighted gradient echo (GRE) in OXVASC and using susceptibility weighted imaging (SWI) in HKU. Further details of scan parameters are provided in Table 2.1. Presence and burden of cerebral microbleeds, visible perivascular spaces (PVSs), lacunes and white matter hyperintensity (WMH) were coded as described in section 2.3. Both cohorts had similar antiplatelet treatment policies and antiplatelet treatment was started routinely irrespective of microbleed burden. However, patients who presented with an amyloid spell (with concomitant cortical superficial siderosis and/or multiple microbleeds), were not considered as having TIAs and were not included in this study.

All patients in OXVASC and HKU were followed-up regularly and assessed for the following clinical outcomes: 1) recurrent stroke (ischaemic and haemorrhagic), 2) acute coronary events (acute coronary syndrome and sudden cardiac death), 3) major extracranial bleeding and 4) mortality (vascular and non-vascular) (see section 2.4 for definitions of adverse clinical outcomes). Modified Rankin Scale (mRS) at 1 month after recurrent stroke was determined and disabling stroke defined as mRS>2.

3.3.2 Statistical analysis

I first conducted separate analyses for the OXVASC and HKU cohorts. As there was no significant heterogeneity (microbleed-burden by cohort interaction p=0.52 for prediction of recurrent stroke), these were then combined in a pooled analysis. The clinical and imaging predictors of ≥5 microbleeds were determined using logistic regression model. Variables including age, male sex, vascular risk factors (hypertension, hyperlipidaemia, diabetes, smoking, atrial fibrillation), glomerular filtration rate, premorbid use of antiplatelets and anticoagulants were

entered into a univariate analysis model and all variables were subsequently entered into a multivariate analysis model to determine the independent predictors of ≥5 microbleeds. The associations of ≥5 microbleeds with other neuroimaging markers of SVD was also determined.

In the primary analysis, I used Kaplan-Meier survival analysis to calculate the 5-year risk of adverse events amongst 1811 antiplatelet users from OXVASC and HKU, censored at death or March 31, 2015. Risks of adverse events by burden of microbleeds were compared with log-rank test. I compared the risk of adverse events within 1-year versus those occurring from 1-5 years of index event by Chi-squared test. I also determined by Cox regression analysis the unadjusted and adjusted (age, sex, and vascular risk factors) risks of adverse outcome amongst patients with 1, 2-4 and ≥5 microbleeds, compared with no microbleeds as reference. The following outcomes were studied: recurrent stroke, recurrent ischaemic stroke, ICH, acute coronary events, major extracranial haemorrhage, death and vascular death. I also compared the severity of recurrent ischaemic stroke and ICH based on mRS with ordinal regresson (mRS shift) analysis.

I performed a meta-analysis using a random effects analysis by pooling the results from the OXVASC and HKU cohorts with those from a recent systematic review.[9] TIA and ischaemic stroke cohorts that were predominantly (≥70% of the study population) on antiplatelets were included.[9] I also stratified the analysis by ethnicity (Caucasians vs. Asians), MRI strength (0.5/1/1.5-T vs. 3-T) and sequence (T2*GRE vs. SWI). Heterogeneity was determined with Chi-squared tests.

Sensitivity analyses was also performed to include all 2083 patients, regardless of antithrombotic status, from OXVASC and HKU and all studies from the systematic review.[9]

All analyses were done with SPSS version 22.

3.4 Results

3.4.1 Baseline clinical and neuroimaging characteristics

After excluding 73 patients with incomplete follow-up data, a total of 2083 patients were included in the final analysis. Characteristics and outcomes of the 1080 patients from OXVASC (572 TIA, 508 ischaemic stroke) and 1003 patients with ischaemic stroke from HKU are shown in Table 3.1. HKU patients were more often male (p=0.001), and were more likely to have hypertension and diabetes (p<0.0001); whilst OXVASC patients were more likely to have hyperlipidemia or a history of smoking (p<0.0001). The median delay from event to MRI was 3 days in OXVASC and 4 days in HKU. Cause of TIA or ischaemic stroke, according to the modified Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria is provided in Table 3.2. Antithrombotic treatment on discharge in the two cohorts was similar (Table 3.1), with 86.9% on antiplatelets only (19.6% dual antiplatelets, 67.3% single antiplatelet), 9.4% anticoagulants only (7.4% warfarin, 2% non-vitamin K antagonist oral anticoagulants), 1.3% anticoagulant plus antiplatelet, and 2.4% on no antithrombotic agents. There were no differences in microbleed-burden in patients with or without antithrombotic treatment (p=0.58, Table 3.3).

Microbleeds were more frequent in the HKU cohort versus OXVASC (45% vs. 15%; p<0.0001), but risk factors for microbleeds were similar (Table 3.4). However, on multivariate analysis, only age [adjusted odds ratio (OR) 1.02, 95% confidence interval (CI) 1.00-1.03, p=0.035] and premorbid anticoagulation use (2.77, 1.00-7.70, p=0.05) remained significant independent predictors of a high-burden (≥5) of microbleeds (Table 3.4). Lacunes and increasing burden of visible basal-ganglia PVSs, periventricular and subcortical WMH were all associated with a high-burden of microbleeds after adjusting for age and sex (all p<0.05) (Table 3.5).

3.4.2 Long-term prognostic implications of microbleeds in TIA and ischaemic stroke patients

On 4265 patient-years follow-up in OXVASC and 3168 patient-years in HKU, associations between microbleed-burden and risk were similar in the two cohorts for all outcomes (Table 3.6) and so further analyses are pooled. After a mean follow-up of 43±25 months (7433 patient-years),

220 patients developed a recurrent stroke (3.0%/year; 180 ischaemic strokes, 2.45%/year; 30 ICHs, 0.41%/year) (Table 3.1). One patient developed a subarachnoid haemorrhage due to an underlying cerebral aneurysm. 78 patients developed an acute coronary event (1.1%/year) and 144 patients an extracranial bleed (1.96%/year; 40 major, 0.55%/year). 294 patients died during follow-up (4.0%/year, 34% vascular deaths).

Among 1811 patients (OXVASC n=949, HKU n=862) who were prescribed with antiplatelet agents (26 with concomitant anticoagulant use excluded), the 5-year risks of recurrent ischaemic stroke and ICH in patients with no, 1, 2-4 and ≥5 microbleeds were 8.7%, 14.1%, 13.7% and 17.4%, (Figure 3.1B, log-rank test p=0.002) and 0.6%, 0.9%, 3.7% and 10.2% respectively (Figure 3.1C, p<0.0001). After adjusting for age, sex and vascular risk factors, a high microbleed-burden was an independent predictor of recurrent ischaemic stroke (p_{trend}=0.013), ICH (p_{trend}<0.0001), all cause mortality (p_{trend}=0.012) and non-vascular death (p_{trend}=0.044) (Table 3.7). A high microbleed-burden was not associated with risk of coronary events (p_{trend}=0.85), major extracranial bleed (p_{trend}=0.87) or vascular death (p_{trend}=0.18) (Table 3.7). Similar findings were noted in 1403 patients (OXVASC n=677, HKU n=726) on a single antiplatelet drug (Table 3.8).

In patients with microbleeds, the 5-year absolute risks of a non-disabling ischaemic stroke exceeded that of a non-disabling ICH (9.4% vs. 1.2%, p<0.0001), even amongst those with ≥5 microbleeds (9.8% vs. 2.1%, p=0.008) (Figure 3.2). Similarly, the 5-year risk of a disabling or fatal ischaemic stroke exceeded that of a disabling or fatal ICH in patients with 1-4 microbleeds (8.3% vs.1.3%, p=0.0004) (Figure 3.2). However, in patients with ≥5 microbleeds, risks of a disabling or fatal ICH increased substantially, such that the 5-year absolute risks of a disabling or fatal ischaemic stroke and ICH were similar (9.0% vs. 9.4%, p=0.81) (Figure 3.2). Moreover, in patients with ≥5 microbleeds, a greater proportion of patients who developed a subsequent ICH were disabled or dead compared to those who developed a recurrent ischaemic stroke (81.8% vs. 40.0; mRS shift OR 6.75, 95% CI 1.14-39.80, p=0.035) (Figure 3.3).

In a time-course analysis, amongst patients with <5 microbleeds, the absolute risks of ischaemic stroke and coronary events combined exceeded that of an ICH and major extracranial bleed, both

within and beyond 1-year of the index event (1-year risk: 4.9% vs. 0.9%; 1-5 year risk: 9.8% vs. 1.6%) (Figure 3.4). In patients with ≥5 microbleeds, risks of a combined ischaemic event also exceeded that of a combined haemorrhagic event during the first year (11.7% vs. 3.7%). However, in years 1-5, the risks of ICH increased steeply such that the risks of ICH matched that of ischaemic stroke (10.5% vs. 11.0%)(time course $p_{interaction}$=0.034) (Figure 3.4).

I pooled the results from OXVASC and HKU with those from a recent meta-analyses (Table 3.9).[9] After excluding cohorts with <70% of the study population on antiplatelet agents, the pooled unadjusted relative risk estimates of recurrent ischaemic stroke in patients with 1, 2-4 and ≥5 versus no microbleeds was 1.68 (95% CI 1.14-2.48, p=0.009, p_{het}=0.18), 2.51 (1.41-4.47, p=0.002, p_{het}=0.0003) and 2.75 (1.75-4.34, p<0.0001, p_{het}=0.031) (Figure 3.5). The pooled relative risk estimates of ICH in patients with 1, 2-4 and ≥5 versus no microbleeds was 3.14 (1.17-8.42, p=0.023, p_{het}=0.52), 5.81 (2.63-12.84, p<0.0001, p_{het}=0.85) and 13.35 (6.75-26.39, p<0.0001, p_{het}=0.92) (Figure 3.6). No significant heterogeneity was noted between the OXVASC and HKU cohorts and pooled relative risk estimates of previous cohorts, and when all studies were stratified by ethnicity, MRI magnet strength or sequence (Figure 3.7). However, risk of recurrent ischaemic stroke in patients with microbleeds versus no microbleeds was significantly greater in the two TIA-only cohorts that had ≤1-year follow-up compared with the other TIA/ischaemic stroke cohorts that had >1-year follow-up (relative risk 4.80, 2.36-9.76 vs. 1.62, 1.32-1.99, p_{het}=0.004) (Figure 3.8). Sensitivity analysis of all patients revealed broadly similar results (Table 3.6, Figures 3.9 and 3.10).

3.5 Discussion

This study comprises the two largest cohorts so far from the West and the East to report the long-term prognostic implications of cerebral microbleed-burden in patients with TIA or ischaemic stroke, adding approximately 6500 patient-years of follow-up data to the 9534 patient-years included in a recent systematic review of 15 smaller studies.[9] This study is also the first to report the long-term prognostic implications amongst patients treated with a policy of routine antiplatelet use, the first to report the other non-stroke determinants of the balance of risks and benefits of antiplatelet drugs (extracranial bleeds and coronary events) stratified by microbleed-burden, the first to determine the severity and time-course of recurrent events and the first to determine whether risks of microbleeds differ in TIA versus ischaemic stroke cohorts.

My results support those from previous studies that microbleeds represents an imaging biomarker of cerebal SVD.[20, 21] Similar to previous studies,[1] I showed that prevalence and burden of microbleeds was significantly greater in Chinese than Caucasians. Whilst this may be due to a greater proportion of hypertension amongst Chinese as noted in my results, patients from HKU also consisted entirely of ischaemic stroke patients, whilst ~53% of OXVASC patients had TIA, in whom the prevalence of microbleeds is known to be lower.[1] Furthermore, all patients from the HKU cohort were scanned using a 3-T MRI with a SWI sequence, in contrast to using a combination of 1.5 and 3-T MRI with T2*-GRE in the OXVASC cohort. MRIs of higher magnetic field strength,[22] and SWI as compared with T2*-GRE[23, 24] are known to be more sensitive in detecting cerebral microbleeds, and this may also have attributed to the higher prevalence of cerebral microbleeds noted in the HK cohort. Nevertheless, when indiviudals from OXVASC and HKU were stratified by microbleed burden, risk of recurrent stroke was similar regardless of ethnicity, and no heterogeneity was observed when all cohorts were pooled and stratified by ethnicity.

Compared with patients with no microbleeds, TIA and ischaemic stroke patients with ≥5 microbleeds on antiplatelet agents were at 3-fold increased risk of recurrent ischaemic stroke and at 13-fold increased risk of ICH. However, the relative risk of ischaemic versus haemorrhagic events was time-dependent, with a 3-fold excess of risk of recurrent ischaemic stroke versus ICH

in the first year (9.5% vs. 3.7%), but an increasing relative risk of ICH thereafter. Furthermore, the disability accrued due to ICH was significantly greater than that due to recurrent ischaemic stroke. The relative risk estimates for ICH in relation to microbleed burden were fairly consistent with the pooled estimates from previous meta-analyses, but my relative risk estimates for recurrent ischaemic stroke were more modest.[9] The pooled estimates from previous meta-analyses were nevertheless undemined by substantial heterogeneity (Figure 3.8), due to a 3-fold higher risk of ischaemic stroke in TIA-only cohorts compared with cohorts that consisted predominantly of ischaemic stroke patients (Figure 3.8). Excluding the TIA-only cohorts[25, 26] substantially reduced the heterogeneity of pooled relative risk estimates of previous studies on recurrent ischaemic stroke risk in 1, 2-4 and ≥5 microbleeds versus no microbleeds (p_{het}=0.095 to 0.24; p_{het}=0.0005 to 0.99 and p_{het}=0.025 to 0.52 respectively).

My findings therefore have clinical implications on the use of antiplatelet drugs in TIA and ischaemic stroke patients with a high microbleed burden. Taken together with the recent meta-analysis of aspirin trials showing considerable early benefit of aspirin in reducing the risk and severity of recurrent ischaemic events after TIA or ischaemic stroke but limited benefit after 12 weeks,[17] my results suggest that patients with non-cardioembolic TIA or ischaemic stroke with <5 microbleeds should be prescribed antiplatelet drugs unless contraindicated. In patients with ≥5 microbleeds however, in view of the high early risk of ischaemic events, particularly in patients presenting as a TIA, antiplatelet agents should also be prescribed within the first year of index event. Antiplatelet agents should perhaps be withdrawn thereafter due to the increasing long-term risks of ICH that are more likely to be disabling or fatal than ischaemic events. However, randomised controlled trials of antiplatelet use in patients with ≥5 microbleeds microbleeds may be required, before definitive recommendations could be made.

Although I consider my findings to be valid, my study has limitations. First, a number of different MRI scanners with different field strengths, echo times and haemosiderin-sensitive sequences were used for detection of microbleeds. Although all these factors may have affected the sensitivity of microbleed detection,[22-24] I have shown that scanner strength and sequence did not result in significant heterogeneity of the results (Figure 3.7). However, studies utilising SWI in

detection of microbleeds are scarce, and power to demonstrate heterogeneity between MRI performed using SWI or T2*-GRE may be limited. Second, I was only able to provide preliminary insights as to whether risks of adverse events amongst patients with ≥5 microbleeds were time-dependent. Although my results were significant, the sample size was small and confirmation of my results by pooling individual patient data from multiple cohorts such as the Microbleeds International Collaborative Network[27] would be required before formal clinical recommendations could be made. Third, I was not able to determine the prognostic implications of patients with strictly deep, lobar, infratentorial or mixed microbleeds based on their burden due to small numbers in each subgroup. Previous meta-analysis have nevertheless shown that patients with microbleeds of mixed location are at greatest risk of a recurrent stroke.[9] My findings support this as amongst the 140 patients with ≥5 microbleeds on antiplatelet agents, 85.7% of them were of mixed location, suggesting that patients with the most severe forms of SVD tend to be of mixed location.

In conclusion, in TIA and ischaemic stroke patients with ≥5 microbleeds, antiplatelets are likely to be beneficial for secondary prevention of ischaemic events within 1-year of index event, especially amongst those presenting with a TIA where the early ischaemic risks are high. However, the associated haemorrhagic risks appear to outweigh its benefits thereafter. Whilst withholding antiplatelet drugs during the acute phase of TIA or ischaemic stroke based on microbleed burden may therefore be inappropriate, the benefits of gradual withdrawal of antiplatelets afterwards needs to be further studied.

Table 3.1. Characteristics and outcomes of OXVASC and HKU cohorts

	OXVASC, UK n=1080 (572 TIA, 508 ischaemic stroke)	HKU, HK n=1003 (1003 ischaemic stroke)	p
Baseline clinical characteristics			
Mean (SD) age (years)	68 (14)	69 (12)	0.17
Males (%)	566 (52)	601 (60)	0.001
Hypertension (%)	588 (55)	657 (66)	<0.0001
Diabetes (%)	143 (13)	284 (28)	<0.0001
Hyperlipidaemia (%)	399 (37)	256 (26)	<0.0001
Ever-smokers (%)	543 (50)	297 (30)	<0.0001
Atrial fibrillation (%)	167 (16)	130 (13)	0.10
Prior TIA or stroke (%)	201 (19)	154 (15)	0.047
Angina or myocardial infarction (%)	146 (14)	92 (9)	0.002
Prior antiplatelet use (%)	137 (13)	218 (22)	<0.0001
Prior warfarin use (%)	12 (1)	20 (2)	0.10
Prior NOAC use (%)	1 (0.1)	3 (0.3)	0.28
Imaging characteristics			
Magnet strength (Tesla)	1.5 (N=628) 3 (N=452)	3	
Method of detecting microbleeds	T2* GRE	SWI	
Median delay (interquartile range) to scan, days	3 (0-29)	4 (3-6)	0.71
N with microbleeds (%)	158 (15)	450 (45)	<0.0001
N with 1 microbleed (%)	79 (7)	184 (18)	<0.0001
N with 2-4 microbleeds (%)	46 (4)	147 (15)	<0.0001
N with ≥5 microbleeds (%)	40 (4)	119 (12)	<0.0001
N with strictly deep micrboleeds (%)	14 (1)	61 (6)	<0.0001
N with strictly lobar microbleeds (%)	73 (7)	161 (16)	<0.0001
N with strictly infratentorial microbleeds (%)	16 (2)	41 (4)	<0.0001
N with microbleeds of mixed location (%)	54 (5)	187 (19)	<0.0001
Post-event antithrombotic use			
Antiplatelets only			
Single antiplatelet (%)	677 (63)	725 (72)	<0.0001
Dual antiplatelet (%)	272 (25)	137 (14)	<0.0001
Anticoagulants only			
Warfarin (%)	105 (10)	50 (5)	0.001
NOAC (%)	6 (1)	35 (4)	<0.0001
Combined anticoagulant and antiplatelet (%)	7 (1)	19 (1)	0.010
Not on antithrombotic agents (%)	13 (1)	37 (4)	<0.0001
Clinical outcome			
Mean follow-up time, months (SD)	47 (27)	3 (20)	
Patient-years follow-up	4265	3168	
Recurrent stroke (%)	97 (9)	113 (11)	0.084
Ischaemic (%)	87 (8)	93 (9)	0.32
Fatal (%)	9 (10)	13 (14)	0.30
Intracerebral haemorrhage (%)	10 (1)	20 (2)	0.041
Fatal (%)	3 (30)	6 (30)	0.27
Acute coronary event (%)	26 (2)	52 (5)	0.001
Extracranial bleed[a] (%)	80 (7)	64 (6)	0.36
Major bleed (%)	19 (24)	21 (33)	0.58
Deaths (%)	161 (15)	130 (13)	0.20
Vascular deaths (%)	41 (25)	60 (46)	0.20

[a]Bleeding events from oral and nasal cavity excluded

TIA=transient ischaemic attack; GRE=gradient-recalled echo; NOAC=non-vitamin K antagonist oral anticoagulant; SWI=susceptibility weighted imaging

Table 3.2. Cause of TIA or ischaemic stroke according to TOAST criteria

	OXVASC (n=1080)	HKU (n=1003)
Small vessel occlusion (%)	132 (12.2)	425 (42.4)
Large artery atherosclerosis (%)	145 (13.4)	342 (34.1)
Cardio-embolism (%)	167 (15.5)	124 (12.4)
Multiple causes (%)	36 (3.3)	28 (2.8)
Others (%)	34 (3.2)	18 (1.8)
Undetermined (%)	539 (50.0)	44 (4.4)
Unknown (%)	26 (2.4)	22 (2.2)

TIA=transient ischaemic attack; TOAST=Trial of Org 10172 in Acute Stroke Treatment

Table 3.3. Burden of microbleeds in patients with or without antithrombotic medications after TIA or ischaemic stroke

	On antithrombotic medications (n=2032)	Not on antithrombotic medications (n=50)
Microbleeds		
0	1442 (71.0)	33 (66.0)
1-4	436 (21.5)	11 (22.0)
5-9	79 (3.9)	2 (4.0)
10-24	49 (2.4)	3 (6.0)
≥25	26 (1.3)	1 (2.0)

TIA=transient ischaemic attack

Table 3.4 Clinical predictors of a high-burden (≥5) of cerebral microbleeds, compared with <5 microbleeds as reference

	Univariate			Age and sex adjusted	Multivariate[a] adjusted	
	OXVASC	HKU	Combined[b]	Combined[b]	Combined[b]	p
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	
Age	1.05 (1.02-1.08)	1.01 (1.00-1.03)	1.02 (1.01-1.04)	1.02 (1.01-1.04)	1.02 (1.00-1.03)	0.035
Male sex	1.00 (0.53-1.89)	1.26 (0.85-1.88)	1.18 (0.84-1.65)	1.27 (0.91-1.79)	1.25 (0.86-1.80)	0.24
Hypertension	1.14 (0.60-2.15)	1.65 (1.07-2.55)	1.47 (1.03-2.10)	1.32 (0.92-1.90)	1.38 (0.93-2.03)	0.11
Diabetes	1.41 (0.61-3.25)	0.88 (0.57-1.36)	0.96 (0.65-1.42)	0.92 (0.62-1.35)	0.83 (0.55-1.26)	0.39
Hyperlipidaemia	1.27 (0.67-2.41)	0.93 (0.60-1.46)	1.03 (0.72-1.48)	1.02 (0.71-1.47)	0.89 (0.61-1.31)	0.57
Ever-smoker	1.09 (0.58-2.06)	1.04 (0.68-1.57)	1.05 (0.74-1.49)	1.02 (0.71-1.49)	0.99 (0.68-1.45)	0.96
Atrial fibrillation	1.38 (0.63-3.06)	0.96 (0.54-1.72)	1.08 (0.68-1.73)	0.90 (0.56-1.46)	0.74 (0.43-1.26)	0.26
GFR <60ml/min/1.73m^2	1.23 (0.60-2.50)	1.95 (1.28-2.98)	1.72 (1.20-2.47)	1.47 (1.00-2.15)	1.37 (0.93-2.03)	0.12
Premorbid antiplatelet use	1.76 (0.80-3.91)	1.38 (0.89-2.14)	1.46 (0.99-2.14)	1.28 (0.87-1.90)	1.42 (0.93-2.15)	0.10
Premorbid anticoagulation use	2.19 (0.28-17.30)	2.11 (0.77-5.79)	2.13 (0.86-5.27)	1.94 (0.78-4.81)	2.77 (1.00-7.70)	0.050

[a]Adjusted for all variables in univariate analysis
[b]Adjusted for for centre
OR=odds ratio; CI=confidence interval; GFR=glomerular filtration rate

Table 3.5 Associations of a high-burden (≥5) of cerebral microbleeds with other neuroimaging markers of small vessel disease

	Univariate			Age and sex adjusted	
	OXVASC	**HKU**	**Combined[a]**	**Combined[a]**	**p**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	
Periventricular white matter hyperintensity	3.25 (2.28-4.65)	3.32 (2.66-4.14)	3.30 (2.73-3.98)	3.45 (2.82-4.21)	<0.0001
Subcortical white matter hyperintensity	3.05 (2.17-4.28)	2.77 (2.18-3.52)	2.86 (2.35-3.48)	2.87 (2.35-3.51)	<0.0001
Lacunes	2.74 (1.39-5.38)	1.28 (0.87-1.89)	1.53 (1.08-2.15)	1.49 (1.06-2.11)	0.023
Basal-ganglia perivascular spaces[b]	2.56 (1.71-3.84)	2.87 (2.18-3.76)	2.77 (2.21-3.48)	2.80 (2.18-3.60)	<0.0001
Centrum-semiovale perivascular spaces[b]	1.88 (1.16-3.06)	0.76 (0.56-1.03)	1.01 (0.80-1.29)	0.96 (0.75-1.22)	0.72

[a]Adjusted for centre
[b]Missing data in 81 patients
OR=odds ratio; CI=confidence interval

Table 3.6 Cox regression analyses of risk of adverse events in all TIA and ischaemic stroke patients with increasing burden of microbleeds versus no microbleeds

	Unadjusted HR (95% CI)			HR (95% CI) adjusted for age and sex			HR (95% CI) adjusted for age, sex and vascular risk factors[a]			
Microbleed number	**1**	**2-4**	**≥5**	**1**	**2-4**	**≥5**	**1**	**2-4**	**≥5**	**p_{trend}**
Recurrent stroke										
OXVASC	2·06 (1·09-3·89)	2·32 (1·07-5·05)	3·02 (1·45-6·28)	1·75 (0·92-3·34)	2·01 (0·92-4·41)	2·49 (1·18-5·23)	1·70 (0·89-3·24)	1·83 (0·83-4·03)	2·41 (1·14-5·10)	0·0005
HKU	1·32 (0·79-2·21)	1·59 (0·93-2·70)	2·86 (1·77-4·61)	1·25 (0·74-2·09)	1·38 (0·81-2·36)	2·54 (1·57-4·11)	1·30 (0·78-2·19)	1·36 (0·79-2·33)	2·81 (1·73-4·57)	0·0002
Combined[b]	1.56 (1.04-2.33)	1.82 (1.17-2.83)	3.03 (2.04-4.51)	1.40 (0.93-2.09)	1.56 (1.00-2.43)	2.59 (1.74-3.86)	1.38 (0.93-2.07)	1.46 (0.94-2.28)	2.67 (1.80-3.98)	<0·0001
Ischaemic stroke										
OXVASC	1·98 (1·02-3·86)	1·34 (0·49-3·68)	2·84 (1·30-6·19)	1·72 (0·88-3·39)	1·16 (0·42-3·21)	2·42 (1·09-5·35)	1·65 (0·84-3·24)	0·98 (0·35-2·74)	2·32 (1·04-5·16)	0·060
HKU	1·41 (0·83-2·41)	1·49 (0·84-2·65)	1·78 (0·99-3·21)	1·33 (0·78-2·27)	1·29 (0·72-2·30)	1·58 (0·88-2·86)	1·40 (0·82-2·39)	1·28 (0·71-2·30)	1·83 (1·01-3·33)	0·054
Combined[b]	1.61 (1.06-2.44)	1.52 (0.93-2.49)	2.10 (1.31-3.38)	1.44 (0.95-2.19)	1.30 (0.79-2.14)	1.81 (1.13-2.91)	1.42 (0.94-2.16)	1.19 (0.73-1.96)	1.87 (1.16-3.01)	0.015
Intracerebral haemorrhage										
OXVASC	2·69 (0·31-23·06)	14·37 (3·42-60·34)	4·94 (0·58-42·41)	1·96 (0·22-17·11)	14·40 (3·28-63·11)	3·09 (0·35-27·16)	1·95 (0·21-18·04)	15·43 (3·21-74·31)	3·80 (0·41-35·33)	0·011
HKU	0·58 (0·07-4·92)	2·33 (0·56-9·73)	11·13 (3·86-32·04)	0·54 (0·06-4·65)	2·08 (0·49-8·78)	10·12 (3·49-29·35)	0·59 (0·07-5·05)	2·14 (0·50-9·12)	9·51 (3·25-27·81)	<0·0001
Combined[b]	1.05 (0.22-4.93)	4.53 (1.57-13.10)	11.35 (4.66-27.65)	0.93 (0.20-4.34)	3.93 (1.35-11.42)	9.52 (3.89-23.30)	0.91 (0.20-4.26)	4.09 (1.40-11.95)	9.81 (3.98-24.15)	<0·0001
Acute coronary event										
OXVASC	1·27 (0·30-5·43)	-	3·64 (1·08-12·26)	0·82 (0·19-3·52)	-	2·03 (0·59-6·94)	0·90 (0·21-3·87)	-	1·96 (0·57-6·81)	0·75
HKU	0·80 (0·36-1·74)	1·11 (0·51-2·43)	1·19 (0·52-2·71)	0·73 (0·33-1·60)	0·89 (0·40-1·95)	0·95 (0·42-2·18)	0·75 (0·34-1·64)	0·88 (0·40-1·95)	1·01 (0·44-2·33)	0·86
Combined[b]	0.88 (0.44-1.77)	0.99 (0.46-2.13)	1.53 (0.76-3.08)	0.73 (0.37-1.47)	0.76 (0.36-1.63)	1.15 (0.58-2.30)	0.73 (0.36-1.46)	0.72 (0.33-1.55)	1.23 (0.61-2.47)	0.98

	Unadjusted HR (95% CI)			HR (95% CI) adjusted for age and sex			HR (95% CI)* adjusted for age, sex and vascular risk factors[a]			
Microbleed number	**1**	**2-4**	**≥5**	**1**	**2-4**	**≥5**	**1**	**2-4**	**≥5**	**p_{trend}**
Ischaemic stroke and acute coronary event										
OXVASC	1·72 (0·92-3·23)	1·05 (0·39-2·86)	3·17 (1·65-6·11)	1·39 (0·74-2·62)	0·83 (0·30-2·26)	2·48 (1·27-4·84)	1·35 (0·72-2·55)	0·74 (0·27-2·05)	2·33 (1·19-4·56)	0·066
HKU	1·15 (0·74-1·80)	1·32 (0·82-2·11)	1·39 (0·84-2·31)	1·08 (0·69-1·68)	1·11 (0·69-1·78)	1·20 (0·72-2·00)	1·11 (0·71-1·74)	1·10 (0·68-1·77)	1·37 (0·82-2·28)	0·27
Combined[b]	1.33 (0.92-1.92)	1.33 (0.88-2.03)	1.80 (1.20-2.73)	1.16 (0.81-1.68)	1.09 (0.72-1.67)	1.50 (0.99-2.25)	1.15 (0.80-1.66)	1.02 (0.67-1.55)	1.56 (1.04-2.34)	0.085
Major extracranial bleeding[c]										
OXVASC	0·77 (0·10-5·82)	1·34 (0·18-10·10)	1·47 (0·20-11·07)	0·53 (0·07-4·06)	1·05 (0·14-8·05)	0·96 (0·13-7·33)	0·47 (0·06-3·64)	1·02 (0·13-7·90)	1·00 (0·13-7·73)	0·85
HKU	0·49 (0·11-2·17)	0·64 (0·14-2·88)	2·03 (0·72-5·77)	0·46 (0·10-2·05)	0·56 (0·13-2·53)	1·81 (0·63-5·15)	0·42 (0·09-1·91)	0·70 (0·15-3·18)	2·19 (0·75-6·38)	0·36
Combined[b]	0.57 (0.17-1.90)	0.79 (0.23-2.67)	1.93 (0.77-4.80)	0.49 (0.15-1.64)	0.67 (0.20-2.25)	1.57 (0.63-3.91)	0.47 (0.14-1.58)	0.71 (0.21-2.40)	1.71 (0.69-4.26)	0.59
All-cause mortality										
OXVASC	2·22 (1·37-3·61)	2·20 (1·22-4·00)	2·21 (1·16-4·21)	1·44 (0·88-2·35)	1·62 (0·89-2·95)	1·24 (0·65-2·38)	1·30 (0·79-2·14)	1·41 (0·76-2·62)	1·26 (0·65-2·43)	0·20
HKU	0·97 (0·59-1·59)	1·48 (0·92-2·39)	1·81 (1·12-2·92)	0·86 (0·52-1·41)	1·16 (0·72-1·88)	1·48 (0·92-2·39)	0·89 (0·54-1·46)	1·19 (0·73-1·93)	1·54 (0·95-2·49)	0·094
Combined[b]	1.43 (1.00-2.04)	1.80 (1.24-2.63)	2.07 (1.41-3.04)	1.10 (0.77-1.56)	1.33 (0.92-1.94)	1.43 (0.98-2.09)	1.05 (0.74-1.50)	1.24 (0.85-1.81)	1.46 (1.00-2.14)	0.041
Vascular death										
OXVASC	1·54 (0·55-4·35)	-	1·46 (0·35-6.09)	1·01 (0·36-2·87)	-	0·84 (0·20-3·52)	0·98 (0·34-2·80)	-	0·81 (0·19-3·41)	0·34
HKU	1·15 (0·57-2·31)	1·74 (0·88-3·42)	1·59 (0·75-3·37)	1·04 (0·52-2·10)	1·39 (0·70-2·73)	1·27 (0·60-2·70)	1·09 (0·54-2·20)	1·37 (0·69-2·74)	1·38 (0·65-2·95)	0·29
Combined[b]	1.21 (0.68-2.17)	1.38 (0.73-2.61)	1.53 (0.79-2.96)	1.00 (0.56-1.79)	1.07 (0.57-2.03)	1.11 (0.58-2.14)	1.00 (0.56-1.78)	1.02 (0.54-1.93)	1.17 (0.61-2.25)	0.71
Non-vascular death										
OXVASC	2·42 (1·34-4·36)	3·17 (1·68-5.99)	2·10 (0·91-4·82)	1·57 (0·87-2·85)	2·30 (1·21-4·37)	1·20 (0·52-2·78)	1·41 (0·77-2·59)	2·08 (1.07-4·05)	1·28 (0·55-2·99)	0·084
HKU	0·81 (0·40-1·63)	1·26 (0·64-2·48)	1·95 (1·05-3·62)	0·68 (0·34-1·39)	0·97 (0·49-1·91)	1·59 (0·86-2·97)	0·70 (0·34-1·42)	1·04 (0·52-2·07)	1·57 (0·84-2·93)	0·24
Combined[b]	1.48 (0.93-2.35)	2.05 (1.27-3.29)	2.27 (1.39-3.72)	1.11 (0.70-1.76)	1.48 (0.92-2.38)	1.56 (0.96-2.54)	1.06 (0.67-1.68)	1.41 (0.87-2.28)	1.60 (0.98-2.61)	0.037

[a]Hypertension, hyperlipidemia, diabetes, atrial fibrillation, smoking [b]Adjusted for centre [c]Dental and nasal bleeds excluded
TIA=transient ischaemic attack; HR, hazards ratio; CI, confidence interval

Table 3.7 Cox regression analyses of risk of adverse events amongst antiplatelet users with increasing burden of microbleeds versus no microbleeds

	Unadjusted HR (95% CI)			HR (95% CI) adjusted for age and sex			HR (95% CI) adjusted for age, sex and vascular risk factors[a]			
Microbleed number	**1**	**2-4**	**≥5**	**1**	**2-4**	**≥5**	**1**	**2-4**	**≥5**	**p_{trend}**
Recurrent stroke										
OXVASC	2·00 (0·99-4·03)	2·63 (1·14-6·08)	3·47 (1·66-7·24)	1·72 (0·85-3·50)	2·27 (0·97-5·30)	2·92 (1·38-6·17)	1·55 (0·76-3·15)	1·83 (0·78-4·31)	2·81 (1·31-6·00)	0·003
HKU	1·38 (0·79-2·41)	1·44 (0·78-2·66)	3·07 (1·83-5·15)	1·27 (0·72-2·22)	1·27 (0·69-2·36)	2·71 (1·61-4·56)	1·30 (0·74-2·29)	1·28 (0·69-2·38)	2·89 (1·70-4·90)	0·001
Combined[b]	1.61 (1.03-2.50)	1.77 (1.07-2.92)	3.35 (2.20-5.10)	1.43 (0.92-2.22)	1.53 (0.93-2.53)	2.87 (1.88-4.39)	1.38 (0.89-2.13)	1.39 (0.84-2.30)	2.89 (1.89-4.41)	<0·0001
Ischaemic stroke										
OXVASC	2·13 (1·06-4·31)	1·76 (0·64-4·84)	3·24 (1·48-7·09)	1·88 (0·92-3·83)	1·51 (0·54-4·21)	2·80 (1·26-6·21)	1·66 (0·81-3·40)	1·13 (0·40-3·21)	2·65 (1·18-5·95)	0·025
HKU	1·41 (0·79-2·52)	1·34 (0·69-2·57)	1·81 (0·96-3·43)	1·30 (0·73-2·33)	1·18 (0·61-2·27)	1·60 (0·84-3·03)	1·35 (0·75-2·42)	1·21 (0·62-2·36)	1·81 (0·95-3·45)	0·096
Combined[b]	1.69 (1.08-2.66)	1.53 (0.88-2.65)	2.29 (1.39-3.79)	1.51 (0.96-2.38)	1.32 (0.76-2.30)	1.98 (1.20-3.28)	1.44 (0.92-2.26)	1.16 (0.67-2.02)	2.01 (1.22-3.32)	0.013
Intracerebral haemorrhage										
OXVASC	-	12·90 (2·36-70·47)	6·35 (0·71-56·99)	-	12·74 (2·22-72·97)	4·20 (0·46-38·62)	-	10·02 (1·67-60·18)	4·51 (0·43-47·36)	0·042
HKU	0·95 (0·10-9·12)	2·57 (0·43-15·40)	16·62 (4·57-60·41)	0·86 (0·09-8·32)	2·30 (0·38-13·85)	14·91 (4·07-54·65)	0·88 (0·09-8·55)	2·22 (0·37-13·42)	13·15 (3·54-48·93)	<0·0001
Combined[b]	0.73 (0.09-6.05)	4.21 (1.16-15.27)	14.34 (5.22-39.39)	0.63 (0.08-5.24)	3.67 (1.01-13.33)	11.86 (4.29-32.81)	0.62 (0.07-5.13)	3.81 (1.04-13.96)	11.52 (4.09-32.43)	<0·0001
Acute coronary event										
OXVASC	1·69 (0·39-7·34)	-	3·01 (0·69-13·10)	1·14 (0·26-4·99)	-	1·76 (0·40-7·76)	1·18 (0·27-5·17)	-	1·62 (0·36-7·34)	0·95
HKU	1·15 (0·51-2·61)	1·03 (0·39-2·74)	1·47 (0·59-3·66)	1·02 (0·45-2·33)	0·85 (0·32-2·28)	1·16 (0·46-2·89)	1·06 (0·46-2·42)	0·92 (0·34-2·48)	1·08 (0·42-2·76)	0·95
Combined[b]	1.25 (0.61-2.58)	0.89 (0.34-2.32)	1.72 (0.78-3.78)	1.01 (0.49-2.07)	0.71 (0.27-1.83)	1.28 (0.58-2.80)	0.99 (0.48-2.04)	0.67 (0.26-1.74)	1.34 (0.61-2.95)	0.85

	Unadjusted HR (95% CI)			HR (95% CI) adjusted for age and sex			HR (95% CI) adjusted for age, sex and vascular risk factors[a]			
Microbleed number	**1**	**2-4**	**≥5**	**1**	**2-4**	**≥5**	**1**	**2-4**	**≥5**	**p_{trend}**
Ischaemic stroke and acute coronary event										
OXVASC	1·92 (0·99-3·73)	1·40 (0·51-3·83)	3·33 (1·66-6·65)	1·59 (0·82-3·11)	1·09 (0·40-3·00)	2·68 (1·33-5·42)	1·43 (0·73-2·80)	0·88 (0·31-2·45)	2·46 (1·21-5·03)	0·041
HKU	1·29 (0·80-2·09)	1·25 (0·73-2·16)	1·48 (0·84-2·58)	1·18 (0·73-1·91)	1·08 (0·62-1·86)	1·27 (0·72-2·22)	1·22 (0·75-1·98)	1·10 (0·63-1·92)	1·40 (0·80-2·48)	0·28
Combined[b]	1.52 (1.02-2.25)	1.36 (0.84-2.20)	1.97 (1.26-3.07)	1.32 (0.89-1.95)	1.13 (0.70-1.82)	1.63 (1.05-2.54)	1.28 (0.86-1.89)	1.02 (0.63-1.64)	1.68 (1.08-2.61)	0.058
Major extracranial bleed[c]										
OXVASC	1·25 (0·16-9·81)	-	-	0·92 (0·12-7·31)	-	-	0·79 (0·10-6·52)	-	-	0·35
HKU	0·71 (0·15-3·36)	-	2·43 (0·73-8·07)	0·62 (0·13-2·92)	-	2·08 (0·62-6·94)	0·66 (0·14-3·22)	-	2·32 (0·64-8·40)	0·53
Combined[b]	0.81 (0.23-2.85)	-	1.85 (0.60-5.66)	0.68 (0.20-2.39)	-	1.50 (0.49-4.60)	0.66 (0.19-2.31)	-	1.53 (0.49-4.75)	0.87
All-cause mortality										
OXVASC	3·00 (1·83-4·91)	2·16 (1·09-4·29)	2·42 (1·22-4·80)	1·97 (1·20-3·23)	1·55 (0·78-3·09)	1·42 (0·71-2·83)	1·66 (1·00-2·76)	1·22 (0·60-2·51)	1·54 (0·77-3·08)	0·10
HKU	1·08 (0·61-1·91)	1·73 (1·00-3·00)	2·18 (1·27-3·74)	0·88 (0·49-1·57)	1·42 (0·82-2·47)	1·75 (1·02-3·01)	0·90 (0·51-1·61)	1·56 (0·89-2·72)	1·63 (0·93-2·86)	0·046
Combined[b]	1.80 (1.22-2.66)	2.02 (1.31-3.10)	2.46 (1.61-3.74)	1.33 (0.90-1.95)	1.50 (0.98-2.30)	1.66 (1.09-2.51)	1.23 (0.84-1.80)	1.36 (0.88-2.09)	1.66 (1.09-2.54)	0.012
Vascular death										
OXVASC	2·41 (0·83-6.98)	-	2·16 (0·51-9.17)	1·62 (0·56-4·71)	-	1·38 (0·32-5·89)	1·49 (0·50-4·40)	-	1·35 (0·31-5·85)	0·97
HKU	1·37 (0·59-3·18)	2·46 (1·12-5·37)	2·04 (0·84-4·91)	1·20 (0·52-2·80)	2·06 (0·94-4·51)	1·59 (0·66-3·85)	1·24 (0·53-2·89)	2·17 (0·98-4·81)	1·55 (0·63-3·84)	0·12
Combined[b]	1.59 (0.81-3.10)	1.92 (0.94-3.95)	2.03 (0.96-4.28)	1.29 (0.66-2.51)	1.56 (0.76-3.19)	1.45 (0.69-3.05)	1.27 (0.65-2.46)	1.50 (0.73-3.08)	1.48 (0.70-3.14)	0.18
Non-vascular death										
OXVASC	3·10 (1·70-5·65)	3·26 (1·61-6.59)	2.02 (0·81-5.03)	2·05 (1·12-3·75)	2·27 (1·11-4·62)	1·19 (0·48-2.99)	1·74 (0·94-3·23)	1.84 (0·88-3.89)	1·36 (0·54-3·44)	0·089
HKU	0·87 (0·39-1·92)	1·25 (0·57-2·77)	2·26 (1·14-4·48)	0·65 (0·29-1·45)	1·00 (0·45-2·22)	1·87 (0·94-3·70)	0·66 (0·30-1·48)	1·10 (0·49-2·46)	1·59 (0·76-3·31)	0·27
Combined[b]	1.81 (1.10-2.97)	2.13 (1.24-3.65)	2.50 (1.46-4.27)	1.30 (0.79-2.12)	1.52 (0.89-2.60)	1.66 (0.98-2.83)	1.18 (0.72-1.94)	1.36 (0.79-2.35)	1.68 (0.98-2.89)	0.044

[a]Hypertension, hyperlipidemia, diabetes, atrial fibrillation, smoking [b]Adjusted for centre [c]Dental and nasal bleeds excluded
TIA=transient ischaemic attack; HR, hazards ratio; CI, confidence interval

Table 3.8 Cox regression analyses of risk of adverse events amongst single antiplatelet users with increasing burden of microbleeds versus no microbleeds

	Unadjusted HR (95% CI)[a]			HR (95% CI) adjusted for age and sex[a]			HR (95% CI) adjusted for age, sex and vascular risk factors[a,b]			
Microbleed number	**1**	**2-4**	**≥5**	**1**	**2-4**	**≥5**	**1**	**2-4**	**≥5**	**p_{trend}**
Recurrent stroke	1.83	1.92	4.17	1.62	1.64	3.53	1.52	1.52	3.46	<0·0001
	(1.11-3.02)	(1.09-3.38)	(2.63-6.63)	(0.98-2.66)	(0.93-2.90)	(2.22-5.62)	(0.93-2.49)	(0.86-2.68)	(2.17-5.51)	
Ischaemic stroke	1.89	1.96	2.82	1.66	1.67	2.37	1.55	1.51	2.36	0.002
	(1.14-3.16)	(1.09-3.52)	(1.63-4.86)	(1.00-2.76)	(0.93-3.00)	(1.38-4.10)	(0.93-2.57)	(0.84-2.72)	(1.37-4.08)	
Intracerebral haemorrhage	1.34	1.78	22.19	1.25	1.64	19.89	1.33	1.88	19.53	<0·0001
	(0.14-13.14)	(0.18-17.68)	(5.84-84.28)	(0.13-12.33)	(0.16-16.40)	(5.15-76.82)	(0.13-13.25)	(0.19-19.08)	(4.83-79.02)	
Acute coronary event	1.48	0.45	1.88	1.18	0.36	1.39	1.15	0.34	1.40	0.99
	(0.68-3.21)	(0.11-1.91)	(0.81-4.41)	(0.55-2.55)	(0.09-1.54)	(0.60-3.23)	(0.53-2.49)	(0.08-1.45)	(0.59-3.30)	
Ischaemic stroke and	1.76	1.48	2.32	1.51	1.22	1.89	1.45	1.12	1.93	0.017
acute coronary event	(1.14-2.72)	(0.87-2.53)	(1.43-3.75)	(0.98-2.33)	(0.71-2.08)	(1.17-3.07)	(0.94-2.22)	(0.65-1.92)	(1.19-3.12)	
Major extracranial	0.65	-	2.11	0.55	-	1.73	0.52	-	1.64	0.95
bleeding[c]	(0.14-2.91)		(0.67-6.64)	(0.12-2.46)		(0.55-5.44)	(0.12-2.36)		(0.50-5.36)	
All-cause mortality	2.10	2.05	2.84	1.52	1.55	1.90	1.40	1.56	1.98	0.002
	(1.37-3.22)	(1.24-3.39)	(1.78-4.52)	(0.99-2.32)	(0.94-2.55)	(1.20-3.02)	(0.91-2.14)	(0.95-2.59)	(1.24-3.18)	
Vascular death	2.24	1.81	2.44	1.80	1.48	1.73	1.83	1.54	1.87	0.094
	(1.10-4.53)	(0.77-4.27)	(1.08-5.51)	(0.89-3.63)	(0.63-3.47)	(0.77-3.89)	(0.90-3.69)	(0.65-3.65)	(0.82-4.25)	
Non-vascular death	1.77	2.18	2.65	1.25	1.60	1.74	1.11	1.58	1.81	0.033
	(1.00-3.12)	(1.17-4.06)	(1.45-4.82)	(0.71-2.20)	(0.86-2.97)	(0.96-3.16)	(0.63-1.95)	(0.85-2.94)	(0.98-3.31)	

[a]Adjusted for centre [b]Hypertension, hyperlipidemia, diabetes, atrial fibrillation, smoking [c]Dental and nasal bleeds excluded HR=hazards ratio; CI=confidence interval

Table 3.9 Summary of studies included in meta-analysis

Author	Region	Year	N and disease type (% TIA)	MRI	Mean age, yr	Males (%)	Antiplatelets (%)	Anticoagulants (%)	Follow-up (patient yrs)
Western cohorts									
Lau, Lovelock *et al*.	UK	2017	1080 IS/TIA (53)	1.5/3T T2* GRE	68	53	88	11	4265
CROMIS I[a]	UK	NA	68 IS/TIA	1.5T T2*GRE	66	66	81	16	136
Heidelberg[a]	NA	NA	265 IS	SWI	65	67	78	20	265
Kwa *et al*.[28]	Netherlands	2013	397 IS/TIA (49)	0.5/1/1.5T T2* GRE	65	58	90	10	1522
Fluri *et al*.[25]	Switzerland	2011	176 TIA	T2* GRE	69	61	77	12	44
Thijs *et al*.[29]	Belgium	2010	487 IS/TIA (27)	1/1.5/3T T2* GRE	72	61	73	27	812
Boulanger *et al*.[30]	Canada	2006	236 IS/TIA	3T T2* GRE	NA	55	NA	NA	354
Asian cohorts									
Lau *et al*.	Hong Kong, China	2017	1003 IS	3T SWI	69	60	88	10	3168
Lim, *et al*.[26]	Korea	2015	500 TIA	NA T2* GRE	64	58	91	15	125
Song, *et al*.[31]	Korea	2013	550 IS	3T MRI	70	59	35	87	1375
Mok *et al*.[32]	Hong Kong, China	2009	75 IS	1.5T T2* GRE	71	52	96	0	375
Soo *et al*.[33]	Hong Kong, China	2008	908 IS	1.5T T2* GRE	68	58	93	3	2059
Huang *et al*.[34]	China	2008	636 IS	1.5T T2* GRE	60	69	100	0	742
Naka *et al*.[35]	Japan	2006	183 Lacunar and atherothrombotic stroke	1T T2* GRE	NA	NA	93	2	275
Fan *et al*.[36]	China	2003	121 IS	1.5T T2* GRE	68	68	80	6	272
Imaizumi *et al*.[37]	Japan	NA	138 IS	1.5T T2* GRE	66	66	33	2	253

[a]Unpublished cohorts
IS=ischaemic stroke; TIA=transient ischaemic attack; GRE=gradient echo; SWI=susceptibility weighted imaging; NA=not available

Figure 3.1. Risk of A) recurrent stroke, B) recurrent ischaemic stroke, C) intracerebral haemorrhage, D) acute coronary events, E) intracerebral haemorrhage and major extracranial bleeding and F) all-cause mortality amongst TIA and ischaemic stroke patients on antiplatelets. Statistical significance of differences in risk is determined by log-rank test.

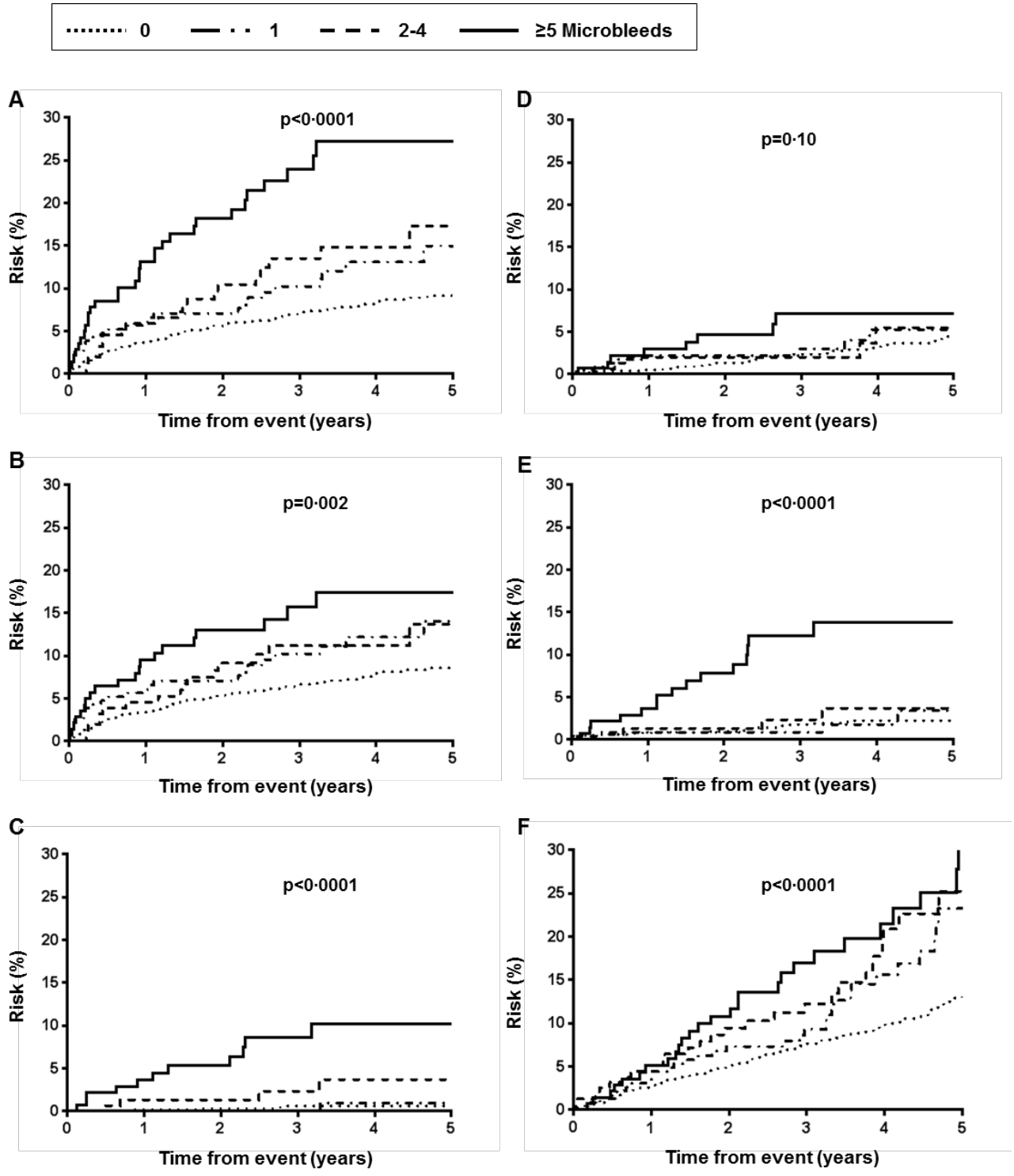


Figure 3.2. Risk of A) disabling or fatal and B) nondisabling ischaemic stroke and intracerebral haemorrhage by microbleed burden in TIA and ischaemic stroke patients on antiplatelets

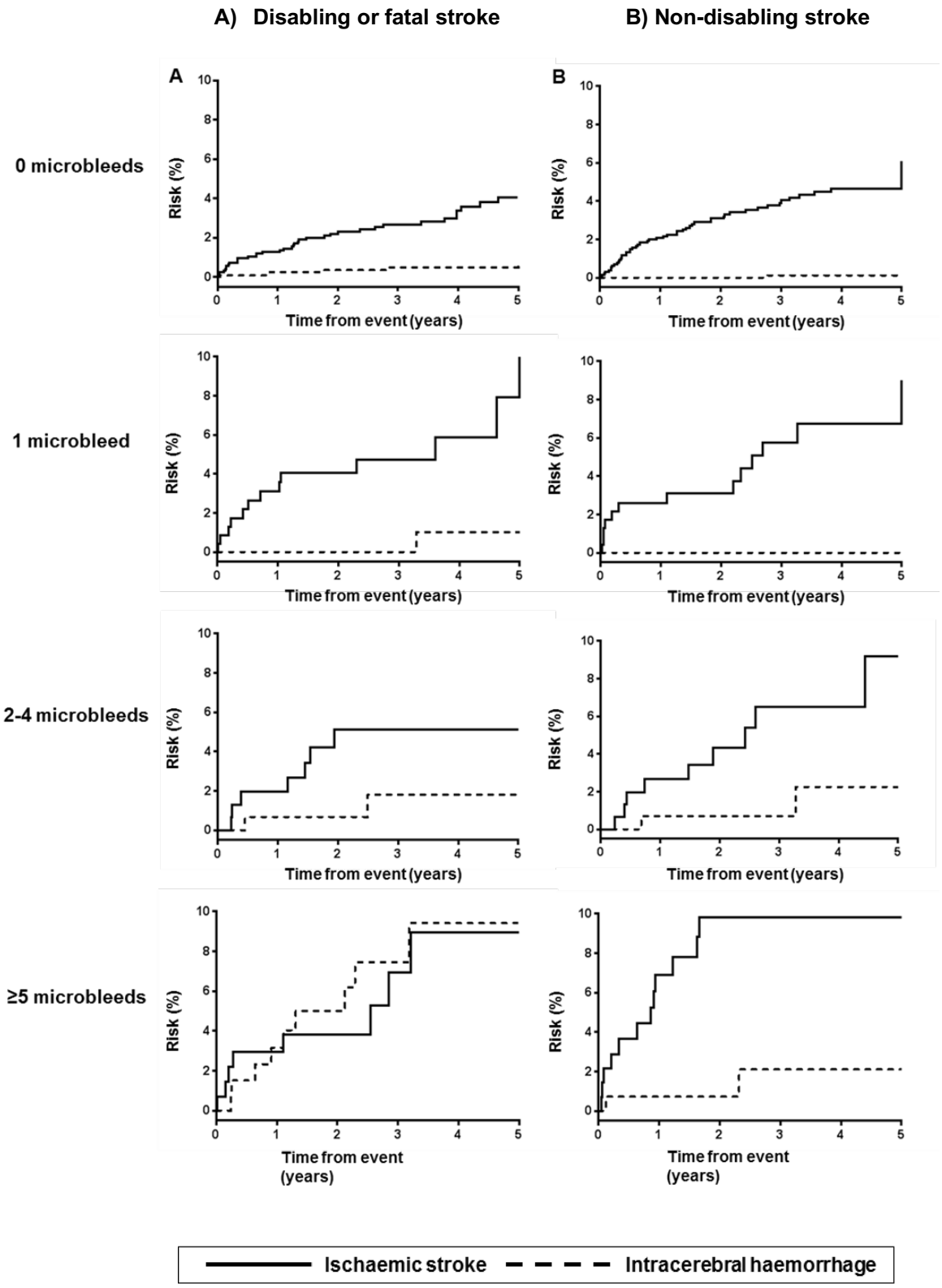
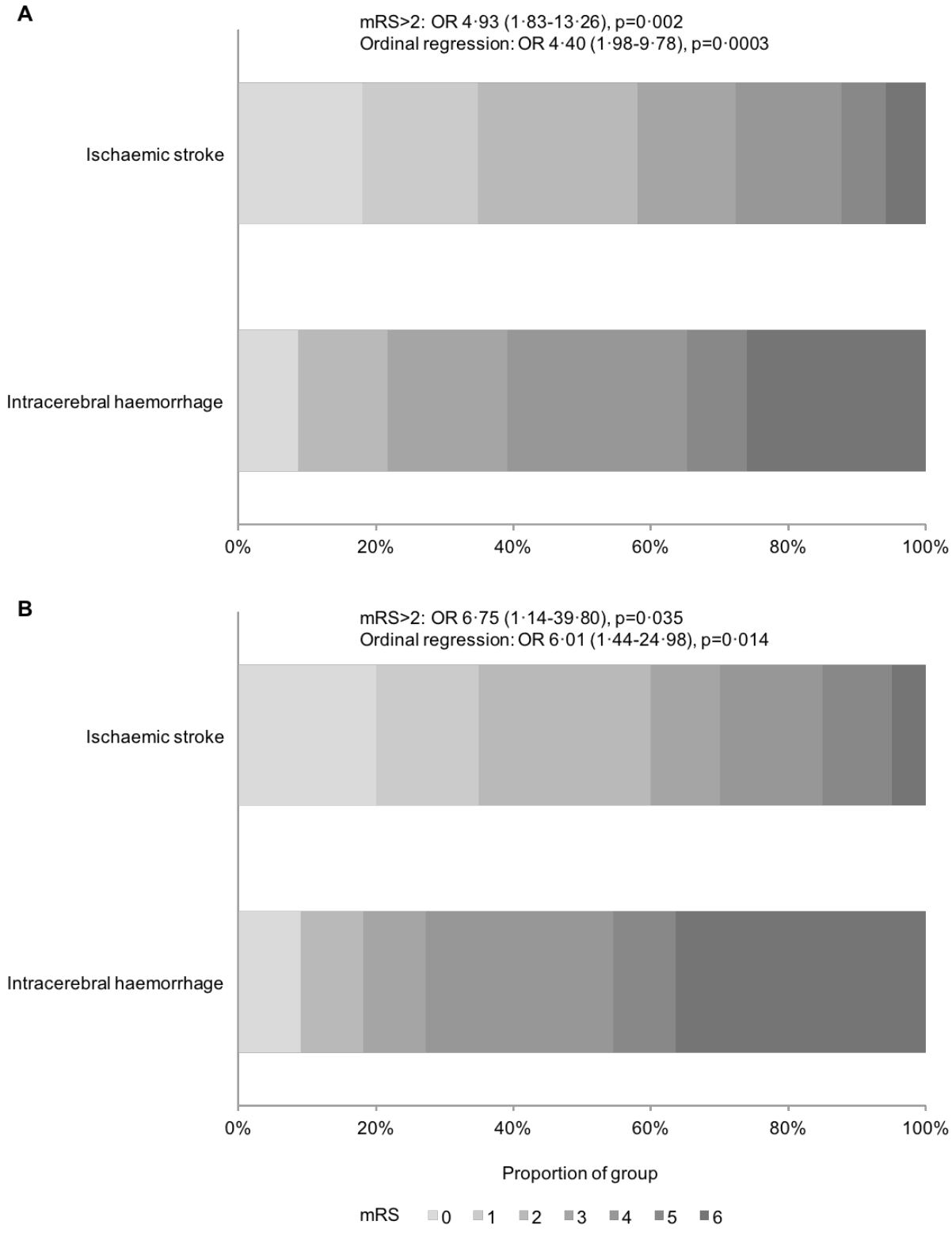


Figure 3.3 Severity of recurrent stroke (mRS at 1-month) in A) all antiplatelet users and in B) antiplatelet users with ≥5 microbleeds



OR=odds ratio; mRS=modified Rankin Scale

Figure 3.4 Risk of ischaemic and haemorrhagic events in TIA and ischaemic stroke patients with <5 and ≥5 microbleeds on antiplatelets, within 1 year of index event and between 1 and 5 years after index event

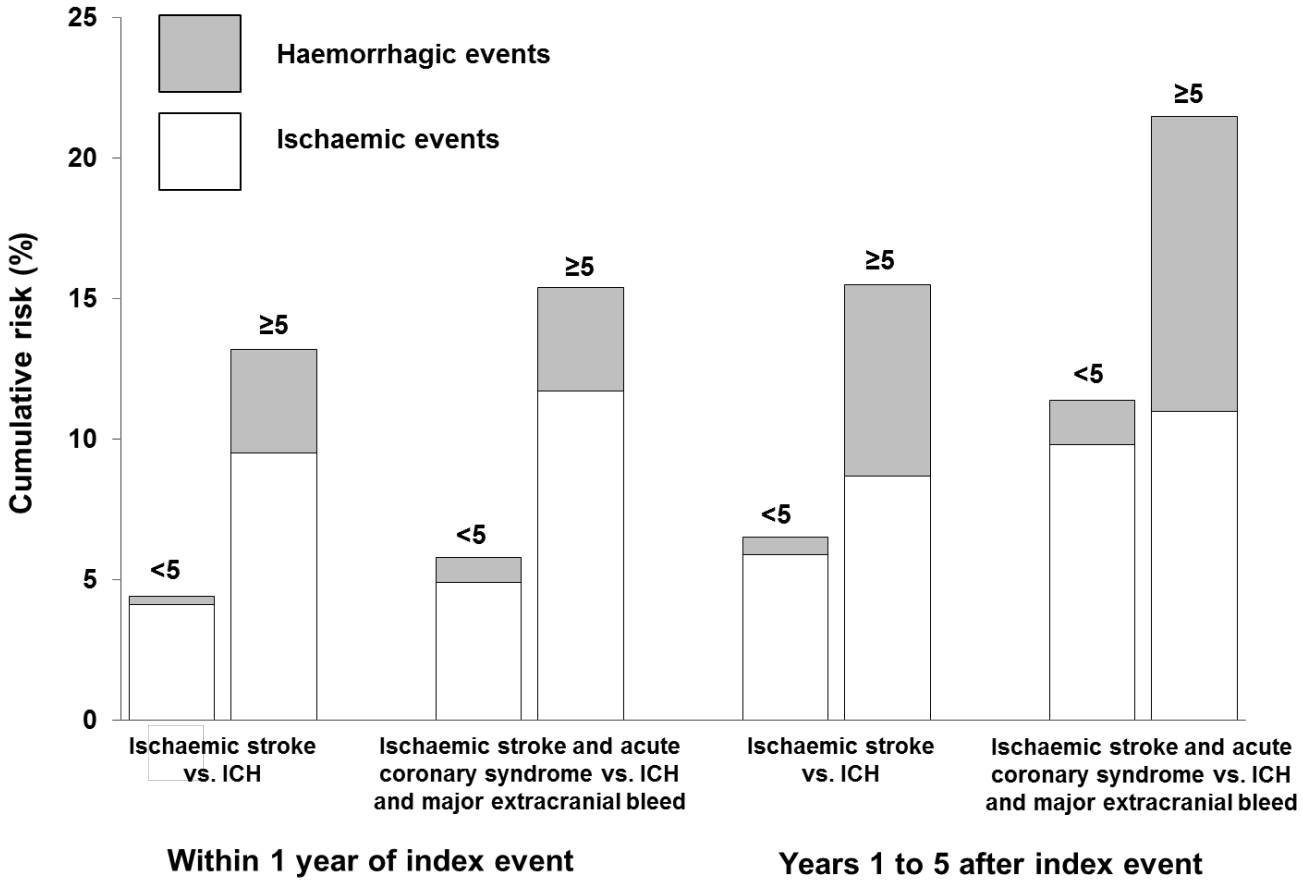


Figure 3.5 Pooled analyses of relative risk estimates from the current and previous studies showing risk of recurrent ischaemic stroke amongst TIA and ischaemic stroke patients on antiplatelet agents with microbleeds versus those without

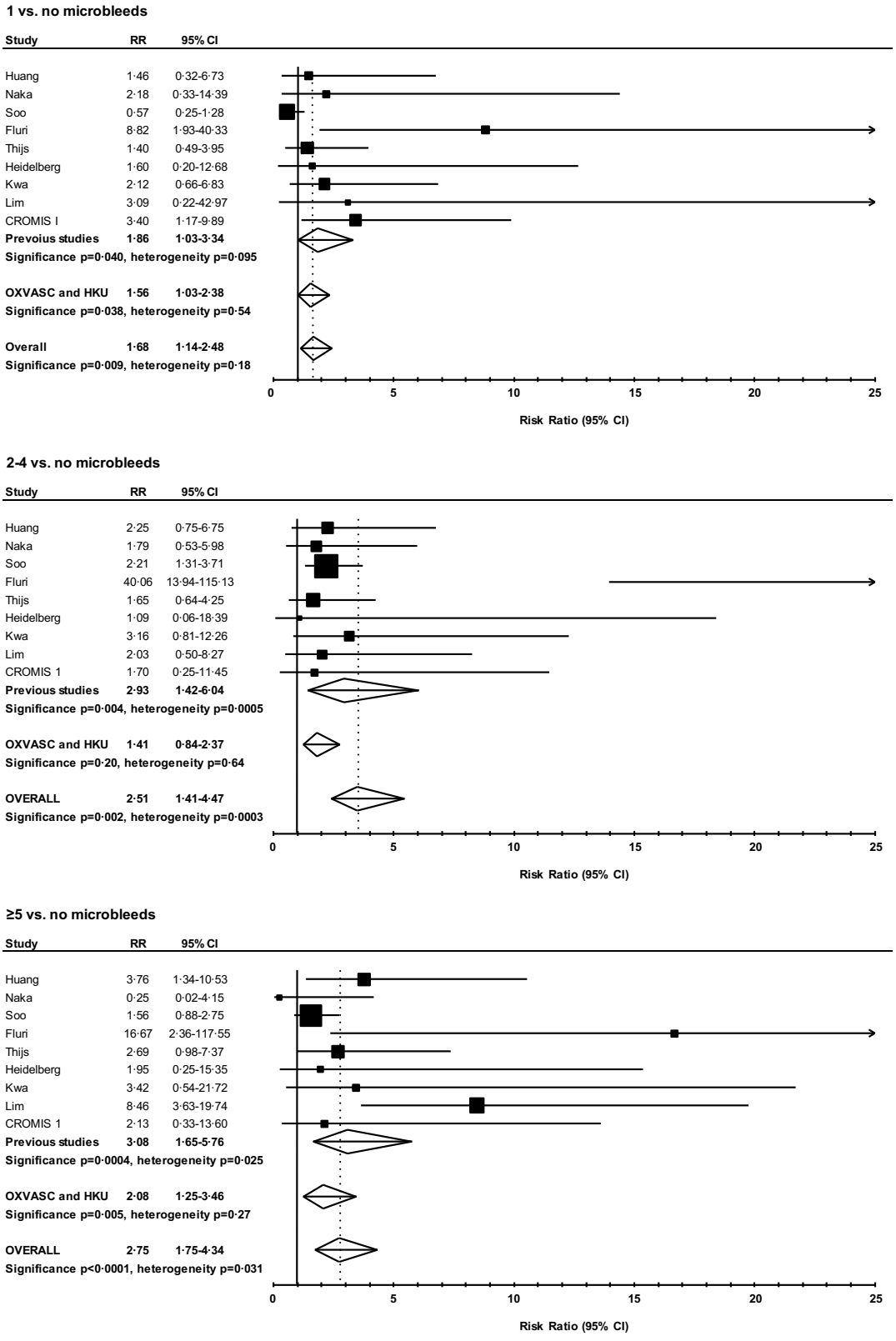


Figure 3.6 Pooled analyses of relative risk estimates from the current and previous studies showing risk of intracerebral haemorrhage amongst TIA and ischaemic stroke patients on antiplatelet agents with microbleeds versus those without

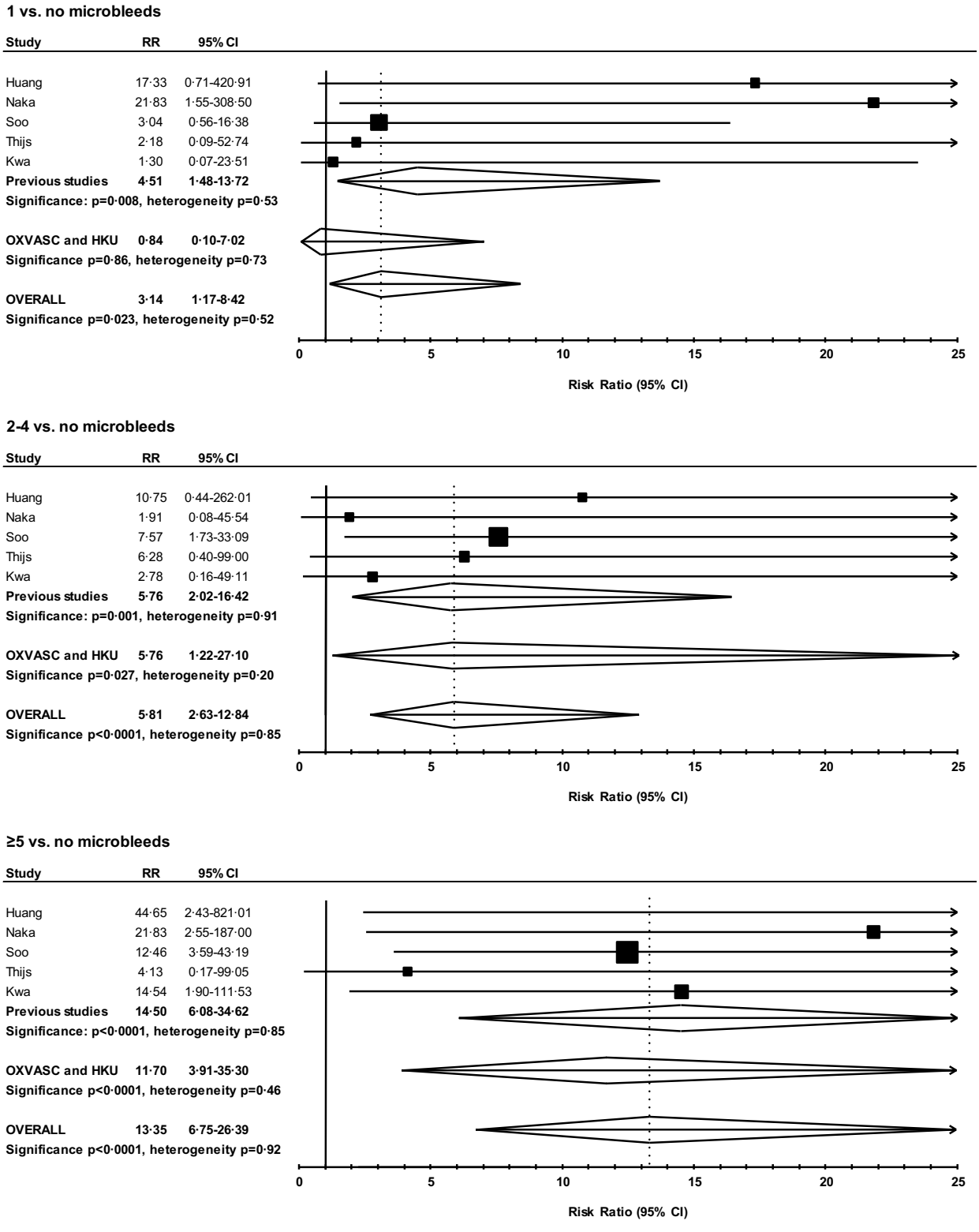
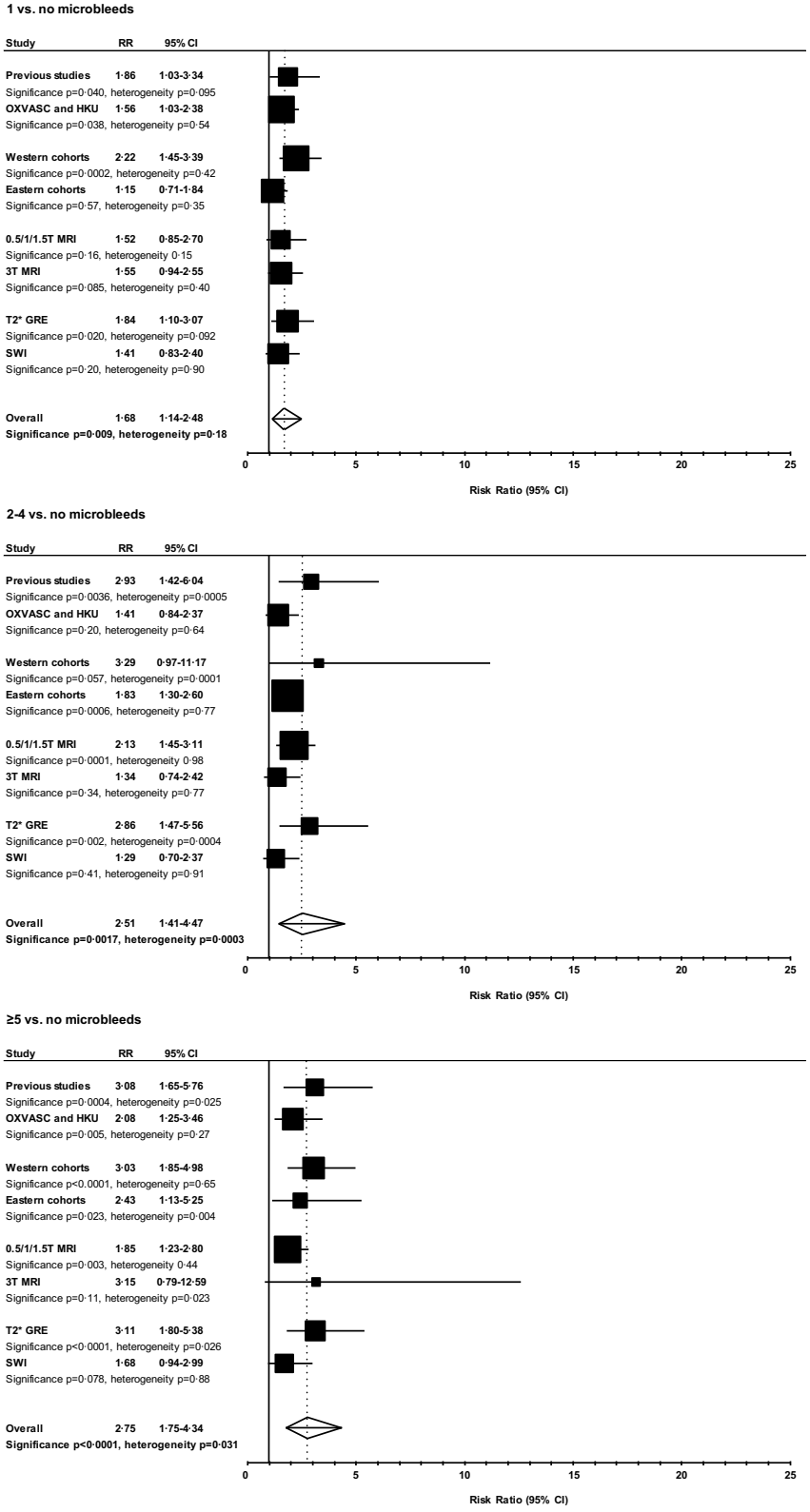


Figure 3.7 Pooled analyses of relative risk estimates from the current and previous studies showing risk of A) recurrent ischaemic stroke and of B) intracerebral haemorrhage amongst TIA and ischaemic stroke patients on antiplatelet agents with microbleeds versus those without, stratified by geographical origin, MRI scanner magnet strength, MRI sequence, and number of microbleeds

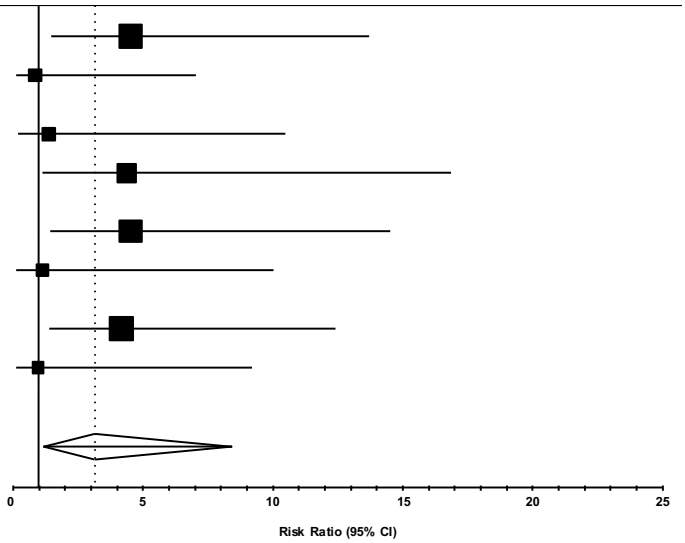
(A)



(B)

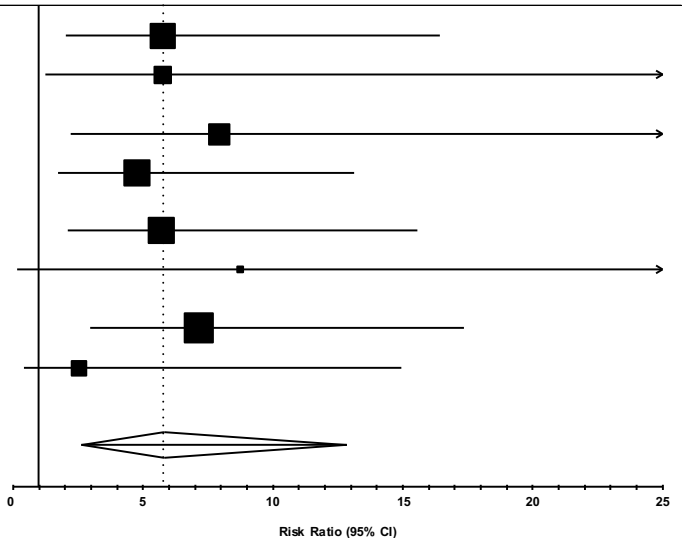
1 vs. no microbleeds

Study	RR	95% CI
Previous studies	**4·51**	**1·48-13·72**
Significance p=0·008, heterogeneity p=0·53		
OXVASC and HKU	**0·84**	**0·10-7·02**
Significance p=0·86, heterogeneity p=0·73		
Western cohorts	**1·37**	**0·18-10·47**
Significance p=0·76, heterogeneity p=0·85		
Eastern cohorts	**4·38**	**1·14-16·87**
Significance p=0·032, heterogeneity p=0·26		
0.5/1/1.5T MRI	**4·52**	**1·41-14·50**
Significance p=0·011, heterogeneity 0·45		
3T MRI	**1·13**	**0·13-10·04**
Significance p=0·92, heterogeneity p=0·58		
T2* GRE	**4·15**	**1·39-12·41**
Significance p=0·011, heterogeneity p=0·57		
SWI	**0·96**	**0·10-9·18**
Significance p=0·97		
Overall	**3·14**	**1·17-8·42**
Significance p=0·023, heterogeneity p=0·52		



2-4 vs. no microbleeds

Study	RR	95% CI
Previous studies	**5·76**	**2·02-16·42**
Significance p=0·001, heterogeneity p=0·91		
OXVASC and HKU	**5·76**	**1·22-27·10**
Significance p=0·027, heterogeneity p=0·20		
Western cohorts	**7·94**	**2·22-28·37**
Significance p=0·001, heterogeneity p=0·67		
Eastern cohorts	**4·77**	**1·73-13·13**
Significance p=0·003, heterogeneity p=0·70		
0.5/1/1.5T MRI	**5·71**	**2·10-15·56**
Significance p=0·0007, heterogeneity 0·91		
3T MRI	**8·73**	**0·16-491·03**
Significance p=0·29, heterogeneity p=0·17		
T2* GRE	**7·15**	**2·95-17·34**
Significance p<0·0001, heterogeneity p=0·90		
SWI	**2·53**	**0·43-14·96**
Significance p=0·31		
Overall	**5·81**	**2·63-12·84**
Significance p<0·0001, heterogeneity p=0·85		



≥5 vs. no microbleeds

Study	RR	95% CI
Previous studies	**14·50**	**6·08-34·62**
Significance p<0·0001, heterogeneity p=0·85		
OXVASC and HKU	**11·70**	**3·91-35·03**
Significance p<0·0001, heterogeneity p=0·46		
Western cohorts	**8·15**	**2·13-31·23**
Significance p=0·002, heterogeneity p=0·75		
Eastern cohorts	**15·84**	**7·18-34·92**
Significance p<0·0001, heterogeneity p=0·87		
0.5/1/1.5T MRI	**14·93**	**6·10-36·54**
Significance p<0·0001, heterogeneity 0·75		
3T MRI	**16·77**	**4·81-58·46**
Significance p<0·0001, heterogeneity p=0·35		
T2* GRE	**12·76**	**5·69-28·61**
Significance p<0·0001, heterogeneity p=0·85		
SWI	**14·92**	**4·18-53·28**
Significance p<0·0001		
Overall	**13·35**	**6·75-26·39**
Significance p<0·0001, heterogeneity p=0·92		

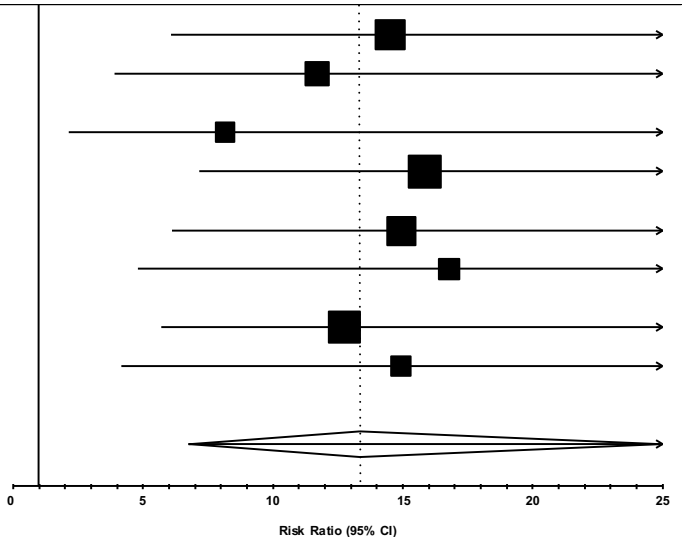
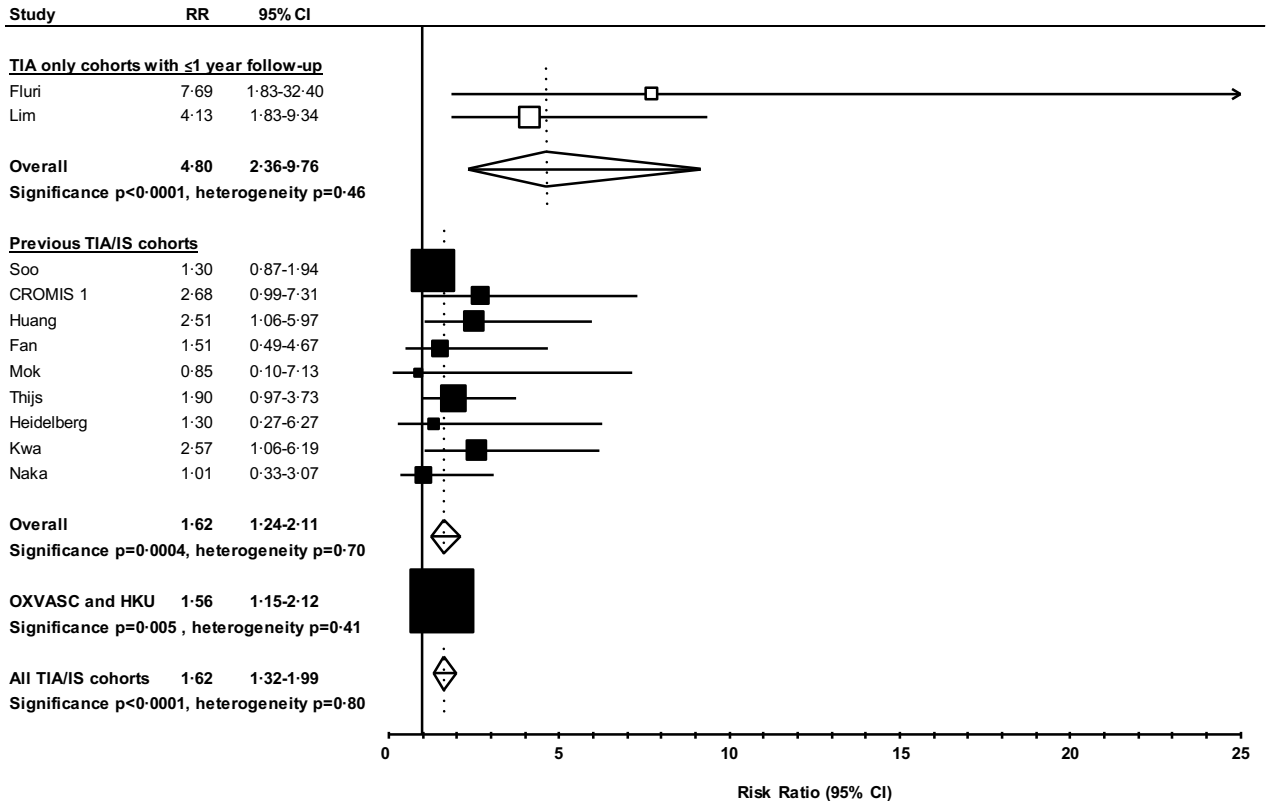


Figure 3.8 Pooled analyses of relative risk estimates from the current and previous studies showing risk of A) recurrent ischaemic stroke and B) intracerebral hemorrhage in TIA and ischaemic stroke patients on antiplatelet agents, stratified by presence versus absence of microbleeds

(A) Ischaemic stroke



(B) Intracerebral haemorrhage

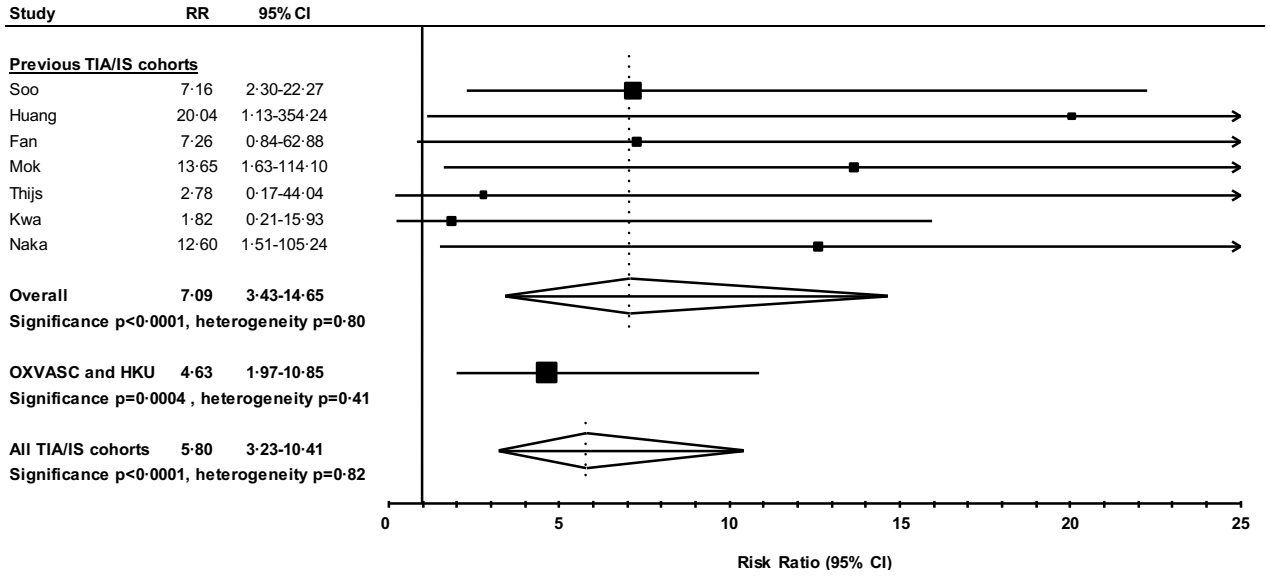


Figure 3.9 Pooled analyses of relative risk estimates from the current and previous studies showing risk of recurrent ischaemic stroke amongst TIA and ischaemic stroke patients with microbleeds versus those without

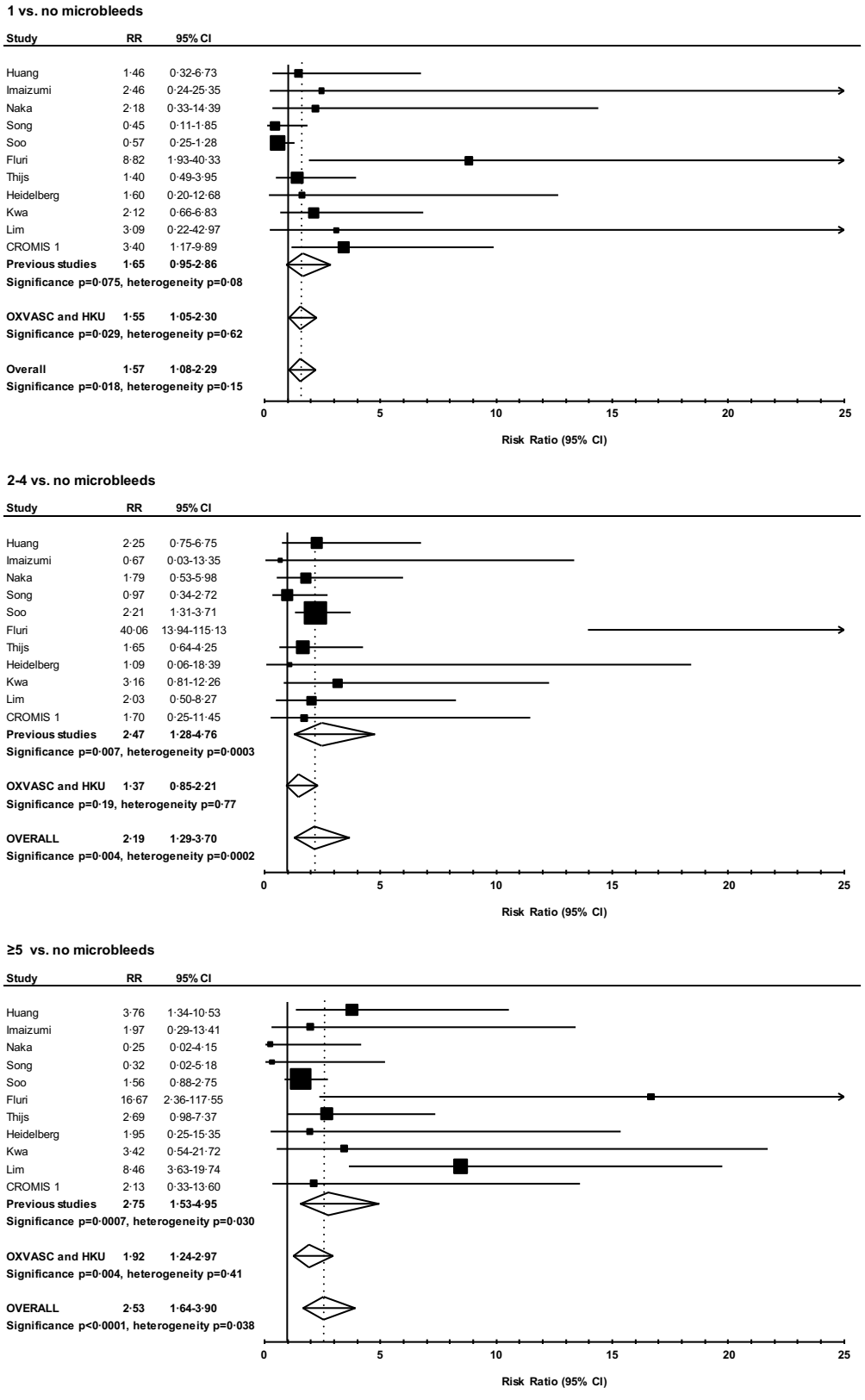
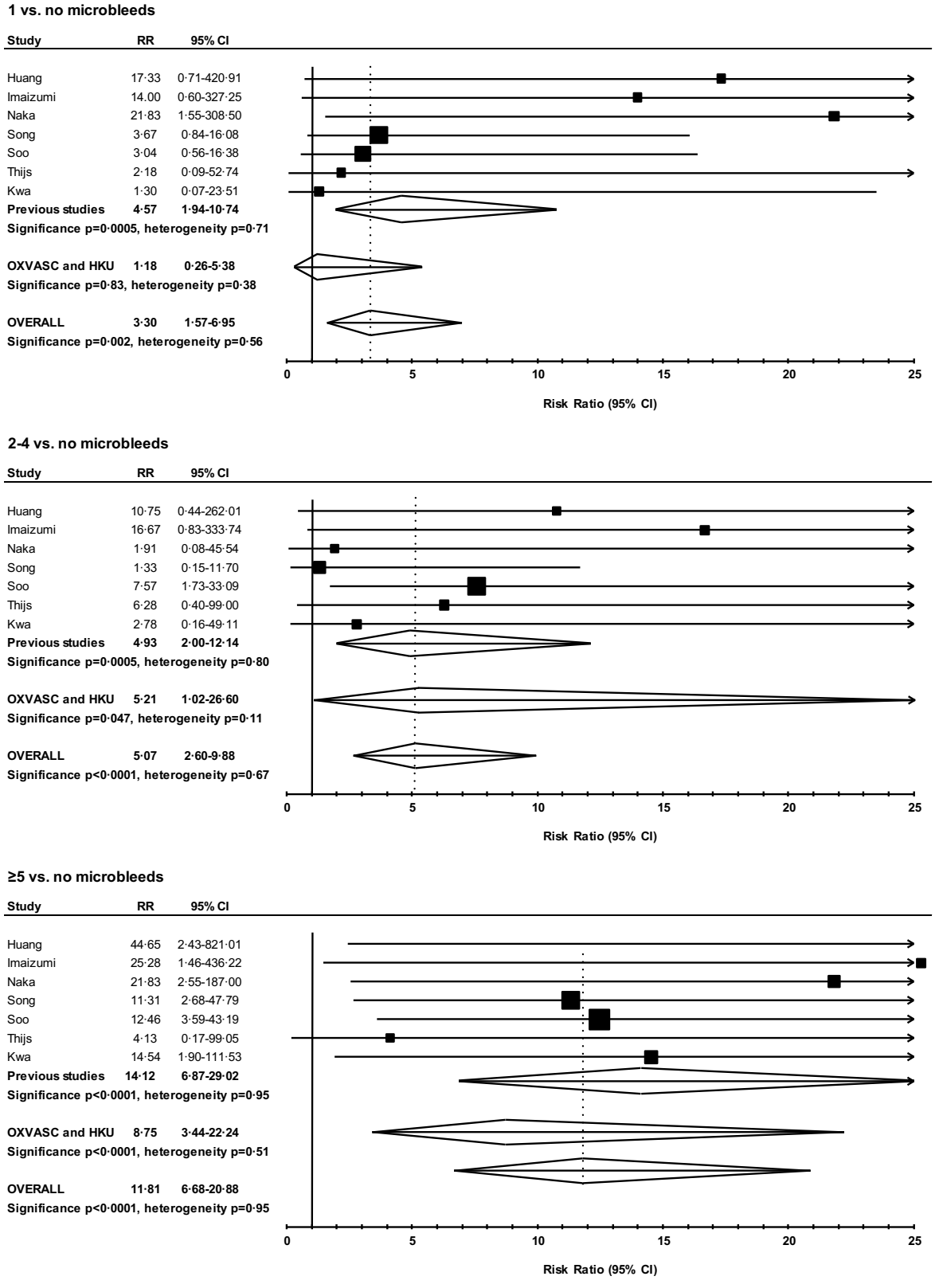


Figure 3.10 Pooled analyses of relative risk estimates from the current and previous studies showing risk of intracerebral haemorrhage amongst TIA and ischaemic stroke patients with microbleeds versus those without



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Chapter 4

Long-term outcomes in anticoagulant users with microbleeds after TIA and ischaemic stroke

4.1 Chapter outline

Compared with antiplatelet use, warfarin is associated with an increased risk of intracerebral haemorrhage (ICH). However, in patients with transient ischaemic attack (TIA) or ischaemic stroke with microbleeds, whether the risk of ICH is similarly greater than in antiplatelet users is uncertain.

Therefore, in this Chapter, I determined the risks of recurrent ischaemic stroke and ICH in warfarin users, with and without microbleeds in two independent cohorts - The Oxford Vascular Study (OXVASC) and The University of Hong Kong (HKU). I also pooled my results with those from other cohorts and compared the absolute annualised rate of recurrent ischaemic stroke and ICH in warfarin versus antiplatelet users.

In 222/2083 patients (OXVASC n=118/1080, HKU n=104/1003) who were prescribed with warfarin (n=175, OXVASC n=112, HKU n=63) or a non-vitamin K antagonist oral anticoagulant (NOAC) (n=47, OXVASC n=6, HKU n=41) after TIA or ischaemic stroke, microbleeds were present in 69/222 (31.1%) of the population. Amongst warfarin users, microbleed presence was independently associated with subsequent risk of ICH [multivariate-adjusted odds ratio (OR) 6.44, 95% confidence interval (CI) 1.07-38.69, p=0.042) but not ischaemic stroke (p=0.93) after 42±25 months of follow-up. In patients with microbleeds, the pooled annual rate of ischaemic stroke and ICH in 652 warfarin users and 5627 antiplatelet users was 1.84% (95% CI 0.92-3.29%) versus 4.82% (4.09-5.64%)(p=0.005) and 1.84% (0.92-3.29%) versus 1.49% (1.10-1.98%)(p=0.52). No patients on NOACs (17/47 with microbleeds) developed an ICH after 25±14 months of follow-up.

I concluded that, although TIA or ischaemic stroke patients with microbleeds on warfarin are at increased risk of ICH, this risk did not differ from that of antiplatelet users.

4.2 Introduction

Cerebral microbleeds are small, perivascular haemosiderin deposits that result from blood leakage from pathologically fragile small vessels affected by hypertensive or cerebral amyloid angiopathy, or occur secondary to an ischaemic insult.[1-3] A high burden of microbleeds is associated with an increased risk of intracerebral haemorrhage (ICH) and possibly also of ischaemic stroke.[4-6]

Warfarin is highly effective in reducing the risk of ischaemic stroke in patients with atrial fibrillation, but is associated with an ICH risk of ~0.5% per year.[7] Compared with antiplatelet users and non-antithrombotic users, an excess of microbleeds have been noted in warfarin users with ICH, suggesting that microbleeds increase the risk of warfarin-associated ICH.[4] Whilst these findings may have implications on atrial fibrillation management,[8] long-term prognostic data of the risk of recurrent stroke in warfarin users with microbleeds are lacking and recent meta-analysis on prognostic implications of microbleeds in transient ischaemic attack (TIA) or ischaemic stroke patients have not stratified patients according to antithrombotic use.[6] Although current guidelines suggest that warfarin for stroke prevention in atrial fibrillation should probably be avoided in patients with lobar ICH and probable or confirmed cerebral amyloid angiopathy,[9, 10] they make no recommendations on the safety of warfarin in TIA or ischaemic stroke patients with microbleeds.[11] In these instances, some clinicians may be tempted to avoid anticoagulation and prescribe antiplatelet agents, due to a perceived lower risk of ICH.[4, 7] However, whether the risk of ICH in antiplatelet users with microbleeds is indeed lower than that of warfarin users has not been studied. More importantly, antiplatelet agents are not effective in reducing the risk of ischaemic stroke in patients with atrial fibrillation, and may result in overall net harm.[7, 12]

In order to understand the long-term risk of recurrent ischaemic stroke and ICH in TIA and ischaemic stroke patients with microbleeds and on warfarin, I analysed data from The Oxford Vascular Study (OXVASC) and The University of Hong Kong (HKU) that consisted of more than 2000 Caucasians and Chinese with TIA and ischaemic stroke, 222 of whom were subsequently prescribed with anticoagulants. I also pooled my results with those from a recent meta-analysis[6]

and compared the risks of ischaemic stroke and ICH in warfarin and antiplatelet users with and without microbleeds and according to microbleed burden. I hypothesise that in TIA and ischaemic stroke patients with microbleeds, anticoagulant-users may be associated with a similar or higher risk of ICH compared with antiplatelet-users, but the risk of recurrent ischaemic stroke may be lower in patients on warfarin compared with antiplatelet-users.

4.3 Methods

4.3.1 Study populations

1080 consecutive cases with TIA or ischaemic stroke who were predominantly Caucasians and 1076 consecutive cases with ischaemic stroke who were predominantly Chinese were recruited from OXVASC and HKU (see section 2.1). After excluding patients who had incomplete clinical data or were lost to follow-up, the analysis in this Chapter includes 222/2083 patients who was diagnosed with a TIA or ischaemic stroke, received a magnetic resonance imaging (MRI) incorporating a haemosiderin-sensitive sequence and was prescribed with warfarin or a non-vitamin K antagonist oral anticoagulant (NOAC) within 1-month of TIA or ischaemic stroke. Baseline data was collected as described in section 2.1 Further details of scan parameters are provided in Table 2.1. Presence and burden of cerebral microbleeds were coded as described in section 2.3. In both cohorts, antithrombotic treatment was started routinely irrespective of microbleed burden. However, patients who presented with an amyloid spell (with concomitant cortical superficial siderosis and/or multiple microbleeds), were not considered as having TIAs and were not included in this study.

4.3.2 Statistical analysis

I compared differences in baseline and imaging characteristics in the OXVASC and HKU cohorts using Student's t-test for continuous variables and Chi-squared test for categorical variables. I used Kaplan-Meier survival analysis to compare the risks of a recurrent stroke (ischaemic and haemorrhagic) and all-cause mortality amongst the 175 warfarin users, censored at death or on March 31, 2015, according to presence or absence of microbleeds and burden of microbleeds. I determined, by Cox regression analysis, the unadjusted and adjusted (for age, sex, vascular risk factors and centre), risks of adverse events amongst patients with presence of, and 1 and ≥2 microbleeds, compared with no microbleeds as reference. The following outcomes were studied: recurrent stroke, recurrent ischaemic stroke, ICH, major extracranial haemorrhage, death and vascular death.

I also performed a meta-analysis to obtain a pooled risk estimate of recurrent ischaemic stroke or ICH according to presence of, and 1, 2-4 and ≥5 microbleeds, compared with no microbleeds as reference, in patients on warfarin or on antiplatelet agents using a random effects analysis. I pooled results from OXVASC and HKU with other cohorts from a recent systematic review that included patients that were predominantly (≥70% of the study population) on warfarin (1 cohort) or antiplatelet agents (11 cohorts).[6] In patients on warfarin and antiplatelet agents, I also calculated the pooled annualised incidence rates of recurrent ishaemic stroke and ICH, stratified by presence or absence of microbleeds and by burden (1-4 and ≥5) of microbleeds.

All analyses were done with SPSS version 22.

4.4 Results

4.4.1 Baseline clinical and neuroimaging characteristics

222/2083 patients (OXVASC n=118/1080, HKU n=104/1003) who were prescribed with warfarin or a NOAC within 1-month of TIA or ischaemic stroke were included in the final analysis. Clinical characteristics and outcomes of patients prescribed with warfarin or a NOAC are shown in Table 4.1 (clinical characteristics of entire cohort shown in Table 3.1). There were no differences in mean age, sex, prevalence of hypertension, hyperlipidaemia or atrial fibrillation between the two cohorts (all $p>0.05$) (Table 4.1). Patients from HKU had a higher prevalence of diabetes (26% vs. 13%, $p=0.012$), whilst patients from OXVASC were more likely to be ever-smokers (44% vs. 20%, $p=0.0002$). The prevalence of microbleeds was greater in HKU (39% vs. 24%, $p=0.012$), but there were no significant differences in the overall burden of microbleeds ($p=0.071$). Patients from HKU had a greater proportion of patients with microbleeds of mixed location (16% vs. 8%, $p=0.044$), but the proportion of strictly deep ($p=0.86$) or strictly lobar microbleeds ($p=0.33$) was similar in the 2 cohorts.

Eighty nine percent of the population had a history of atrial fibrillation. In all patients on anticoagulants, warfarin was more commonly used in OXVASC (95% vs. 61%, $p<0.0001$), whilst NOACs were more commonly used in HKU [39% vs. 5%, $p<0.0001$; dabigatran (HKU n=34, OXVASC n=3); rivaroxaban (HKU n=7, OXVASC n=1); apixaban (HKU n=0, OXVASC n=2)].

4.4.2 Long-term prognostic implications of microbleeds in TIA and ischaemic stroke patients on anticoagulants

In the 175 patients on warfarin (OXVASC n=112, 386 patient-years follow-up; HKU n=63, 218 patient-years follow-up), 21 patients developed a recurrent stroke (3.5%/year; 15 ischaemic strokes, 2.5%/year; 6 ICHs, 1.0%/year) (Table 4.1) after a mean follow-up of 42±25 months (604 patient-years). Sixteen patients developed an extracranial bleed (2.8%/year; 6 major, 1.0%/year) and 33 patients died during follow-up (5.3%/year, 52% vascular deaths). Presence of microbleeds

was associated with risk of ICH (log-rank test p=0.019), but there were no associations between presence of microbleeds with risk of recurrent ischaemic stroke (p=0.65) and all-cause mortality (p=0.98) (Figure 4.1). Cox-regression analysis showed that independent of vascular risk factors, presence of microbleeds was significantly associated with subsequent risk of ICH [hazard ratio (HR) 6.44, 95% confidence interval (CI) 1.07-38.69, p=0.042], but not ischaemic stroke, extracranial bleeding, all cause, or vascular mortality (all p>0.05) (Table 4.2). Similarly, compared with patients with no microbleeds, an increasing burden of microbleeds was independently associated with subsequent risk of ICH [1: HR 2.63 (0.22-32.03); ≥2: HR 13.00 (1.76-96.23), p_{trend}=0.014], but not other adverse outcomes (Table 4.3, Figure 4.2).

In the 47 patients on NOACs (OXVASC n=6, 6 patient-years follow-up; HKU n=41, 92 patient-years follow-up; combined mean follow-up of 25±14 months, 98 patient-years), 6 patients developed a recurrent stroke (6.4%/year), all of which were ischaemic strokes. No patients on NOACs [17/47 (36%) with microbleeds, 9% with ≥5 microbleeds] developed an ICH.

I pooled the results from OXVASC and HKU with a study[13] from a recent meta-analysis[6] that included 477 patients on warfarin (total: 725 patients, 1484 patient years follow-up). The pooled relative risk estimates of a recurrent ischaemic stroke in 225 TIA or ischaemic stroke patients with microbleeds versus 500 patients without was 0.76 (95% CI 0.38-1.53, p=0.44) (Figure 4.3), which was lower than the pooled relative risk estimates of recurrent ischaemic stroke in 5629 patients (12823 patient years follow-up) on antiplatelet agents [1.84 (1.45-2.34), p<0.0001; p_{het}=0.019]. However, the pooled relative risks of ICH in TIA or ischaemic stroke patients with microbleeds versus those without was not significantly different amongst warfarin [3.98 (1.49-10.62), p=0.006] and antiplatelet users [5.80 (3.23-10.41), p<0.0001; p_{het}=0.52].

Figure 4.4 shows the annual rate of recurrent ischaemic stroke and ICH in TIA or ischaemic stroke patients on warfarin and antiplatelet agents, with and without microbleeds. In patients with no microbleeds, the annual rate of recurrent ischaemic stroke in warfarin users was 2.33% (95% CI 1.59-3.29%), which was similar to the rate observed in antiplatelets users [2.66% (2.34-

3.01%), p=0.48]. The annual rate for ICH in patients without microbleeds appeared higher in warfarin users [0.44% (0.16-0.95%) vs. 0.21% (0.13-0.32%)] although these rates were not statistically different from one another (p=0.10). In patients with microbleeds, the annual rate of recurrent ischaemic stroke in antiplatelet users [4.82% (4.09-5.64%)] was more than two-fold that of warfarin users [1.84% (0.92-3.29%), p=0.0005]. However, there were no differences in rate of ICH in patients with microbleeds on warfarin [1.84% (0.92-3.29%)] or on antiplatelets agents [1.47% (1.10-1.98%), p=0.52].

When all patients were stratified by burden of microbleeds, the absolute rates of recurrent ischaemic stroke in warfarin users did not differ from that of patients with no microbleeds [0 microbleeds: 2.33% (1.59-3.29%); 1-4: 1.75% (0.80-3.32%); ≥5: 2.38% (0.27-8.60%)]; but the risk of recurrent ishaemic stroke increased steadily in antiplatelet users [0: 2.66% (2.34-3.01%); 1-4: 4.37% (3.55-5.32%), ≥5: 6.53% (4.86-8.59%)] (Figures 4.5 and 4.6). In contrast, risk of ICH increased with burden of microbleeds in both warfarin [0: 0.44% (0.16-0.95%); 1-4: 1.36% (0.55-2.81%); ≥5: 4.76% (1.28-12.19%)] and antiplatelet users [0: 0.21% (0.13-0.32%); 1-4: 0.62% (0.34-1.05%); ≥5: 3.33% (2.17-4.88%)] with no heterogeneity (p>0.05 for all comparisons) (Figures 4.5 and 4.7).

4.5 Discussion

I have shown that in TIA or ischaemic stroke patients with increasing burden of microbleeds, warfarin use was associated with an increased risk of ICH compared with patients without microbleeds, but that the risk of ICH was not significantly different from that of antiplatelet users. However, unlike patients on antiplatelet agents, an increasing burden of microbleeds in warfarin users was not associated with an increased risk of recurrent ischaemic stroke compared with patients with no microbleeds.

The pooled absolute annual rate of ICH in TIA or ischaemic stroke patients with microbleeds on warfarin or antiplatelets was 1.84% versus 1.49%, and 4.76% versus 3.33% in those with ≥5 microbleeds with no heterogeneity (p_{het}=0.52 and 0.49). In view of the similar rates of ICH in warfarin and aspirin users, my results suggest that in TIA or ischaemic stroke patients with microbleeds necessitating anticoagulants, antiplatelets should not be preferably prescribed as a 'safer alternative'.[14] Whilst this practice may be justified by some in view of the overall lower risks of ICH amongst antiplatelet users on the whole [annual rate of ICH in antiplatelet users regardless of microbleed status: 0.29% (0.13-0.30%) vs. 0.86% (0.50-1.38%) in warfarin users in OXVASC and HKU (p<0.0001)], I have provided evidence that the risks of ICH amongst patients with different microbleed burden is no different amongst antiplatelet and warfarin users. Starting TIA or ischaemic patients and microbleeds who require anticoagulation on antiplatelet agents may cause overall net harm[12] - antiplatelet agents are minimally effective in preventing against cardioembolic events in patients with atrial fibrillation,[7, 12, 15] is associated with a significantly increased risk of severe upper gastrointestinal bleeding,[16] especially in the elderly,[16] and as I have demonstrated, has comparable intracranial bleeding risks as warfarin in this group of patients.

Although this study was limited by the small number (n=47) of patients who were prescribed with NOACs on discharge and short duration of follow-up in these patients (~2years), none of the patients on NOACs [17/47 (36%) with microbleeds, 9% with ≥5 microbleeds] developed an ICH on follow-up. These findings are concordant with those from other studies that have shown that in contrast to warfarin users,[4, 17] prior NOAC use does not appear to increase the prevalence of

microbleeds.[18] Furthermore, meta-analysis of randomised controlled trials in TIA or ischaemic stroke patients with atrial fibrillation (n=14527) have shown that compared with warfarin, use of NOACs is associated with a significantly lower risk of stroke or systemic embolism (OR 0.85, 95% 0.74-0.99), mainly driven by a lower risk of ICH (0.44, 0.32-0.62).[19] Whilst the OXVASC and HKU cohorts has lacked power in confirming these findings amongst patients with microbleeds, the prevalence of microbleeds amongst TIA or ischaemic stroke patients with atrial fibrillation is approximately 31%.[13, 17] In other words, ~4500/14527 patients in the meta-analysis of previous trials[19] would probably have had underlying microbleeds, providing indirect evidence that even amongst patients with microbleeds, NOACs are probably less likely to be associated with ICH than warfarin users,[14, 20] although these postulations would need to be confirmed in dedicated studies.

My study has a number of limitations. Despite pooling the OXVASC and HKU cohort data with the only one available prospective study[13] in recent systematic reviews[6] that has investigated the implications of microbleeds amongst stroke patients with atrial fibrillation, the total number of patients on warfarin and NOACs remained small at ~650 and ~50 patients respectively. Recent expert recommendations[20] have suggested that in patients with atrial fibrillation and <5 microbleeds, the benefits of warfarin will likely outweigh the potential risks and patients should be treated according to current guidelines. In contrast, in patients with ≥5 microbleeds, antithrombotic agents should be given in caution as the absolute ischaemic stroke risk appears to be similar to the absolute ICH risk, and NOACs or left atrial appendage occlusion should be considered.[20] My results somewhat supports these recommendations in view of the slightly higher rates of ischaemic stroke and ICH in warfarin users with 1-4 microbleeds (1.75% vs. 1.36%), but doubling in rates of ICH versus ischaemic stroke in warfarin users with ≥5 microbleeds (4.76% vs. 2.38%). However, the pooled cohorts were limited with regards to the number of patients with the outcomes of interest to confirm these recommendations (only 2 patients with recurrent ischaemic strokes and 4 with ICH in warfarin users with ≥5 microbleeds).[20] Similarly, due to the limited number of outcomes, I was also unable to perform further sensitivity analyses in OXVASC and HKU based on the 8/69 patients with strictly deep microbleeds or 27/69 patients with strictly lobar

microbleeds. Whilst strictly lobar microbleeds is more likely to be associated with cerebral amyloid angiopathy and strictly deep microbleeds with hypertensive angiopathy, it remains uncertain at present whether the location of microbleeds has bearings on the risk of ICH in patients on warfarin.[21] I look forward to results from the Microbleeds International Collaborative Network[22] and clinical trials such as The Clinical Relevance of Microbleeds in Stroke Study (CROMIS-2)[23] and Intracerebral Haemorrhage Due to Oral Anticoagulants: Prediction of the Risk by Magnetic Resonance (HERO) that would be able to address the shortcomings of my study.

Table 4.1 Characteristics and outcomes of OXVASC and HKU cohorts on anticoagulants

	OXVASC, UK n=118 (63 TIA, 55 ischaemic Stroke)	HKU, HK n=104 (104 ischaemic stroke)	p
Baseline clinical characteristics			
Mean (SD) age (years)	73 (12)	75 (8)	0.20
Males (%)	53 (45)	55 (53)	0.24
Hypertension (%)	73 (62)	71 (68)	0.32
Diabetes (%)	15 (13)	27 (26)	0.012
Hyperlipidaemia (%)	42 (36)	30 (29)	0.28
Ever-smokers (%)	52 (44)	21 (20)	0.0002
Atrial fibrillation (%)	105 (89)	92 (89)	0.90
Prior TIA or stroke (%)	27 (23)	20 (19)	0.51
Angina or myocardial infarction (%)	15 (13)	20 (19)	0.18
Imaging characteristics			
Magnet strength (Tesla)	1·5 (N=66) 3 (N=52)	3	
Method of detecting microbleeds	T2* GRE	SWI	
Median delay (interquartile range) to scan, days	2 (0-16)	5 (3-7)	0.095
N with microbleeds (%)	28 (24)	41 (39)	0.012
N with 1 microbleed (%)	10 (9)	18 (17)	0.071
N with 2-4 microbleeds (%)	13 (11)	15 (14)	
N with ≥5 microbleeds (%)	5 (4)	8 (8)	
N with strictly deep microbleeds (%)	4 (3)	4 (4)	0.86
N with strictly lobar microbleeds (%)	12 (10)	15 (14)	0.33
N with strictly infratentorial microbleeds (%)	3 (3)	5 (5)	0.37
N with microbleeds of mixed location (%)	9 (8)	17 (16)	0.044
Post-event antithrombotic use			
Warfarin (%)	112 (95)	63 (61)	<0.0001
NOAC (%)	6 (5)	41 (39)	<0.0001
Combined anticoagulant and antiplatelet (%)	7 (6)	19 (18)	0.004
Clinical outcome			
Mean follow-up time, months	41±27	37±20	
Patient-years follow-up	407	319	
Recurrent stroke (%)	11 (9)	16 (15)	0.17
Ischaemic (%)	8 (7)	13 (13)	0.15
Fatal (%)	3 (38)	5 (38)	0.37
Intracerebral haemorrhage (%)	3 (3)	3 (3)	0.88
Fatal (%)	1 (33)	1 (33)	0.93
Extracranial bleed[a] (%)	12 (10)	7 (7)	0.36
Major bleed (%)	5 (4)	2 (2)	0.33
Deaths (%)	22 (19)	15 (14)	0.40
Vascular deaths (%)	9 (41)	10 (67)	0.60

[a]Bleeding events from oral and nasal cavity excluded
SD=standard deviation; TIA=transient ischaemic attack; NOAC=non-vitamin K antagonist oral anticoagulant; GRE=gradient-recalled echo; SWI=susceptibility weighted imaging

Table 4.2 Cox regression analyses of risk of adverse events amongst anticoagulant users with microbleeds versus those without

	Univariate HR (95% CI)	p	HR (95% CI) adjusted for age, sex and vascular risk factors[a]	p
Recurrent stroke				
OXVASC	1.65 (0.43-6.31)	0.47	1.94 (0.42-9.03)	0.40
HKU	2.18 (0.62-7.74)	0.23	2.30 (0.55-9.64)	0.26
Combined[b]	1.91 (0.78-4.67)	0.16	1.84 (0.72-4.69)	0.20
Ischaemic stroke				
OXVASC	0.58 (0.07-4.73)	0.61	0.57 (0.06-5.29)	0.62
HKU	1.92 (0.43-8.59)	0.39	1.80 (0.32-10.06)	0.51
Combined[b]	1.18 (0.39-3.54)	0.77	1.06 (0.33-3.41)	0.93
Intacerebral haemorrhage				
OXVASC	10.14 (0.90-114.91)	0.061	-	0.80
HKU	3.00 (0.27-33.03)	0.37	-	0.83
Combined[b]	5.88 (1.02-33.95)	0.048	6.44 (1.07-38.69)	0.042
Major extracranial bleed[c]				
OXVASC	2.45 (0.41-14.75)	0.33	3.24 (0.47-22.11)	0.23
HKU	-	0.66	-	0.56
Combined[b]	1.53 (0.27-8.60)	0.63	1.55 (0.24-10.16)	0.65
All cause mortality				
OXVASC	0.87 (0.29-2.59)	0.81	0.61 (0.19-1.99)	0.41
HKU	1.26 (0.38-4.12)	0.71	0.78 (0.19-3.25)	0.74
Combined[b]	1.02 (0.46-2.23)	0.97	0.77 (0.35-1.72)	0.53
Vascular mortality				
OXVASC	0.03 (0.00-35.24)	0.34	-	0.97
HKU	2.43 (0.58-10.16)	0.23	1.08 0.19-6.03)	0.93
Combined[b]	0.99 (0.34-2.88)	0.99	0.76 (0.25-2.27)	0.62

[a]Hypertension, hyperlipidemia, diabetes, atrial fibrillation, smoking
[b]Adjusted for centre
[c]Dental and nasal bleeds excluded
HR=hazards ratio; CI=confidence interval

Table 4.3 Cox regression analyses of risk of adverse events amongst anticoagulant users with 1 or ≥2 microbleeds versus those without

	Unadjusted HR (95% CI)			HR (95% CI) adjusted for age, sex and vascular risk factors[a]		
Microbleed number	**1**	**≥2**	**p_{trend}**	**1**	**≥2**	**p_{trend}**
Recurrent stroke						
OXVASC	2.31 (0.49-10.92)	1.04 (0.13-8.47)	0.68	2.68 (0.47-15.44)	1.36 (0.15-11.91)	0.53
HKU	1.03 (0.19-5.64)	4.95 (1.23-19.94)	0.043	1.37 (0.22-8.43)	3.65 (0.73-18.19)	0.13
Combined[b]	1.35 (0.42-4.35)	2.75 (0.95-7.90)	0.073	1.48 (0.44-4.94)	2.49 (0.85-7.33)	0.10
Ischaemic stroke						
OXVASC	1.17 (0.14-9.53)	0.00 (0.00-0.00)	0.46	1.06 (0.11-10.36)	0.00 (0.00-0.00)	0.52
HKU	1.40 (0.23-8.40)	3.06 (0.51-18.43)	0.25	3.05 (0.29-31.74)	1.26 (0.16-9.95)	0.70
Combined[b]	1.17 (0.31-4.45)	1.18 (0.26-5.44)	0.79	1.21 (0.30-4.88)	0.89 (0.18-4.43)	0.98
Intacerebral haemorrhage						
OXVASC	10.66 (0.66-173.17)	9.66 (0.59-159.51)	0.071	-	-	0.069
HKU	0.00 (0.00-0.00)	10.19 (0.92-112.91)	0.079	-	-	0.29
Combined[b]	2.26 (0.19-26.53)	11.05 (1.80-67.69)	0.010	2.63 (0.22-32.03)	13.00 (1.76-96.23)	0.014
Major extracranial bleed[c]						
OXVASC	-	4.55 (0.74-28.01)	0.15	-	12.54 (1.07-147.66)	0.062
HKU	-	-	0.64	-	-	0.48
Combined[b]	-	3.00 (0.54-16.81)	0.31	-	4.16 (0.51-33.76)	0.27
All cause mortality						
OXVASC	-	2.03 (0.66-6.23)	0.61	-	1.45 (0.44-4.81)	0.98
HKU	0.76 (0.15-3.75)	2.25 (0.56-9.03)	0.38	0.56 (0.08-4.10)	0.96 (0.20-4.67)	0.90
Combined[b]	0.38 (0.09-1.62)	1.91 (0.81-4.52)	0.38	0.30 (0.07-1.31)	1.40 (0.57-3.42)	0.89
Vascular mortality						
OXVASC	-	-	0.35	-	-	0.96
HKU	1.43 (0.24-8.56)	4.55 (0.91-22.66)	0.081	0.54 (0.05-5.87)	1.43 (0.24-8.55)	0.67
Combined[b]	0.65 (0.14-2.98)	1.50 (0.42-5.42)	0.73	0.63 (0.13-2.99)	0.88 (0.23-3.36)	0.74

[a]Hypertension, hyperlipidemia, diabetes, atrial fibrillation, smoking
[b]Adjusted for centre
[c]Dental and nasal bleeds excluded
HR=hazards ratio; CI=confidence interval

Figure 4.1 Risk of A) recurrent stroke, B) recurrent ischaemic stroke, C) intracerebral haemorrhage and D) all-cause mortality amongst TIA and ischaemic stroke patients on warfarin with and without cerebral microbleeds. Statistical significance of differences in risk is determined by log-rank test.

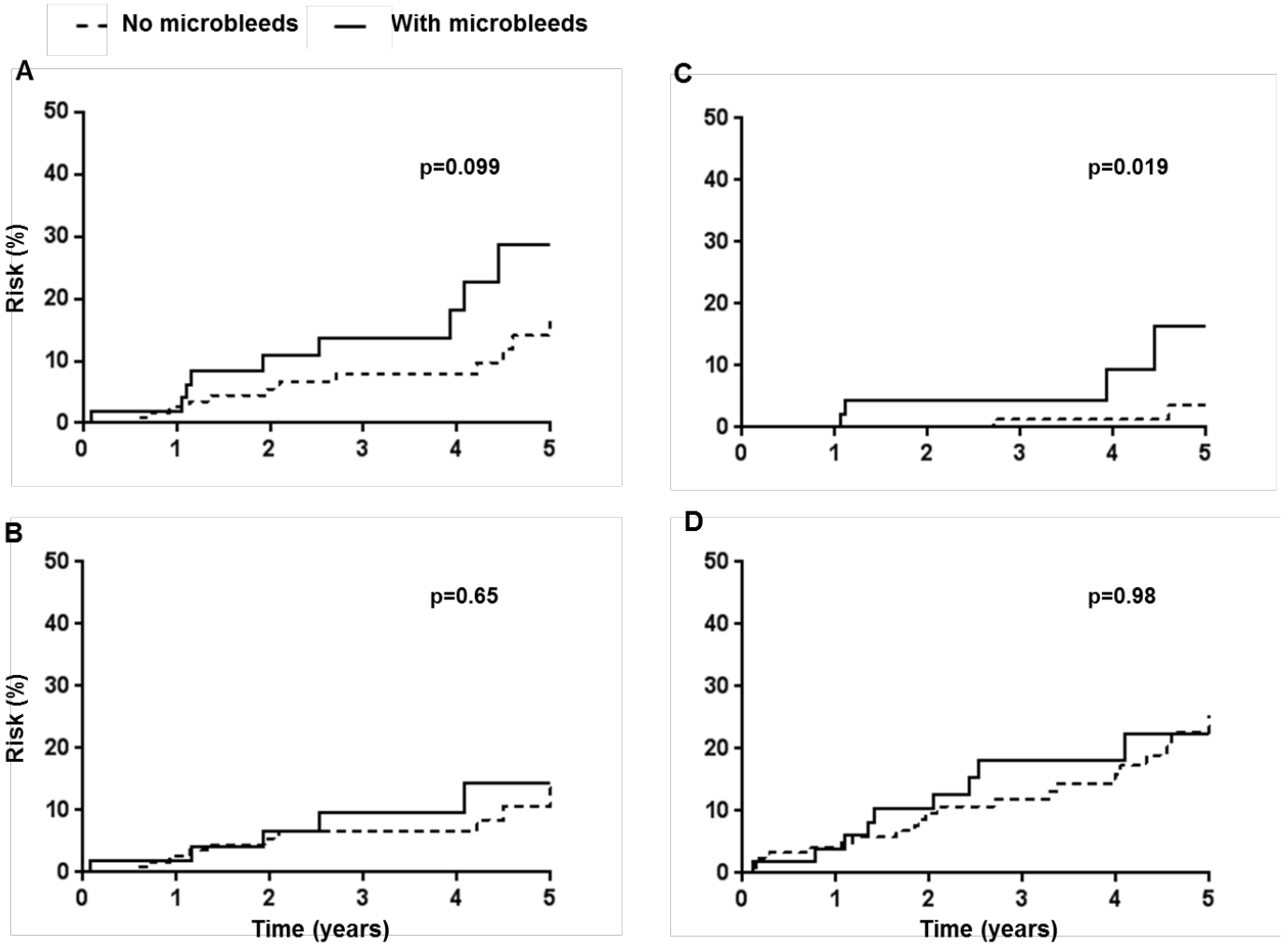


Figure 4.2 Risk of A) recurrent stroke, B) recurrent ischaemic stroke, C) intracerebral haemorrhage and D) all-cause mortality in TIA and ischaemic stroke patients on warfarin with increasing burden of cerebral microbleeds

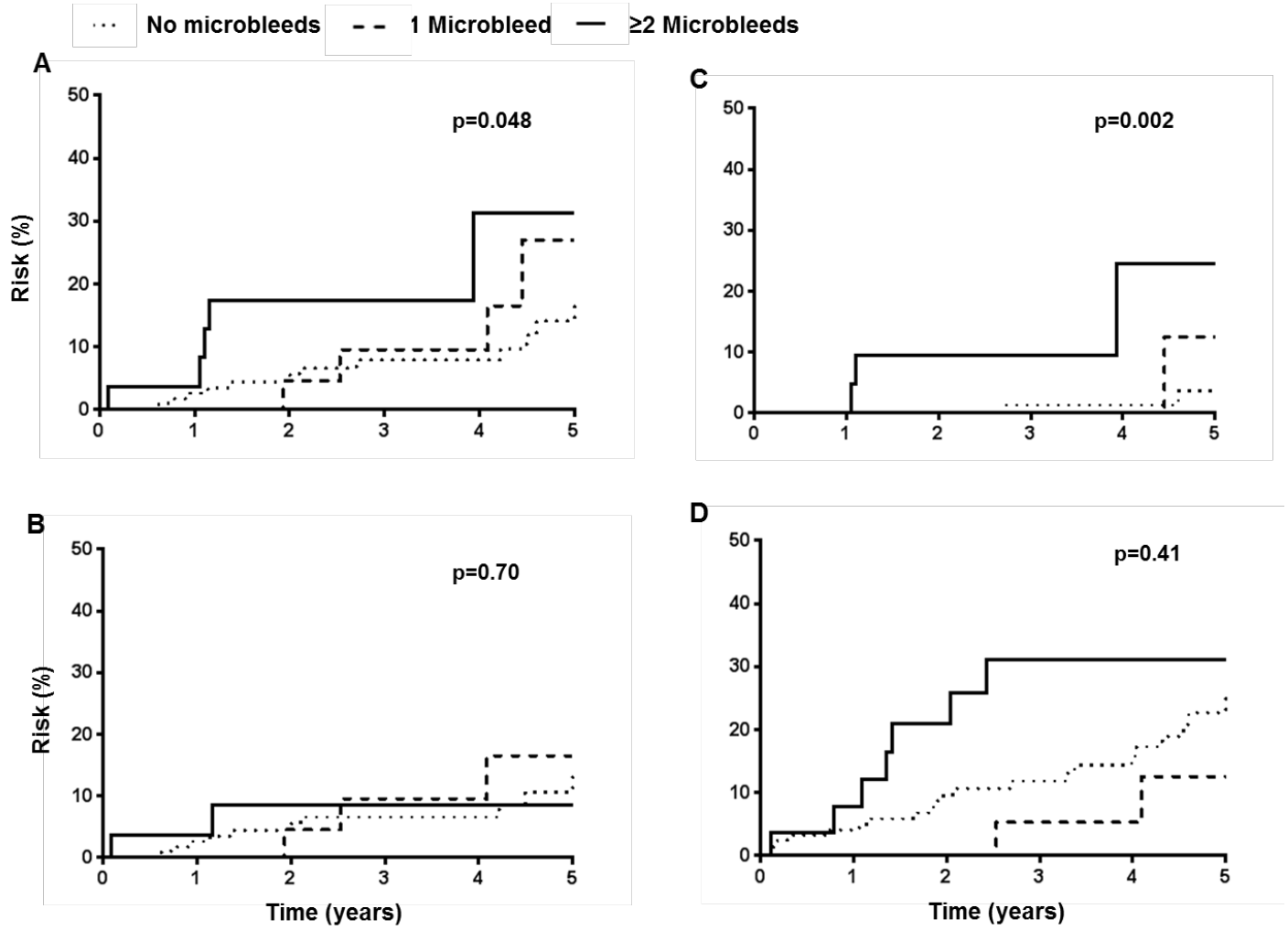


Figure 4.3 Pooled analyses of relative risk estimates from current and previous studies showing risk of A) recurrent ischaemic stroke and B) intracerebral haemorrhage in TIA and ischaemic stroke patients on warfarin or antiplatelet treatment, with microbleeds versus those without

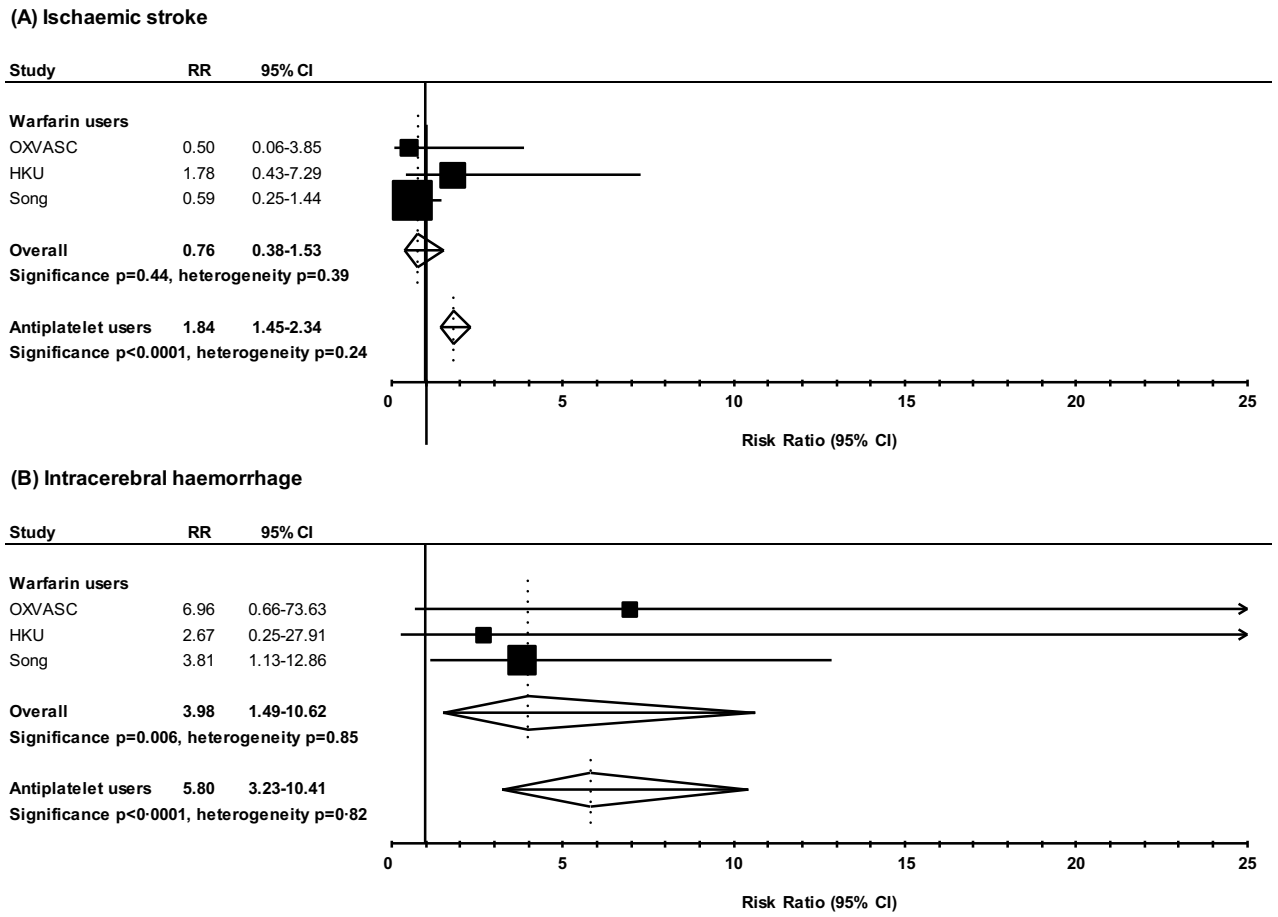


Figure 4.4 Annual rate of recurrent ischaemic stroke and intracerebral haemorrhage in TIA or ischaemic stroke patients on warfarin or antiplatelet treatment, according to presence or absence of microbleeds. Rates for patients on antiplatelet therapy estimated from Wilson *et al*.[6] and data from OXVASC and HKU. Rates for patients on warfarin therapy estimated from Song *et al*.[13] and data from OXVASC and HKU. Error bars are 95% confidence intervals.

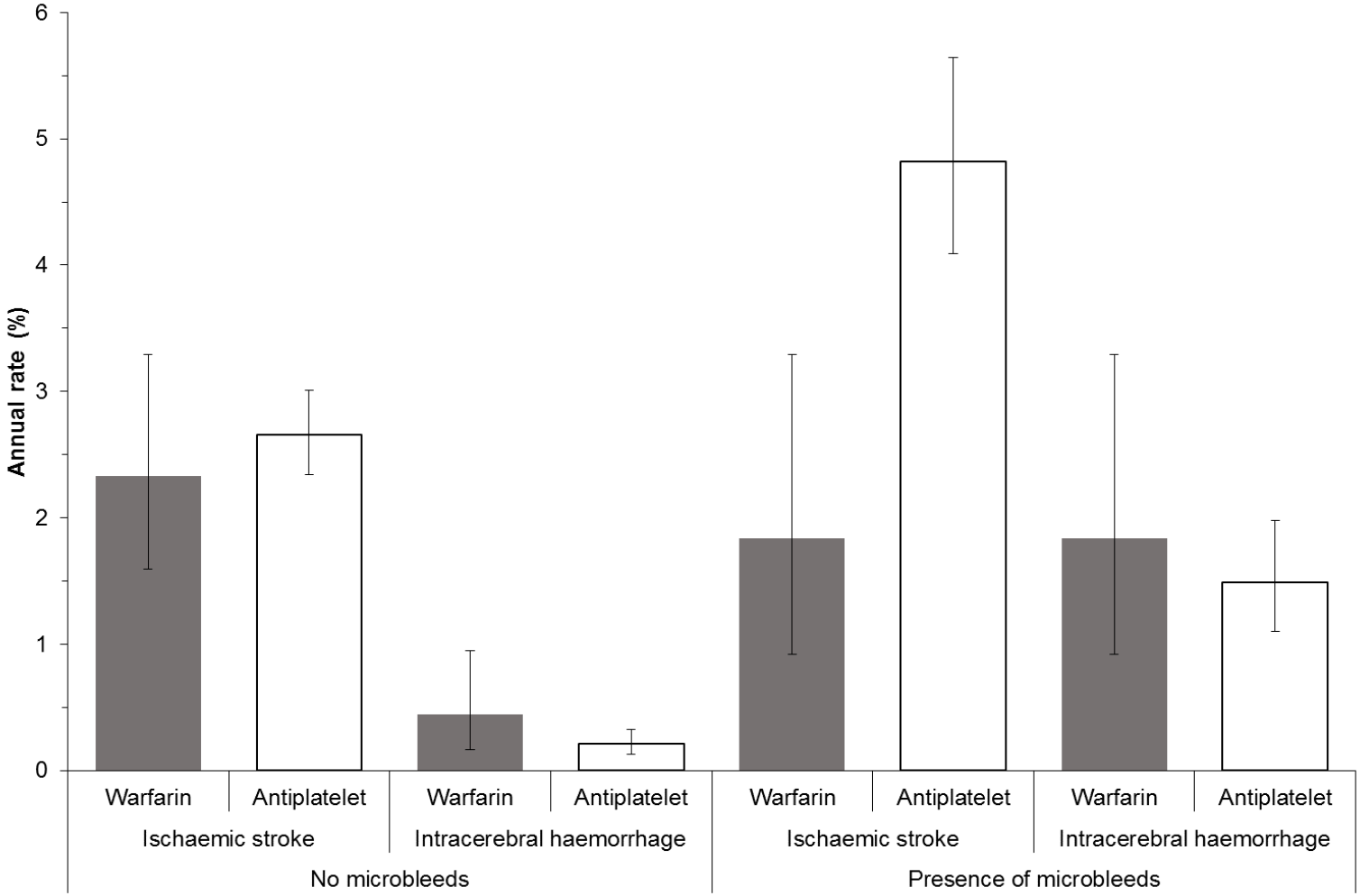


Figure 4.5 Annual rate of recurrent ischaemic stroke and intracerebral haemorrhage in TIA or ischaemic stroke patients on warfarin or antiplatelet treatment, according to burden of microbleeds. Rates for patients on antiplatelet therapy estimated from Wilson *et al*.[6] and data from OXVASC and HKU. Rates for patients on warfarin therapy estimated from Song *et al*.[13] and data from OXVASC and HKU. Error bars are 95% confidence intervals.

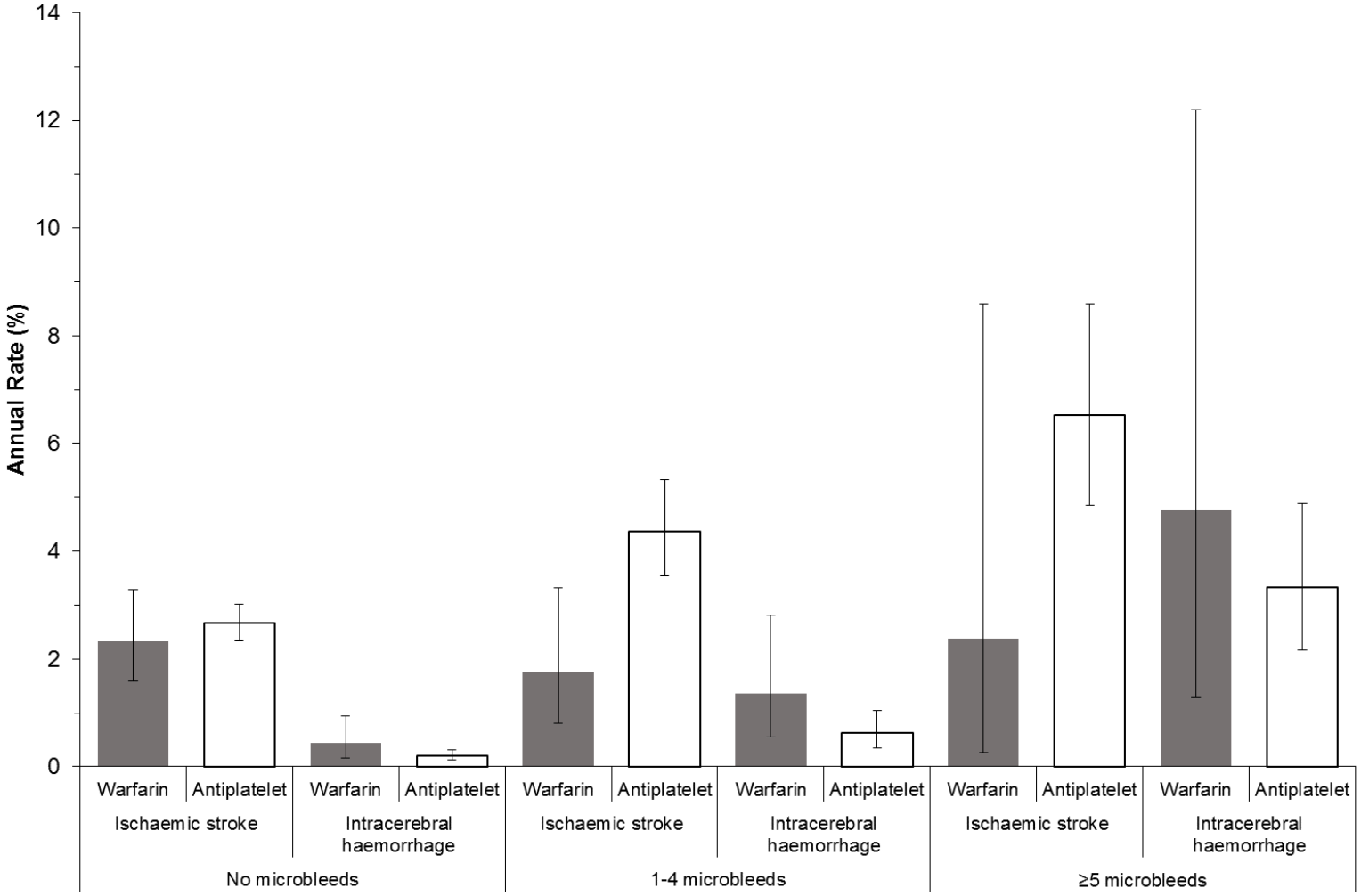


Figure 4.6 Pooled analyses of relative risk estimates from current and previous studies showing risk of recurrent ischaemic stroke in TIA or ischaemic stroke patients on warfarin or antiplatelet treatment, with 1, 2-4 and ≥5 microbleeds versus those without

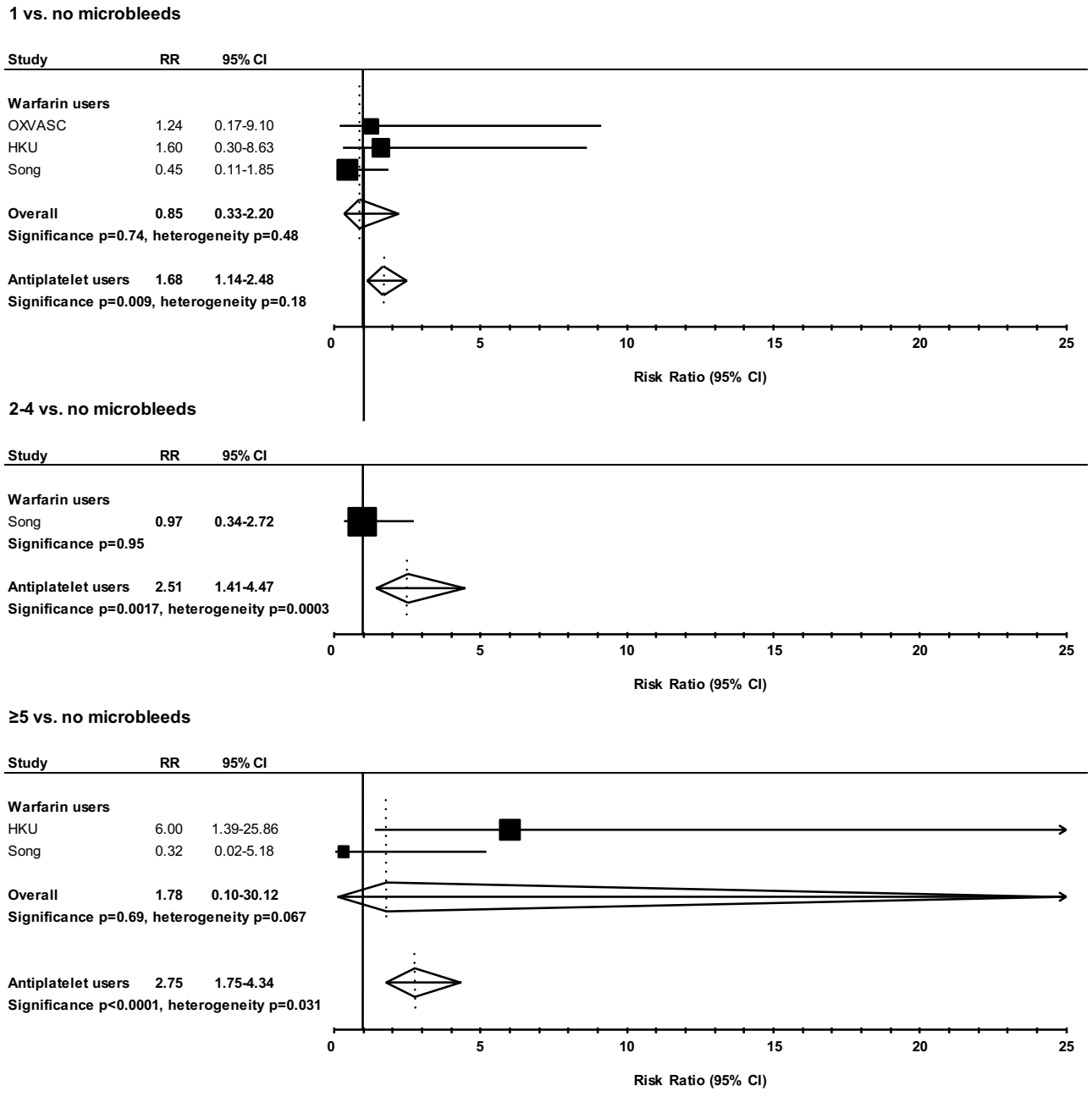
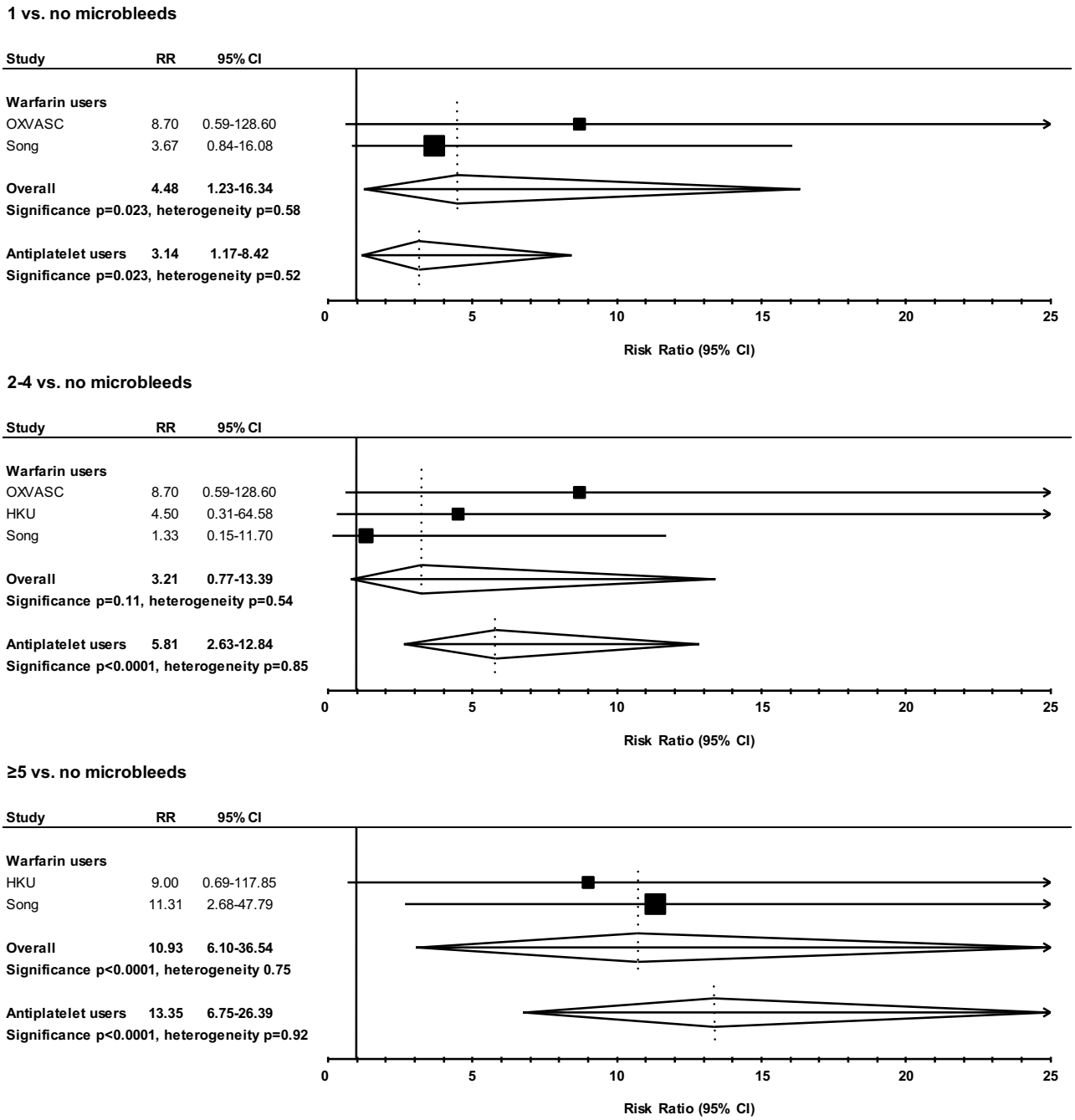


Figure 4.7 Pooled analyses of relative risk estimates from current and previous studies showing risk of intracerebral haemorrhage in TIA and ischaemic stroke patients on warfarin or antiplatelet treatment, with 1, 2-4 and ≥5 microbleeds versus those without



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Chapter 5

Clinical correlates, ethnic differences and prognostic implications of MRI-visible perivascular spaces in TIA and ischaemic stroke

5.1 Chapter outline

MRI-visible enlarged perivascular spaces (PVSs) are considered markers of small vessel disease (SVD). However, the long-term prognostic implications of visible PVSs in transient ischaemic attack (TIA) or ischaemic stroke patients are unknown. Ethnic differences in visible PVS prevalence are also unknown. Therefore, in this Chapter, I aimed to study the ethnic differences, clinical and imaging correlates of visible basal ganglia (BG) and centrum semiovale (CS) PVSs. I also aimed to determine the long-term prognostic implications of visible BG and CS-PVSs in patients with TIA or ischaemic stroke. I adjusted analyses for age, sex vascular risk factors and scanner strength.

I found that Caucasians with TIA or ischaemic stroke had a higher prevalence of both visible BG and CS-PVSs compared with Chinese (>20 BG-PVSs: 22.4% vs. 7.1%; >20 CS-PVSs: 45.8% vs. 10.4%, $p<0.0001$). >20 visible BG or CS-PVSs were both associated with increasing age and white matter hyperintensity, although associations with BG-PVSs were stronger (all $p<0.0001$). During 6924 patient-years follow-up, an increasing burden of visible BG-PVSs was independently associated with an increased risk of recurrent ischaemic stroke [adjusted hazard ratio (HR) compared with <11 PVSs, 11-20 PVSs: 1.15, 95% confidence interval (CI) 0.78-1.68; >20 PVSs: 1.82, 1.18-2.80, $p_{trend}=0.011$] but not ICH ($p_{trend}=0.10$) or all-cause mortality ($p_{trend}=0.16$). An increasing burden of visible CS-PVSs was not associated with recurrent stroke ($p_{trend}=0.57$) nor mortality ($p_{trend}=0.072$) in patients with TIA or ischaemic stroke.

I concluded that over and above the ethnic differences in frequency of visible PVSs in TIA or ischaemic stroke patients, visible BG and CS-PVSs had similar risk factors, but whilst >20 visible BG-PVSs was associated with an increased risk of recurrent ischemic stroke, CS-PVSs were not.

5.2 Introduction

Perivascular spaces (PVSs) are tiny cavities of cerebrospinal fluid that surround arterioles that penetrate the brain parenchyma.[1] They are most frequently found in the inferior basal ganglia (BG), centrum semiovale (CS) and midbrain.[2] Although it is normal to have a few visible PVSs on neuroimaging,[3] an increased burden of BG and CS-PVSs have been associated with increasing age,[4-7] hypertension,[4-6, 8] renal impairment,[9] white matter hyperintensity (WMH),[4-6, 8, 10] and lacunes.[4, 6, 8, 10] An increasing burden of visible BG-PVSs have in addition also been associated with male sex,[6] mean systolic and diastolic blood pressure,[8, 11] deep or infratentorial cerebral microbleeds,[7, 8] and also stroke due to small vessel occlusion.[10] A high burden of visible BG-PVSs have hence been considered a marker of hypertensive arteriopathy secondary to endothelial dysfunction,[7, 8] and has recently been proposed as one of the four components of the 'Total Small Vessel Disease (SVD) score'.[12] In contrast, a high burden of visible CS-PVSs have been associated with lobar microbleeds in healthy adults and those with cognitive impairment.[5, 8] A high burden of visible CS-PVSs have also been noted in patients with cerebral amyloid angiopathy (CAA).[7, 13] It has therefore been hypothesised that in contrast to BG-PVSs, a high burden of visible CS-PVSs may be a neuroimaging marker of CAA by representing fluid and metabolic waste clearance dysfunction due to vascular amyloid deposition.[7, 8, 14]

Although visible PVSs have shown potential as an imaging biomarker of hypertensive angiopathy and CAA, the long-term prognostic implications of visible PVSs amongst patients with transient ischaemic attack (TIA) or ischaemic stroke have yet to be determined. Ethnic differences in visible PVSs are also uncertain. To address these unanswered questions, I determined the associations of visible PVSs with ethnicity, vascular risk factors, other neuroimaging markers of SVD and long-term risks of stroke and death in two independent cohorts. I hypothesise that a high-burden of visible BG and CS-PVSs may be associated with other neuroimaging markers of SVD. I also postulate that in TIA and ischaemic stroke patients, a high-burden of visible BG-PVSs may be associated with an increased risk of recurrent stroke, especially recurrent ischaemic stroke, and that a high-burden of visible CS-PVSs may be associated with an increased risk of ICH.

5.3 Methods

5.3.1 Study populations

1080 consecutive cases with TIA or ischaemic stroke who were predominantly Caucasians and 1076 consecutive cases with ischaemic stroke who were predominantly Chinese were recruited from The Oxford Vascular Study (OXVASC) and The University of Hong Kong (HKU) respectively (see section 2.1). Baseline data was collected as described in section 2.1. All patients received a cerebral magnetic resonance imaging (MRI) scan at baseline (see section 2.2) and presence and burden of visible BG and CS-PVSs, cerebral microbleeds, WMH and lacunes coded as described in section 2.3. 75 MRI scans from HKU were cross-interpreted by investigators in OXVASC with an inter-rater κ of 0.64 for both BG and CS-PVSs.

All patients in OXVASC and HKU were followed-up regularly and assessed for recurrent stroke (ischaemic and haemorrhagic) and death (vascular and non-vascular) (see section 2.4).

5.3.2 Statistical analysis

I compared differences in baseline and imaging characteristics in the OXVASC and HKU cohorts using Student's t-test for continuous variables and Chi-squared test for categorical variables. The predictors of >20 visible BG and CS-PVSs were determined using a logistic regression model adjusted for centre and MRI scanner strength. Variables including age, male sex, vascular risk factors (hypertension, hyperlipidaemia, diabetes, smoking, atrial fibrillation), renal impairment (defined as glomerular filtration rate <60ml/min/1.73m^2, see section 2.1), periventricular and subcortical WMH, deep and lobar microbleed number and lacunes were entered into a univariate analysis model. All variables were subsequently entered into a multivariate analysis model to determine the independent predictors of >20 visible BG and CS-PVSs. The multivariate model to determine the independent predictors of >20 visible BG-PVSs was also adjusted for CS-PVSs and vice-versa.

In a logistic regression model, I determined the odds of a TIA or ischaemic stroke due to SVD with increasing burden of visible BG and CS-PVSs, adjusted for age, sex, vascular risk factors, centre and MRI scanner strength.

I used Kaplan-Meier survival analysis to calculate the 5-year risk of a recurrent stroke (ischaemic and haemorrhagic) and all-cause mortality, censored at death or March 31, 2015, according to the burden of visible PVSs. I also determined, by Cox regression analysis, the unadjusted and adjusted (for age, sex, vascular risk factors, centre and MRI scanner strength) risks of recurrent stroke (ischaemic and haemorrhagic), mortality (vascular and non-vascular) in patients with 11-20 and >20 visible BG and CS-PVSs compared with <11 PVSs as reference. I also determined by logistic regression (forward stepwise model) the independent predictors of recurrent stroke adjusting for all neuroimaging markers of SVD (periventricular and subcortical WMH, lacunes, cerebral microbleeds, visible BG and CS-PVSs). Finally, I performed a stratified analysis to determine whether the prognosis of visible PVSs differed in patients with no or mild, versus moderate or severe periventricular and subcortical WMH.

All analyses were done with SPSS version 22.

5.4 Results

5.4.1 Baseline clinical and neuroimaging characteristics

The two study populations contributed a total of 2156 patients. After excluding 154 patients with incomplete clinical and/or imaging data, 2002 (OXVASC n=1028, 542 TIA, 486 ischaemic stroke; HKU n=974, all ischaemic stroke) were included in the final analysis. Baseline clinical and imaging characteristics of patients are shown in Table 5.1. Proportion of patients according to Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification is shown in Table 5.2. HKU patients had a higher proportion of males (p=0.001), and were more likely to have hypertension and diabetes (p<0.0001), whilst OXVASC patients were more likely to have hyperlipidaemia or a history of smoking (p<0.0001) (Table 5.1).

Patients from OXVASC had a higher burden of >20 visible BG (22.4% vs. 7.1%, p<0.0001) and CS-PVSs (45.8% vs. 10.4%, p<0.0001) compared with those from HKU (Table 5.1). These differences in PVS burden remained despite stratification of individuals into stroke subtypes (Table 5.3). OXVASC patients also had more severe periventricular WMH (p<0.0001) (Table 5.1). In contrast, those from HKU had a greater burden of subcortical WMH (p<0.0001) and microbleeds (p<0.0001) (Table 5.1). These differences remained in analyses confined to patients who received a MRI with a 3-T scanner (Table 5.4). However, within OXVASC, patients who received a 3T MRI (n=446) had a greater burden of >20 visible BG-PVSs (25.8% vs. 19.8%, p=0.022) and CS-PVSs (55.6% vs. 38.3%, p<0.0001) compared with patients who received a 1.5-T MRI (n=582).

5.4.2 Clinical correlates of visible perivascular spaces

Burden of visible BG and CS-PVSs increased with age, baseline history of hypertension, atrial fibrillation and renal impairment (p<0.05) (Table 5.5). Burden of visible BG and CS-PVSs was also greater in patients with lacunes and severe WMH (p<0.05) (Table 5.5). In a multivariate analysis, >20 visible BG or CS-PVSs were associated with increasing age [multivariate adjusted odds ratio (OR), BG: 1.05, 95% confidence interval (CI) 1.03-1.07, p<0.0001; CS: 1.01, 1.00-1.03,

p=0.020] and subcortical WMH (BG: 1.44, 1.20-1.72, p<0.0001; CS: 1.28, 1.09-1.50, p=0.003). >20 BG-PVSs was also associated with atrial fibrillation (1.58, 1.10-2.29, p=0.014), and periventricular WMH (2.01, 1.66-2.44, p<0.0001) (Table 5.6). Whilst underlying significant (>50%) large artery atherosclerosis was not related to >20 visible BG-PVSs (multivariate adjusted OR 1.10, 0.77-1.56, p=0.61), an independent association between >20 visible CS-PVSs with significant large artery disease was noted (1.44, 1.07-1.93, p=0.015). Caucasians, as compared with Chinese, were at increased odds of >20 visible BG (multivariate-adjusted OR 2.09, 1.35-3.22, p=0.001) and CS-PVSs (8.82, 6.25-12.46, p<0.0001). These ORs remained similar after additional adjustment of MRI magnet strength (BG: 2.50, 1.56-4.02, p=0.0002; CS: 11.93, 8.15-17.47, p<0.0001).

5.4.3 Relationships of visible perivascular spaces with TIA and ischaemic stroke subtypes

26.8% of the study population were classified to have TIA or ischaemic stroke due to SVD (Table 5.2). These patients were associated with a higher BG and CS-PVS burden (multivariate-adjusted OR compared with <11 PVSs, 11-20 BG-PVSs: 2.44, 1.45-4.10; >20 BG-PVSs: 2.82, 1.60-4.97, p_{trend}=0.0002; 11-20 CS-PVSs: 2.54, 1.32-4.88; >20 CS-PVSs 4.20, 2.19-8.06; p_{trend}<0.0001).

5.4.4 Prognostic implications of visible perivascular spaces

After a mean follow-up of 42±23 months (OXVASC 45±26 months, HKU 37±19 months, 6924 patient-years of follow-up), 199 recurrent strokes occurred (85.4% ischaemic) (Table 5.1). 266 patients died, 34.5% of which were vascular deaths. The 5-year risk of recurrent ischaemic stroke and intracerebral haemorrhage (ICH) in patients with <11, 11-20 and >20 visible BG-PVSs was 8.5%, 11.5%, 19.3% (log-rank test: p<0.0001) and 1.6%, 2.3% and 3.7% respectively (p=0.038) (Figure 5.1). An increasing burden of visible BG-PVSs was also associated with a higher all-cause mortality (p<0.0001) (Figure 5.1). In contrast, burden of visible CS-PVSs was not associated with recurrent ischaemic stroke (p=0.76), ICH (p=0.96) or all-cause mortality (p=0.33) (Figure 5.2).

On Cox regression analysis, very strong univariate associations between increasing burden of visible BG-PVSs with all-cause mortality was noted (p_{trend}<0.0001), but this association disappeared after adjustment for age and sex (p_{trend}=0.058) and on multivariate adjustment (p_{trend}=0.16) (Table 5.7). In contrast, the strong univariate associations between increasing burden of visible BG-PVSs with recurrent ischaemic stroke persisted with multivariate adjustment [hazard ratio (HR) compared with <11 BG-PVSs, 11-20: 1.15, 0.78-1.68; >20: 1.82, 95% CI 1.18-2.80; p_{trend}=0.011] (Table 5.8). BG-PVS burden was not independently associated with ICH (p_{trend}=0.10) (Table 5.8). An increasing burden of visible CS-PVSs was not related to ischaemic stroke (p_{trend}=0.42), ICH (p_{trend}=0.69), or mortality (p_{trend}=0.072) (Tables 5.7 and 5.8). When patients were stratified by MRI scanner in OXVASC, the prognostic value of visible BG and CS-PVSs remained similar for prediction of recurrent stroke (BG-PVS: p_{het}=0.15; CS-PVS: p_{het}=0.45) (Table 5.9). Similarly, no heterogeneity was observed when analyses for risk of recurrent stroke were stratified by patients with no or mild versus moderate-severe WMH (BG-PVS: p_{het}=0.92; CS-PVS: p_{het}=0.076) (Table 5.10). In an unadjusted model, burden of visible BG-PVSs, microbleeds, periventricular WMH, subcortical WMH and presence of lacunes were all associated with recurrent ischaemic stroke (p<0.05) (Table 5.11). Forward stepwise multivariate Cox regression model adjusting for all neuroimaging markers of SVD revealed that burden of visible BG-PVSs (p_{trend}=0.001) and microbleeds (p_{trend}=0.001) as independent predictors of recurrent ischaemic stroke (Table 5.11).

5.5 Discussion

This study has combined the current two largest cohorts from the West and the East of the clinical implications of visible BG and CS-PVSs in patients with TIA or ischaemic stroke and is the first to determine the ethnic differences in prevalence. In this study, visible PVSs were coded according to a validated rating scale,[15] with excellent intra-rater variability and good inter-rater variability when scans were cross-interpreted between the two centres. This study is also the first to determine the long-term prognostic implications of visible PVSs in patients with TIA or ischaemic stroke.

The results from this study support those from previous studies that a high burden of visible BG and CS-PVSs are both markers of hypertensive angiopathy.[6, 8, 14] I too found that visible PVSs were associated with age[4-6] and WMH.[5, 6] Concordant with previous studies,[4-8, 10] I also found that compared with CS-PVSs, visible BG-PVSs was a stronger marker of hypertensive angiopathy, with greater associations with periventricular and subcortical WMH and that visible BG-PVSs was more strongly associated with TIA or ischaemic stroke due to SVD. Whilst previous studies have also noted significant associations of BG-PVS with deep microbleeds,[7, 8] this finding did not reach statistical significance in OXVASC and HKU (p=0.063).

The stronger association of visible BG-PVSs with hypertensive angiopathy was also reflected in the long-term follow-up data of OXVASC and HKU. Compared with <11 visible PVSs, TIA or ischaemic stroke patients with >20 visible BG-PVSs were at 1.8-fold increased risk of recurrent ischaemic stroke on multivariate analysis. There was a trend towards patients with increasing burden of visible BG-PVSs being similarly at increased risk of subsequent ICH and mortality. Furthermore, I was able to demonstrate that the prognostic implications of visible BG-PVSs were independent to that of other neuroimaging markers of SVD.

In contrast, although previous studies have revealed an association of visible CS-PVSs with lobar microbleeds[5, 8] and CAA,[7] suggesting that visible CS-PVSs may be an imaging biomarker of CAA,[5, 7, 8] CS-PVSs were not associated with lobar microbleeds nor adverse clinical events

including ICH in OXVASC and HKU. It should be noted however, that studies that have ascertained the relationship of CS-PVSs with lobar microbleeds were based on either healthy adults or subjects recruited from a memory clinic,[5, 8] with an expected lower prevalence of vascular risk factors and hence less severe imaging markers of SVD compared with patients in OXVASC and HKU. It is widely accepted that PVSs may be difficult to identify in patients with extensive WMH.[15] This is particularly the case for CS-PVSs that are often masked by subcortical WMHs. Indeed, in OXVASC and HKU, where up to 36% of individuals had moderate-severe subcortical WMH, the true prevalence of visible CS-PVSs would without doubt be under-estimated.

My results also demonstrate that significant ethnic differences in PVS prevalence exist. I showed a similar prevalence of >20 visible BG and CS-PVSs in OXVASC to a previous study of Caucasians with TIA or ischaemic stroke.[4] In contrast, however, I showed that Chinese with TIA or ischaemic stroke had a much lower prevalence of visible PVSs. The prevalence of visible BG and CS-PVSs amongst Chinese with ischaemic stroke have previously been reported.[9, 16] One study showed that 10.7% of subjects had >40 visible BG-PVSs[16] and in another, about 40% of subjects had >10 visible BG or CS-PVSs.[9] These two studies however, were purely based on patients with lacunar stroke subtype.[9, 16] In a large cohort of Japanese neurologically healthy individuals, a low prevalence of >20 visible BG-PVS and CS-PVS of 2.5% and 22.6% was similarly noted.[8] Such low prevalence of >20 visible PVSs in the HKU cohort would have limited the statistical power when determining the clinical correlates of PVSs and attributed to some of the differences observed when compared to OXVASC. Atrial fibrillation was also noted to be significantly associated with >20 visible BG-PVSs, whilst underlying large artery disease was significantly associated with >20 visible CS-PVSs in OXVASC and HKU. Further studies to confirm, as well as to delineate the underlying mechanisms of my findings would be required. Finally, my findings are also limited by patients in OXVASC being scanned on 4 different scanners over the 10-year study period. However, although this could have been a potential source of heterogeneity, the prognostic value for prediction of recurrent stroke with increasing burden of visible PVSs were similar across the 4 scanners, suggesting that the prognostic value of visible PVSs is robust to variations in scanner type and sequences. PVS size, symmetry or

ventricular size was not studied in OXVASC and HKU. Hence, I was only able to study clinical and imaging correlates and prognostic implications according to visible PVS number,[18] but not its size or symmetry.

My results have a number of clinical implications. First, in two large cohorts, my results confirm visible BG-PVSs as a marker of SVD, independent to WMH. These results therefore justify the inclusion of visible BG-PVSs into the recently derived 'Total SVD score'.[12] In the current version,[12] patients with >11 visible BG-PVSs are given 1 point, as are patients with severe periventricular WMH or moderate-severe subcortical WMH. Whether alternative cut-offs (e.g. >20 BG-PVSs) should be utilised instead in view of the relatively low prognostic value of patients with 11-20 BG-PVSs noted in this study, would require further research. Second, although the burden of CS-PVSs may possibly have prognostic implications in healthy individuals or those seen in the Memory Clinic, the role of visible CS-PVSs as a prognostic imaging marker in the TIA or ischaemic stroke population appears to be limited.

In conclusion, in addition to identifying ethnic differences in frequency of visible PVSs, I found that visible BG-PVSs are markers of hypertensive angiopathy and predict risk of recurrent ischaemic stroke in patients with TIA or ischaemic stroke, independent to that of WMH. In contrast, the prognostic value of visible CS-PVSs in TIA or ischaemic stroke is limited.

Table 5.1 Clinical and imaging characteristics of the study populations

	OXVASC n=1028 (542 TIA, 486 ischaemic stroke)	HKU n=974 (974 ischaemic stroke)	p
Baseline clinical characteristics			
Mean age, yr (SD)	68 (14)	69 (12)	0.25
Males (%)	538 (52.3)	583 (59.9)	0.001
Hypertension (%)	563 (54.8)	640 (65.7)	<0.0001
Diabetes (%)	136 (13.2)	275 (28.2)	<0.0001
Hyperlipidaemia (%)	381 (37.1)	249 (25.6)	<0.0001
Ever-smokers (%)	521 (50.7)	291 (29.9)	<0.0001
Atrial fibrillation (%)	160 (15.6)	128 (13.1)	0.12
Prior TIA or stroke (%)	187 (18.2)	154 (15.8)	0.16
Imaging characteristics			
Magnet strength, T	1.5-T n=582, 3-T n=446	3-T n=974	
N with DWI positive lesion (%)	233 (22.7)	759 (77.9)	<0.0001
N with visible basal ganglia PVSs (%)			
<11 (%)	527 (51.3)	659 (67.7)	<0.0001
11-20 (%)	271 (26.4)	246 (25.3)	
>20 (%)	230 (22.4)	69 (7.1)	
N with visible centrum semiovale PVSs (%)			
<11 (%)	226 (22.0)	410 (42.1)	<0.0001
11-20 (%)	331 (32.2)	463 (47.5)	
>20 (%)	471 (45.8)	101 (10.4)	
N with lacunes (%)	182 (17.7)	430 (44.1)	<0.0001
N with microbleeds (%)	156 (15.2)	441 (45.3)	<0.0001
1 microbleed (%)	79 (7.7)	179 (18.4)	
2-4 microbleeds (%)	44 (4.3)	145 (14.9)	
≥5 microbleeds (%)	39 (3.8)	117 (12.0)	
N with periventricular WMH (%)			
Grade 1 (%)	386 (37.5)	213 (21.9)	<0.0001
Grade 2 (%)	201 (19.6)	75 (7.7)	
Grade 3 (%)	96 (9.3)	30 (3.1)	
N with subcortical WMH (%)			
Grade 1 (%)	338 (32.9)	475 (48.8)	<0.0001
Grade 2 (%)	177 (17.2)	278 (28.5)	
Grade 3 (%)	117 (11.4)	155 (15.9)	
Outcome			
Mean follow-up time, months	45±26	37±19	
Patient-years follow-up	3884	3040	
Recurrent stroke (%)	90 (8.8)	109 (11.2)	0.069
Ischaemic stroke (%)	81 (7.9)	89 (9.1)	0.31
Fatal (%)	6 (7.4)	12 (13.4)	0.13
Intracerebral haemorrhage (%)	9 (0.9)	20 (2.1)	0.027
Fatal (%)	3 (33.3)	6 (30.0)	0.28
Deaths (%)	142 (13.8)	124 (12.7)	0.48
Vascular deaths (%)	35 (24.6)	57 (46.0)	0.009

TIA=transient ischaemic attack; DWI=diffusion weighted imaging; PVS=perivascular space; WMH=white matter hyperintensity

Table 5.2 Aetiology of TIA or ischaemic strokes according to TOAST classification

	OXVASC, UK n=1028	HKU, HK n=974	All n=2002
Small vessel disease (%)	124 (12.1)	413 (42.4)	537 (26.8)
Large artery atherosclerosis (%)	137 (13.3)	334 (34.3)	471 (23.5)
Cardio-embolic (%)	160 (15.6)	118 (12.1)	278 (13.9)
Undetermined (%)	514 (50.0)	42 (4.3)	556 (27.8)
Multiple (%)	35 (3.4)	28 (2.9)	63 (3.1)
Unknown (%)	26 (2.5)	22 (2.3)	48 (2.4)
Others (%)	32 (3.1)	17 (1.7)	49 (2.4)

TIA=transient ischaemic attack; TOAST=Trial of Org 10172 Acute Stroke Treatment

Table 5.3 Proportion of TIA or ischaemic stroke patients with visible perivascular spaces according to TOAST classification

	OXVASC n=1028	HKU n=974	p
Small vessel disease	n=124	n=413	
N with visible basal ganglia PVSs (%)			
<11 (%)	53 (42.7)	269 (65.1)	p<0.0001
11-20 (%)	39 (31.5)	109 (26.4)	
>20 (%)	32 (25.8)	35 (8.5)	
N with visible centrum semiovale PVSs (%)			
<11 (%)	15 (12.1)	172 (41.6)	p<0.0001
11-20 (%)	39 (31.5)	200 (48.4)	
>20 (%)	70 (56.5)	41 (9.9)	
Large artery atherosclerosis	n=137	n=334	
N with visible basal ganglia PVSs (%)			
<11 (%)	52 (38.0)	232 (69.5)	p<0.0001
11-20 (%)	46 (33.6)	82 (24.6)	
>20 (%)	39 (28.5)	20 (6.0)	
N with visible centrum semiovale PVSs (%)			
<11 (%)	17 (12.4)	134 (40.1)	p<0.0001
11-20 (%)	40 (29.2)	162 (48.5)	
>20 (%)	80 (58.4)	38 (11.4)	
Cardio-embolic	n=160	n=118	
N with visible basal ganglia PVSs (%)			
<11 (%)	66 (41.3)	76 (64.4)	p<0.0001
11-20 (%)	42 (26.3)	33 (28.0)	
>20 (%)	52 (32.5)	9 (7.6)	
N with visible centrum semiovale PVSs (%)			
<11 (%)	27 (16.9)	56 (47.5)	p<0.0001
11-20 (%)	48 (30.0)	47 (39.8)	
>20 (%)	85 (53.1)	15 (12.7)	

TIA=transient ischaemic attack; TOAST=Trial of Org 10172 Acute Stroke Treatment; PVS=perivascular space

Table 5.4 Clinical and imaging characteristics in OXVASC and HKU patients who were scanned by a 3-T MRI

	OXVASC, UK n=446 (267 TIA, 179 ischaemic stroke)	HKU, HK n=974 (974 ischaemic stroke)	p
Baseline clinical characteristics			
Mean age, yr (SD)	70 (14)	69 (12)	0.39
Males (%)	235 (52.7)	583 (59.9)	0.011
Hypertension (%)	237 (53.1)	640 (65.7)	<0.0001
Diabetes (%)	53 (11.9)	275 (28.2)	<0.0001
Hyperlipidaemia (%)	154 (34.5)	249 (25.6)	0.001
Ever-smokers (%)	190 (42.6)	291 (29.9)	<0.0001
Atrial fibrillation (%)	69 (15.5)	128 (13.1)	0.24
Prior TIA or stroke (%)	77 (17.3)	154 (15.8)	0.49
Imaging characteristics			
N with DWI positive lesion (%)	101 (22.6)	759 (77.9)	<0.0001
N with visible basal ganglia PVSs (%)			
<11 (%)	181 (40.6)	659 (67.7)	<0.0001
11-20 (%)	150 (33.6)	246 (25.3)	
>20 (%)	115 (25.8)	69 (7.1)	
N with visible centrum semiovale PVSs (%)			
<11 (%)	60 (13.5)	410 (42.1)	<0.0001
11-20 (%)	138 (30.9)	463 (47.5)	
>20 (%)	248 (55.6)	101 (10.4)	
N with lacunes (%)	58 (13.0)	430 (44.1)	<0.0001
N with microbleeds (%)	64 (14.3)	441 (45.3)	<0.0001
1 microbleed (%)	35 (7.8)	179 (18.4)	
2-4 microbleeds (%)	17 (3.8)	145 (14.9)	
≥5 microbleeds (%)	12 (2.7)	117 (12.0)	
N with periventricular WMH (%)			
Grade 1 (%)	182 (40.8)	213 (21.9)	<0.0001
Grade 2 (%)	89 (20.0)	75 (7.7)	
Grade 3 (%)	31 (7.0)	30 (3.1)	
N with subcortical WMH (%)			
Grade 1 (%)	141 (31.6)	475 (48.8)	<0.0001
Grade 2 (%)	70 (15.7)	278 (28.5)	
Grade 3 (%)	53 (11.9)	155 (15.9)	

TIA=transient ischaemic attack; DWI=diffusion weighted imaging; PVS=perivascular space; WMH=white matter hyperintensity

Table 5.5 Clinical and imaging correlates of TIA or ischaemic stroke patients with increasing burden of visible perivascular spaces

	Number of visible basal ganglia PVSs				Number of visible centrum semiovale PVSs			
	<11 n=1186	**11-20 n=517**	**>20 n=299**	**p_{trend}**	**<11 n=636**	**11-20 n=794**	**>20 n=572**	**p_{trend}**
Age, yr (SD)	64 (13)	74 (10)	77 (9)	<0.0001	65 (14)	69 (13)	73 (11)	<0.0001
Male sex (%)	679 (57.3)	291 (56.3)	151 (50.5)	0.11	360 (56.6)	472 (59.4)	289 (50.5)	0.004
Hypertension (%)	645 (54.4)	360 (69.6)	198 (66.2)	<0.0001	337 (53.0)	504 (63.5)	362 (63.3)	<0.0001
Diabetes (%)	247 (20.8)	110 (21.3)	54 (18.1)	0.51	136 (21.4)	174 (21.9)	101 (17.7)	0.13
Hyperlipidaemia (%)	366 (30.9)	166 (32.1)	98 (32.8)	0.76	186 (29.2)	241 (30.4)	203 (35.5)	0.045
Ever-smoker (%)	485 (40.9)	208 (40.2)	119 (39.8)	0.92	249 (39.2)	314 (39.6)	249 (43.5)	0.23
Atrial fibrillation (%)	123 (10.4)	93 (18.0)	72 (24.1)	<0.0001	88 (13.8)	100 (12.6)	100 (17.5)	0.035
GFR<60ml/min/1.73m^2	210 (17.9)	145 (28.4)	89 (29.9)	<0.0001	124 (19.7)	173 (22.0)	147 (25.9)	0.033
Severe periventricular WMH (%)	30 (2.5)	30 (5.8)	66 (22.1)	<0.0001	27 (4.2)	33 (4.2)	66 (11.5)	<0.0001
Severe subcortical WMH (%)	87 (7.3)	91 (17.6)	94 (31.4)	<0.0001	68 (10.7)	99 (12.5)	105 (18.4)	0.0003
≥5 Microbleeds (%)	58 (4.9)	47 (9.1)	51 (17.1)	<0.0001	63 (9.9)	57 (7.2)	36 (6.3)	0.046
Lacunes (%)	337 (28.4)	176 (34.0)	99 (33.1)	0.040	184 (28.9)	269 (33.9)	159 (27.8)	0.031

TIA=transient ischaemic attack; PVS=perivascular space; GFR=glomerular filtration rate; WMH=white matter hyperintensity

Table 5.6 Clinical correlates of >20 visible perivascular spaces

	Univariate		Age and sex adjusted		Multivariate[b] adjusted	
	OXVASC OR (95% CI)	HKU OR (95% CI)	Combined[a] OR (95% CI)	Combined[a] OR (95% CI)	Combined[a] OR (95% CI)	p
Basal ganglia PVSs						
Age	1.08 (1.06-1.10)	1.09 (1.06-1.12)	1.08 (1.07-1.10)	1.08 (1.07-1.10)	1.05 (1.03-1.07)	<0.0001
Male sex	0.79 (0.59-1.06)	0.98 (0.60-1.62)	0.84 (0.65-1.08)	1.03 (0.78-1.34)	1.17 (0.86-1.59)	0.33
Hypertension	1.71 (1.26-2.31)	1.30 (0.76-2.23)	1.60 (1.23-2.09)	1.09 (0.82-1.44)	0.98 (0.70-1.37)	0.91
Diabetes	1.41 (0.94-2.12)	0.75 (0.42-1.34)	1.13 (0.81-1.57)	1.00 (0.71-1.41)	1.09 (0.74-1.62)	0.67
Hyperlipidaemia	1.07 (0.79-1.45)	0.47 (0.24-0.94)	0.91 (0.70-1.20)	0.78 (0.59-1.04)	0.81 (0.58-1.13)	0.22
Ever-smoker	0.77 (0.57-1.03)	0.58 (0.32-1.06)	0.73 (0.56-0.94)	0.82 (0.62-1.10)	0.86 (0.62-1.19)	0.37
Atrial fibrillation	2.46 (1.72-3.54)	1.43 (0.75-2.75)	2.15 (1.57-2.93)	1.35 (0.97-1.87)	1.58 (1.10-2.29)	0.014
GFR<60ml/min/1.73m^2	1.31 (0.93-1.82)	2.50 (1.49-4.18)	1.57 (1.18-2.08)	0.76 (0.55-1.04)	0.81 (0.57-1.15)	0.24
Periventricular WMH	3.14 (2.62-3.76)	2.72 (2.12-3.48)	2.99 (2.58-3.45)	2.43 (2.08-2.84)	2.01 (1.66-2.44)	<0.0001
Subcortical WMH	2.61 (2.23-3.07)	2.55 (1.89-3.45)	2.60 (2.26-3.00)	2.16 (1.86-2.50)	1.44 (1.20-1.72)	<0.0001
Deep microbleed number	1.23 (1.01-1.50)	1.05 (1.02-1.09)	1.06 (1.03-1.10)	1.07 (1.03-1.11)	1.05 (1.00-1.10)	0.063
Lobar microbleed number	1.05 (1.00-1.10)	1.02 (1.00-1.03)	1.02 (1.01-1.04)	1.03 (1.01-1.04)	1.00 (0.98-1.02)	0.84
Lacunes	3.22 (2.29-4.54)	1.64 (0.97-2.74)	1.87 (1.40-2.49)	1.67 (1.24-2.26)	1.18 (0.84-1.65)	0.34
Centrum semiovale PVSs						
Age	1.05 (1.04-1.06)	1.01 (1.00-1.03)	1.04 (1.03-1.05)	1.04 (1.03-1.05)	1.01 (1.00-1.03)	0.020
Male sex	0.78 (0.61-1.00)	0.89 (0.59-1.35)	0.81 (0.66-1.00)	0.89 (0.72-1.11)	0.88 (0.69-1.12)	0.29
Hypertension	1.64 (1.28-2.10)	1.41 (0.89-2.23)	1.59 (1.27-1.97)	1.23 (0.97-1.54)	1.13 (0.87-1.48)	0.36
Diabetes	1.17 (0.82-1.68)	1.32 (0.85-2.05)	1.23 (0.93-1.63)	1.12 (0.84-1.49)	1.15 (0.83-1.60)	0.39
Hyperlipidaemia	1.01 (0.78-1.30)	1.12 (0.71-1.78)	1.04 (0.83-1.29)	0.92 (0.73-1.16)	0.94 (0.72-1.23)	0.67
Ever-smoker	0.77 (0.60-0.98)	0.84 (0.53-1.34)	0.78 (0.63-0.97)	0.88 (0.70-1.11)	0.91 (0.70-1.17)	0.45
Atrial fibrillation	1.60 (1.14-2.24)	0.79 (0.41-1.53)	1.35 (1.01-1.80)	0.98 (0.72-1.32)	0.85 (0.61-1.19)	0.34
GFR<60ml/min/1.73m^2	1.26 (0.94-1.68)	1.40 (0.87-2.25)	1.30 (1.01-1.66)	0.81 (0.61-1.06)	0.87 (0.64-1.18)	0.37
Periventricular WMH	1.90 (1.65-2.19)	1.00 (0.77-1.31)	1.62 (1.45-1.82)	1.35 (1.18-1.53)	0.90 (0.76-1.08)	0.26
Subcortical WMH	1.87 (1.64-2.14)	1.28 (1.00-1.63)	1.71 (1.53-1.92)	1.48 (1.31-1.67)	1.28 (1.09-1.50)	0.003
Deep microbleed number	1.25 (0.99-1.58)	0.98 (0.91-1.05)	1.00 (0.96-1.04)	1.00 (0.96-1.04)	0.95 (0.86-1.04)	0.25
Lobar microbleed number	1.02 (0.98-1.05)	0.98 (0.92-1.03)	1.00 (0.98-1.02)	1.00 (0.98-1.02)	0.98 (0.94-1.02)	0.26
Lacunes	1.89 (1.37-2.62)	1.39 (0.92-2.10)	1.68 (1.30-2.17)	1.55 (1.19-2.01)	1.26 (0.95-1.69)	0.11

[a]Adjusted for Centre
[b]Also adjusted for MRI scanner strength, centrum semiovale PVSs for prediction of basal ganglia PVSs and basal ganglia PVSs for prediction of centrum semiovale PVSs
OR=odds ratio; CI=confidence interval; GFR=glomerular filtration rate; WMH=white matter hyperintensity; PVS=perivascular space

Table 5.7 Cox regression analyses of risk of death in patients with increasing burden of visible perivascular spaces versus <11 perivascular spaces

	Unadjusted HR (95% CI)[a]		HR (95% CI) adjusted for age and sex[a]		HR (95% CI) adjusted for age, sex, vascular risk factors[b] and MRI scanner strength[a]		
Number of PVSs:	**11-20**	**>20**	**11-20**	**>20**	**11-20**	**>20**	**p_{trend}**
Basal ganglia PVSs							
All-cause mortality							
OXVASC	1.68 (1.10-2.58)	3.23 (2.20-4.74)	0.81 (0.52-1.26)	1.42 (0.94-2.13)	0.74 (0.47-1.17)	1.25 (0.83-1.89)	0.27
HKU	1.59 (1.08-2.35)	2.67 (1.55-4.61)	1.03 (0.69-1.54)	1.36 (0.78-2.39)	1.04 (0.69-1.55)	1.32 (0.75-2.31)	0.41
Combined[c]	1.63 (1.23-2.18)	3.04 (2.24-4.13)	0.92 (0.68-1.24)	1.44 (1.05-1.99)	0.89 (0.66-1.20)	1.33 (0.96-1.85)	0.16
Vascular death							
OXVASC	1.53 (0.63-3.71)	3.32 (1.55-7.12)	0.79 (0.32-2.00)	1.55 (0.68-3.50)	0.73 (0.29-1.85)	1.31 (0.58-3.00)	0.49
HKU	1.66 (0.94-2.94)	2.54 (1.12-5.77)	1.03 (0.58-1.85)	1.22 (0.53-2.83)	1.06 (0.59-1.93)	1.21 (0.52-2.81)	0.67
Combined[c]	1.62 (1.01-2.62)	2.98 (1.74-5.12)	0.95 (0.58-1.55)	1.43 (0.81-2.50)	0.93 (0.57-1.54)	1`.31 (0.74-2.31)	0.47
Nonvascular death							
OXVASC	1.62 (0.96-2.73)	2.95 (1.84-4.75)	0.80 (0.46-1.37)	1.33 (0.80-2.20)	0.72 (0.41-1.24)	1.17 (0.70-1.95)	0.55
HKU	1.53 (0.90-2.62)	2.79 (1.34-5.78)	1.03 (0.60-1.77)	1.50 (0.71-3.18)	1.01 (0.58-1.76)	1.42 (0.67-3.02)	0.47
Combined[c]	1.58 (1.09-2.29)	2.80 (1.91-4.10)	0.90 (0.61-1.32)	1.39 (0.93-2.07)	0.86 (0.58-1.27)	1.32 (0.87-2.00)	0.30
Centrum semi-ovale PVSs							
All-cause mortality							
OXVASC	1.08 (0.67-1.74)	1.90 (1.23-2.92)	0.54 (0.33-0.89)	0.78 (0.50-1.22)	0.50 (0.30-0.81)	0.68 (0.43-1.07)	0.40
HKU	0.80 (0.56-1.16)	0.53 (0.26-1.06)	0.81 (0.56-1.17)	0.49 (0.24-0.98)	0.87 (0.60-1.27)	0.50 (0.25-1.01)	0.068
Combined[c]	0.90 (0.67-1.20)	1.28 (0.94-1.76)	0.69 (0.51-0.93)	0.77 (0.55-1.07)	0.70 (0.52-0.94)	0.74 (0.53-1.03)	0.072
Vascular death							
OXVASC	1.06 (0.37-3.07)	2.57 (1.03-6.45)	0.54 (0.18-1.63)	1.11 (0.43-2.88)	0.49 (0.16-1.48)	0.95 (0.36-2.48)	0.59
HKU	0.89 (0.52-1.51)	0.40 (0.12-1.33)	0.90 (0.53-1.53)	0.36 (0.11-1.20)	0.99 (0.57-1.71)	0.36 (0.11-1.20)	0.19
Combined[c]	0.91 (0.57-1.47)	1.27 (0.73-2.20)	0.77 (0.48-1.25)	0.85 (0.47-1.52)	0.80 (0.49-1.30)	0.81 (0.45-1.45)	0.43
Nonvascular death							
OXVASC	0.91 (0.52-1.60)	1.52 (0.91-2.53)	0.47 (0.26-0.83)	0.63 (0.37-1.08)	0.43 (0.24-0.77)	0.56 (0.33-0.95)	0.14
HKU	0.74 (0.45-1.22)	0.63 (0.26-1.49)	0.74 (0.45-1.22)	0.58 (0.24-1.40)	0.78 (0.47-1.31)	0.61 (0.26-1.47)	0.20
Combined[c]	0.81 (0.56-1.18)	1.20 (0.80-1.78)	0.61 (0.41-0.89)	0.70 (0.46-1.07)	0.61 (0.42-0.90)	0.68 (0.44-1.03)	0.067

[a]Compared with <11 PVSs as reference
[b]Hypertension, hyperlipidaemia, diabetes mellitus, atrial fibrillation, smoking history
[c]Also adjusted for Centre
HR=hazard ratio; CI=confidence interval; PVS=perivascular space

Table 5.8 Cox regression analyses of recurrent stroke with increasing burden of visible perivascular spaces versus <11 perivascular spaces

	Unadjusted HR (95% CI)[a]		HR (95% CI) adjusted for age and sex[a]		HR (95% CI) adjusted for age, sex, vascular risk factors[b] and MRI scanner strength[a]		
Number of PVSs:	**11-20**	**>20**	**11-20**	**>20**	**11-20**	**>20**	**p_{trend}**
Basal ganglia PVSs							
Recurrent stroke							
OXVASC	1.36 (0.79-2.32)	2.45 (1.52-3.95)	1.16 (0.65-2.05)	2.03 (1.19-3.46)	1.11 (0.62-1.97)	1.90 (1.11-3.25)	0.021
HKU	1.46 (0.95-2.24)	2.95 (1.70-5.11)	1.11 (0.72-1.73)	1.97 (1.11-3.49)	1.20 (0.77-1.87)	2.32 (1.29-4.15)	0.013
Combined[c]	1.40 (1.00-1.95)	2.62 (1.83-3.77)	1.11 (0.78-1.57)	1.92 (1.30-2.83)	1.13 (0.79-1.61)	1.94 (1.31-2.89)	0.002
Ischaemic stroke							
OXVASC	1.24 (0.70-2.20)	2.36 (1.43-3.89)	1.11 (0.60-2.04)	2.08 (1.18-3.65)	1.04 (0.56-1.92)	1.99 (1.13-3.49)	0.020
HKU	1.56 (0.98-2.47)	2.38 (1.24-4.58)	1.15 (0.72-1.86)	1.55 (0.79-3.04)	1.30 (0.80-2.11)	1.94 (0.98-3.85)	0.056
Combined[c]	1.40 (0.98-2.01)	2.39 (1.61-3.54)	1.12 (0.77-1.63)	1.77 (1.16-2.70)	1.15 (0.78-1.68)	1.82 (1.18-2.80)	0.011
Intracerebral haemorrhage							
OXVASC	2.55 (0.51-12.69)	3.31 (0.66-16.59)	1.39 (0.25-7.75)	1.63 (0.29-9.35)	1.03 (0.17-6.16)	0.95 (0.14-6.22)	0.95
HKU	1.01 (0.32-3.17)	5.00 (1.73-14.43)	0.87 (0.27-2.83)	4.07 (1.30-12.76)	0.79 (0.24-2.58)	3.84 (1.20-12.29)	0.089
Combined[c]	1.33 (0.54-3.29)	4.02 (1.63-9.94)	1.02 (0.40-2.63)	2.77 (1.05-7.35)	0.95 (0.37-2.46)	2.58 (0.97-6.89)	0.10
Centrum semiovale PVSs							
Recurrent stroke							
OXVASC	1.07 (0.58-1.98)	1.84 (1.06-3.19)	0.90 (0.48-1.70)	1.43 (0.79-2.59)	0.83 (0.44-1.57)	1.30 (0.71-2.37)	0.21
HKU	1.02 (0.69-1.50)	0.39 (0.15-0.97)	1.02 (0.70-1.50)	0.37 (0.15-0.92)	1.07 (0.73-1.58)	0.37 (0.15-0.94)	0.16
Combined[c]	1.02 (0.73-1.41)	1.15 (0.78-1.68)	0.92 (0.66-1.28)	0.91 (0.61-1.35)	0.92 (0.66-1.28)	0.89 (0.60-1.33)	0.57
Ischaemic stroke							
OXVASC	1.20 (0.63-2.26)	1.72 (0.95-3.10)	1.04 (0.54-2.01)	1.41 (0.75-2.67)	0.94 (0.49-1.82)	1.28 (0.68-2.41)	0.33
HKU	1.04 (0.68-1.59)	0.29 (0.09-0.93)	1.04 (0.68-1.59)	0.27 (0.08-0.87)	1.12 (0.73-1.72)	0.28 (0.09-0.92)	0.18
Combined[c]	1.07 (0.75-1.52)	1.07 (0.70-1.63)	0.96 (0.67-1.37)	0.85 (0.55-1.31)	0.96 (0.67-1.37)	0.83 (0.54-1.28)	0.42
Intracerebral haemorrhage							
OXVASC	-	2.89 (0.59-14.17)	-	1.37 (0.25-7.46)	-	1.29 (0.19-8.69)	0.35
HKU	0.90 (0.36-2.27)	0.83 (0.18-3.83)	0.90 (0.36-2.26)	0.80 (0.17-3.71)	0.86 (0.34-2.17)	0.75 (0.16-3.51)	0.67
Combined[c]	0.71 (0.30-1.72)	1.65 (0.65-4.20)	0.67 (0.28-1.61)	1.35 (0.51-3.57)	0.69 (0.28-1.67)	1.36 (0.51-3.59)	0.69

[a]Compared with <11 PVSs as reference
[b]Hypertension, hyperlipidaemia, diabetes mellitus, atrial fibrillation, smoking history
[c]Also adjusted for Centre
HR=hazard ratio; CI=confidence interval; PVS=perivascular space

Table 5.9 Cox regression analyses of risk of recurrent stroke with increasing burden of visible perivascular spaces versus <11 perivascular spaces, stratified by OXVASC MRI scanner

	Unadjusted HR (95% CI)[a]			HR (95% CI) adjusted for age and sex[a]		
Number of PVSs:	**11-20**	**>20**	**p_{trend}**	**11-20**	**>20**	**p_{trend}**
Basal ganglia PVSs						
OXVASC scanner 1 (3-T)	1.20 (0.35-4.15)	3.06 (1.05-8.96)	0.035	1.07 (0.29-4.01)	2.60 (0.77-8.83)	0.090
OXVASC scanner 2 (3-T)	2.69 (0.38-19.13)	10.82 (2.06-56.90)	0.004	2.67 (0.34-20.71)	17.60 (2.11-146.52)	0.009
OXVASC scanner 3 (1.5-T)	4.43 (1.13-17.28)	1.11 (0.13-9.54)	0.37	2.67 (0.59-12.05)	0.62 (0.07-5.85)	0.94
OXVASC scanner 4 (1.5-T)	1.11 (0.54-2.31)	1.94 (1.02-3.67)	0.056	0.97 (0.44-2.12)	1.66 (0.81-3.38)	0.19
Centrum semiovale PVSs						
OXVASC scanner 1 (3-T)	0.66 (0.06-7.33)	3.19 (0.42-24.08)	0.037	0.56 (0.05-6.28)	2.54 (0.32-20.03)	0.057
OXVASC scanner 2 (3-T)	0.76 (0.13-4.56)	2.67 (0.58-12.23)	0.22	0.66 (0.11-4.09)	2.26 (0.48-10.70)	0.30
OXVASC scanner 3 (1.5-T)	3.39 (0.66-17.52)	4.05 (0.64-25.64)	0.11	2.39 (0.42-13.65)	1.97 (0.26-14.82)	0.53
OXVASC scanner 4 (1.5-T)	1.04 (0.49-2.23)	1.50 (0.75-3.01)	0.21	0.90 (0.40-2.01)	1.21 (0.55-2.65)	0.54

[a]Compared with <11 PVSs as reference
HR=hazard ratio; CI=confidence interval; PVS=perivascular space

Table 5.10 Cox regression analyses of risk of recurrent stroke with increasing burden of visible perivascular spaces versus <11 perivascular spaces, stratified by white matter hyperintensity burden

	Unadjusted HR (95% CI)[a]		HR (95% CI) adjusted for age and sex[b]		HR (95% CI) adjusted for age, sex, vascular risk factors[b] and MRI scanner strength[a]		
Number of PVSs:	**11-20**	**>20**	**11-20**	**>20**	**11-20**	**>20**	**p_{trend}**
No or mild WMH							
Basal ganglia PVSs							
OXVASC	0.67 (0.26-1.75)	2.47 (1.12-5.46)	0.55 (0.20-1.48)	1.92 (0.81-4.56)	0.65 (0.24-1.76)	2.17 (0.89-5.34)	0.24
HKU	1.27 (0.61-2.65)	3.84 (1.18-12.51)	0.92 (0.43-1.94)	2.63 (0.79-8.72)	0.99 (0.46-2.11)	2.88 (0.85-9.78)	0.33
Combined[c]	0.97 (0.54-1.73)	2.89 (1.48-5.62)	0.73 (0.40-1.33)	1.99 (0.99-4.00)	0.80 (0.44-1.47)	2.20 (1.07-4.52)	0.20
Centrum semi-ovale PVSs							
OXVASC	1.12 (0.48-2.58)	1.99 (0.91-4.35)	0.97 (0.41-2.32)	1.64 (0.71-3.80)	0.90 (0.38-2.17)	1.87 (0.81-4.33)	0.11
HKU	1.01 (0.55-1.84)	0.92 (0.31-2.67)	0.97 (0.53-1.78)	0.90 (0.31-2.63)	0.93 (0.51-1.72)	0.83 (0.28-2.45)	0.72
Combined[c]	1.04 (0.64-1.71)	1.49 (0.84-2.64)	0.92 (0.56-1.51)	1.19 (0.66-2.15)	0.88 (0.53-1.44)	1.27 (0.71-2.29)	0.54
Moderate to severe WMH							
Basal ganglia PVSs							
OXVASC	1.30 (0.60-2.81)	1.49 (0.72-3.09)	1.37 (0.62-3.06)	1.57 (0.74-3.36)	1.23 (0.55-2.76)	1.37 (0.64-2.95)	0.42
HKU	1.32 (0.76-2.30)	2.32 (1.20-4.51)	1.10 (0.62-1.96)	1.74 (0.87-3.50)	1.09 (0.61-1.95)	1.87 (0.91-3.84)	0.13
Combined[c]	1.28 (0.82-2.01)	1.79 (1.09-2.94)	1.17 (0.73-1.86)	1.59 (0.95-2.66)	1.15 (0.72-1.83)	1.53 (0.91-2.59)	0.12
Centrum semiovale PVSs							
OXVASC	0.64 (0.25-1.62)	0.81 (0.35-1.84)	0.64 (0.25-1.64)	0.81 (0.35-1.88)	0.63 (0.25-1.62)	0.74 (0.32-1.73)	0.72
HKU	0.95 (0.57-1.56)	0.10 (0.01-0.76)	0.98 (0.60-1.62)	0.10 (0.01-0.73)	1.10 (0.67-1.85)	0.10 (0.01-0.75)	0.066
Combined[c]	0.82 (0.52-1.28)	0.60 (0.35-1.04)	0.81 (0.52-1.26)	0.57 (0.33-1.00)	0.85 (0.54-1.33)	0.57 (0.33-1.00)	0.051

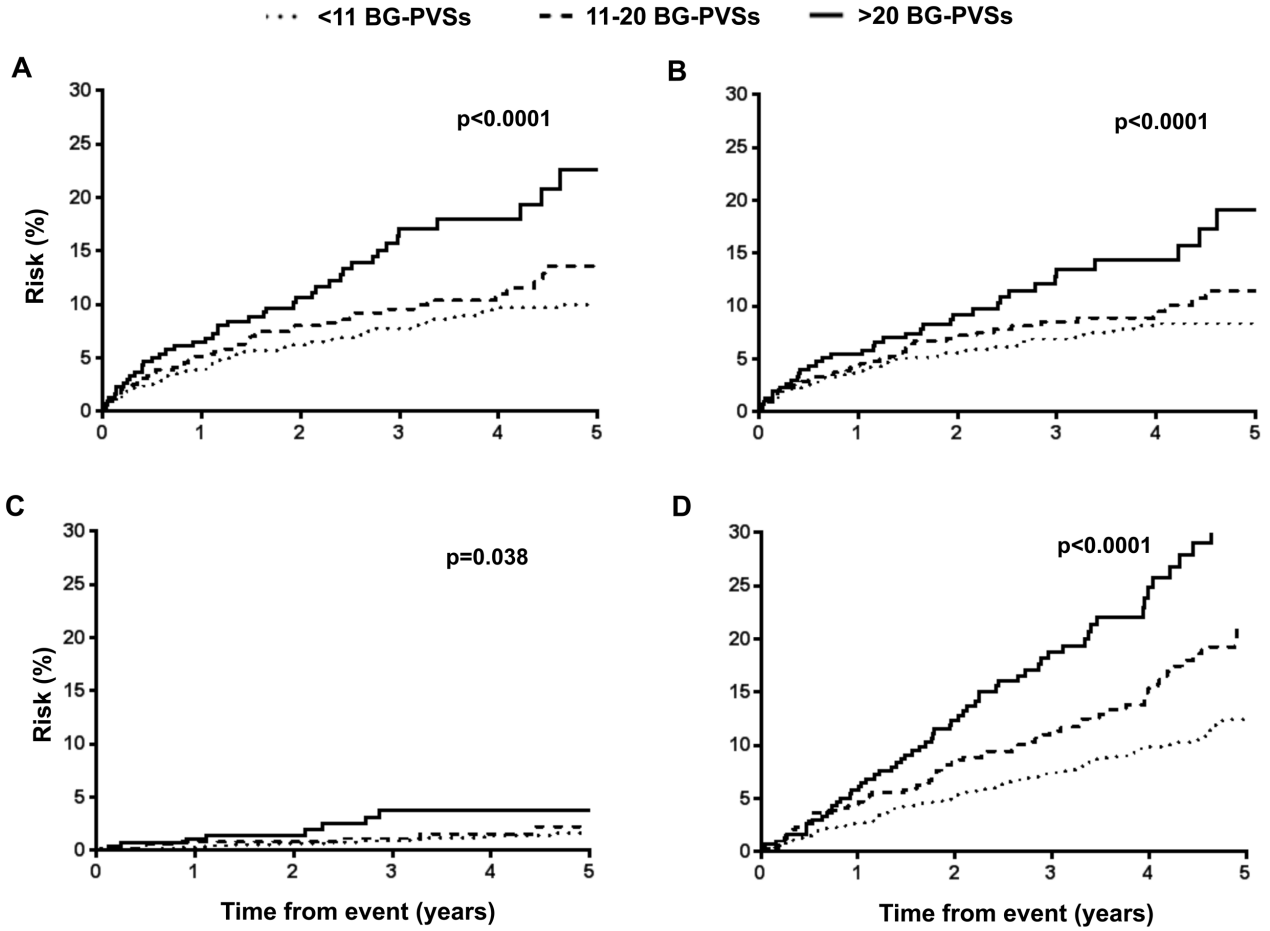
[a]Compared with <11 PVSs as reference
[b]Hypertension, hyperlipidaemia, diabetes mellitus, atrial fibrillation, smoking history
[c]Also adjusted for Centre
HR=hazard ratio; CI=confidence interval; PVS=perivascular space; WMH=white matter hyperintensity

Table 5.11 Cox regression analyses of risk of recurrent ischaemic stroke with increasing burden of neuroimaging markers of small vessel disease

	Unadjusted HR (95% CI)	p_{trend}	Multivariate adjusted HR (95% CI) (Forward stepwise)	p_{trend}
Number of visible BG-PVSs[a]				
11-20	1.40 (0.98-2.01)	<0.0001	1.22 (0.84-1.77)	0.001
>20	2.39 (1.61-3.54)		1.75 (1.17-2.63)	
Number of visible CS-PVSs[a]				
11-20	1.07 (0.75-1.52)	0.73	-	
>20	1.07 (0.70-1.63)		-	
Lacunes[b]	1.42 (1.02-1.96)	0.036	-	
Microbleed[b]				
1 microbleed	1.72 (1.13-2.62)	0.001	1.67 (1.10-2.53)	0.001
2-4 microbleed	1.64 (1.00-2.69)		1.43 (0.87-2.35)	
≥5 microbleeds	2.14 (1.32-3.49)		1.76 (1.06-2.92)	
Periventricular WMH[b]				
Grade 1	1.48 (1.02-2.13)	<0.0001	-	
Grade 2	1.91 (1.22-2.97)		-	
Grade 3	2.57 (1.49-4.44)		-	
Subcortical WMH[b]				
Grade 1	1.14 (0.72-1.83)	0.001	1.04 (0.66-1.64)	0.17
Grade 2	2.36 (1.48-3.76)		1.85 (1.15-2.98)	
Grade 3	1.64 (0.95-2.85)		1.09 (0.60-1.98)	

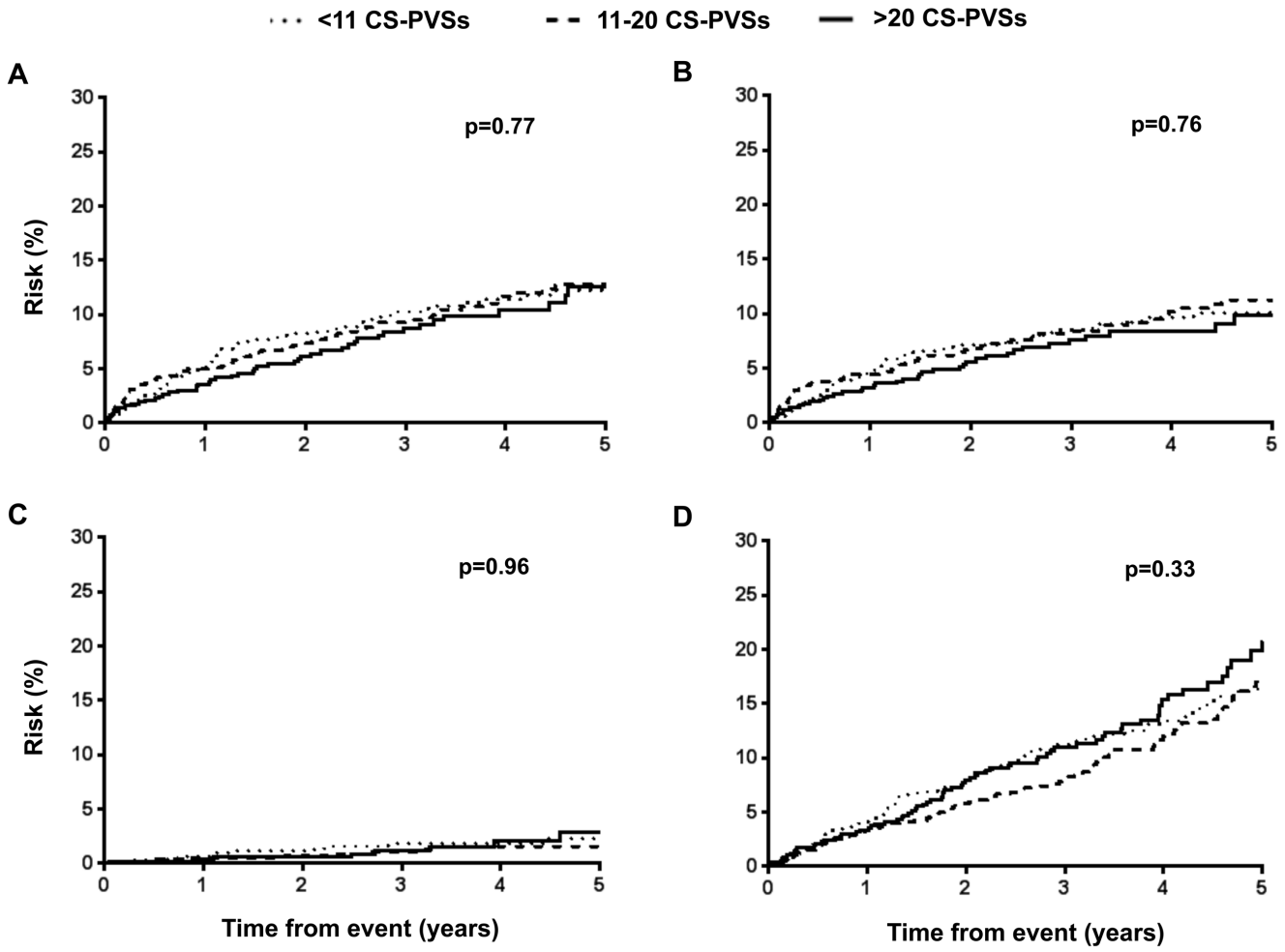
[a]Compared with <11 PVSs as reference
[b]Compared with no lacunes, microbleeds or WMH as reference
HR=hazard ratio, CI=confidence interval, BG=basal ganglia, CS=centrum semi-ovale, PVS=perivascular space, WMH=white matter hyperintensity

Figure 5.1 Risk of A) recurrent stroke, B) recurrent ischaemic stroke, C) intracerebral haemorrhage and D) all-cause mortality amongst TIA or ischaemic stroke patients with increasing visible basal ganglia perivascular space burden



TIA=transient ischaemic attack; BG=basal ganglia; PVS=perivascular space

Figure 5.2 Risk of A) recurrent stroke, B) recurrent ischaemic stroke, C) intracerebral haemorrhage and D) all-cause mortality amongst TIA or ischaemic stroke patients with increasing visible centrum semiovale perivascular space burden



TIA=transient ischaemic attack; CS=centrum semiovale; PVS=perivascular space

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Chapter 6

Total small vessel disease score and risk of recurrent stroke – validation in two large cohorts

6.1 Chapter outline

The Total Small Vessel Disease (SVD) Score incorporates four established neuroimaging markers of SVD and aims to capture the overall burden of SVD. Although the score has been associated with age, hypertension, ambulatory blood pressure and lacunar stroke subtype, the prognostic value of the Total SVD Score has yet to be validated in a cohort of patients with transient ischaemic attack (TIA) and ischaemic stroke. Therefore in this Chapter, I validated the Total SVD Score by determining its prognostic value for recurrent stroke.

I determined the Total SVD Score for 2002 patients [Oxford Vascular Study (OXVASC) n=1028, The University of Hong Kong (HKU) n=974) with TIA or ischaemic stroke and calculated its predictive value for risk of recurrent stroke (ischaemic and haemorrhagic). I also evaluated whether refinements to the score improved its prognostic value.

I found that in patients with TIA or ischaemic stroke, a higher Total SVD Score was associated with an increased risk of recurrent ischaemic stroke [adjusted hazard ratio (HR) per unit increase: 1.32, 95% confidence interval (CI) 1.16-1.51, $p<0.0001$; c-statistic 0.61, 0.56-0.65, $p<0.0001$) and intracerebral haemorrhage (ICH) (HR 1.54, 1.11-2.13, $p=0.009$; c-statistic 0.65, 0.54-0.76, $p=0.006$). A higher score predicted recurrent stroke in SVD and non-SVD TIA or ischaemic stroke subtypes (c-statistic 0.67, 0.59-0.74, $p<0.0001$ and 0.60, 0.55-0.65, $p<0.0001$). Including burden of microbleeds and white matter hyperintensity and adjusting the cut-off of basal ganglia perivascular spaces potentially improved predictive power for ICH (c-statistic 0.71, 0.60-0.81, $p_{het}=0.45$), but not for recurrent ischaemic stroke (c-statistic 0.60, 0.56-0.65, $p_{het}=0.76$) on internal validation.

I concluded that the Total SVD Score has predictive value for recurrent stroke after TIA or ischaemic stroke. However, the Total SVD Score is unable to differentiate patients at high risk of ICH from those at high risk of recurrent ischaemic events. Prediction of recurrence in patients with non-lacunar events highlights the potential role of SVD in wider stroke aetiology.

6.2 Introduction

Cerebral small vessel disease (SVD) is a common cause of stroke, cognitive impairment and gait disturbances.[1, 2] Recently, a "Total SVD Score" was proposed,[3-5] which incorporates four established neuroimaging biomarkers of SVD and aims to capture the overall burden of cerebral SVD. In this score, one point is allocated to each of the following: 1) presence of lacunes, 2) presence of microbleeds, 3) moderate-severe (>10) visible basal ganglia perivascular spaces (BG-PVS) and 4) severe periventricular and/or moderate-severe deep white matter hyperintensity (WMH).[3] The score has been associated with age, male sex, hypertension, smoking and lacunar stroke subtype in ischaemic stroke patients.[3] In patients with lacunar infarct, the Total SVD Score has also been associated with increased ambulatory blood pressure[4] and cognitive impairment.[5]

However, although the "Total SVD Score" has subsequently shown to be associated with cognitive impairment,[6, 7] its long-term prognostic implications for recurrent stroke in patients with transient ischaemic attack (TIA) or ischaemic stroke have yet to be determined.[3] Whether the score also predicts risk of recurrent stroke in non-lacunar stroke subtypes is unknown. Moreover, whether refinements to the score (e.g. by incorporating different weightings based on microbleed[8] and WMH burden) may improve its predictive value has not been explored.[3, 9] Therefore, in two large prospective studies, one comprising predominantly Caucasians with TIA and ischaemic stroke and one predominantly Chinese with ischaemic stroke, I 1) validated the current Total SVD score by determining its long-term prognostic implications and 2) determined whether minor refinements to the score might improve its prognostic value. I hypothesise that given the higher prevalence of hypertension in Asia, the Total SVD Score will be higher in the Hong Kong cohort compared to residents of Oxfordshire. I also hypothesise that the Total SVD Score may be predictive of recurrent stroke and that minor modifications to the score by increasing the granularity of various components may potentially increase its prognostic value.

6.3 Methods

6.3.1 Study populations

1080 consecutive cases with TIA or ischaemic stroke who were predominantly Caucasians and 1076 consecutive cases with ischaemic stroke who were predominantly Chinese were recruited from the Oxford Vascular Study (OXVASC) and The University of Hong Kong (HKU) respectively (see section 2.1). Baseline data was collected as described in section 2.1. All patients received a cerebral magnetic resonance imaging (MRI) scan at baseline (see section 2.2) and presence and burden of visible BG and centrum-semiovale (CS)-PVSs, cerebral microbleeds, WMH and lacunes coded as described in section 2.3.

All patients in OXVASC and HKU were followed-up regularly and assessed for recurrent stroke (ischaemic and haemorrhagic) as described in section 2.4. The modified Rankin Scale of recurrent strokes was determined at 1 month after recurrent event and a disabling stroke was defined as a modified Rankin Scale ≥3.

6.3.2 Statistical analysis

I compared differences in baseline and imaging characteristics in OXVASC and HKU using Student's t-test for continuous variables and Chi-squared test for categorical variables. I determined, by Cox regression analysis, the unadjusted and adjusted (for age, sex and vascular risk factors) risks of recurrent ischaemic stroke and intracerebral haemorrhage (ICH) in patients with 1) lacunes, 2) increasing burden (1, 2-4 and ≥5 vs. 0) of microbleeds, 3) increasing burden (11-20 and >20 vs. <11) of visible BG and CS-PVSs and 4) increasing burden (Fazekas grade 1, 2 and 3 vs. grade 0) of periventricular and subcortical WMH. I calculated the Total SVD Score for all patients[3, 4] and determined the unadjusted and adjusted (for age, sex and vascular risk factors) odds of TIA or ischaemic stroke due to SVD and subsequent risks of recurrent ischaemic stroke and ICH with increasing Total SVD Score. The c-statistic for area under receiver operating characteristic curve for prediction of a recurrent stroke based on the Total SVD Score was

calculated. Prediction of a non-disabling and disabling or fatal recurrent stroke based on increasing Total SVD Score was also determined.

All analyses were done with SPSS version 22.

6.4 Results

6.4.1 Baseline clinical and neuroimaging characteristics

The two study populations contributed a total of 2156 patients. After excluding 154 patients with incomplete clinical and/or imaging data, 2002 patients (OXVASC n=1028, 542 TIA, 486 ischaemic stroke; and HKU n=974, all ischaemic stroke) were included in the final analysis. Baseline clinical and imaging characteristics of patients are shown in Table 6.1. HKU patients had a higher proportion of males (p=0.001), and were more likely to have hypertension and diabetes (p<0.0001), whilst patients from OXVASC were more likely to have hyperlipidaemia or a history of smoking (p<0.0001).

Patients from OXVASC had a higher burden of visible BG-PVSs, CS-PVSs and periventricular WMH (p<0.0001) (Table 6.1). In contrast, those from HKU had a greater burden of lacunes, microbleeds and subcortical WMH (p<0.0001) (Table 6.1). Consequently, the Total SVD Score was higher in the HKU cohort than in the OXVASC cohort (p<0.0001, Table 6.1). These differences remained in analyses confined to OXVASC and HKU patients who received a MRI with a 3-T scanner (Table 6.2). There were also no differences in mean Total SVD Scores amongst OXVASC patients scanned on different MRI scanners (p=0.69) (Table 6.3).

6.4.2 Relationships of Total Small Vessel Disease Score with TIA or ischaemic stroke subtype

26.8% of the study population (OXVASC n=124/1028, HKU n=413/974) were classified to have TIA or ischaemic stroke due to SVD or occlusion by Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria (Table 6.4). An increasing Total SVD Score was associated with a greater odds of having presented at baseline with a small vessel TIA or ischaemic stroke (TOAST classification) in both cohorts [OXVASC: age, sex and vascular risk factor adjusted odds ratio (OR) per unit increase in score 2.02, 95% confidence interval (CI) 1.66-2.46, p<0.0001; HKU: 1.25, 1.11-1.42, p=0.0004) (Table 6.5).

6.4.3 Prognostic implications of the Total Small Vessel Disease Score

A total of 199 recurrent strokes occurred (85.4% ischaemic) after a mean follow-up of 42±23 months (OXVASC: 45±26 months, HKU: 37±19 months, total 6924 patient-years) (Table 6.1). The hazard ratios (HR) of recurrent ischaemic stroke and ICH based on individual markers of SVD are shown in Tables 6.6 – 6.10 and summarised in Figure 6.1.

An increasing Total SVD Score was associated with an increased risk of recurrent stroke in OXVASC and HKU (OXVASC c-statistic: 0.60, 0.54-0.67, p=0.001; HKU: 0.61, 0.56-0.67, p=0.0001; p_{het}=0.82) (Table 6.11). When the two cohorts were pooled, patients with increasing Total SVD Score were at greater risk of recurrent stroke, recurrent ischaemic stroke and ICH. The multivariate adjusted HRs of developing a recurrent stroke, ischaemic stroke and ICH per unit increase in score was 1.36 (1.21-1.54, p<0.0001), 1.32 (1.16-1.51, p<0.0001) and 1.54 (1.11-2.13, p=0.009) (Table 6.12). The c-statistics were 0.62 (0.57-0.66, p<0.0001), 0.61 (0.56-0.65, p<0.0001) and 0.65 (0.54-0.76, p=0.006) respectively (Table 6.12). Patients with an increasing Total SVD Score were at increased risk of a recurrent non-disabling ischaemic stroke (age, sex and vascular risk factor adjusted HR per unit increase: 1.28, 1.07-1.54, p=0.008) and recurrent disabling or fatal ischaemic stroke (1.39, 1.14-1.69, p=0.001) (Table 6.13). They were also at increased risk of non-disabling ICH (2.34, 1.15-4.74, p=0.019). However, the risk-association of Total SVD Score with disabling or fatal ICH did not reach statistical significance (1.42, 0.99-2.05, p=0.060) (Table 6.13).

When all patients were stratified by their baseline TOAST subtype, the Total SVD Score predicted risk of recurrent stroke in both individuals presenting with a baseline TIA or ischaemic stroke classified as SVD (537/2002) [age and sex adjusted HR for recurrent stroke per unit increase in score in SVD subtype: 1.43 (1.12-1.83), p=0.004], and non-SVD (1465/2002) [HR: 1.39 (1.19-1.61) p<0.0001 (p_{het}=0.85)], the relationship of which was significant amongst patients with baseline TIA or ischaemic stroke due to large artery atherosclerosis [large artery atherosclerosis 1.32 (1.03-1.69), cardio-embolic 1.27 (0.91-1.75), undetermined: 1.40 (1.00-1.95)] (Table 6.14). Furthermore, amongst 1028 OXVASC patients, the prognostic value of the Total SVD Score did

not differ for the prediction of recurrent ischaemic strokes that were classified as SVD (8/81) or non-SVD (73/81) (p=0.72).

Compared with <11 visible BG-PVSs, patients with 11-20 visible BG-PVSs were not at increased risk of recurrent stroke (adjusted HR 1.13, 0.79-1.60) (Table 6.10). However, risk of recurrent stroke was approximately doubled (1.94, 1.30-2.88) in patients with >20 visible BG-PVSs (Table 6.10, Figure 6.1). Risk of recurrent stroke was also greater with increasing burden of microbleeds (p_{trend}<0.0001), especially those with ≥5 microbleeds (Table 6.7, Figure 6.1). Similarly, risk of recurrent stroke was also greater in patients with more severe WMH, especially those with periventricular WMH (p_{trend}=0.0001) (Tables 6.8 and 6.9, Figure 6.1). I therefore considered the following three refinements to the Total SVD Score: first, I allocated 1 point to those with >20 visible BG-PVSs rather than >11 BG-PVSs. Second, burden of microbleeds was accounted for by assigning 1 point to patients with 1-4 microbleeds and 2 points to those with ≥5 microbleeds. Third, burden of total WMH (combined periventricular and subcortical WMH) was also accounted for by allocating 1 point to those with a moderate degree of WMH (combined score of 3 or 4) and 2 points to those with severe WMH (combined score of 5 or 6).

The corresponding c-statistic for the modified Total SVD Score in predicting recurrent stroke (0.62, 0.58-0.66, p<0.0001) and recurrent ischaemic stroke (0.60, 0.56-0.65, p<0.0001) did not differ from the Total SVD Score in prediction of recurrent stroke (p_{het}=1.00) and recurrent ischaemic stroke (p_{het}=0.76) (Table 6.15). The modified Total SVD Score also did not improve the prediction of non-disabling versus disabling or fatal events compared with the Total SVD Score (Table 6.13). However, the modified Total SVD Score might possibly be better in identifying patients at high risk of ICH (adjusted HR per unit increase: 1.65, 1.34-2.02, p<0.0001; c-statistic 0.71, 0.60-0.81, p=0.0001), but on comparison of c-statistics, this difference was far from being statistically significant (p_{het}=0.45) (Table 6.15).

6.5 Discussion

I validated the prognostic value of the Total SVD Score in TIA and ischaemic stroke patients in 2 large prospective cohorts involving over 2000 Caucasians and Chinese with approximately 7000 patient-years follow-up. Similar to an earlier report,[3] the Total SVD Score was strongly associated with TIA and ischaemic stroke due to SVD in both of these cohorts. In addition, I demonstrated that patients with a higher score were at increasing risk of a recurrent ischaemic stroke and ICH and that the Total SVD Score predicted both non-disabling and disabling recurrent ischaemic strokes. I also showed that the Total SVD Score predicted recurrent strokes both in patients with SVD and non-SVD subtype of TIA or ischaemic stroke at baseline.

The overall SVD burden, as reflected by the Total SVD Score was significantly greater in the Hong Kong cohort compared with the Oxford cohort. This is likely to be accounted by a number of factors, including a higher prevalence of hypertension in the Hong Kong cohort, a much higher proportion of patients with ischaemic stroke in the Hong Kong cohort compared with the Oxford cohort (~50% being TIAs), but also differences in MRI scanner strength and sequences between the two cohorts. Differences in environmental factors between the two cohorts are also present, and may also account for the differences in SVD burden.

Although previous studies have demonstrated that a high burden of visible BG-PVSs are markers of hypertensive angiopathy,[10, 11] the need for data on long-term prognostic implications of visible BG-PVSs has been highlighted.[12] My results demonstrated that compared with patients with <11 visible BG-PVSs, those with 11-20 visible BG-PVSs were not at significantly increased risk of recurrent ischaemic stroke or ICH. In contrast, TIA or ischaemic stroke patients with >20 visible BG-PVSs were at 1.8-fold increased risk of recurrent ischaemic stroke and 2.6-fold increased risk of ICH; potentially justifying adjustment of cut-off for visible BG-PVSs in the Total SVD Score from >11 to >20. I also found that the risk of recurrent stroke, in particular ICH, varied substantially with burden of microbleeds, consistent with previous studies.[8] Rather than dichotomising the grading of microbleeds as present or absent, I considered allocating different weightings for patients with increasing burden of microbleeds. Similarly, I considered increasing the points attributed for

WMH, in view of the clear stepwise increase in risk noted for increasing burden of WMH, in particular periventricular WMH. However, rather than having different cut-offs for periventricular and subcortical WMH, which may over-complicate the score, I decided to attribute points to moderate or severe total WMH, by combining the total burden of periventricular and subcortical WMH.

My results also support the initial decision of not incorporating visible CS-PVSs within the Total SVD Score. Similar to BG-PVSs, CS-PVSs have too been associated with age, hypertension, WMH and lacunes.[10, 11, 13] However, visible CS-PVSs have also been associated with lobar microbleeds[10] and cerebral amyloid angiopathy (CAA)[14] and hence has been hypothesised to be a neuroimaging marker of CAA by representing fluid and metabolic waste clearance dysfunction due to vascular amyloid deposition.[10, 15] Whilst a high burden of visible CS-PVSs may possibly be associated with subsequent risk of ICH in healthy individuals[10] and in patients with cognitive impairment,[16] my results demonstrate that a high burden of visible CS-PVSs was not predictive of recurrent ischaemic stroke nor ICH in TIA or ischaemic stroke patients. Indeed, interpretation of CS-PVSs is often affected by the presence of concomitant subcortical WMH which was present in 77% of patients in our cohort, and hence the usefulness of visible CS-PVSs would without doubt be limited in a TIA or ischaemic stroke population.

My study has a number of limitations. First, the two cohorts were different in several respects – OXVASC consists of predominantly Caucasians with 50% being TIAs, whilst the HKU cohort comprises predominantly of Chinese with ischaemic stroke. Although this would probably explain some differences in stroke subtypes between the two populations, and there was inevitably some selection in both cohorts in relation to MR imaging, particularly in the earlier stages of the studies, when the two cohorts were analysed separately (Table 6.11), I was able to demonstrate that the Total SVD Score had very similar prognostic value in predicting risk of recurrent stroke (c-statistic 0.60 and 0.61) with no heterogeneity. Similarly, when I stratified my results according to period of recruitment (first 5 years vs. latter 5 years), the Total SVD Score had similar prognostic value in prediction of recurrent stroke with no significant heterogeneity (p_{het}=0.25). These analyses suggest that the utility of the Total SVD Score is robust to ethnicity and is applicable to patients

with TIA and ischaemic stroke. Second, my study was limited by the small number of some clinical outcomes, with only 29 ICH on long-term follow-up, amongst 199 total recurrent strokes. Third, the TOAST subtypes of recurrent strokes were only available amongst OXVASC patients. Whether the Total SVD Score predicts recurrent strokes of different subtypes would require further study. Fourth, patients in OXVASC were scanned on 4 different scanners over the 10-year study period. However, although this could have been a potential source of heterogeneity, Total SVD scores were similar across the 4 scanners and appeared to have similar predictive value for recurrent stroke (p_{het}=0.42), accepting the limited statistical power to address this for individual scanners. Overall, therefore, the robustness of my findings to different scanner types is a potential strength of the Total SVD score. Fifth, any modifications to the Total SVD Score require further external validation in other much larger cohorts. Finally, although the Total SVD Score has been shown to predict cognitive impairment in the elderly population[6] and in patients with hypertension,[7] whether the score also predicts cognitive decline in patients with TIA or ischaemic stroke remains uncertain.

Table 6.1 Clinical and imaging characteristics of the study populations

	OXVASC, UK n=1028 (542 TIA, 486 ischaemic stroke)	HKU, HK n=974 (974 ischaemic stroke)	p
Baseline clinical characteristics			
Mean age, yr (SD)	68 (14)	69 (12)	0.25
Males (%)	538 (52.3)	583 (59.9)	0.001
Hypertension (%)	563 (54.8)	640 (65.7)	<0.0001
Diabetes (%)	136 (13.2)	275 (28.2)	<0.0001
Hyperlipidaemia (%)	381 (37.1)	249 (25.6)	<0.0001
Ever-smokers (%)	521 (50.7)	291 (29.9)	<0.0001
Atrial fibrillation (%)	160 (15.6)	128 (13.1)	0.12
Prior TIA / stroke (%)	187 (18.2)	154 (15.8)	0.16
Imaging characteristics			
Magnet strength, T	1.5T n=580, 3T n=448	3T n=974	
N with DWI positive lesion (%)	233 (22.7)	759 (77.9)	<0.0001
N with visible basal ganglia PVSs (%)			
<10 (%)	527 (51.3)	659 (67.7)	<0.0001
10-20 (%)	271 (26.4)	246 (25.3)	
>20 (%)	230 (22.4)	69 (7.1)	
N with visible centrum semiovale PVSs (%)			
<10 (%)	226 (22.0)	410 (42.1)	<0.0001
10-20 (%)	331 (32.2)	463 (47.5)	
>20 (%)	471 (45.8)	101 (10.4)	
N with lacunes (%)	182 (17.7)	430 (44.1)	<0.0001
N with microbleeds (%)	156 (15.2)	441 (45.3)	<0.0001
1 microbleed (%)	79 (7.7)	179 (18.4)	
2-4 microbleeds (%)	44 (4.3)	145 (14.9)	
≥5 microbleeds (%)	39 (3.8)	117 (12.0)	
Strictly deep microbleeds (%)	14 (1.4)	60 (6.2)	<0.0001
Strictly lobar microbleeds (%)	73 (7.1)	157 (16.1)	<0.0001
Strictly infratentorial microbleeds (%)	16 (1.6)	38 (3.9)	0.001
Microbleeds of mixed location (%)	53 (5.2)	186 (19.1)	<0.0001
N with periventricular WMH (%)			
Grade 1 (%)	386 (37.5)	213 (21.9)	<0.0001
Grade 2 (%)	201 (19.6)	75 (7.7)	
Grade 3 (%)	96 (9.3)	30 (3.1)	
N with subcortical WMH (%)			
Grade 1 (%)	338 (32.9)	475 (48.8)	<0.0001
Grade 2 (%)	177 (17.2)	278 (28.5)	
Grade 3 (%)	117 (11.4)	155 (15.9)	
Mean Total SVD Score[a]	1.12±1.11	1.67±1.15	<0.0001
Mean modified Total SVD Score[b]	1.01±1.31	1.27±1.31	<0.0001
Outcome			
Mean follow-up time, months	45±26	37±19	
Patient-years follow-up	3884	3040	
Recurrent stroke (%)	90 (8.8)	109 (11.2)	0.069
Ischaemic stroke (%)	81 (7.9)	89 (9.1)	0.31
Disabling or fatal (%)	26 (32.1)	52 (58.4)	0.001
Fatal (%)	6 (7.4)	12 (13.4)	0.13
Intracerebral haemorrhage (%)	9 (0.9)	20 (2.1)	0.027
Disabling or fatal (%)	6 (66.7)	17 (85.0)	0.015
Fatal (%)	3 (33.3)	6 (30.0)	0.28

[a]1 point allocated for: presence of lacunes, microbleeds, moderate-severe (>10) PVSs in basal ganglia, periventricular WMH Fazekas 3 and/or deep WMH Fazekas 2-3

[b]1 point allocated for: presence of lacunes, 1-4 microbleeds, frequent-severe (>20) PVSs in basal ganglia, moderate WMH (total periventricular + subcortical WMH grade 3-4), 2 points allocated for ≥5 microbleeds and severe WMH (total periventricular + subcortical WMH grade 5-6)

TIA=transient ischaemic attack; DWI=diffusion weighted imaging; PVS=perivascular space; WMH=white matter hyperintensity; SVD=small vessel disease

Table 6.2 Clinical and imaging characteristics in OXVASC and HKU patients who were scanned by a 3-T MRI

	OXVASC, UK n=446 (267 TIA , 179 ischaemic stroke)	HKU, HK n=974 974 ischaemic stroke	p
Baseline clinical characteristics			
Mean age, yr (SD)	70 (14)	69 (12)	0.39
Males (%)	235 (52.7)	583 (59.9)	0.011
Hypertension (%)	237 (53.1)	640 (65.7)	<0.0001
Diabetes (%)	53 (11.9)	275 (28.2)	<0.0001
Hyperlipidaemia (%)	154 (34.5)	249 (25.6)	0.001
Ever-smokers (%)	190 (42.6)	291 (29.9)	<0.0001
Atrial fibrillation (%)	69 (15.5)	128 (13.1)	0.24
Prior TIA / stroke (%)	77 (17.3)	154 (15.8)	0.49
Imaging characteristics			
N with DWI positive lesion (%)	101 (22.6)	759 (77.9)	<0.0001
N with visible basal ganglia PVSs (%)			
<10 (%)	181 (40.6)	659 (67.7)	<0.0001
10-20 (%)	150 (33.6)	246 (25.3)	
>20 (%)	115 (25.8)	69 (7.1)	
N with visible centrum semiovale PVSs (%)			
<10 (%)	60 (13.5)	410 (42.1)	<0.0001
10-20 (%)	138 (30.9)	463 (47.5)	
>20 (%)	248 (55.6)	101 (10.4)	
N with lacunes (%)	58 (13.0)	430 (44.1)	<0.0001
N with microbleeds (%)	64 (14.3)	441 (45.3)	<0.0001
1 microbleed (%)	35 (7.8)	179 (18.4)	
2-4 microbleeds (%)	17 (3.8)	145 (14.9)	
≥5 microbleeds (%)	12 (2.7)	117 (12.0)	
Periventricular WMH grade (%)			
Grade 1 (%)	182 (40.8)	213 (21.9)	<0.0001
Grade 2 (%)	89 (20.0)	75 (7.7)	
Grade 3 (%)	31 (7.0)	30 (3.1)	
N with subcortical WMH (%)			
Grade 1 (%)	141 (31.6)	475 (48.8)	<0.0001
Grade 2 (%)	70 (15.7)	278 (28.5)	
Grade 3 (%)	53 (11.9)	155 (15.9)	
Mean Total SVD Score[a]	1.14±1.01	1.67±1.15	<0.0001
Mean modified Total SVD Score[b]	0.94±1.24	1.27±1.31	<0.0001

[a]1 point allocated for: presence of lacunes, microbleeds, moderate-severe (>10) PVSs in basal ganglia, periventricular WMH Fazekas 3 and/or deep WMH Fazekas 2-3

[b]1 point allocated for: presence of lacunes, 1-4 microbleeds, frequent-severe (>20) PVSs in basal ganglia, moderate WMH (total periventricular + subcortical WMH grade 3-4), 2 points allocated for ≥5 microbleeds and severe WMH (total periventricular + subcortical WMH grade 5-6)

TIA=transient ischaemic attack; DWI=diffusion weighted imaging; PVS=perivascular space; MB=microbleed; WMH=white matter hyperintensity; SVD=small vessel disease

Table 6.3 Distribution of Total Small Vessel Disease Score amongst OXVASC and HKU patients

	HKU Achieva, Philips Healthcare (n=974)	OXVASC scanner 1 Magnetom Verio, Siemens Healthcare (n=384)	OXVASC scanner 2 Discovery MR750, GE Healthcare (n=62)	OXVASC scanner 3 Achieva, Philips Healthcare (n=489)	OXVASC scanner 4 Signa HDxt, GE Healthcare (n=93)
Total SVD Score					
0 (%)	172 (17.7)	11.4 (29.7)	24 (38.7)	208 (42.5)	41 (44.1)
1 (%)	286 (29.4)	145 (37.8)	13 (21.0)	114 (23.3)	21 (22.6)
2 (%)	263 (27.0)	94 (24.5)	15 (24.2)	86 (17.6)	20 (21.5)
3 (%)	199 (20.4)	24 (6.3)	7 (11.3)	61 (12.5)	11 (11.8)
4 (%)	54 (5.5)	7 (1.8)	3 (4.8)	20 (4.1)	0 (0.0)

SVD=small vessel disease

Table 6.4 Aetiology of TIA and ischaemic strokes according to TOAST classification in the OXVASC and HKU cohorts

	OXVASC, UK n=1028 (542 TIA, 486 ischaemic stroke)	HKU, HK n=974 (974 ischaemic stroke)	All n=2002
Small vessel disease (%)	124 (12.1)	413 (42.4)	537 (26.8)
Large artery atherosclerosis (%)	137 (13.3)	334 (34.3)	471 (23.5)
Cardio-embolic (%)	160 (15.6)	118 (12.1)	278 (13.9)
Undetermined (%)	514 (50.0)	42 (4.3)	556 (27.8)
Multiple (%)	35 (3.4)	28 (2.9)	63 (2.1)
Unknown (%)	26 (2.5)	22 (2.3)	48 (2.4)
Others (%)	32 (3.1)	17 (1.7)	49 (2.4)

TIA=transient ischaemic attack; TOAST=Trial of Org 10172 in Acute Stroke Treatment

Table 6.5 Association of Total Small Vessel Disease Score[a] with TOAST subtype of baseline TIA or ischaemic stroke: small vessel events vs. other subtypes

Total Small Vessel Disease Score	Events / patients	Unadjusted HR (95% CI)	p_{trend}	Age and sex adjusted HR (95% CI)	p_{trend}	Multivariate[b] adjusted HR (95% CI)	p_{trend}
OXVASC							
0	35/387	1	<0.0001	1	<0.0001	1	<0.0001
1	27/293	1.02 (0.60-1.73)		1.67 (0.94-2.99)		1.84 (1.02-3.32)	
2	29/215	1.57 (0.93-2.65)		3.11 (1.68-5.77)		3.78 (1.99-7.19)	
3	22/103	2.73 (1.52-4.91)		5.93 (2.97-11.84)		7.07 (3.47-14.42)	
4	11/30	5.82 (2.57-13.22)		13.21 (5.28-33.04)		20.09 (7.45-54.20)	
HKU							
0	59/172	1	0.002	1	<0.0001	1	0.0004
1	116/286	1.31 (0.88-1.94)		1.38 (0.93-2.05)		1.21 (0.80-1.82)	
2	117/263	1.54 (1.03-2.29)		1.77 (1.18-2.67)		1.58 (1.04-2.42)	
3	90/199	1.58 (1.04-2.41)		1.94 (1.25-3.00)		1.69 (1.07-2.66)	
4	31/54	2.58 (1.38-4.82)		3.35 (1.76-6.37)		3.20 (1.62-6.35)	

[a]1 point allocated for: presence of lacunes, microbleeds, moderate-severe (>10) PVSs in basal ganglia, periventricular WMH Fazekas 3 and/or deep WMH Fazekas 2-3
[b]Adjusted for age, sex, hypertension, hyperlipidaemia, diabetes, atrial fibrillation and smoking
TIA=transient ischaemic attack; TOAST=Trial of Org 10172 in Acute Stroke Treatment; HR=hazards ratio; CI=confidence interval; PVS=perivascular space; WMH=white matter hyperintensity

Table 6.6 Cox regression analyses of risk of recurrent stroke for lacunes versus no lacunes

	Univariate HR (95% CI)[a]	HR (95% CI) adjusted for age and sex [a]	HR (95% CI) adjusted for age, sex and vascular risk factors [a,b]	p
Recurrent stroke				
OXVASC	1.62 (1.01-2.60)	1.47 (0.91-2.38)	1.39 (0.86-2.24)	0.18
HKU	1.28 (0.88-1.86)	1.27 (0.87-1.86)	1.28 (0.87-1.87)	0.21
Combined[c]	1.40 (1.04-1.89)	1.34 (0.99-1.80)	1.30 (0.97-1.75)	0.084
Ischaemic stroke				
OXVASC	1.55 (0.93-2.57)	1.44 (0.86-2.39)	1.33 (0.80-2.22)	0.28
HKU	1.32 (0.87-2.00)	1.33 (0.87-2.02)	1.34 (0.88-2.05)	0.17
Combined[c]	1.42 (1.02-1.96)	1.34 (0.97-1.86)	1.30 (0.94-1.79)	0.12
Intracerebral haemorrhage				
OXVASC	2.18 (0.54-8.70)	1.61 (0.40-6.55)	1.52 (0.35-6.73)	0.58
HKU	1.06 (0.44-2.56)	1.03 (0.42-2.49)	1.04 (0.43-2.53)	0.93
Combined[c]	1.29 (0.60-2.76)	1.26 (0.59-2.69)	1.28 (0.60-2.73)	0.53

[a]Compared with no lacunes as reference
[b]Hypertension, hyperlipidaemia, diabetes mellitus, atrial fibrillation smoking history
[c]Also adjusted for Centre
HR=hazard ratio; CI=confidence interval

Table 6.7 Cox regression analyses of risk of recurrent stroke for different burden of microbleeds versus no microbleeds

	Unadjusted HR (95% CI)[a]			HR (95% CI) adjusted for age and sex [a]			HR (95% CI) adjusted for age, sex and vascular risk factors [a,b]			
Microbleeds	**1**	**2-4**	**≥5**	**1**	**2-4**	**≥5**	**1**	**2-4**	**≥5**	**p_{trend}**
Recurrent stroke										
OXVASC	2.08 (1.10-3.95)	2.07 (0.90-4.78)	3.28 (1.57-6.85)	1.84 (0.96-3.52)	1.87 (0.80-4.35)	2.83 (1.34-5.98)	1.81 (0.95-3.47)	1.73 (0.74-4.06)	3.00 (1.41-6.36)	0.002
HKU	1.42 (0.84-2.38)	1.68 (0.98-2.87)	2.88 (1.76-4.71)	1.32 (0.78-2.22)	1.43 (0.83-2.46)	2.52 (1.54-4.12)	1.39 (0.82-2.35)	1.41 (0.82-2.44)	2.82 (1.72-4.65)	0.0002
Combined[c]	1.64 (1.09-2.46)	1.84 (1.17-2.88)	3.09 (2.06-4.64)	1.47 (0.98-2.21)	1.58 (1.01-2.49)	2.66 (1.77-4.00)	1.46 (0.97-2.19)	1.49 (0.95-2.35)	2.77 (1.84-4.16)	<0.0001
Ischaemic stroke										
OXVASC	2.03 (1.04-3.97)	1.48 (0.54-4.07)	3.10 (1.42-6.79)	1.83 (0.93-3.62)	1.33 (0.48-3.70)	2.77 (1.25-6.15)	1.76 (0.89-3.49)	1.16 (0.42-3.24)	2.87 (1.29-6.39)	0.014
HKU	1.53 (0.89-2.62)	1.58 (0.88-2.83)	1.76 (0.95-3.24)	1.42 (0.83-2.43)	1.34 (0.75-2.41)	1.53 (0.83-2.82)	1.51 (0.87-2.59)	1.34 (0.74-2.43)	1.81 (0.97-3.35)	0.057
Combined[c]	1.72 (1.13-2.62)	1.64 (1.00-2.69)	2.14 (1.32-3.49)	1.54 (1.01-2.35)	1.41 (0.85-2.32)	1.85 (1.14-3.01)	1.53 (1.00-2.33)	1.31 (0.79-2.16)	1.94 (1.19-3.16)	0.007
Intracerebral haemorrhage										
OXVASC	2.44 (0.28-20.91)	8.87 (1.72-45.80)	4.91 (0.57-42.26)	1.88 (0.22-16.35)	10.12 (1.88-54.39)	3.45 (0.39-30.44)	1.82 (0.19-17.20)	11.42 (1.83-71.19)	5.20 (0.52-52.34)	0.024
HKU	0.58 (0.07-4.93)	2.29 (0.55-9.59)	10.85 (3.77-31.24)	0.54 (0.06-4.64)	2.05 (0.49-8.63)	9.84 (3.39-28.54)	0.58 (0.07-4.99)	2.07 (0.49-8.86)	9.18 (3.13-26.92)	<0.0001
Combined[c]	0.98 (0.21-4.57)	3.47 (1.14-10.61)	10.55 (4.33-25.71)	0.88 (0.19-4.09)	3.06 (0.99-9.43)	8.99 (3.67-22.05)	0.85 (0.18-3.96)	3.25 (1.05-10.06)	9.24 (3.74-22.81)	<0.0001

[a]Compared with no microbleeds as reference
[b]Hypertension, hyperlipidaemia, diabetes mellitus, atrial fibrillation smoking history
[c]Also adjusted for Centre
HR=hazard ratio; CI=confidence interval

Table 6.8 Cox regression analyses of risk of recurrent stroke for different burden of periventricular white matter hyperintensity versus no periventricular white matter hyperintensity

	Unadjusted HR (95% CI)[a]			HR (95% CI) adjusted for age and sex [a]			HR (95% CI) adjusted for age, sex and vascular risk factors[a,b]			
Periventricular WMH	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**p_{trend}**
Recurrent stroke										
OXVASC	2.42 (1.33-4.43)	3.31 (1.76-6.32)	4.59 (2.24-9.42)	2.31 (1.22-4.39)	3.09 (1.52-6.28)	4.21 (1.90-9.36)	2.03 (1.07-3.86)	2.79 (1.38-5.66)	4.14 (1.88-9.10)	0.0002
HKU	1.21 (0.76-1.92)	1.80 (0.99-3.27)	3.12 (1.49-6.51)	0.91 (0.57-1.47)	1.25 (0.68-2.30)	2.43 (1.15-5.12)	0.94 (0.59-1.52)	1.51 (0.81-2.82)	2.57 (1.21-5.44)	0.030
Combined[c]	1.54 (1.09-2.17)	2.21 (1.48-3.32)	3.30 (2.04-5.33)	1.27 (0.89-1.81)	1.69 (1.11-2.59)	2.39 (1.44-3.98)	1.24 (0.87-1.78)	1.76 (1.14-2.70)	2.53 (1.53-4.21)	0.0001
Ischaemic stroke										
OXVASC	2.27 (1.23-4.16)	2.85 (1.47-5.53)	3.28 (1.50-7.16)	2.25 (1.17-4.31)	2.78 (1.34-5.77)	3.13 (1.32-7.40)	1.91 (1.00-3.67)	2.43 (1.17-5.02)	3.00 (1.28-6.99)	0.006
HKU	1.12 (0.67-1.88)	1.43 (0.71-2.89)	2.70 (1.16-6.28)	0.82 (0.49-1.39)	0.97 (0.47-2.00)	2.03 (0.86-4.77)	0.86 (0.51-1.46)	1.28 (0.62-2.65)	2.22 (0.94-5.25)	0.19
Combined[c]	1.48 (1.02-2.13)	1.91 (1.22-2.97)	2.57 (1.49-4.44)	1.21 (0.83-1.77)	1.44 (0.91-2.30)	1.83 (1.03-3.27)	1.17 (0.80-1.72)	1.50 (0.94-2.41)	1.93 (1.08-3.43)	0.015
Intracerebral haemorrhage										
OXVASC[d]	-	-	-	-	-	-	-	-	-	
HKU	1.67 (0.56-4.99)	3.80 (1.17-12.36)	5.44 (1.17-25.18)	1.49 (0.48-4.61)	3.10 (0.91-10.51)	5.06 (1.06-24.13)	1.54 (0.49-4.80)	2.75 (0.79-9.60)	4.62 (0.97-22.01)	0.027
Combined[c]	1.81 (0.66-4.91)	4.54 (1.66-12.47)	9.85 (3.39-28.62)	1.50 (0.53-4.19)	3.66 (1.28-10.46)	7.57 (2.45-23.42)	1.54 (0.55-4.32)	3.48 (1.20-10.08)	7.96 (2.58-24.56)	0.0002

[a]Compared with no periventricular WMH as reference
[b]Hypertension, hyperlipidaemia, diabetes mellitus, atrial fibrillation smoking history
[c]Also adjusted for Centre
[d]No intracerebral haemorrhages occurred in OXVASC patients with periventricular WMH grade 0
HR=hazard ratio; CI=confidence interval; WMH=white matter hyperintensity

Table 6.9 Cox regression analyses of risk of recurrent stroke for different burden of subcortical white matter hyperintensity versus no subcortical white matter hyperintensity

	Unadjusted HR (95% CI)[a]			HR (95% CI) adjusted for age and sex [a]			HR (95% CI) adjusted for age, sex and vascular risk factors[a,b]			
Subcortical WMH	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**p_{trend}**
Recurrent stroke										
OXVASC	1.34 (0.77-2.33)	2.90 (1.67-5.01)	2.04 (1.02-4.08)	1.21 (0.68-2.13)	2.45 (1.35-4.42)	1.69 (0.81-3.55)	1.23 (0.70-2.18)	2.26 (1.25-4.08)	1.69 (0.80-3.54)	0.024
HKU	1.19 (0.50-2.82)	2.02 (0.85-4.80)	1.64 (0.65-4.14)	1.06 (0.45-2.54)	1.64 (0.68-3.94)	1.23 (0.48-3.14)	1.09 (0.46-2.60)	1.74 (0.72-4.19)	1.34 (0.52-3.44)	0.16
Combined[c]	1.36 (0.87-2.13)	2.55 (1.62-3.99)	1.95 (1.16-3.27)	1.13 (0.72-1.77)	1.90 (1.20-3.02)	1.40 (0.82-2.39)	1.10 (0.70-1.73)	1.83 (1.15-2.90)	1.41 (0.82-2.41)	0.022
Ischaemic stroke										
OXVASC	1.12 (0.62-2.01)	2.74 (1.56-4.83)	1.92 (0.94-3.94)	1.05 (0.57-1.93)	2.47 (1.33-4.56)	1.74 (0.80-3.76)	1.06 (0.58-1.94)	2.20 (1.20-4.07)	1.66 (0.77-3.59)	0.027
HKU	0.93 (0.39-2.24)	1.71 (0.71-4.10)	1.19 (0.45-3.12)	0.82 (0.34-2.00)	1.36 (0.56-3.31)	0.87 (0.32-2.31)	0.86 (0.35-2.09)	1.49 (0.61-3.64)	1.00 (0.38-2.69)	0.31
Combined[c]	1.14 (0.72-1.83)	2.36 (1.48-3.76)	1.64 (0.95-2.85)	0.95 (0.59-1.53)	1.79 (1.11-2.88)	1.20 (0.68-2.12)	0.93 (0.58-1.50)	1.69 (1.05-2.73)	1.21 (0.68-2.14)	0.058
Intracerebral haemorrhage										
OXVASC	6.48 (0.75-55.61)	5.33 (0.48-58.87)	4.68 (0.29-75.67)	3.99 (0.46-34.70)	2.57 (0.22-29.72)	1.78 (0.10-30.91)	4.72 (0.51-43.82)	2.58 (0.20-34.12)	2.23 (0.12-42.74)	0.79
HKU[d]	-	-	-	-	-	-	-	-	-	
Combined[c]	7.22 (0.92-59.97)	7.54 (0.91-62.71)	9.89 (1.14-85.57)	5.75 (0.72-45.68)	5.38 (0.64-45.37)	6.64 (0.75-58.89)	5.66 (0.71-44.94)	5.37 (0.64-45.20)	6.69 (0.75-59.61)	0.19

[a]Compared with no subcortical WMH as reference
[b]Hypertension, hyperlipidaemia, diabetes mellitus, atrial fibrillation smoking history
[c]Also adjusted for Centre
[d]No intracerebral haemorrhages occurred in HKU patients with subcortical WMH grade 0
HR=hazard ratio; CI=confidence interval; WMH=white matter hyperintensity

Table 6.10 Cox regression analyses of risk of recurrent stroke for different burden of perivascular spaces versus <11 perivascular spaces

	Unadjusted HR (95% CI)[a]		HR (95% CI) adjusted for age and sex[a]		HR (95% CI) adjusted for age, sex and vascular risk factors[a,b]		
Perivascular spaces	**11-20**	**>20**	**11-20**	**>20**	**11-20**	**>20**	**p_{trend}**
Basal ganglia PVSs							
Recurrent stroke							
OXVASC	1.36 (0.79-2.32)	2.45 (1.52-3.95)	1.16 (0.65-2.05)	2.03 (1.19-3.46)	1.10 (0.62-1.95)	1.89 (1.10-3.23)	0.023
HKU	1.46 (0.95-2.24)	2.95 (1.70-5.11)	1.11 (0.72-1.73)	1.97 (1.11-3.49)	1.20 (0.77-1.87)	2.32 (1.29-4.15)	0.013
Combined[c]	1.40 (1.00-1.95)	2.62 (1.83-3.77)	1.11 (0.78-1.57)	1.92 (1.30-2.83)	1.13 (0.79-1.60)	1.94 (1.30-2.88)	0.003
Ischaemic stroke							
OXVASC	1.24 (0.70-2.20)	2.36 (1.43-3.89)	1.11 (0.60-2.04)	2.08 (1.18-3.65)	1.04 (0.57-1.92)	1.98 (1.13-3.49)	0.02
HKU	1.56 (0.98-2.47)	2.38 (1.24-4.58)	1.15 (0.72-1.86)	1.55 (0.79-3.04)	1.30 (0.80-2.11)	1.94 (0.98-3.85)	0.056
Combined[c]	1.40 (0.98-2.01)	2.39 (1.61-3.54)	1.12 (0.77-1.63)	1.77 (1.16-2.70)	1.14 (0.78-1.67)	1.81 (1.18-2.79)	0.012
Intracerebral haemorrhage							
OXVASC	2.55 (0.51-12.69)	3.31 (0.66-16.59)	1.39 (0.25-7.75)	1.63 (0.29-9.35)	1.05 (0.18-6.17)	0.98 (0.15-6.31)	0.98
HKU	1.01 (0.32-3.17)	5.00 (1.73-14.43)	0.87 (0.27-2.83)	4.07 (1.30-12.76)	0.79 (0.24-2.58)	3.84 (1.20-12.29)	0.089
Combined[c]	1.33 (0.54-3.29)	4.02 (1.63-9.94)	1.02 (0.40-2.63)	2.77 (1.05-7.35)	0.95 (0.37-2.45)	2.56 (0.96-6.84)	0.11
Centrum semiovale PVSs							
Recurrent stroke							
OXVASC	1.07 (0.58-1.98)	1.84 (1.06-3.19)	0.90 (0.48-1.70)	1.43 (0.79-2.59)	0.83 (0.44-1.56)	1.29 (0.71-2.35)	0.22
HKU	1.02 (0.69-1.50)	0.39 (0.15-0.97)	1.02 (0.70-1.50)	0.37 (0.15-0.92)	1.07 (0.73-1.58)	0.37 (0.15-0.94)	0.16
Combined[c]	1.02 (0.73-1.41)	1.15 (0.78-1.68)	0.92 (0.66-1.28)	0.91 (0.61-1.35)	0.92 (0.66-1.28)	0.89 (0.59-1.32)	0.54
Ischaemic stroke							
OXVASC	1.20 (0.63-2.26)	1.72 (0.95-3.10)	1.04 (0.54-2.01)	1.41 (0.75-2.67)	0.94 (0.49-1.82)	1.27 (0.68-2.40)	0.33
HKU	1.04 (0.68-1.59)	0.29 (0.09-0.93)	1.04 (0.68-1.59)	0.27 (0.08-0.87)	1.12 (0.73-1.72)	0.28 (0.09-0.92)	0.18
Combined[c]	1.07 (0.75-1.52)	1.07 (0.70-1.63)	0.96 (0.67-1.37)	0.85 (0.55-1.31)	0.96 (0.67-1.37)	0.83 (0.54-1.28)	0.42
Intracerebral haemorrhage							
OXVASC	-	2.89 (0.59-14.17)	-	1.37 (0.25-7.46)	-	1.37 (0.21-8.93)	0.32
HKU	0.90 (0.36-2.27)	0.83 (0.18-3.83)	0.90 (0.36-2.26)	0.80 (0.17-3.71)	0.86 (0.34-2.17)	0.75 (0.16-3.51)	0.67
Combined[c]	0.71 (0.30-1.72)	1.65 (0.65-4.20)	0.67 (0.28-1.61)	1.35 (0.51-3.57)	0.68 (0.28-1.66)	1.34 (0.50-3.55)	0.71

[a]Compared with <11 PVSs as reference
[b]Hypertension, hyperlipidaemia, diabetes mellitus, atrial fibrillation smoking history
[c]Also adjusted for Centre
HR=hazard ratio; CI=confidence interval; PVS=perivascular space

Table 6.11 Prognostic value of the Total Small Vessel Disease Score[a] in prediction of recurrent strokes in OXVASC and HKU (external validation)

Total Small Vessel Disease Score	Events / patients	Unadjusted HR (95% CI)	Age and sex adjusted HR (95% CI)	Multivariate[b] adjusted HR (95% CI)	p_{trend}	c-statistic (95% CI)	p
OXVASC							
0	27/387	1	1	1	0.001	0.60 (0.54-0.67)	0.001
1	16/293	0.99 (0.53-1.84)	0.87 (0.45-1.67)	0.83 (0.43-1.60)			
2	24/215	1.98 (1.14-3.46)	1.67 (0.90-3.10)	1.51 (0.81-2.81)			
3	16/103	2.94 (1.58-5.48)	2.44 (1.22-4.87)	2.18 (1.10-4.34)			
4	7/30	4.45 (1.93-10.27)	3.73 (1.54-9.03)	3.38 (1.39-8.21)			
HKU							
0	13/172	1	1	1	0.001	0.61 (0.56-0.67)	0.0001
1	22/286	1.05 (0.53-2.08)	1.00 (0.50-1.98)	1.06 (0.53-2.13)			
2	29/263	1.59 (0.83-3.06)	1.29 (0.67-2.49)	1.38 (0.71-2.69)			
3	31/199	2.25 (1.18-4.30)	1.73 (0.90-3.33)	1.96 (1.01-3.82)			
4	14/54	4.01 (1.89-8.54)	2.78 (1.29-6.01)	3.15 (1.44-6.86)			

[a]1 point allocated for: presence of lacunes, microbleeds, moderate-severe (>10) PVSs in basal ganglia, periventricular WMH Fazekas 3 and/or deep WMH Fazekas 2-3
[b]Adjusted for age, sex, hypertension, hyperlipidaemia, diabetes, atrial fibrillation and smoking
HR=hazards ratio; CI=confidence interval; PVS=perivascular space; WMH=white matter hyperintensity

Table 6.12 Prognostic value of the Total Small Vessel Disease Score[a] (external validation)

Total Small Vessel Disease Score	Events / patients	Unadjusted HR (95% CI)	Age and sex adjusted HR (95% CI)	Multivariate[b] adjusted HR (95% CI)	p_{trend}	c-statistic (95% CI)	p
Recurrent stroke							
0	40/559	1	1	1	<0.0001	0.62 (0.57-0.66)	<0.0001
1	38/579	1.08 (0.69-1.69)	0.90 (0.57-1.42)	0.89 (0.57-1.41)			
2	53/478	1.88 (1.24-2.85)	1.43 (0.93-2.21)	1.38 (0.89-2.13)			
3	47/302	2.71 (1.77-4.15)	2.02 (1.29-3.17)	2.02 (1.29-3.18)			
4	21/84	4.58 (2.69-7.78)	3.32 (1.91-5.78)	3.20 (1.83-5.59)			
Recurrent ischaemic stroke							
0	35/559	1	1	1	<0.0001	0.61 (0.56-0.65)	<0.0001
1	32/579	1.03 (0.63-1.66)	0.86 (0.53-1.42)	0.86 (0.53-1.41)			
2	48/478	1.93 (1.24-2.99)	1.48 (0.93-2.35)	1.43 (0.90-2.28)			
3	40/302	2.60 (1.65-4.12)	1.96 (1.21-3.18)	1.98 (1.22-3.23)			
4	15/84	3.60 (1.96-6.60)	2.65 (1.41-4.98)	2.60 (1.38-4.91)			
Intracerebral haemorrhage							
0	5/559	1	1		0.009	0.65 (0.54-0.76)	0.006
1	6/579	1.42 (0.43-4.65)	1.13 (0.34-3.79)	1.10 (0.33-3.69)			
2	5/478	1.43 (0.41-4.94)	1.03 (0.28-3.74)	0.94 (0.26-3.43)			
3	7/302	3.20 (1.01-10.09)	2.25 (0.67-7.55)	2.11 (0.62-7.11)			
4	6/84	9.97 (3.03-32.81)	6.79 (1.93-23.93)	5.86 (1.62-21.23)			

[a]1 point allocated for: presence of lacunes, microbleeds, moderate-severe (>10) PVSs in basal ganglia, periventricular WMH Fazekas 3 and/or deep WMH Fazekas 2-3
[b]Adjusted for age, sex, hypertension, hyperlipidaemia, diabetes, atrial fibrillation and smoking
HR=hazards ratio; CI=confidence interval; PVS=perivascular space; WMH=white matter hyperintensity

Table 6.13 Prognostic value of the Total Small Vessel Disease Score[a] and Modified Total Small Vessel Disease Score[b] in prediction of non-disabling and disabling or fatal recurrent strokes

	Total Small Vessel Disease Score	p	Modified Total Small Vessel Disease Score	p	p_{het}
Nondisabling recurrent ischaemic stroke					
Adjusted[c] HR per unit increase in score (95% CI)	1.28 (1.07-1.54)	0.008	1.23 (1.07-1.42)	0.005	0.73
c-statistic (95% CI)	0.56 (0.49-0.62)	0.066	0.56 (0.50-0.62)	0.056	1.00
Disabling / fatal recurrent ischaemic stroke					
Adjusted[c] HR per unit increase in score (95% CI)	1.39 (1.14-1.69)	0.001	1.20 (1.03-1.39)	0.016	0.24
c-statistic (95% CI)	0.65 (0.59-0.71)	<0.0001	0.64 (0.58-0.70)	<0.0001	0.82
Nondisabling intracerebral haemorrhage					
Adjusted[b] HR per unit increase in score (95% CI)	2.34 (1.15-4.74)	0.019	1.87 (1.21-2.89)	0.005	0.60
c-statistic (95% CI)	0.72 (0.46-0.97)	0.068	0.81 (0.66-0.95)	0.009	0.58
Disabling / fatal intracerebral haemorrhage					
Adjusted[c] HR per unit increase in score (95% CI)	1.42 (0.99-2.05)	0.060	1.61 (1.27-2.04)	<0.0001	0.57
c-statistic (95% CI)	0.63 (0.51-0.75)	0.032	0.68 (0.55-0.81)	0.003	0.58

[a]1 point allocated for: presence of lacunes, microbleeds, moderate-severe (>10) PVSs in basal ganglia, periventricular WMH Fazekas 3 and/or deep WMH Fazekas 2-3
[b]1 point allocated for: presence of lacunes, 1-4 microbleeds, frequent-severe (>20) PVSs in basal ganglia, moderate WMH (total periventricular + subcortical WMH grade 3-4), 2 points given for ≥5 microbleeds and severe WMH (total periventricular + subcortical WMH grade 5-6)
[c]Adjusted for age, sex, hypertension, hyperlipidaemia, diabetes, atrial fibrillation and smoking
HR=hazards ratio; CI=confidence interval; PVS=perivascular space; WMH=white matter hyperintensity

Table 6.14 Risk of recurrent stroke in patients with increasing Total Small Vessel Disease Score[a] stratified by TIA or ischaemic stroke TOAST subtype on baseline

TOAST classification	Events / patients	Unadjusted HR (95% CI)	Age and sex adjusted HR per unit increase in score (95% CI)	p	c-statistic (95% CI)	p
Small vessel disease	54/537	1.60 (1.27-2.01)	1.43 (1.12-1.83)	0.004	0.67 (0.59-0.74)	<0.0001
Non-small vessel disease	145/1465	1.47 (1.28-1.69)	1.39 (1.19-1.61)	<0.0001	0.60 (0.55-0.65)	<0.0001
Large artery atherosclerosis	53/471	1.37 (1.08-1.74)	1.32 (1.03-1.69)	0.030	0.59 (0.51-0.67)	0.031
Cardioembolic	35/278	1.34 (1.00-1.78)	1.27 (0.91-1.75)	0.16	0.57 (0.46-0.68)	0.17
Undetermined	33/556	1.48 (1.11-1.97)	1.40 (1.00-1.95)	0.049	0.57 (0.46-0.68)	0.18

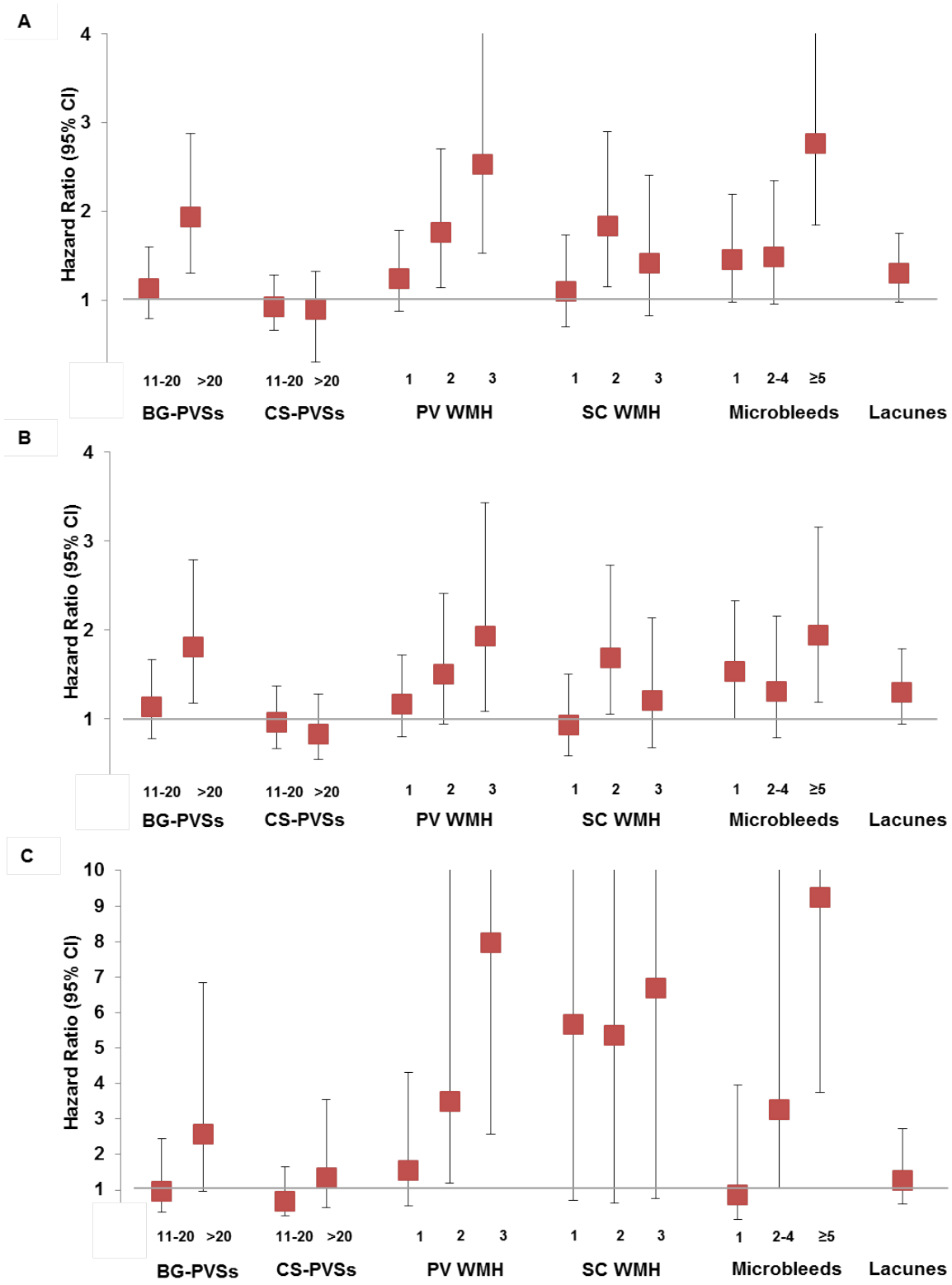
[a]1 point allocated for: presence of lacunes, microbleeds, moderate-severe (>10) PVSs in basal ganglia, periventricular WMH Fazekas 3 and/or deep WMH Fazekas 2-3
TIA=transient ischaemic attack; TOAST=Trial of Org 10172 in Acute Stroke Treatment; HR=hazards ratio; CI=confidence interval; PVS=perivascular space; WMH=white matter hyperintensity

Table 6.15 Prognostic value of modified Total Small Vessel Disease Score[a] (internal validation)

Modified Total Small Vessel Disease Score	Events / patients	Unadjusted HR (95% CI)	Age and sex adjusted HR (95% CI)	Multivariate[b] adjusted HR (95% CI)	p_{trend}	c-statistic (95% CI)	p
Recurrent stroke							
0	52/824	1	1	1	<0.0001	0.62 (0.58-0.66)	<0.0001
1	54/593	1.60 (1.09-2.35)	1.42 (0.96-2.09)	1.35 (0.92-1.99)			
2	41/280	2.65 (1.76-3.99)	2.09 (1.37-3.21)	1.95 (1.27-3.00)			
3	26/170	2.84 (1.77-4.56)	2.30 (1.42-3.73)	2.30 (1.41-3.73)			
4	11/79	2.83 (1.47-5.43)	2.35 (1.22-4.54)	2.34 (1.21-4.53)			
5	9/42	4.13 (2.03-8.39)	3.13 (1.52-6.45)	3.10 (1.50-6.40)			
6	6/14	7.73 (3.32-18.01)	6.01 (2.55-14.15)	6.41 (2.70-15.20)			
Recurrent ischaemic stroke							
0	46/824	1	1	1	0.0003	0.60 (0.56-0.65)	<0.0001
1	49/593	1.63 (1.09-2.44)	1.44 (0.96-2.17)	1.38 (0.91-2.08)			
2	37/280	2.67 (1.73-4.13)	2.11 (1.35-3.32)	1.97 (1.25-3.10)			
3	20/170	2.42 (1.43-4.09)	1.96 (1.14-3.35)	1.94 (1.13-3.32)			
4	10/79	2.88 (1.45-5.71)	2.38 (1.19-4.76)	2.40 (1.20-4.80)			
5	5/42	2.48 (0.98-6.24)	1.90 (0.74-4.83)	1.92 (0.75-4.92)			
6	3/14	4.23 (1.32-13.62)	3.27 (1.01-10.61)	3.62 (1.11-11.84)			
Intracerebral haemorrhage							
0	6/824	1	1	1	<0.0001	0.71 (0.60-0.81)	0.0001
1	5/593	1.28 (0.39-4.20)	1.13 (0.34-3.77)	1.05 (0.32-3.51)			
2	4/280	2.23 (0.63-7.94)	1.79 (0.48-6.65)	1.70 (0.46-6.35)			
3	6/170	5.59 (1.80-17.35)	4.55 (1.40-14.71)	4.64 (1.43-15.07)			
4	1/79	2.21 (0.27-18.42)	1.91 (0.23-16.08)	1.86 (0.22-15.71)			
5	4/42	15.87 (4.45-56.55)	12.10 (3.19-45.97)	9.91 (2.59-37.93)			
6	3/14	27.55 (6.87-110.50)	21.57 (5.12-90.80)	21.48 (4.96-93.02)			

[a]1 point allocated for: presence of lacunes, 1-4 microbleeds, frequent-severe (>20) perivascular spaces in basal ganglia, moderate WMH (total periventricular + subcortical WMH grade 3-4), 2 points given for ≥5 microbleeds and severe WMH (total periventricular + subcortical WMH grade 5-6)
[b]Adjusted for age, sex, hypertension, hyperlipidaemia, diabetes, atrial fibrillation and smoking
HR=hazards ratio; CI=confidence interval

Figure 6.1 Risk of A) recurrent stroke B) recurrent ischaemic stroke and C) intracerebral haemorrhage with increasing burden of small vessel disease



Hazard ratios adjusted for age, sex, vascular risk factors and centre and compared with patients with <11 BG-PVSs, <11 CS-PVSs and no PV WMH, SC WMH, microbleeds or lacunes respectively
BG=basal ganglia; CS=centrum semi-ovale; PVS=perivascular space; PV=periventricular; WMH=white matter hyperintensity; SC=subcortical

6.6 References

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Chapter 7

Long-term premorbid blood pressure and total small vessel disease burden in TIA and ischaemic stroke

7.1 Chapter outline

Studies of causes of cerebral small vessel disease (SVD) are required, and would need to fully adjust for blood pressure (BP), but most epidemiological studies are based on a single blood pressure (BP) measurement or "history of hypertension", which might underestimate the role of hypertension in SVD. In patients with transient ischaemic attack (TIA) and ischaemic stroke, I therefore compared the risk associations of baseline and long-term premorbid mean BP with the "Total SVD Score".

I studied 1009 patients with TIA or ischaemic stroke who had a brain magnetic resonance imaging, in the population-based Oxford Vascular Study (OXVASC). I related the Total SVD Score with baseline and mean premorbid BP (total: 22096 readings, median: 15/patient, interquartile range: 7-33).

I found that baseline BP [odds ratio (OR) of top vs. bottom systolic BP (SBP) quartile: 2.28, 95% CI 1.62-3.21, p<0.0001; diastolic BP (DBP): 0.71, 0.51-1.00, p=0.050] and hypertension (2.53, 2.01-3.20, p<0.0001) were weakly associated with an increasing Total SVD Score. However, the associations with mean premorbid BP (SBP: 6.09, 4.34-8.55, p<0.0001; DBP: 1.59, 1.16-2.18, p=0.004), especially DBP measurements taken 10-20 years before TIA or ischaemic stroke (SBP: 5.92, 4.05-8.65; DBP: 3.35, 2.33-4.84; both p<0.0001), were much stronger. Relationships between premorbid BP and SVD burden was strongest in patients age<70 compared with patients age≥70 (SBP: 6.99, 4.11-11.86, p<0.0001 vs. 2.37, 1.42-3.94, p=0.001; DBP: 3.13, 1.95-5.07, p<0.0001 vs.1.16, 0.74-1.84, p=0.52).

I concluded that mean premorbid BP, especially measurements 10-20 years prior to TIA or ischaemic stroke, is most strongly associated with global SVD burden than a baseline measurement of BP suggesting a latency effect of hypertension on the pathogenesis of SVD. Epidemiological studies of other aetiological factors in SVD should therefore aim to adjust for long-term BP.

7.2 Introduction

Cerebral small vessel disease (SVD) accounts for 20-25% of all strokes and up to 45% of all dementias.[1] Although the pathogenesis of SVD is largely unknown,[1] and novel pathological mechanisms have been postulated,[1, 2] hypertension remains one of the leading causes of SVD.[1, 3, 4] Individual neuroimaging biomarkers of SVD – lacunes,[5] white matter hyperintensity (WMH),[6] microbleeds[7] and MRI-visible enlarged perivascular spaces (PVSs),[8] as well as the global burden of SVD as assessed by the Total SVD Score[9] have all been associated with hypertension.

Whilst non-hypertensive cases of SVD without obvious genetic aetiology have been described,[10] studies that have investigated the relationship of hypertension and other novel risk factors with SVD have a number of short-comings. SVD is a slowly progressive disorder, often with mild, subtle and neglected features initially,[11] but frequently progressing into a chronic disabling vasculopathy presenting with recurrent transient ischaemic attacks (TIA) or strokes, vascular cognitive impairment, depression and gait disturbances.[1, 3] However, the majority of previous studies on risk associations of hypertension and SVD (or other novel risk factors where hypertension has been adjusted for) have been based on single clinic or ambulatory blood pressure (BP) measurements at baseline, known history of hypertension and/or prior use of antihypertensive agents,[5, 6, 8, 9] potentially underestimating the effect of BP changes accrued during the many years prior to clinical presentation. Studying this 'latency effect' of long-term premorbid BP on SVD is important, especially as systolic BP (SBP) increases, and diastolic BP (DBP) decreases with age.[12] Only relating baseline BPs with SVD without age stratification may therefore potentially undermine significant age-specific associations. Furthermore, current hypertension guidelines[13] are primarily based on randomised trials that have studied the role of BP-lowering in reducing the risk of 'hard' clinical events, such as myocardial infarction, stroke, revascularisation procedures and death. Few trials have studied end-points specific to SVD, potentially underestimating the effect of hypertension on SVD.

To better understand the pathogenesis of SVD and its association with long-term BP control in patients with TIA or ischaemic stroke, I studied the age-specific relationships of premorbid BP

with the Total SVD Score[14] in patients with TIA or ischaemic stroke, in the population-based Oxford Vascular Study (OXVASC). I hypothesise that long-term mean premorbid BP, especially SBP, may be more strongly associated with the Total SVD Score than a baseline measurement of BP. I also hypothesise that in view of the general decrease of DBP with age, a negative association between recent DBP with the Total SVD Score may be present and that the associations between DBP and SVD burden may only be evident amongst readings taken during mid-life.

7.3 Methods

7.3.1 Study populations

1080 consecutive cases with TIA or ischaemic stroke were recruited from OXVASC (see section 2.1). Baseline data was collected as described in section 2.1. All patients had their BPs measured during ascertainment using an oscillometric BP measurement device (A&D Medical, Japan). BPs were taken after 5 minutes of rest in the sitting or lying position and a single BP reading was used for analysis. Hypertension was defined as a known history of hypertension or prior use of antihypertensive agents. Premorbid BP readings from the primary care records was collected for all patients during the preceding 20 years prior to ascertainment and the mean of all readings was calculated. Mean BP readings taken between 1-5 years, 5-10 years and 10-20 years prior to TIA or ischaemic stroke were also used for analysis. All patients received a cerebral magnetic resonance imaging (MRI) scan at baseline (see section 2.2) and presence and burden of visible basal ganglia and centrum semiovale PVSs, cerebral microbleeds, WMH and lacunes coded as described in section 2.3.

7.3.2 Statistical analysis

I determined by binary and ordinal logistic regression, the relationships of hypertension, baseline BP (top vs. bottom quartile as referent) and mean premorbid BP (top vs. bottom quartile) with presence of lacunes and the Total SVD Score (as calculated in Chapter 6), in univariate analysis and analyses adjusted for age and sex. I calculated the c-statistics for area under characteristic curve for prediction of a high burden of SVD, defined as a Total SVD Score of 4, based on a diagnosis of hypertension, baseline and mean premorbid BP as a continuous variable. I also determined by ordinal logistic regression, the relationships of mean premorbid BP taken within 1 year, 1-5 years, 5-10 years and 10-20 years prior to TIA or ischaemic stroke with the Total SVD Score. I compared the differences in baseline and imaging characteristics amongst patients aged <70 versus ≥70 using Student's t-test for continuous variables and Chi-squared test for categorical variables. Finally, I determined the relationships of premorbid BP with Total SVD

Score, stratified by age<70 vs. ≥70 and also by premorbid use of antihypertensive agents.

All analyses were done with SPSS version 22.

7.4 Results

7.4.1 Baseline clinical and neuroimaging characteristics

1080 patients were recruited during the study period. After excluding 71 patients (6.6%) with missing clinical, premorbid BP or imaging data, 1009 patients (TIA n=528, ischaemic stroke n=481) were included in the final analysis. Details of baseline clinical and imaging characteristics are shown in Table 7.1. The mean (SD) age of the study population was 68.6 (13.8) years and 52% were male. 55% of the study population had a history of hypertension or were on antihypertensive agents. A total of 22096 premorbid BP readings [median 15 readings/patient, interquartile range 7-33; 9 (4-21) in age<70 and 23 (12-41) in aged≥70] were obtained. The mean (SD) premorbid BP was 139(14)/80(8) mmHg whilst the mean BP on assessment was 150(24)/84(13) mmHg. The mean (SD) Total SVD Score was 1.12 (1.11). Compared with patients ≥70years, those aged<70 were more likely to be men and smokers (p<0.01) (Table 7.1). Individuals <70years had a lower prevalence of vascular risk factors and had better renal function (p<0.01). Those aged<70 also had a lower premorbid and baseline mean SBP, but higher premorbid and baseline mean DBP (p<0.01) (Table 7.1). The overall prevalence of individual neuroimaging markers and burden of SVD was also lower in patients aged<70 (Table 7.1).

7.4.2 Relationships between blood pressure and neuroimaging markers of small vessel disease

The relationships of lacunes with baseline BP, history of hypertension and premorbid BP are shown in Table 7.2. History of hypertension was significantly associated with presence of lacunes [age and sex adjusted odds ratio (OR) 1.74, 95% confidence interval (CI) 1.22-2.49, p=0.002], but there were no relationships between baseline SBP (age and sex adjusted OR of top vs. bottom quartile 1.52, 0.91-2.53, p=0.11) or DBP (0.67, 0.40-1.12, p=0.12) with lacunes after adjusting for age and sex. However, the associations of mean premorbid SBP and DBP with lacunes was stronger (SBP: 2.92, 1.69-5.03, p=0.0001; DBP: 1.99, 1.26-3.16, p=0.003).

Similar findings were noted for the relationships of baseline and premorbid BP with Total SVD Score (Table 7.2). The associations between baseline BP and hypertension with the Total SVD Score were weaker (SBP: 1.46, 1.02-2.10, p=0.039; DBP: 1.16, 1.20-1.89, p=0.43; hypertension: 1.61, 1.26-2.06, p=0.0001) than those for mean premorbid BP (SBP: 2.53, 1.76-3.65, p<0.0001; DBP: 2.00, 1.42-2.80, p<0.0001) (Table 7.2).

7.4.3 Identifying a latency effect between long-term premorbid blood pressure and small vessel disease burden

I determined the relationships between premorbid BP and Total SVD Score taken at different time points – within 1, 1-5, 5-10 and 10-20 years prior to index TIA or ischaemic stroke (Table 7.3, Figure 7.1). A clear stepwise increase in severity between premorbid SBP and DBP taken within 1-year, 1-5 years, 5-10 years and 10-20 years with the Total SVD score was noted in univariate analysis [SBP: 2.17 (1.48-3.17); 3.94 (2.78-5.56); 4.67 (3.23-6.76); 5.92 (4.05-8.65); DBP: 0.91 (0.62-1.33); 0.76 (0.54-1.06); 1.26 (0.89-1.79); 3.35 (2.33-4.84)] (Table 7.3, Figure 7.1). These associations remained similar after adjusting for sex (Table 7.3) and in the 466/1009 antihypertensive naïve patients in univariate analysis (Table 7.4).

7.4.4 Age-specific associations between premorbid blood pressure and small vessel disease burden

When I stratified my results by age, the associations between premorbid SBP taken at different timepoints with an increasing Total SVD Score was similar in the 484/1009 patients aged <70 compared with the entire population (10-20 years: 5.73, 2.97-11.07, p<0.0001), but were attenuated, in patients aged ≥70 (10-20 years: 2.30, 1.30-4.10, p=0.004) (Table 7.3). However, the associations between mean premorbid DBP (especially those taken 10-20 years prior to TIA or ischaemic stroke) with an increasing Total SVD Score were strong amongst patients age <70 (10-20 years: 5.94, 3.36-10.52, p<0.0001) but not significant in those aged ≥70 (10-20 years: 1.16, 0.69-1.94, p=0.58) (Table 7.3). In the 251/1009 patients aged <60 (Table 7.5), the risk associations between premorbid mean DBP with an increasing Total SVD Score was stronger still

(10-20 years: 6.56, 2.47-17.41, p=0.0002) and outweighed the risk associations between SBP and Total SVD Score (10-20 years: 3.65, 0.98-13.61, p=0.054).

7.5 Discussion

I have demonstrated in a large population-based study of TIA and ischaemic stroke patients, the relationships of baseline BP, hypertension history and long-term mean premorbid BP with global SVD burden. I noted only weak associations between baseline BP, history of hypertension and SVD burden, but the association between premorbid BP and SVD burden was much stronger. A latency effect between BP and SVD burden was also present, such that the risk associations with SVD burden were stronger with BP readings taken within more distant time periods from TIA or ischaemic stroke. Furthermore, I demonstrated significant age-specific associations between premorbid BP and SVD burden, with stronger associations between premorbid BP, especially DBP, and SVD burden in younger individuals.

My findings have the following implications. First, my results suggest that the importance of hypertension as a risk factor towards SVD is likely to have been underestimated in the past. The majority of previous research on SVD has either studied the relationships of SVD with baseline BP or diagnosis of hypertension (often defined as a known history of hypertension or on antihypertensive agents).[5, 6, 8] However, as I have demonstrated, multiple premorbid BP readings (median of 15 readings per patient over 20 years in our cohort) are much stronger correlates with global SVD burden than a single BP measurement or a known history of hypertension. It may well be possible that a proportion of previously noted cases of non-hypertensive cerebral SVD[10] had 'masked hypertension', and a diagnosis of hypertension might have been made with repeated measurements of BP. My findings therefore suggest that studies investigating etiological factors in SVD should also aim to adjust for repeated measurements of BP or long-term premorbid BP.

Second, my results reinforce the importance of BP control in early or mid-life. We noted a significant latency effect of BP on SVD burden. The risk associations between premorbid BP with SVD burden decreased progressively with time such that the associations with SVD burden were greatest with BP measurements taken 10-20 years prior to TIA or ischaemic stroke, especially amongst individuals aged less than 60-70 (i.e. when they were aged 40-60). Whilst current international guidelines on BP management[13] recommend a target BP of <150/90mmHg and

<140/90mmHg in the general population aged ≥60 and in those aged <60 respectively, BP guidelines are primarily based on results from randomised controlled trials that have studied clinical outcomes such as mortality, cardiovascular events (myocardial infarction, heart failure), stroke, revascularisation procedures and renal function decline. Seldom have clinical trials investigated the benefits of BP lowering on end-points relevant to cerebral SVD such as vascular cognitive impairment or gait disturbances, which can be equally disabling, or used neuroimaging surrogate markers of SVD.[15] Furthermore, randomised controlled trials on benefits of BP lowering have rarely followed-up patients for more than 5 years, and hence the benefits of lowering BP in early or mid-life in reducing the long-term consequences of SVD is likely to have been underestimated. Basing BP recommendations on outcomes such as mortality or cardiovascular events has often led to large numbers of needed to treat (NNT) values. For example, in the recent Systolic Blood Pressure Intervention Trial (SPRINT) which included a population with mean age of 68 years, followed-up for a median of 3.26 years, the absolute risk reduction for stroke in patients randomised to intensive BP lowering (SBP target <120mmHg) versus standard treatment (SBP target <140mmHg) was 0.06% per year, corresponding to a NNT of 1667.[16] I postulate that the NNT to prevent the long-term consequences of SVD with aggressive BP lowering, especially during early to mid-life is likely to be much lower.

Third, my results demonstrate the importance of studying the age-specific associations between hypertension and SVD. As we age, our arteries stiffen and this is accompanied by a reduction in DBP.[12] Analysing all patients together without stratification by age may therefore undermine potential strong age-specific associations of DBP with SVD. Indeed, whilst it remains unclear whether SBP or DBP is more important in the pathogenesis of SVD,[1] I was able to demonstrate that DBP was more strongly associated with SVD burden in younger individuals; but in the elderly, the associations between SBP and SVD burden was more significant.

Although I consider my results valid, my study has a number of limitations. First, premorbid measurements of BPs were obtained in a retrospective manner through thorough tracing of medical records from individual primary care practices. Without doubt, there would have been

inconsistencies with regards to the measurement of BPs from practice to practice and from visit to visit. Although methods of recording premorbid BP was not standardised and this may potentially have resulted in bias, my findings provide novel insights in the importance of mid-life BP control in the pathogenesis of SVD. My results could be confirmed in other large prospective cohorts with long-term follow-up that have included standardised BP measurements and incorporated detailed neuroimaging. Second, I used the Total SVD Score to represent the global SVD burden. As noted in Chapter 6, the Total SVD Score is not without limitations. In its current form, the Total SVD Score is able to predict risk of recurrent stroke, but is not able to differentiate individuals who are at higher risk of developing intracerebral haemorrhage than ischaemic stroke, even after accounting for burden of cerebral microbleeds. Nevertheless, by using the Total SVD Score to study the relationships of BP with SVD burden, I have been able to encapsulate a wide range of neuroimaging markers of SVD, in contrast to other studies that has often only determined the role of BP with an individual marker of SVD.[15, 17-19] Third, patients were scanned on 4 different scanners during the study period. Although this may have been a potential source of heterogeneity, I have shown in Chapter 6 that the mean Total SVD Scores was similar amongst patients scanned across the 4 scanners, and the prognostic value of the Total SVD Score was robust to scanner type or strength. Finally, my analysis was limited to patients predominantly with a hypertensive form of SVD with only small numbers of patients with cerebral amyloid angiopathy (72 patients with strictly lobar microbleeds). Nevertheless, within this small subset of patients, both mean premorbid SBP and DBP remained very strong predictors of an increasing Total SVD Score (age and sex adjusted OR of top vs. bottom quartile of SBP: 24.78, 4.22-145.57, p=0.0004; DBP: 9.42, 2.50-35.45, p=0.001). However, these observations would need to be confirmed in larger cohorts.

Table 7.1 Baseline clinical and neuroimaging characteristics of the study population

Baseline clinical characteristics	All N=1009	Age <70 N=484	Age ≥70 N=525	p
Mean age, yr (SD)	68.6 (13.8)	57.0 (10.1)	79.3 (6.0)	<0.0001
Males (%)	528 (52.3)	273 (56.4)	255 (48.6)	0.013
Hypertension (%)	557 (55.2)	209 (43.2)	348 (66.3)	<0.0001
Hyperlipidaemia (%)	378 (37.5)	156 (32.2)	222 (42.3)	0.001
Diabetes (%)	134 (13.3)	47 (9.7)	87 (16.6)	0.001
Ever-smokers (%)	512 (50.7)	269 (55.7)	243 (46.3)	0.003
Atrial fibrillation (%)	158 (15.7)	44 (9.1)	114 (21.7)	<0.0001
Prior TIA or stroke (%)	186 (18.4)	68 (14.0)	118 (22.5)	0.001
Glomerular filtration rate, ml/min/1.73m^2	75.9 (23.2)	84.8 (21.9)	67.8 (21.2)	<0.0001
Baseline SBP (SD)	150.3 (24.4)	145.9 (21.8)	154.4 (26.0)	<0.0001
Baseline DBP (SD)	83.8 (13.2)	86.1 (12.8)	81.7 (13.3)	<0.0001
Mean premorbid SBP (SD)	138.5 (14.1)	133.4 (14.4)	143.2 (12.1)	<0.0001
Mean premorbid DBP (SD)	80.0 (7.6)	80.7 (8.5)	79.3 (6.6)	0.004
Median number of premorbid BP readings (IQR)	15 (7-33)	9 (4-21)	23 (12-41)	<0.0001
Premorbid use of antihypertensives (%)	543 (55.2)	46 (9.5)	108 (20.6)	<0.0001
Premorbid use of antithrombotics (%)	133 (13.2)	34 (7.0)	99 (18.9)	<0.0001
Premorbid use of statins (%)	85 (8.4)	28 (5.8)	57 (10.9)	0.004
Imaging characteristics				
N with lacunes (%)	178 (17.6)	67 (13.8)	111 (21.1)	0.002
Subcortical WMH (%)				
Grade 1 (%)	328 (32.5)	146 (30.2)	182 (34.7)	<0.0001
Grade 2 (%)	177 (17.5)	49 (10.1)	128 (24.4)	
Grade 3 (%)	116 (11.5)	27 (5.6)	89 (17.0)	
Periventricular WMH (%)				
Grade 1 (%)	383 (38.0)	163 (33.7)	220 (41.9)	<0.0001
Grade 2 (%)	196 (19.4)	49 (10.1)	147 (28.0)	
Grade 3 (%)	95 (9.4)	19 (3.9)	76 (14.5)	
Basal ganglia perivascular spaces (%)				
1-10 (%)	518 (51.3)	344 (71.1)	174 (33.1)	<0.0001
11-20 (%)	265 (26.3)	88 (18.2)	177 (33.7)	
>20 (%)	226 (22.4)	52 (10.7)	174 (33.1)	
N with microbleeds (%)	154 (15.3)	47 (9.7)	107 (20.4)	<0.0001
1 microbleed (%)	77 (7.6)	24 (5.0)	53 (10.1)	0.0002
2-4 microbleeds (%)	44 (4.4)	15 (3.1)	29 (5.5)	
≥5 microbleeds (%)	39 (3.9)	12 (2.5)	27 (5.1)	
Strictly deep microbleeds (%)	14 (1.4)	6 (1.2)	8 (1.5)	0.70
Strictly lobar microbleeds (%)	72 (7.1)	25 (5.2)	47 (9.0)	0.020
Microbleeds of mixed location (%)	53 (5.3)	14 (2.9)	39 (7.4)	0.001
Mean Total Small Vessel Disease Score	1.12 (1.11)	0.69 (0.96)	1.52 (1.09)	<0.0001

SD=standard deviation; SBP=systolic blood pressure; DBP=diastolic blood pressure; BP=blood pressure; WMH=white matter hyperintensity

Table 7.2 Relationships of lacunes and Total Small Vessel Disease Score with baseline blood pressure, premorbid blood pressure (top versus bottom quartile) and history of hypertension

	Univariate OR (95% CI)	p	Age and sex adjusted OR (95% CI)	p	c-statistic (95% CI)[a]	p
Lacunes						
Baseline SBP	1.82 (1.10-3.00)	0.019	1.52 (0.91-2.53)	0.11	0.57 (0.52-0.62)	0.008
Baseline DBP	0.56 (0.34-0.93)	0.025	0.67 (0.40-1.12)	0.12	0.44 (0.39-0.50)	0.031
Hypertension	2.05 (1.45-2.89)	<0.0001	1.74 (1.22-2.49)	0.002	0.58 (0.54-0.63)	0.0004
Mean premorbid SBP	3.79 (2.27-6.31)	<0.0001	2.92 (1.69-5.03)	0.0001	0.63 (0.59-0.67)	<0.0001
Mean premorbid DBP	1.83 (1.17-2.88)	0.008	1.99 (1.26-3.16)	0.003	0.57 (0.52-0.62)	0.004
Total Small Vessel Disease Score						
Baseline SBP	2.28 (1.62-3.21)	<0.0001	1.46 (1.02-2.10)	0.039	0.51 (0.38-0.65)	0.81
Baseline DBP	0.71 (0.51-1.00)	0.050	1.16 (1.20-1.89)	0.43	0.37 (0.25-0.49)	0.024
Hypertension	2.53 (2.01-3.20)	<0.0001	1.61 (1.26-2.06)	0.0001	0.62 (0.53-0.72)	0.023
5-yr mean premorbid SBP	3.71 (2.64-5.20)	<0.0001	2.06 (1.44-2.96)	<0.0001	0.69 (0.58-0.80)	0.001
5-yr mean premorbid DBP	0.81 (0.58-1.12)	0.20	1.52 (1.07-2.16)	0.020	0.60 (0.49-0.72)	0.079
Mean premorbid SBP	6.09 (4.34-8.55)	<0.0001	2.53 (1.76-3.65)	<0.0001	0.68 (0.58-0.77)	0.001
Mean premorbid DBP	1.59 (1.16-2.18)	0.004	2.00 (1.42-2.80)	<0.0001	0.62 (0.52-0.72)	0.030

[a]Total Small Vessel Disease Score=4 used as cut-off
OR=odds ratio; CI=confidence interval; SBP=systolic blood pressure; DBP=diastolic blood pressure

Table 7.3 Relationships of Total Small Vessel Disease Score with premorbid blood pressures measured (top versus bottom quartile) within 1 year, 1-5 years, 5-10 years and 10-20 years of TIA or ischaemic stroke

	All patients (n=1009)				Age <70 (n=484)		Age ≥70 (n=525)	
	Univariate OR (95% CI)	**p**	**Sex adjusted OR (95% CI)**	**p**	**Univariate OR (95% CI)**	**p**	**Univariate OR (95% CI)**	**p**
Mean premorbid SBP								
Within 1 year	2.17 (1.48-3.17)	<0.0001	2.15 (1.47-3.15)	<0.0001	2.93 (1.61-5.33)	0.0004	1.31 (0.78-2.19)	0.31
1-5 years	3.94 (2.78-5.56)	<0.0001	3.92 (2.77-5.55)	<0.0001	4.32 (2.49-7.52)	<0.0001	2.16 (1.35-3.47)	0.001
5-10 years	4.67 (3.23-6.76)	<0.0001	4.66 (3.22-6.75)	<0.0001	5.06 (2.76-9.31)	<0.0001	2.03 (1.19-3.45)	0.009
10-20 years	5.92 (4.05-8.65)	<0.0001	5.92 (4.05-8.65)	<0.0001	5.73 (2.97-11.07)	<0.0001	2.30 (1.30-4.10)	0.004
Mean premorbid DBP								
Within 1 year	0.91 (0.62-1.33)	0.62	0.92 (0.63-1.34)	0.65	1.51 (0.83-2.75)	0.18	1.21 (0.70-2.09)	0.50
1-5 years	0.76 (0.54-1.06)	0.11	0.76 (0.54-1.07)	0.12	1.38 (0.80-2.36)	0.24	1.08 (0.66-1.78)	0.75
5-10 years	1.26 (0.89-1.79)	0.19	1.28 (0.90-1.83)	0.17	2.81 (1.60-4.93)	0.0003	0.83 (0.51-1.35)	0.44
10-20 years	3.35 (2.33-4.84)	<0.0001	3.48 (2.41-5.03)	<0.0001	5.94 (3.36-10.52)	<0.0001	1.16 (0.69-1.94)	0.58

OR=odds ratio; CI=confidence interval; SBP=systolic blood pressure; DBP=diastolic blood pressure

Table 7.4 Sensitivity analysis of Total Small Vessel Disease Score with mean premorbid blood pressure (top versus bottom quartile) excluding antihypertensive users

	Univariate OR (95% CI)	p	Age and sex adjusted OR (95% CI)	p
Mean premorbid SBP				
Within 1 year	4.81 (2.40-9.68)	<0.0001	2.69 (1.28-5.65)	0.009
1-5 years	5.90 (3.25-10.70)	<0.0001	3.53 (1.89-6.61)	<0.0001
5-10 years	6.92 (3.39-14.15)	<0.0001	2.30 (1.07-4.96)	0.033
10-20 years	8.11 (3.86-17.03)	<0.0001	2.16 (0.98-4.77)	0.056
Mean premorbid DBP				
Within 1 year	1.24 (0.65-2.35)	0.52	1.94 (0.96-3.90)	0.064
1-5 years	0.97 (0.57-1.67)	0.93	1.25 (0.69-2.25)	0.46
5-10 years	1.36 (0.76-2.44)	0.31	1.30 (0.69-2.46)	0.41
10-20 years	3.71 (1.98-6.95)	<0.0001	2.07 (1.07-4.01)	0.031

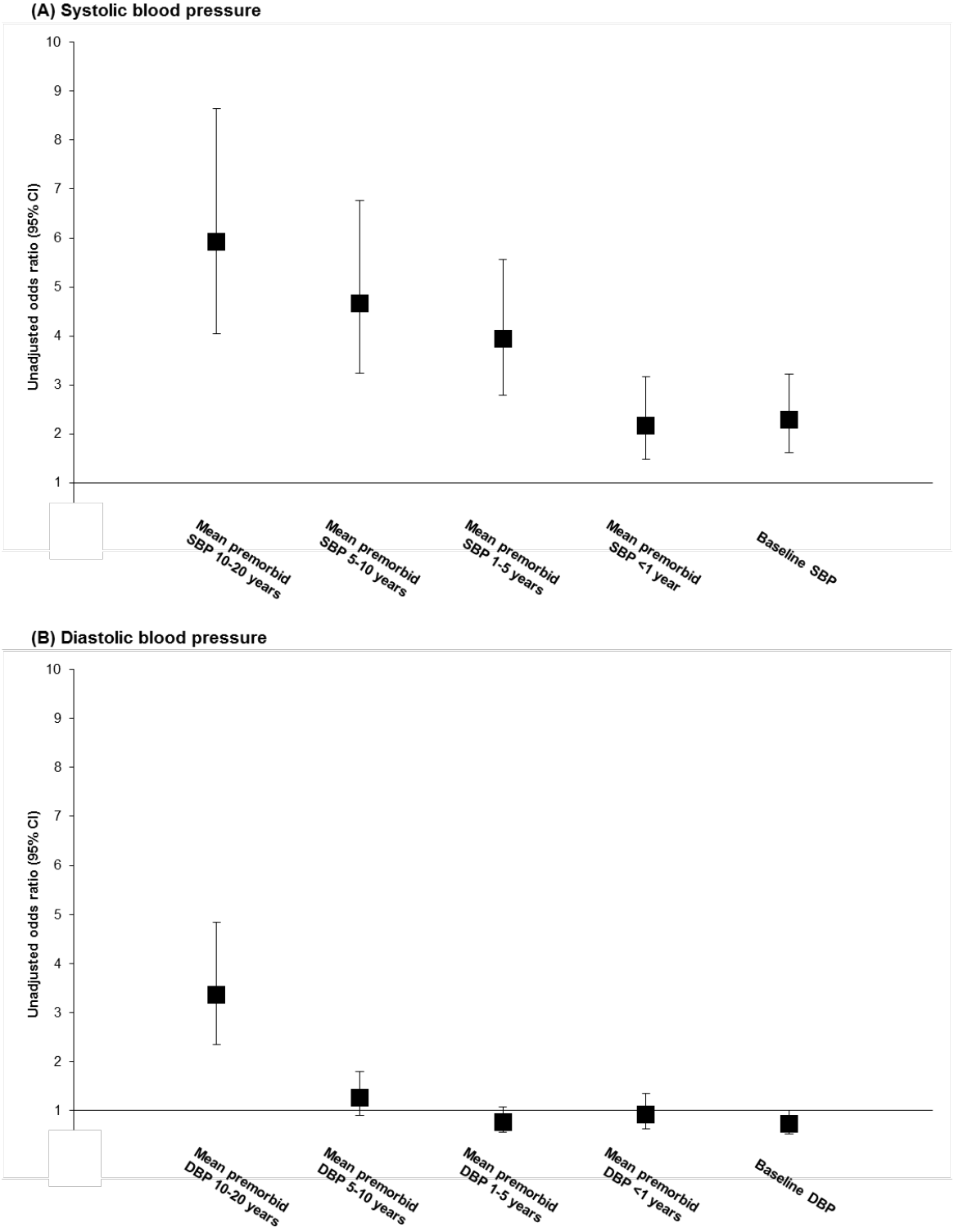
OR=odds ratio; CI=confidence interval; SBP=systolic blood pressure; DBP=diastolic blood pressure

Table 7.5 Relationships of Total Small Vessel Disease Score with premorbid blood pressures measured (top versus bottom quartile) within 1year, 1-5 years, 5-10 years and 10-20 years of TIA or ischaemic stroke in patients aged <60

	Age <60 (n=251)	
	Univariate OR (95% CI)	**p**
Mean premorbid SBP		
Within 1 year	2.07 (0.78-5.47)	0.14
1-5 years	2.32 (0.97-5.55)	0.060
5-10 years	2.34 (0.82-6.68)	0.11
10-20 years	3.65 (0.98-13.61)	0.054
Mean premorbid DBP		
Within 1 year	1.03 (0.38-2.80)	0.95
1-5 years	0.96 (0.40-2.27)	0.92
5-10 years	2.34 (0.90-6.08)	0.080
10-20 years	6.56 (2.47-17.41)	0.0002

OR=odds ratio; CI=confidence interval; SBP=systolic blood pressure; DBP=diastolic blood pressure

Figure 7.1 Unadjusted odds ratios for an increasing Total Small Vessel Disease Score with baseline and premorbid A) systolic blood pressure and B) diastolic blood pressure (odds ratios of baseline and premorbid blood pressure taken as top versus bottom quartile as referent)



SBP=systolic blood pressure; DBP=diastolic blood pressure

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Chapter 8

Age-specific associations between renal impairment and small vessel disease burden in Chinese with ischaemic stroke

8.1 Chapter outline

Renal impairment has been associated with small vessel disease (SVD) on neuroimaging, as represented by presence of silent brain infarcts or white matter hyperintensity (WMH). In patients with lacunar infarct, renal impairment has also been associated with global SVD burden. Nevertheless, whether renal impairment is similarly associated with SVD burden in all stroke subtypes remain uncertain. Whether there are age-specific associations between renal impairment and SVD burden also has not been studied previously.

Therefore, in this Chapter, I determined the relationships between renal impairment and individual neuroimaging markers of SVD as well as the Total SVD Score in 959 predominantly Chinese with ischaemic stroke who received a cerebral magnetic resonance imaging at the University of Hong Kong. I also stratified analyses by stroke subtype and by age.

I found that although renal impairment was associated with the Total SVD Score in univariate analysis in all patients, this was not significant after adjusting for age and sex [odds ratio (OR) 1.13, 95% confidence interval (CI) 0.86-1.50, p=0.38]. Similar findings were noted in patients with SVD (n=405/959, OR: 1.36, 95% CI 0.88-2.12, p=0.17) and non-SVD (n=554/959, OR: 1.00, 0.69-1.43, p=0.99) ischaemic stroke subtypes. However, in 222/959 patients aged <60, renal impairment was independently associated with microbleed, subcortical and periventricular WMH burden (all $p<0.05$) and also with the Total SVD Score (age, sex and vascular risk factor adjusted OR 3.41, 1.16-10.04, p=0.026). There were nevertheless no associations between renal impairment and individual neuroimaging markers of SVD nor with SVD burden in patients aged ≥60 after adjusting for age and sex (all $p>0.05$).

I concluded that in Chinese with ischaemic stroke, renal impairment was independently associated with individual neuroimaging markers of SVD as well as overall global burden of SVD in patients aged <60, but not in those aged 60 years or above.

8.2 Introduction

Renal impairment is associated with an increased risk of stroke,[1] the risk associations of which appear to be particularly strong amongst Asian populations.[2] As the microvasculature of the brain and kidneys are similar anatomically and functionally,[3] and hence may be equally susceptible to vascular injury, there has been considerable interest in determining the relationships between renal impairment and cerebral small vessel disease (SVD).

In patients without stroke, renal impairment has been associated with presence of silent brain infarcts, white matter hyperintensity (WMH)[4] as well as presence and burden of cerebral microbleeds.[5] Amongst those with ischaemic stroke, renal impairment has also been associated with presence of microbleeds,[6] especially deep-seated microbleeds,[7] and also with WMH progression.[8] Recently, in a cohort of Chinese with lacunar infarct, renal impairment has been associated with visible basal ganglia (BG) and centrum semiovale perivascular spaces (CS-PVSs)[9] as well as the global SVD burden as assessed by the Total SVD Score.[9, 10] However, whilst one might expect patients with renal impairment would similarly be associated with lacunar strokes, a recent meta-analysis did not find an association between renal impairment with lacunar versus non-lacunar stroke subtypes.[4] Nevertheless, the majority of previous studies have not stratified analyses by age and whether there are any age-specific associations between renal impairment and global SVD burden is uncertain. Most studies have only studied a specific neuroimaging marker of SVD and in the few studies that have studied the relationship of renal impairment and global SVD burden, focused only on patients with lacunar infarct.[9]

Therefore, in a large cohort of predominantly Chinese with ischaemic stroke, I determined the age-specific associations of renal impairment and a range of neuroimaging markers of SVD as well as the Total SVD Score. I hypothesise that any independent associations between cerebral SVD burden with renal impairment would be strongest amongst individuals of young age, possibly due to shared genetic susceptibility to disease of the small vessels of the cerebral and renal vasculatures.

8.3 Methods

8.3.1 Study population

I prospectively studied 1076 predominantly Chinese with a diagnosis of acute ischemic stroke who received a magenetic resonance imaging (MRI) scan incorporating a haemosiderin-sensitive sequence at The University of Hong Kong (HKU) MRI Unit during March 1, 2008, to September 30, 2014 (see section 2.1). Baseline data was collected as described in section 2.1. Blood tests for renal function were taken upon admission for ischaemic stroke and renal impairment was defined as a glomerular filtration rate (GFR) of <60/ml/min/1.73m^2 according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation for Asian populations.[11] Presence and burden of visible BG and CS-PVSs, cerebral microbleeds, WMH and lacunes were coded as described in section 2.3 and the Total SVD Score calculated for all patients.[10]

8.3.2 Statistical analysis

I compared the clinical and imaging characteristics of patients with and without renal impairment using t-test for continuous variables and Chi-squared test for categorical variables. Using ordinal regression, I determined the relationships of renal impairment with burden of microbleeds (0, 1, 2-4, ≥5), subcortical and periventricular WMH (Fazekas grade 0, 1, 2, 3) and visible BG-PVSs (<11, 11-20, ≥20) as well as the Total SVD Score (0, 1, 2, 3, 4), in a univariate model, model adjusted for age and sex and multivariate model adjusted for age, sex and vascular risk factors (hypertension, hyperlipidaemia, diabetes, atrial fibrillation, smoking history). I stratified the analysis according to ischaemic stroke subtype (SVD vs. non-SVD) as classified by the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria,[12] and also by age (<60 vs. ≥60).

All analyses were done with SPSS version 22.

8.4 Results

A total of 1076 patients with ischaemic stroke received a MRI stroke protocol at the HKU MRI Unit during the period March 1, 2008, to September 30, 2014. After excluding 117 patients who had incomplete imaging data or did not have renal function measured prior to MRI scan, 959 patients were included in the final analysis. Clinical and neuroimaging characteristics of the study population are shown in Table 8.1. The mean (SD) age of the study population was 69 (12) years and 60% were men. Sixty six percent had a past history of hypertension. The mean GFR was 78(24)ml/min/1.73m^2. Patients with renal impairment were older [76 (9) vs. 67 (12) years] and were more likely to have underlying hypertension (79.8% vs. 60.6%), diabetes (39.1% vs. 24.4%) and atrial fibrillation (22.2% vs. 9.8%)(all p<0.0001).

There were no differences in proportion of patients with a diffusion weighted imaging-positive lesion in patients with or without renal impairment (p=0.21). However, patients with renal impairment were associated with a greater prevalence (51.0% vs. 43.3%, p=0.037) and burden (16.9% vs. 10.3% with ≥5, p=0.008) of microbleeds. There were no differences in prevalence of strictly deep or strictly lobar microbleeds, but patients with renal impairment were more likely to have microbleeds of mixed location (26.7% vs. 16.6%, p=0.001). Patients with renal impairment were also associated with a greater burden of periventricular WMH (p<0.0001) as well as burden of visible BG-PVSs (12.3% vs. 5.3% with >20, p<0.001), but no differences in prevalence of subcortical WMH (p=0.069) nor lacunes (p=0.94) were noted in patients with or without renal impairment. Consequently, the mean Total SVD Score was greater in patients with renal impairment compared with patients without renal impairment [1.90 (1.19) vs. 1.60 (1.12), p<0.0001].

In univariate analysis, renal impairment was significantly associated with burden of microbleeds, WMH (subcortical and periventricular) and visible BG-PVSs (all p<0.05, Table 8.2). However, all associations of renal impairment with SVD burden were attenuated after adjusting for age and sex (Table 8.2).

When patients were stratified according to the TOAST classification (Table 8.3), there were no

significant associations between renal impairment and SVD burden in patients with ischaemic stroke due to SVD (n=405/959) and non-SVD subtypes (n=554/959), after adjusting for age and sex (all p>0.05, Table 8.3). However, in patients with ischaemic stroke due to non-SVD, renal impairment was associated with a reduced risk of strictly deep microbleeds [age and sex adjusted odds ratio (OR) 0.33, 95% confidence interval (CI) 0.12-0.89, p=0.029], but increased risk of microbleeds of mixed location (OR 1.94, 1.17-3.20, p=0.010, Table 8.3).

In an analysis stratified by age, strong univariate associations were noted between renal impairment and burden of microbleeds, subcortical and periventricular WMH, and Total SVD Score in the 222/959 patients aged <60 (all p<0.05, Table 8.4). These associations remained after adjusting for age and sex and also after adjusting for other vascular risk factors (Table 8.4). Patients with renal impairment were independently associated with an increasing microbleed (multivariate adjusted OR 6.82, 2.26-20.59, p=0.001), subcortical WMH (4.97, 1.62-15.24, p=0.005) and periventricular WMH burden (3.96, 1.08-14.51, p=0.038) as well as an increasing Total SVD Score (3.41, 1.16-10.04, p=0.026). No significant associations between renal impairment and visible BG-PVS burden nor presence of lacunes were noted. However, in patients aged ≥60, no relationships between renal impairment and an increasing burden of individual neuroimaging markers of SVD nor global SVD burden was noted after adjusting for age and sex (Total SVD Score: 1.03, 0.77-1.38, p=0.85; p_{het}=0.036) (Table 8.5).

8.5 Discussion

In a large cohort of Chinese patients with ischaemic stroke, I noted that although renal impairment was strongly associated with a range of neuroimaging markers of SVD and an increasing global burden of SVD, these associations were all attenuated after adjusting for age and sex. There were however, significant age-specific associations between renal impairment and SVD burden and renal impairment was significantly associated with microbleed, subcortical and periventricular WMH burden as well as the Total SVD Score, independent of other vascular risk factors in patients aged <60, but not in those aged ≥60. The relationships between renal impairment and SVD burden was also not significantly different in patients with ischaemic stroke classified as small vessel occlusion or non-small vessel occlusion.

The burden of SVD has previously been associated with a range of vascular risk factors including age, male sex, hypertension and smoking in ischaemic stroke patients.[10, 13] Recently, Xiao *et al*. noted that in 413 Chinese patients with lacunar infarct, renal impairment (as defined by a GFR of <60/ml/min/1.73m^2 or presence of proteinuria) was independently associated with the Total SVD Score as well as burden of visible PVSs.[9] In contrast, renal impairment was not associated with visible PVSs nor with global SVD burden in our cohort, even in the 405 patients with ischaemic stroke due to small vessel occlusion. However, I noted a significant interaction in the risk associations of renal impairment and SVD burden with age. Independent of other vascular risk factors, renal impairment was significantly associated with global SVD burden in patients age <60, but not in patients with age ≥60 (p_{het}=0.036), probably due to fewer competing risk factors for SVD in younger individuals. Indeed, the cohort from Xiao *et al*.[9] was on the whole younger with a mean age of 64 compared with patients in our cohort with mean age 69. Therefore, although recent meta-analysis did not reveal a significant association between renal impairment and lacunar stroke,[4] an age-specific association between renal impairment and SVD may be present if the results were stratified by age.

Xiao *et al*. also noted significant associations between lacunar stroke patients with renal impairment and burden of visible PVSs[9] which was not noted in this study, even amongst patients aged <60. However, there were only 108/405 patients with stroke due to small vessel occlusion

who were aged <60, and hence I lacked power to determine if such a relationship remained valid in young patients with lacunar stroke.

A number of biological processes occur in patients with renal impairment that may lead to SVD, independent of hypertension. In particular, insult to the renal endothelium may activate the renin-angiotensin system resulting in an increased production of angiotensin II.[14] Angiotensin II has significant proinflammatory effects and induces the production of reactive oxygen species, inflammatory cytokines and adhesion molecules.[14] Current evidence suggest that the blood brain barrier plays an important role in the pathogenesis and development of SVD.[15] With age, the cerebrovascular endothelium becomes more permeable.[16] However, blood brain barrier dysfunction also occurs in processes such as inflammation[17] and oxidative stress,[18] and hence may occur in patients with renal impairment. Increased permeability of the blood brain barrier with diffuse cerebrovascular endothelial failure would lead to leakage of plasma components and inflammatory cells into the vessel wall and perivascular tissue.[15] Smooth muscle cells of arterioles are then replaced by collagenous tissue, foamy macrophages and inflammatory cells (lipohyalinosis). This results in thickening and narrowing of the vessel, arterial stiffness and impaired autoregulation, subsequently leading to the SVD changes seen on neuroimaging.

My study is limited by the following aspects. First, I based the definition of renal impairment on a serum creatinine-derived equation,[11] from a single renal function measurement taken upon hospitalisation after ischaemic stroke. Such a measurement may be affected by acute inflammation or dehydration secondary to stroke and may potentially overdiagnose renal impairment. Use of premorbid renal function tests may potentially be a more accurate representative of the patients' renal function. Second, unlike other studies, I did not determine the relationships of SVD with proteinuria, another widely used surrogate marker to reflect renal function. Although I consider my results valid, my findings should be replicated in other cohorts using other surrogate markers of renal function, such as proteinuria. Third, although I postulate that my findings may be due to blood brain barrier dysfunction secondary to inflammation and oxidative stress as a result of renal impairment, I did not correlate my findings with markers of inflammation, oxidative stress nor with neuroimaging markers of blood brain barrier function.

Fourth, in this cross sectional study, I was not able to determine whether changes in renal function with time corresponded with changes in the burden of SVD. Finally, as shown in Chapter 7, premorbid blood pressure is a stronger predictor of SVD burden than baseline measurements of blood pressure or a 'history of hypertension'. Whether adjusting for premorbid blood pressure may alter my findings is uncertain and was limited by a lack of available premorbid blood pressure readings in this cohort.

In conclusion, there are age-specific associations between renal impairment and SVD. Independent to age, sex and vascular risk factors, renal impairment is a significant predictor of SVD burden in patients with ischaemic stroke age <60 but not in those aged ≥60.

Table 8.1 Clinical and imaging characterstics of the study population

	All patients n=959	Renal impairment n=243	Normal renal function n=716	p
Baseline clinical characteristics				
Mean (SD) age (years)	69 (12)	76 (9)	67 (12)	<0.0001
Males (%)	576 (60.1)	149 (61.3)	427 (59.6)	0.65
Hypertension (%)	628 (65.5)	194 (79.8)	434 (60.6)	<0.0001
Diabetes (%)	270 (28.2)	95 (39.1)	175 (24.4)	<0.0001
Hyperlipidaemia (%)	245 (25.5)	72 (29.6)	173 (24.2)	0.11
Ever-smokers (%)	287 (29.9)	67 (27.6)	220 (30.7)	0.37
Atrial fibrillation (%)	124 (12.9)	54 (22.2)	70 (9.8)	<0.0001
Imaging characteristics				
N with DWI lesion (%)	750 (78.2)	183 (75.3)	567 (79.2)	0.21
N with microbleeds (%)	434 (45.3)	124 (51.0)	310 (43.3)	0.037
N with ≥5 microbleeds (%)	115 (12.0)	41 (16.9)	74 (10.3)	0.008
N with strictly deep microbleeds (%)	60 (6.3)	11 (4.5)	49 (6.8)	0.22
N with strictly lobar microbleeds (%)	153 (16.0)	44 (18.1)	109 (15.2)	0.31
N with strictly infratentorial microbleeds (%)	37 (3.9)	4 (1.6)	33 (4.6)	0.051
N with microbleeds of mixed location (%)	184 (19.2)	65 (26.7)	119 (16.6)	0.001
N with periventricular WMH				<0.0001
Grade 1 (%)	211 (22.0)	67 (27.6)	144 (20.1)	
Grade 2 (%)	74 (7.7)	32 (13.2)	42 (5.9)	
Grade 3 (%)	30 (3.1)	11 (4.5)	19 (2.7)	
N with subcortical WMH				0.069
Grade 1 (%)	468 (48.8)	105 (43.2)	363 (50.7)	
Grade 2 (%)	274 (28.6)	73 (30.0)	201 (28.1)	
Grade 3 (%)	152 (15.8)	50 (20.6)	102 (14.2)	
N with visible basal ganglia PVSs				<0.0001
<10 (%)	650 (67.8)	143 (58.8)	507 (70.8)	
10-20 (%)	241 (25.1)	70 (28.8)	171 (23.9)	
>20 (%)	68 (7.1)	30 (12.3)	38 (5.3)	
N with lacunes (%)	427 (44.5)	109 (44.9)	318 (44.4)	0.94
Mean Total SVD Score	1.67±1.15	1.90±1.19	1.60±1.12	<0.0001

SD=standard deviation; DWI=diffusion weighted imaging; WMH=white matter hyperintensity; PVS=perivascular spaces; SVD=small vessel disease

Table 8.2 Relationships of renal impairment with individual neuroimaging markers and global burden of small vessel disease

	Unadjusted OR (95% CI)	p	Age and sex adjusted OR (95% CI)	p	Multivariate[a] adjusted OR (95% CI)	p
Microbleed burden	1.51 (1.15-1.99)	0.003	1.31 (0.98-1.75)	0.073	1.30 (0.96-1.75)	0.087
Strictly deep microbleeds	0.65 (0.33-1.26)	0.20	0.65 (0.32-1.32)	0.24	0.67 (0.33-1.38)	0.28
Strictly lobar microbleeds	1.23 (0.84-1.81)	0.29	1.22 (0.81-1.84)	0.35	1.22 (0.80-1.87)	0.35
Microbleeds of mixed location	1.83 (1.30-2.59)	0.001	1.47 (1.01-2.14)	0.042	1.46 (1.00-2.14)	0.050
Subcortical WMH burden	1.41 (1.07-1.84)	0.013	1.09 (0.81-1.45)	0.58	1.11 (0.83-1.49)	0.49
Periventricular WMH burden	2.09 (1.57-2.80)	<0.0001	1.27 (0.92-1.73)	0.14	1.30 (0.94-1.75)	0.087
BG-PVS burden	1.78 (1.33-2.40)	<0.0001	1.00 (0.72-1.38)	1.00	0.99 (0.71-1.38)	0.96
Presence of lacunes	1.02 (0.76-1.36)	0.91	1.06 (0.77-1.46)	0.72	1.04 (0.75-1.43)	0.83
Total SVD Score	1.61 (1.24-2.09)	<0.0001	1.13 (0.86-1.50)	0.38	1.12 (0.84-1.49)	0.44

[a]Adjusted for age, sex, hypertension, hyperlipidaemia, diabetes mellitus, ever-smoking and atrial fibrillation
OR=odds ratio; CI=confidence interval; WMH=white matter hyperintensity; BG=basal ganglia; PVS=perivascular space; SVD=small vessel disease

Table 8.3 Relationships of renal impairment with individual neuroimaging markers and global burden of small vessel disease, stratified by stroke subtype according to the TOAST classification

	Small vessel disease (n=405)				Non-small vessel disease (n=554)			
	Unadjusted OR (95% CI)	p	Age and sex adjusted OR (95% CI)	p	Unadjusted OR (95% CI)	p	Age and sex adjusted OR (95% CI)	p
Microbleed burden	1.60 (1.04-2.47)	0.033	1.34 (0.85-2.12)	0.20	1.48 (1.04-2.11)	0.029	1.28 (0.87-1.88)	0.20
Strictly deep microbleeds	1.63 (0.60-4.43)	0.33	2.14 (0.73-6.27)	0.17	0.35 (0.13-0.90)	0.029	0.33 (0.12-0.89)	0.029
Strictly lobar microbleeds	1.75 (0.98-3.12)	0.059	1.68 (0.91-3.10)	0.10	0.96 (0.57-1.61)	0.88	0.96 (0.54-1.68)	0.88
Microbleeds of mixed location	1.35 (0.79-2.31)	0.28	1.03 (0.58-1.83)	0.92	2.43 (1.54-3.85)	<0.0001	1.94 (1.17-3.20)	0.010
Subcortical WMH burden	1.17 (0.76-1.80)	0.47	0.93 (0.59-1.47)	0.76	1.62 (1.14-2.30)	0.007	1.15 (0.78-1.68)	0.48
Periventricular WMH burden	2.48 (1.58-3.39)	<0.0001	1.59 (0.99-2.57)	0.057	1.95 (1.33-2.86)	0.001	1.05 (0.69-1.61)	0.80
BG-PVS burden	1.82 (1.15-2.89)	0.011	0.98 (0.59-1.63)	0.95	1.81 (1.23-2.66)	0.003	1.01 (0.66-1.54)	0.96
Presence of lacunes	1.41 (0.88-2.26)	0.15	1.36 (0.83-2.24)	0.23	0.86 (0.59-1.27)	0.46	0.89 (0.58-1.35)	0.58
Total SVD Score	1.98 (1.30-3.02)	0.001	1.36 (0.88-2.12)	0.17	1.46 (1.04-2.04)	0.027	1.00 (0.69-1.43)	0.99

TOAST=Trial of Org 10172 in Acute Stroke Treatment; OR=odds ratio; CI=confidence interval; WMH=white matter hyperintensity; BG=basal ganglia; PVS=perivascular space; SVD=small vessel disease

Table 8.4 Relationships of renal impairment with individual neuroimaging markers and global burden of small vessel disease in patients age <60

	Unadjusted OR (95% CI)	p	Age and sex adjusted OR (95% CI)	p	Multivariate[a] adjusted OR (95% CI)	p
Microbleed burden	6.83 (2.33-20.07)	0.0005	6.70 (2.27-19.73)	0.001	6.82 (2.26-20.59)	0.001
Strictly deep microbleeds	1.03 (0.13-8.48)	0.98	1.07 (0.13-8.85)	0.95	0.79 (0.09-6.79)	0.83
Strictly lobar microbleeds	3.54 (1.00-12.58)	0.051	3.57 (1.00-12.78)	0.050	3.55 (0.95-13.30)	0.060
Microbleeds of mixed location	3.54 (1.00-12.58)	0.051	3.44 (0.95-12.50)	0.060	3.75 (0.94-14.95)	0.061
Subcortical WMH burden	4.72 (1.60-13.96)	0.005	4.43 (1.49-13.16)	0.007	4.97 (1.62-15.24)	0.005
Periventricular WMH burden	4.20 (1.21-14.64)	0.024	4.18 (1.20-14.66)	0.025	3.96 (1.08-14.51)	0.038
BG-PVS burden	0.61 (0.08-4.95)	0.64	0.58 (0.07-4.70)	0.61	0.59 (0.07-5.01)	0.63
Presence of lacunes	1.87 (0.57-6.07)	0.30	1.84 (0.56-5.99)	0.31	1.61 (0.48-5.45)	0.44
Total SVD Score	3.72 (1.29-10.74)	0.015	3.50 (1.21-10.10)	0.021	3.41 (1.16-10.04)	0.026

[a]Adjusted for age, sex, hypertension, hyperlipidaemia, diabetes mellitus, ever-smoking and atrial fibrillation

OR=odds ratio; CI=confidence interval; WMH=white matter hyperintensity; BG=basal ganglia; PVS=perivascular space; SVD=small vessel disease

Table 8.5 Relationships of renal impairment with individual neuroimaging markers and global burden of small vessel disease, in patients aged ≥60

	Unadjusted OR (95% CI)	p	Age and sex adjusted OR (95% CI)	p
Microbleed burden	1.26 (0.94-1.69)	0.12	1.16 (0.86-1.58)	0.33
Strictly deep microbleeds	0.67 (0.32-1.39)	0.28	0.58 (0.27-1.23)	0.16
Strictly lobar microbleeds	1.07 (0.71-1.62)	0.76	1.12 (0.73-1.73)	0.61
Microbleeds of mixed location	1.59 (1.10-2.31)	0.013	1.39 (0.94-2.04)	0.098
Subcortical WMH burden	1.13 (0.84-1.50)	0.43	1.01 (0.75-1.37)	0.93
Periventricular WMH burden	1.56 (1.15-2.11)	0.004	1.19 (0.87-1.64)	0.28
BG-PVS burden	1.42 (1.04-1.93)	0.027	1.02 (0.73-1.41)	0.93
Presence of lacunes	0.96 (0.71-1.32)	0.82	1.01 (0.73-1.40)	0.96
Total SVD Score	1.25 (0.95-1.65)	0.12	1.03 (0.77-1.38)	0.85

OR=odds ratio; CI=confidence interval; WMH=white matter hyperintensity; BG=basal ganglia; PVS=perivascular space; SVD=small vessel disease

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Chapter 9

Carotid pulsatility and small vessel disease burden in TIA and ischaemic stroke

9.1 Chapter outline

Although large artery stiffness has been implicated in the pathogenesis of cerebral small vessel disease (SVD), whether carotid pulsatility, a surrogate marker of arterial stiffness, is similarly associated with the global burden of SVD is unknown.

I studied 587 patients with transient ischaemic attack or ischaemic stroke from the Oxford Vascular Study who received a brain magnetic resonance imaging and carotid duplex ultrasound during 2002-2014. I determined the clinical correlates of common carotid (CCA) and internal carotid artery (ICA) pulsatility index (PI) and also determined the associations between CCA and ICA-PI with the Total SVD Score.

I found that CCA and ICA-PI were both independently associated with age, diabetes and mean pulse pressure after adjustment for age, male sex and cardiovascular risk factors (all $p<0.05$). CCA-PI was also associated with male sex ($p<0.0001$), but ICA-PI was not ($p=0.22$). In 261/587 patients aged<70, ICA-PI was associated with an increasing Total SVD Score (age and sex-adjusted odds ratio of top vs. bottom quartile of PI: 95% confidence interval 2.30, 1.01-5.25, $p=0.048$), but no associations between CCA-PI and Total SVD Score were noted (1.08, 0.52-2.25, $p=0.84$), nor were there any associations between CCA-PI ($p=0.55$) and ICA-PI ($p=0.62$) with Total SVD Score in patients aged ≥70. There were also no significant sex differences in associations between CCA and ICA-PI with individual neuroimaging markers and global burden of SVD.

In 94 consecutive patients who also received transcranial Doppler ultrasound, strong associations between middle cerebral artery (MCA)-PI and an increasing SVD Score was noted (sex-adjusted OR - MCA: 4.43, 1.49-13.13, $p=0.007$; ICA: 2.37, 0.82-6.89, $p=0.11$; CCA: 1.29, 0.43-1.88, $p=0.76$).

I concluded that ICA and MCA-PI are significantly associated with global SVD burden, especially in individuals age <70 and may represent a surrogate marker of underlying SVD burden.

9.2 Introduction

Cerebral small vessel disease (SVD) accounts for approximately 20% of all strokes and 45% of patients with dementia.[1] However, although hypertension has been implicated as one of the main risk factors leading to SVD, the pathogenesis of SVD is not fully understood.[1]

Stiffening of the large vessels has been associated with hypertension[2] and in the pathogenesis of cerebral SVD. Previous studies have demonstrated that large artery stiffness is associated with silent cerebral infarcts,[3] cerebral microbleeds,[4] white matter hyperintensity (WMH) volume[3] and strokes of lacunar subtype.[5] Furthermore, stiffness of the aorta is associated with an increased arterial pulsatility measured at the common carotid artery (CCA)[5, 6] and middle cerebral artery (MCA).[7, 8] Previous small studies have also shown that MCA pulsatility is correlated with leukoaraiosis,[7] overall suggesting that leukoaraiosis and lacunar infarcts may be the result of increased arterial pulsatility secondary to large artery stiffness.[6, 7, 9-11]

Studies have also shown that the risk of leukoaraiosis varies with age and sex,[12] being more severe in women than in men at older ages, possibly due to sex differences in premature death.[12] However, sex differences in carotid bifurcation and internal carotid (ICA) anatomy is also known to be present and although men may have larger CCA and ICA lumen sizes compared with women, the ICA/CCA ratio and ICA/external carotid artery ratio is greater in women compared with men.[13] Nevertheless, despite these sex differences in carotid anatomy, and age and sex differences in risk of leukoaraiosis, the age and sex specific associations between carotid pulsatility and global SVD burden is unknown.

I therefore investigated the age and sex specific associations of carotid pulsatility with individual neuroimaging markers and global burden of SVD in 587 patients with transient ischaemic attack (TIA) or ischaemic stroke, nested in the population-based Oxford Vascular Study (OXVASC). In a subset of 94 patients, I also studied the relationship of MCA pulsatility with the Total SVD Score. I hypothesise that carotid pulsatility may be associated with global SVD burden due to transmission of pulsatile flow from the proximal to distal arteries in patients with arterial stiffness. I also

postulate that the associations between carotid pulsatility and SVD burden would be greatest in patients of younger ages and that sex differences in risk associations between carotid pulsatility and SVD burden may exist in view of sex differences in carotid anatomy.

9.3 Methods

9.3.1 Study population

I prospectively studied 606 cases of TIA or ischaemic stroke recruited from OXVASC during the period November 1, 2004 to September 30, 2014 who received a cerebral magnetic resonance imaging (MRI) and also a carotid duplex ultrasound. The study population is described in detail in section 2.1. Briefly, from April 1, 2002 - March 31, 2010 (phase 1), non-contrast computed tomography (CT) brain and carotid duplex ultrasound were the first-line investigations and MRI and MR angiography was performed in selected patients when clinically indicated. From April 1, 2010 onwards (phase 2), brain MRI and MR angiography became the first-line imaging methods. However, carotid duplex ultrasound was also performed in selected patients in instances where MRI was contraindicated, MR angiography was not available or if carotid stenosis was identified on MR angiography and required more accurate quantification of stenosis via ultrasonography. I also studied 94 consecutive patients who also received a transcranial Doppler (TCD) ultrasound upon ascertainment.

Baseline data was collected as described in section 2.1. Presence and burden of MRI-visible enlarged basal ganglia (BG) and centrum semiovale perivascular spaces (CS-PVSs), cerebral microbleeds, WMH and lacunes were coded as described in section 2.3 and global burden of SVD, as represented by the Total SVD Score was calculated as described in Chapter 6. Premorbid blood pressure readings for all 606 patients during the 20 years prior to ascertainment (13404 readings in total, median number of readings per patient: 16, interquartile range [IQR]: 7-33) was also retrieved from the primary care practices and the mean systolic blood pressure, diastolic blood pressure and pulse pressure used for analysis.

Patients were scanned predominantly (488/606) with 2 scanners – Achieva, Philips Healthcare (369/443 patients who received a 1.5T MRI), and Magnetom Verio, Siemens Healthcare (119/163 patients who received a 3T MRI). Details of scan parameters are documented in Table 2.1. 119, 44, 369 and 74 patients were scanned by OXVASC scanners 1, 2, 3 and 4 respectively.

9.3.2 Carotid duplex and transcranial Doppler ultrasound examination

All carotid duplex ultrasounds were performed by trained vascular sonographers at the Vascular Laboratory, Oxford Regional Vascular Unit of John Radcliffe Hospital, Oxford. The carotid duplex ultrasound protocol included B-mode imaging (transverse and longitudinal plane) from the clavicle to mandible and the proximal, mid and distal CCA, carotid bifurcation as well as the proximal, mid and distal ICA were imaged where possible. Doppler ultrasound was also performed over bilateral CCA and ICA and the peak systolic velocity and end-diastolic velocity were calculated for the left and right CCA and ICA respectively. Presence of carotid plaques and plaque morphology were documented.

TCD sonography (Doppler Box, Compumedics DWL, Singen, Germany) was performed by one of three experienced operators. MCA blood flow velocities were recorded with a handheld 2MHz probe through temporal bone window at the depth that provided the best signal. Each session was stored in the hard disk of the TCD device for subsequent off-line analysis.

The CCA, ICA and MCA-PI were calculated for all patients. Pulsatility index (PI) was defined as the (peak systolic velocity – end-diastolic velocity)/mean flow velocity where mean flow velocity was defined as [peak systolic velocity + (end diastolic velocityx2)]/3.[14]

9.3.3 Statistical analysis

The mean CCA, ICA and MCA-PI of the left and right carotid or middle cerebral arteries were used for analysis. In patients who had unilateral >50% stenosis of the CCA, ICA or MCA, the contralateral CCA, ICA or MCA-PI was used for analysis. Patients were excluded from the main analysis if they had bilateral >50% stenosis of the CCA, ICA and/or MCA.

I determined the clinical predictors of CCA and ICA-PI by linear regression in a univariate model, model adjusted for age and sex as well as a multivariate model adjusted for all co-variates (age, sex, vascular risk factors, glomerular filtration rate, and premorbid mean systolic blood pressure, diastolic blood pressure and pulse pressure). I also determined, by ordinal regression, the odds

ratios (OR) of lacunes, an increasing burden of subcortical and periventricular WMH (Fazekas grade 0, 1, 2, 3), cerebral microbleeds (0, 1, 2-4, ≥5), visible BG and CS-PVSs (<11, 11-20, ≥20) and Total SVD Score (0, 1, 2, 3, 4) in patients with the top CCA or ICA-PI quartile, compared with those in the bottom CCA or ICA-PI quartile as reference, in all patients and in an analysis stratified by sex and age. In the 94/606 patients who also had TCD ultrasound performed, I similarly determined by ordinal regression the ORs of an increasing Total SVD Score in patients with the top MCA-PI quartile, compared with those in the bottom quartile as reference.

All analyses were done with SPSS version 22.

9.4 Results

9.4.1 Baseline clinical and imaging characteristics

A total of 606 patients received a MRI and carotid duplex ultrasound during the study period. After excluding 29 patients who had bilateral CCA or ICA stenosis >50%, 587 patients (306 TIA, 281 ischemic stroke) were included in the final analysis. Carotid duplex ultrasound was performed on a median of 3 days (IQR 1-13) after TIA or ischaemic stroke symptom onset whilst cerebral MRI was performed after a median of 11 days (1-40) from TIA or ischemic stroke onset. Baseline clinical and imaging characteristics of patients are shown in Table 9.1. The mean (SD) age of the population was 70 (14) years and 51% were males. Lacunes were present in 20.4% of the study population, 15.7% had microbleeds, 11.2% with Fazekas grade 3 subcortical WMH, 10.9% with Fazekas grade 3 periventricular WMH, 20.8% with >20 visible BG-PVSs and 42.9% with >20 visible CS-PVSs (Table 9.2). The mean (SD) Total SVD Score was 1.16 (1.17). The mean (SD) CCA-PI was 1.57 (0.29), mean ICA-PI was 1.28 (0.27) and mean MCA-PI was 1.08 (0.24).

9.4.2 Clinical correlates of common carotid and internal carotid artery pulsatility index

After adjusting for age, sex, vascular risk factors and premorbid blood pressure, age, diabetes and premorbid mean pulse pressure were identified as independent predictors of CCA-PI (multivariate-adjusted B - age: 0.0002, 95% CI 0.000-0.004, p=0.016; diabetes: 0.079, 0.016-0.143, p=0.014; mean pulse pressure: 0.075, 0.026-0.124, p=0.003) and ICA-PI (age: 0.005, 0.004-0.007, p<0.0001; diabetes: 0.098, 0.042-0.155, p=0.001; mean pulse pressure: 0.063, 0.020-0.107, p=0.004) (Table 9.2). Male sex was associated with an increasing CCA-PI (0.119, 0.074-0.164, p<0.0001). However, no relationships between sex and ICA-PI were noted on univariate analysis (p=0.40) (Table 9.2).

9.4.3 Associations of common carotid and internal carotid artery pulsatility index with individual neuroimaging markers and global burden of small vessel disease

The associations between CCA and ICA-PI with an increasing burden of individual neuroimaging markers of SVD and global burden of SVD are shown in Table 9.3. Strong univariate associations between CCA and ICA-PI with individual neuroimaging markers of SVD, in particular subcortical WMH, periventricular WMH, visible BG-PVSs and CS-PVSs were noted (all $p<0.01$). CCA and ICA-PI was also strongly associated with the Total SVD Score on univariate analysis [OR of top vs. bottom quartile of PI – CCA: 2.28, 95% confidence interval (CI) 1.49-3.47, p=0.0001; ICA: 4.68, 3.01-7.29, $p<0.0001$]. The associations between individual neuroimaging markers and global burden of SVD also appeared to be stronger with ICA-PI than with CCA-PI. However, after adjusting for age and sex, amongst all the neuroimaging markers of SVD, only a significant relationship between ICA-PI and periventricular WMH burden remained (1.68, 95% CI 1.03-2.75, p=0.037) and the relationships between CCA and ICA-PI with the Total SVD Score was attenuated (CCA: 1.12, 0.71-1.76, p=0.64; ICA: 1.49, 0.91-2.45, p=0.11).

When I stratified my analysis by sex (Table 9.4), no significant differences in associations between CCA and ICA-PI with Total SVD Score was noted in the 300/587 men versus 287/587 women (p_{het}=0.98 and 0.94 respectively).

However, when I stratified my analyses by age, in the 261/587 patients aged <70, significant associations between ICA-PI with lacunes (5.35, 1.95-14.70, p=0.001), increasing burden of periventricular WMH (2.86, 1.26-6.46, p=0.012), visible BG-PVSs (2.83, 1.07-7.46, p=0.035) and Total SVD Score (2.30, 1.01-5.25, p=0.048) was noted, even after adjusting for age and sex. However, no relationships between CCA-PI and Total SVD Score were noted in patients aged <70 (1.08, 0.52-2.25, p=0.84), nor were there any significant relationships noted between CCA-PI or ICA-PI with neuroimaging markers of SVD in the 326/587 patients aged ≥70 (Total SVD Score – CCA: 1.20, 0.66-2.21, p=0.55; ICA: 1.20, 0.59-2.42, 0.62) (Table 9.5).

In 94 consecutive patients who also had a TCD ultrasound performed, I noted strong univariate associations between MCA-PI and ICA-PI (B 0.499, 0.341-0.657, p<0.0001) as well as between MCA-PI and CCA-PI (0.353, 0.193-0.512, p<0.0001). Risk associations between CCA, ICA and MCA-PI with Total SVD Score increased progressively when arteries of closest proximity to the brain parenchyma were imaged (sex adjusted OR of top vs. bottom quartile of PI – CCA: 1.29, 0.43-1.88, p=0.76; ICA: 2.37, 0.82-6.89, p=0.11; MCA: 4.43, 1.49-13.13, p=0.007) (Table 9.6). 14.5% (85/587) of the study population were classified as having a TIA or ischaemic stroke due to acutely symptomatic small vessel disease or occlusion by Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria (Table 9.7). However, CCA, ICA and MCA-PI were not associated with TIA or ischaemic stroke due to SVD on univariate analysis (OR of top vs. bottom quartile of PI – CCA: 1.03, 0.54-1.99, p=0.92; ICA: 1.03, 0.83-1.28, p=0.77; MCA: 1.83, 0.45-0.75, p=0.40), even in patients aged <70 (CCA: 1.20, 0.49-2.98, p=0.69; ICA: 1.97, 0.76-5.13, p=0.17; MCA: 1.70, 0.35-8.34, p=0.51).

9.5 Discussion

I showed that in TIA or ischaemic stroke patients, ICA-PI was significantly associated with a range of neuroimaging markers of SVD (lacunes, periventricular WMH and visible BG-PVS burden) as well as the overall burden of SVD in patients aged <70, but not in those aged ≥70. I also demonstrated that the overall associations between PI and SVD burden were in general stronger with more distal arterial beds, and that in a subset of individuals, SVD burden was most strongly associated with MCA-PI, followed by ICA-PI and then CCA-PI.

A number of previous studies have demonstrated that PI, measured at the CCA or MCA is a reflection of underlying large artery stiffness.[5, 7, 8] My results support these findings, as age, diabetes and premorbid pulse pressure, all of which have previously been associated with arterial stiffness,[15-17] were noted to be independent predictors of CCA and ICA-PI in this study. Male sex was also a strong independent predictor of CCA-PI, but I did not note any associations between ICA-PI and sex on univariate analysis (p=0.40). These observations may be due to the underlying sex differences in carotid bifurcation and ICA anatomy, as although men may have larger CCA and ICA lumen sizes compared with women, the ICA/CCA ratio and ICA/external carotid artery ratio have been noted to be greater in women compared with men.[13] Nevertheless, despite known sex differences in carotid anatomy, I was not able to demonstrate and significant sex differences in the associations between CCA or ICA-PI with neuroimaging markers of SVD.

Similar to previous studies that have noted associations between large artery stiffness or arterial pulsatility with leukoaraiosis,[3, 7] I too noted significant associations between ICA-PI with periventricular WMH burden in all patients. However, I also noted significant age-specific associations between carotid PI and SVD, such that in younger individuals age <70, additional associations between ICA-PI with lacunes, visible BG-PVS and global SVD burden were present, but these very strong associations were attenuated in patients aged ≥70. Age is perhaps the most important independent predictor of arterial stiffness[15] and cerebral SVD,[18, 19] and hence any associations between carotid PI and SVD burden would be confounded by age.

In a small subgroup of consecutive patients who also had TCD ultrasound performed, I noted that the association between PI and SVD burden increased progressively with more distal arterial beds (MCA>ICA>CCA). Together with findings from previous studies,[7, 9-11] my results support the hypothesis that arterial stiffening results in an increased pulsatile flow that is propagated distally, along the large arterial beds, and is subsequently transmitted to the cerebral small vessels. This process may then result in the various parenchymal lesions as a consequence of cerebral SVD (e.g. leukoaraiosis, lacunes and BG-PVSs) due to diffuse cerebrovascular endothelial failure, blood brain barrier dysfunction, alterations in perfusion during diastole, increased endothelial shear stress and/or impaired cerebral autoregulation.[1]

My study has a number of limitations. The series of patients who received a TCD ultrasound was small and although my findings have been consistent with those from other studies,[3-5, 7, 8] my results would need to be confirmed in larger cohorts. Only 85/587 patients in the study population were classified as having a TIA or ischaemic stroke due to small vessel disease or occlusion and we were unable to demonstrate significant associations between carotid PI with TIA or ischaemic stroke due to SVD, although there was a trend that a potential association may be present, especially in younger individuals (OR of top vs. bottom quartile of ICA PI: 1.97, 0.76-5.13, p=0.17). Second, in contrast to previous studies,[4] I was not able to demonstrate significant associations between carotid pulsatility and cerebral microbleed burden. Cerebral microbleeds could be secondary to hypertensive or cerebral amyloid angiopathy. This cohort however, lacked power to determine whether carotid PI was a stronger predictor of strictly deep-seated microbleeds (i.e. suggestive of hypertensive angiopathy, only 7/587 patients with strictly deep microbleeds in this cohort) or strictly lobar microbleeds (suggestive of cerebral amyloid angiopathy, 44/587 patients with strictly lobar microbleeds in this cohort). Third, although I demonstrated that ICA and MCA-PI may be a surrogate marker of underlying SVD burden, the prognostic value of ICA and MCA-PI, over and above traditional vascular risk factors or neuroimaging findings in patients with TIA or ischaemic stroke remains uncertain. Similarly, whether ICA or MCA-PI is able to predict progression of SVD burden was not studied in this cohort. Finally, this cohort spanned a 10-year period, during which the neuroimaging protocol of OXVASC has changed. Patients were also scanned with 4 different MRI scanners. Nevertheless,

I have shown in Chapters 5 and 6 that the prognostic value of individual neuroimaging markers of SVD, such as visible PVSs, as well as the Total SVD Score was robust to the variations in scanner type and sequences used within OXVASC.

In conclusion, my findings suggest that ICA-PI and MCA-PI is a surrogate marker of underlying SVD burden and support the hypothesis that stiffening of the large arteries results in increased transmission of pulsatile flow along the carotid and cerebral circulation and may play a role in the pathophysiology of cerebral SVD. I have also demonstrated that significant age-specific associations between ICA-PI with neuroimaging markers of SVD are present.

Table 9.1 Clinical and imaging characteristics of the study population

	N=587 (TIA N=306, ischaemic stroke N=281)
Clinical characteristics	
Mean age, yr (SD)	69.5 (13.6)
Males (%)	300 (51.1)
Hypertension (%)	342 (58.3)
Hyperlipidaemia (%)	245 (41.7)
Diabetes (%)	85 (14.5)
Ever-smokers (%)	333 (56.7)
Atrial fibrillation (%)	81 (13.8)
Prior transient ischaemic attack or stroke (%)	133 (22.7)
Ischaemic heart disease (%)	103 (16.7)
Glomerular filtration rate, ml/min/1.73m^2	71.1 (21.4)
Imaging characteristics	
Common carotid artery pulsatility index (SD)	1.57 (0.29)
Internal carotid artery pulsatility index (SD)	1.28 (0.27)
Middle cerebral artery pulsatility index (SD)[a]	1.08 (0.24)
N with diffusion weighted imaging positive lesion (%)	125 (21.3)
N with visible basal ganglia perivascular spaces (%)	
1-10 (%)	316 (53.8)
11-20 (%)	149 (25.4)
>20(%)	122 (20.8)
N with visible centrum semi-ovale perivascular spaces (%)	
1-10 (%)	147 (25.0)
11-20 (%)	188 (32.0)
>20(%)	252 (42.9)
N with lacunes (%)	120 (20.4)
N with microbleeds (%)	92 (15.7)
1 microbleed (%)	42 (7.2)
2-4 microbleeds (%)	27 (4.6)
≥5 microbleeds (%)	27 (4.6)
Strictly deep microbleeds (%)	7 (1.2)
Strictly lobar microbleeds (%)	44 (7.5)
N with periventricular white matter hyperintensity (%)	
Grade 1 (%)	215 (36.6)
Grade 2 (%)	121 (20.6)
Grade 3 (%)	64 (10.9)
N with subcortical white matter hyperintensity (%)	
Grade 1 (%)	198 (33.7)
Grade 2 (%)	116 (19.8)
Grade 3 (%)	66 (11.2)
Mean Total Small Vessel Disease score	1.16 (1.17)

[a]Middle cerebral artery pulsatility index was measured in a subset of 94/587 patients
TIA=transient ischaemic attack; SD=standard deviation

Table 9.2 Clinical predictors of common carotid and internal carotid artery pulsatility index

	Univarate B (95% CI)	p	Age and sex adjusted B (95% CI)	p	Multivariate adjusted B (95% CI)	p
Common carotid artery pulsatility index						
Age	0.005 (0.004, 0.007)	<0.0001	0.006 (0.004, 0.007)	<0.0001	0.002 (0.000, 0.004)	0.016
Male sex	0.106 (0.061, 0.152)	<0.0001	0.107 (0.063, 0.151)	<0.0001	0.119 (0.074, 0.164)	<0.0001
History of hypertension	0.021 (-0.026, 0.068)	0.38	-0.012 (-0.058, 0.034)	0.60	-0.050 (-0.105, 0.005)	0.075
Hyperlipidaemia	0.032 (-0.015, 0.079)	0.18	0.014 (-0.031, 0.059)	0.54	-0.006 (-0.054, 0.041)	0.80
Diabetes	0.095 (0.030, 0.161)	0.004	0.075 (0.013, 0.138)	0.018	0.079 (0.016, 0.143)	0.014
Ever-smoking	0.006 (-0.041, 0.053)	0.80	-0.008 (-0.054, 0.038)	0.74	-0.022 (-0.067, 0.022)	0.33
Atrial fibrillation	0.097 (0.031, 0.164)	0.004	0.064 (-0.001, 0.128)	0.055	0.063 (0.000, 0.127)	0.051
Glomerular filtration rate	-0.002 (-0.003, -0.001)	0.0004	-0.001 (-0.002, 0.000)	0.16	0.000 (-0.002, 0.001)	0.49
Premorbid mean SBP (per SD increase)	0.049 (0.027, 0.070)	<0.0001	0.023 (0.001, 0.046)	0.045	-0.003 (-0.061, 0.054)	0.91
Premorbid mean DBP (per SD increase)	-0.025 (-0.046, -0.003)	0.028	-0.028 (-0.049, -0.007)	0.009	-0.019 (-0.056, 0.017)	0.30
Premorbid mean PP (per SD increase)	0.081 (0.060, 0.102)	<0.0001	0.063 (0.039, 0.087)	<0.0001	0.075 (0.026, 0.124)	0.003
Internal carotid artery pulsatility index						
Age	0.008 (0.007, 0.010)	<0.0001	0.008 (0.007, 0.010)	<0.0001	0.005 (0.004, 0.007)	<0.0001
Male sex	0.019 (-0.025, 0.062)	0.40	0.020 (-0.020, 0.060)	0.32	0.025 (-0.015, 0.065)	0.22
History of hypertension	0.103 (0.059, 0.146)	<0.0001	0.051 (0.009, 0.092)	0.017	0.012 (-0.037, 0.062)	0.62
Hyperlipidaemia	0.027 (-0.017, 0.072)	0.22	0.005 (-0.035, 0.046)	0.80	-0.032 (-0.074, 0.010)	0.14
Diabetes	0.110 (0.049, 0.171)	0.0005	0.091 (0.034, 0.147)	0.002	0.098 (0.042, 0.155)	0.001
Ever-smoking	-0.008 (-0.052, 0.036)	0.72	0.004 (-0.037, 0.045)	0.86	-0.014 (-0.054, 0.026)	0.49
Atrial fibrillation	0.081 (0.018, 0.144)	0.012	0.018 (-0.041, 0.077)	0.55	0.013 (-0.044, 0.069)	0.66
Glomerular filtration rate	-0.002 (-0.003, -0.001)	<0.0001	0.000 (-0.001, 0.001)	0.72	0.001 (0.000, 0.002)	0.11
Premorbid mean SBP (per SD increase)	0.081 (0.062, 0.101)	<0.0001	0.046 (0.026, 0.066)	<0.0001	0.020 (-0.031, 0.071)	0.44
Premorbid mean DBP (per SD increase)	-0.012 (-0.032, 0.009)	0.28	-0.011 (-0.030, 0.008)	0.26	-0.024 (-0.056, 0.009)	0.16
Premorbid mean PP (per SD increase)	0.110 (0.091, 0.128)	<0.0001	0.076 (0.055, 0.097)	<0.0001	0.063 (0.020, 0.107)	0.004

CI=confidence interval; SBP=systolic blood pressure; DBP=diastolic blood pressure; PP=pulse pressure; SD=standard deviation

Table 9.3 Relationships of common carotid and internal carotid artery pulsatility index (top vs. bottom quartile) with individual neuroimaging markers and global burden of small vessel disease

	Univariate OR (95% CI)	p	Age and sex adjusted OR (95% CI)	p
Lacunes				
CCA-PI	1.35 (0.77-2.38)	0.30	1.08 (0.59-1.96)	0.82
ICA-PI	2.74 (1.47-5.12)	0.002	1.94 (0.97-3.86)	0.061
Subcortical WMH burden				
CCA-PI	2.04 (1.34-3.10)	0.001	1.23 (0.79-1.93)	0.36
ICA-PI	3.50 (2.27-5.39)	<0.0001	1.48 (0.91-2.40)	0.11
Periventricular WMH burden				
CCA-PI	2.40 (1.57-3.66)	<0.0001	1.32 (0.84-2.07)	0.24
ICA-PI	4.71 (3.04-7.31)	<0.0001	1.68 (1.03-2.75)	0.037
Cerebral microbleed burden				
CCA-PI	1.77 (0.91-3.45)	0.093	1.14 (0.57-2.29)	0.71
ICA-PI	2.93 (1.48-5.81)	0.002	1.63 (0.76-3.47)	0.21
BG-PVS burden				
CCA-PI	1.94 (1.25-3.02)	0.003	0.90 (0.55-1.47)	0.66
ICA-PI	4.58 (2.80-7.48)	<0.0001	1.35 (0.77-2.36)	0.29
CS-PVS burden				
CCA-PI	1.96 (1.28-3.01)	0.002	1.13 (0.71-1.79)	0.60
ICA-PI	3.64 (2.35-5.64)	<0.0001	1.52 (0.93-2.47)	0.093
Total Small Vessel Disease Score				
CCA-PI	2.28 (1.49-3.47)	0.0001	1.12 (0.71-1.76)	0.64
ICA-PI	4.68 (3.01-7.29)	<0.0001	1.49 (0.91-2.45)	0.11

OR=odds ratio; CI=confidence interval; CCA=common carotid artery; ICA=internal carotid artery; PI=pulsatility index; WMH=white matter hyperintensity; BG=basal ganglia; CS=centrum semiovale; PVS=perivascular space

Table 9.4 Relationships of common carotid and internal carotid artery pulsatility index (top vs. bottom quartile) with individual neuroimaging markers and global burden of small vessel disease, stratified by sex

	Men (n=300)				Women (n=287)			
	Univariate OR (95% CI)	p	Age adjusted OR (95% CI)	p	Univariate OR (95% CI)	p	Age adjusted OR (95% CI)	p
Lacunes								
CCA-PI	1.58 (0.64-3.91)	0.32	1.39 (0.55-3.51)	0.48	1.51 (0.70-3.27)	0.30	0.94 (0.41-2.16)	0.89
ICA-PI	2.99 (1.17-7.62)	0.022	2.72 (0.99-7.46)	0.052	2.61 (1.12-6.08)	0.026	1.38 (0.53-3.60)	0.52
Subcortical WMH burden								
CCA-PI	1.78 (0.97-3.23)	0.061	1.14 (0.61-2.13)	0.68	2.62 (1.41-4.89)	0.002	1.19 (0.62-2.31)	0.60
ICA-PI	3.20 (1.74-5.91)	0.0002	1.65 (0.84-3.22)	0.15	3.97 (2.14-7.39)	<0.0001	1.31 (0.65-2.64)	0.45
Periventricular WMH burden								
CCA-PI	2.00 (1.10-3.65)	0.024	1.21 (0.64-2.28)	0.56	3.62 (1.92-6.83)	<0.0001	1.55 (0.78-3.05)	0.21
ICA-PI	4.44 (2.39-8.22)	<0.0001	2.03 (1.03-4.00)	0.041	5.30 (2.81-9.98)	<0.0001	1.37 (0.67-2.81)	0.39
Cerebral microbleed burden								
CCA-PI	2.22 (0.77-6.38)	0.14	1.67 (0.57-4.91)	0.35	1.59 (0.64-4.01)	0.33	0.86 (0.32-2.29)	0.76
ICA-PI	2.21 (0.94-5.21)	0.070	1.33 (0.52-3.44)	0.55	4.43 (1.38-14.28)	0.013	2.29 (0.64-8.24)	0.20
BG-PVS burden								
CCA-PI	1.42 (0.76-2.65)	0.27	0.72 (0.36-1.43)	0.35	3.12 (1.62-6.01)	0.001	1.30 (0.63-2.67)	0.48
ICA-PI	2.89 (1.48-5.65)	0.002	0.95 (0.44-2.04)	0.89	7.49 (3.61-15.53)	<0.0001	1.96 (0.86-4.43)	0.11
CS-PVS burden								
CCA-PI	1.41 (0.77-2.59)	0.27	0.87 (0.46-1.64)	0.66	2.82 (1.48-5.37)	0.002	1.49 (0.75-2.96)	0.25
ICA-PI	3.28 (1.78-6.04)	0.0001	1.53 (0.78-2.99)	0.21	4.03 (2.14-7.58)	<0.0001	1.47 (0.72-2.99)	0.29
Total SVD Score								
CCA-PI	1.84 (1.01-3.35)	0.046	1.11 (0.59-2.09)	0.75	3.12 (1.67-5.82)	0.0004	1.10 (0.56-2.15)	0.78
ICA-PI	3.72 (2.01-6.88)	<0.0001	1.46 (0.74-2.88)	0.28	6.06 (3.20-11.47)	<0.0001	1.52 (0.74-3.14)	0.26

OR=odds ratio; CI=confidence interval; CCA=common carotid artery; ICA=internal carotid artery; PI=pulsatility index; WMH=white matter hyperintensity; BG=basal ganglia; CS=centrum semiovale; PVS=perivascular space; SVD=small vessel disease

Table 9.5 Relationships of common carotid and internal carotid artery pulsatility index (top vs. bottom quartile) with individual neuroimaging markers and global burden of small vessel disease, stratified by age

	Age <70 (n=261)				Age ≥70 (n=326)			
	Univariate OR (95% CI)	p	Age and sex adjusted OR (95% CI)	p	Univariate OR (95% CI)	p	Age and sex adjusted OR (95% CI)	p
Lacunes								
CCA-PI	1.68 (0.70-4.04)	0.25	1.43 (0.57-3.55)	0.45	1.03 (0.47-2.28)	0.94	1.00 (0.45-2.25)	1.00
ICA-PI	6.17 (2.29-16.65)	0.0003	5.35 (1.95-14.70)	0.001	1.24 (0.49-3.16)	0.65	1.04 (0.40-2.73)	0.93
Subcortical WMH burden								
CCA-PI	1.55 (0.80-3.00)	0.20	1.23 (0.60-2.49)	0.58	1.30 (0.72-2.36)	0.39	1.19 (0.65-2.18)	0.58
ICA-PI	2.15 (0.99-4.65)	0.052	1.49 (0.66-3.35)	0.34	1.90 (0.96-3.78)	0.067	1.45 (0.72-2.95)	0.30
Periventricular WMH burden								
CCA-PI	1.80 (0.92-3.50)	0.084	1.40 (0.69-2.87)	0.35	1.46 (0.80-2.67)	0.22	1.30 (0.70-2.40)	0.40
ICA-PI	3.87 (1.78-8.42)	0.001	2.86 (1.26-6.46)	0.012	1.36 (0.68-2.71)	0.38	0.99 (0.49-2.02)	0.99
Cerebral microbleed burden								
CCA-PI	1.55 (0.46-5.20)	0.48	1.22 (0.35-4.25)	0.75	1.26 (0.54-2.95)	0.59	1.05 (0.44-2.49)	0.91
ICA-PI	1.50 (0.37-6.01)	0.57	1.14 (0.27-4.71)	0.86	1.99 (0.72-5.53)	0.19	1.57 (0.55-4.46)	0.40
BG-PVS burden								
CCA-PI	1.00 (0.45-2.23)	1.00	0.55 (0.23-1.32)	0.18	1.40 (0.76-2.58)	0.27	1.16 (0.62-2.16)	0.65
ICA-PI	4.25 (1.72-10.52)	0.002	2.83 (1.07-7.46)	0.035	1.57 (0.78-3.19)	0.21	0.99 (0.48-2.07)	0.98
CS-PVS burden								
CCA-PI	1.49 (0.77-2.88)	0.23	1.12 (0.56-2.23)	0.76	1.23 (0.65-2.32)	0.53	1.15 (0.60-2.19)	0.67
ICA-PI	2.71 (1.26-5.81)	0.01	1.96 (0.89-4.32)	0.095	1.67 (0.82-3.40)	0.16	1.48 (0.72-3.08)	0.29
Total SVD Score								
CCA-PI	1.50 (0.75-2.97)	0.25	1.08 (0.52-2.25)	0.84	1.48 (0.82-2.68)	0.19	1.20 (0.66-2.21)	0.55
ICA-PI	3.37 (1.54-7.38)	0.002	2.30 (1.01-5.25)	0.048	1.83 (0.93-3.63)	0.082	1.20 (0.59-2.42)	0.62

OR=odds ratio; CI=confidence interval; CCA=common carotid artery; ICA=internal carotid artery; PI=pulsatility index; WMH=white matter hyperintensity; BG=basal ganglia; CS=centrum semiovale; PVS=perivascular space; SVD=small vessel disease

Table 9.6 Relationships of common carotid, internal carotid and middle cerebral artery pulsatility index with global burden of small vessel disease in 94 patients with TIA or ischaemic stroke

	Univariate OR (95% CI)		Sex adjusted OR (95% CI)	
Total Small Vessel Disease Score				
CCA-PI (top vs. bottom quartile)	1.33 (0.45-3.96)	0.61	1.29 (0.43-1.88)	0.76
ICA-PI (top vs. bottom quartile)	2.37 (0.81-6.87)	0.11	2.37 (0.82-6.89)	0.11
MCA-PI (top vs. bottom quartile)	4.26 (1.45-12.55)	0.008	4.43 (1.49-13.13)	0.007

TIA=transient ischaemic attack; OR=odds ratio; CI=confidence interval; CCA=common carotid artery; ICA=internal carotid artery; PI=pulsatility index

Table 9.7 Aetiology of TIA or ischaemic strokes according to TOAST classification

	n=587 (TIA N=306, ischaemic stroke N=281)
Small vessel disease (%)	85 (14.5)
Large artery atherosclerosis (%)	87 (14.8)
Cardio-embolic (%)	79 (13.5)
Undetermined (%)	291 (49.6)
Multiple (%)	21 (3.6)
Unknown (%)	5 (0.9)
Others (%)	19 (3.2)

TIA=transient ischaemic attack; TOAST=Trial of Org 10172 in Acute Stroke Treatment

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Chapter 10

Conclusions and future research

Several interesting and original observations as a result of this thesis warrants further study.

First of all, I noted marked ethnic differences in underlying prevalence of individual neuroimaging markers of SVD – subjects from the Oxford cohort had a higher prevalence and burden of periventricular WMH, MRI-visible enlarged BG and CS-PVSs whilst those from the Hong Kong cohort had a higher prevalence and burden of microbleeds, lacunes and subcortical WMH. Although the two cohorts were matched by age, the underlying vascular risk factor profile was very different in the two cohorts. Those recruited from Hong Kong had a greater prevalence of hypertension and diabetes and those from the Oxford cohort had a greater prevalence of hyperlipidaemia and ever-smokers. Whether these observed ethnic differences in SVD prevalence and burden are due to underlying genetic factors, vascular risk factors, dietary and salt intake, other environmental (e.g. air pollution) or socio-economic risk factors warrants further study. Furthermore, although MRI-visible enlarged PVSs have been considered a marker of SVD, the fact that visible PVSs and periventricular WMH were more common in the Oxford cohort, whilst other markers of SVD (lacunes, microbleeds and subcortical WMH) were more common in the Hong Kong cohort, suggests that the development of visible PVSs and periventricular WMH may be due to alternative mechanisms. Indeed, we provided data from the Oxford cohort, that in TIA or ischaemic stroke patients aged <70, an increased internal carotid artery pulsatility index (ICA-PI) was associated with an increasing visible BG-PVS and periventricular WMH burden as well as lacunes, but was not associated with subcortical WMH or cerebral microbleed burden.

Second, although I noted significant time-course of risks of recurrent stroke in TIA or ischaemic stroke patients with microbleeds on antiplatelet agents, these findings require confirmation from other large multicenter studies, such as via the Microbleeds International Collaborative Network.[1] In patients with ≥5 microbleeds whose long-term risks of ICH may outweigh its ischaemic risks, whether gradual withdrawal of antiplatelet agents after the first year of TIA or ischaemic stroke would result in clinical benefit also requires further study and justifies a randomised controlled trial. Furthermore, previous studies, such as the Secondary Prevention of Small Subcortical Strokes (SPS3) trial[2] have demonstrated that in patients with recent lacunar strokes, the addition of long-term clopidogrel to aspirin does not significantly reduce the risk of recurrent stroke, but

significantly increases the risk of bleeding and death, suggesting limited benefit of long-term dual antiplatelet therapy in patients with lacunar strokes. However, whether the risks of short or long-term dual antiplatelet therapy similarly outweighs its benefits in patients with cerebral microbleeds, too requires further study.

Third, despite pooling my results with those from a recent meta-analysis,[3] the total number of TIA or ischaemic stroke patients on warfarin included in my study was small. Recent expert recommendations[4] have suggested that in patients with atrial fibrillation and <5 microbleeds, the benefits of warfarin will likely outweigh the potential risks and patients should be treated according to current atrial fibrillation guidelines. In contrast, in patients with ≥5 microbleeds, antithrombotic agents should be given in caution as the absolute ischaemic stroke risk appears to be similar to the absolute intracerebral haemorrhage (ICH) risk, and non-vitamin K oral anticoagulants (NOACs) or left atrial appendage occlusion should be considered.[4] Although my results somewhat supports these recommendations, the two cohorts which my findings were based on were limited with regards to the outcomes of interest (only 2 patients with recurrent ischaemic stroke and 4 with ICH in warfarin users with ≥5 microbleeds). Similarly, due to the limited number of outcomes, I was unable to perform further sensitivity analyses based on the small number of patients with strictly deep or strictly lobar microbleeds. Moreover, our comparison of the hazards and rates of recurrent ischaemic stroke and ICH in TIA or ischaemic stroke patients with atrial fibrillation given anticoagulants, versus TIA or ischaemic stroke patients (with or without atrial fibrillation) given antiplatelet agents may not be entirely valid. A better comparison may have been a randomised controlled trial of TIA/ischaemic stroke patients with atrial fibrillation with ≥5 microbleeds randomised to warfarin, antiplatelet agents and NOACs. Large international collaborations such as the Microbleeds International Collaborative Network[1] and the ongoing clinical trials - The Clinical Relevance of Microbleeds in Stroke Study (CROMIS-2)[5] and Intracerebral Haemorrhage Due to Oral Anticoagulants: Prediction of the Risk by Magnetic Resonance (HERO) would be able to provide more information about the implications of warfarin use in TIA or ischaemic stroke patients with microbleeds.

Fourth, there were only a small number of patients who were prescribed with NOACs in OXVASC and HKU and the duration of follow-up was short in these patients. Although it was reassuring to note that none of the patients with microbleeds on NOACs developed an ICH on follow-up, my study is underpowered to study this and further research to determine the safety of NOACs in patients with microbleeds is warranted.

Fifth, my studies were limited as anti-thrombotic (antiplatelet, warfarin and NOAC) use at one month from discharge was recorded and hence long-term compliance is uncertain. The relationship of long-term anti-thrombotic use with risk, time-course and severity of recurrent events with cerebral microbleed burden could therefore be looked into in future studies.

Sixth, although in my thesis, I studied a wide range of neuroimaging markers of SVD, I have yet to study the ethnic differences, clinical correlates and prognostic implications of cortical superficial siderosis. Cortical superficial siderosis seen on haemosiderin-sensitive sequences of MRI refers to the linear deposits of haemosiderin deposited at the cortical sulci over the convexities of the cerebral hemispheres and is considered a key feature of cerebral amyloid angiopathy.[6] Whether further modifications to the Total SVD Score, such as by incorporating presence of cortical superficial siderosis, in addition to the proposed ammendments in this thesis will identify a very high risk group of individuals who are at risk of developing ICH with antithrombotic agents would require further study.

Seventh, recent studies of individuals without stroke have demonstrated the associations of a range of neuroimaging markers of SVD with various markers of systemic and vascular inflammation as well as oxidative stress, suggesting that biological mechanisms underlying ischaemic (e.g. lacunes and WMH) and haemorrhagic subtypes (e.g. microbleeds) of SVD may differ.[7] However, this study mainly consisted of healthy Caucasians without stroke with a low prevalence of SVD. Whether these markers are similarly implicated in the pathogenesis of SVD in TIA or ischaemic stroke have yet to be studied.

Eighth, blood brain barrier dysfunction has been implicated in the pathogenesis of SVD.[8] Whether differences in blood brain barrier permeability may explain for the differences in prevalence of individual neuroimaging markers of SVD in Caucasians and Chinese remains unknown. Mechanisms (e.g. differences in inflammatory, oxidative stress markers and other dietary and environmental factors etc.) underlying potential differences in blood brain permeability (if any) between the two populations would also require further study.

Ninth, the main outcome measures in this thesis was recurrent ischaemic stroke, ICH and death. However, SVD is a common cause of cognitive impairment and gait disturbances and these outcomes were not studied in this thesis. How global SVD burden predict cognitive impairment and cognitive decline in TIA or ischaemic stroke patients warrants further study.

Tenth, although I managed to show interesting results correlating carotid and cerebral pulsatility with SVD burden, the sample size was small with only 94 TIA or ischaemic stroke patients having received a carotid and transcranial Doppler ultrasound as well as MRI. Larger studies to confirm my findings will be needed. My findings nevertheless support that the hypotehesis that arterial stiffening results in an increased pulsatile flow that is propagated distally, along the large arterial beds, and is subsequently transmitted to the cerebral small vessels. This process may then result in the various parenchymal lesions as a consequence of cerebral SVD (e.g. WMH, lacunes and MRI-visible enlarged basal ganglia perivascular spaces). However, it is uncertain how much middle cerebral artery pulsatility index is also a measure of the resistance characteristics of the distal small vessel vascular bed, and hence how much the middle cerebral artery pulsatility index risk association is due to reverse causation is unknown.

Eleventh, although we demonstrated that premorbid blood pressure is a stronger predictor of SVD burden than a baseline measurement of blood pressure or known history of hypertension, and that a latency effect seemed to exist, such that the risk associations between blood pressure and SVD burden is stronger amongst readings taken 10-20 years prior to TIA/ischaemic stroke, a number of interesting questions remain. There has been studies that have demonstrated that with progressive cognitive decline, blood pressure seems to fall,[9] possibly due to a decrease in

number of C1 neurons in the medulla oblongata and blood pressure dysregulation.[10] Whether our results reflect a drop in blood pressure with time in patients with increasing SVD burden could not be excluded. Further studies can also consider classifying patients by duration and severity of hypertension to determine if these factors may be an even stronger predictor of SVD burden.

Twelfth, it should be noted that the multiple statistical comparisons used in this thesis may have generated potential false positive results. However, some of the analyses are somewhat more hypothesis-generating than hypothesis-testing and in some analyses where no other data is available, an explorative approach is inevitable and our results should subsequently be confirmed and validated in other cohorts.

Finally, the work in this thesis was only limited to utilising structural neuroimaging markers of SVD available on conventional MRI. Further research with new MRI techniques such as measuring white matter structural and functional integrity using diffusion tensor imaging (DTI) or diffusion kurtosis imaging (DKI) and studying cerebrovascular reactivity[11] are now available and would be able to shed light in further understanding the mechanisms and risk factors leading to SVD (especially at the early stages where markers detectable by conventional imaging have yet to develop) and hence provide opprotunities in prevention and treatment of SVD.

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