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Prediction, detection and suppression of cerebral microemboli associated with carotid disease

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I am aware of University regulations governing plagiarism and I declare that this document is all my own work except where I have stated otherwise. I consider myself ready for Ph.D. dissertation submission.

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Abstract

Background
Transient cerebral microemboli detected by transcranial Doppler (TCD) have been demonstrated to be a reliable biomarker for short term stroke risk in two clinical settings; patients with carotid artery stenosis and those following carotid endarterectomy. Suppressing cerebral microemboli using TCD-directed anti-platelet treatment reduces risk of recurrent stroke. The association between classical cardiovascular risk factors and cerebral microemboli has not been studied. Furthermore, standard TCD method to detect cerebral microemboli is limited by lack of acoustic temporal bone window which is not available in approximately 1 in 7 patients. TCD is the only real-time imaging modality for detecting microemboli. The demonstration of the kinetic of the microemboli is invaluable in assessing the efficacy of antiplatelet agents.

Hypotheses
1. Whether Pocock cardiovascular risk score and $ABCD^2$ risk score are able to predict the presence of cerebral microemboli.
2. The feasibility of using transorbital Doppler as an alternative to transcranial Doppler.
3. The effectiveness of Tirofiban in suppressing cerebral microemboli.

Methods
1. Pocock score was assessed for the newly developed Carotid Surgery Registry of 670 patients managed between January 2002 and December 2012.
2. $ABCD^2$ score was determined in 206 patients with hyper-acute symptomatic critical carotid artery stenosis in which 102 of these patients were from Registry. A further 104 consecutive patients were recruited between February 2011 and May 2013 within a new prospective observational study.
3. The feasibility of using transorbital Doppler was assessed based on the Registry data. Further new prospective validation study of transorbital Doppler against standard TCD method involving 100 consecutive patients undergoing elective carotid endarterectomies were undertaken.
4. From the Registry, patients who had microembolic signals acutely following carotid surgery were assessed to evaluate the effectiveness of Tirofiban in suppressing microemboli.

Findings
1. A high Pocock score (≥ 0.8%) predicts presence of cerebral microemboli acutely following carotid endarterectomy (Area Under Curve (AUC) 0.582 95% CI 0.507 – 0.658, P = 0.038). It also showed a high sensitivity for the presence of microemboli in patients with hyper-acute symptomatic carotid artery disease.
2. The $ABCD^2$ score did not predict presence of cerebral microemboli ((AUC 0.49 95% CI 0.41 – 0.57, P = 0.860), or carotid disease in over one-quarter of patients with symptomatic critical carotid artery stenosis.
3. Transorbital Doppler imaging appears a valid alternative to transcranial Doppler for detecting microembolic signals in patients with no suitable temporal acoustic window (sensitivity 80 %, specificity 86 %. Bland and Altman analysis revealed no significant bias [bias 0.11 microemboli (95% CI: -0.52 to 0.74), P = 0.81]).
4. Tirofiban has been shown to be more effective in treating microemboli in comparison to other most commonly used antithrombotic agents. The time for complete microemboli resolution (Tirofiban 68 minutes (53-94); dextran 113 (79-146); or in controls 53(49-68); P<0.001, KW) were shorter with tirofiban.

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Abbreviations

ACA	Anterior cerebral artery
CEA	Carotid endarterectomy
CTA	Computed Tomography Angiogram
CVA	Cerebrovascular accident
DW MR Imaging	Diffusion-weighted magnetic resonance imaging
ECST	European Carotid Surgery Trial
GA	General anaesthesia
LA	Local anaesthesia
MI	Myocardial infarction
MRA	Magnetic Resonance Angiogram
MRI	Magnetic Resonance Imaging
MCA	Middle cerebral artery
MES	Microembolic signals
NASCET	North American Symptomatic Carotid Endarterectomy Trial
TAW	Temporal acoustic window
TCD	Transcranial Doppler
TIA	Transient ischaemic attack
TOD	Transorbital Doppler

Introduction

The risk of recurrent stroke following first transient ischaemic attack (TIA) or minor stroke is highest during the first 7 – 14 days[1]. Atherosclerotic carotid artery disease, whether symptomatic or asymptomatic is a major contributor to recurrent stroke. Thromboembolism is the primary mechanism of recurrent stroke in carotid artery disease. Transcranial Doppler detected microemboli have been shown as a reliable short term stroke risk biomarker in acute carotid syndrome. The context of my thesis is the clinical prediction, detection and suppression of transcranial Doppler detection of microemboli in symptomatic critical carotid artery disease patients. Transient cerebral microemboli associated with carotid disease detected by transcranial Doppler commonly occurred in 2 clinical settings which comprised the research cohorts in my thesis:

1. Hyper-acute symptomatic critical carotid artery stenosis patients – defined as patients who presented with acute symptoms of stroke or transient ischaemic attack with evidence of carotid stenosis within 2 weeks of the index symptoms[2].

2. Patients who underwent carotid endarterectomy.

Background chapters cover an overview of symptomatic carotid atherosclerosis artery disease which includes the mechanism, cardiovascular risk factors, $ABCD^2$ risk score, principles of carotid duplex and carotid endarterectomy. The association between cardiovascular risk factors, $ABCD^2$ risk score and carotid artery disease were reviewed. This will be followed by detailed description of the establishment of microemboli detected by transcranial Doppler as the short term stroke risk in carotid artery disease patients and post carotid endarterectomy patients. In this thesis, the terms 'microembolic signals' detected by the transcranial Doppler and 'microemboli' were used interchangeably.

The process of establishing the carotid Registry and prospective observational studies was described in the methods section. The process of data collection was explained in great detail. The same data definitions were used for the Registry and the prospective observational studies. The

technical aspects of transcranial Doppler and carotid endarterectomy were also described in the chapter.

The results chapters were divided within the context of Registry, hyper-acute prospective observational study of minor stroke/TIA, transorbital imaging and antiplatelet treatment. The analysis of association between Pocock risk score, ABCD^2 risk score and microemboli were covered in Registry, hyper-acute prospective observational study of minor stroke/TIA. Transorbital imaging explained the development of transorbital Doppler in detecting microemboli and the antiplatelet treatment chapter covers the relevance of tirofiban as the treatment of choice for microemboli.

The thesis concludes with an overall discussion of the presented data in the context of other studies and an outlook for future studies.

Thesis flow chart
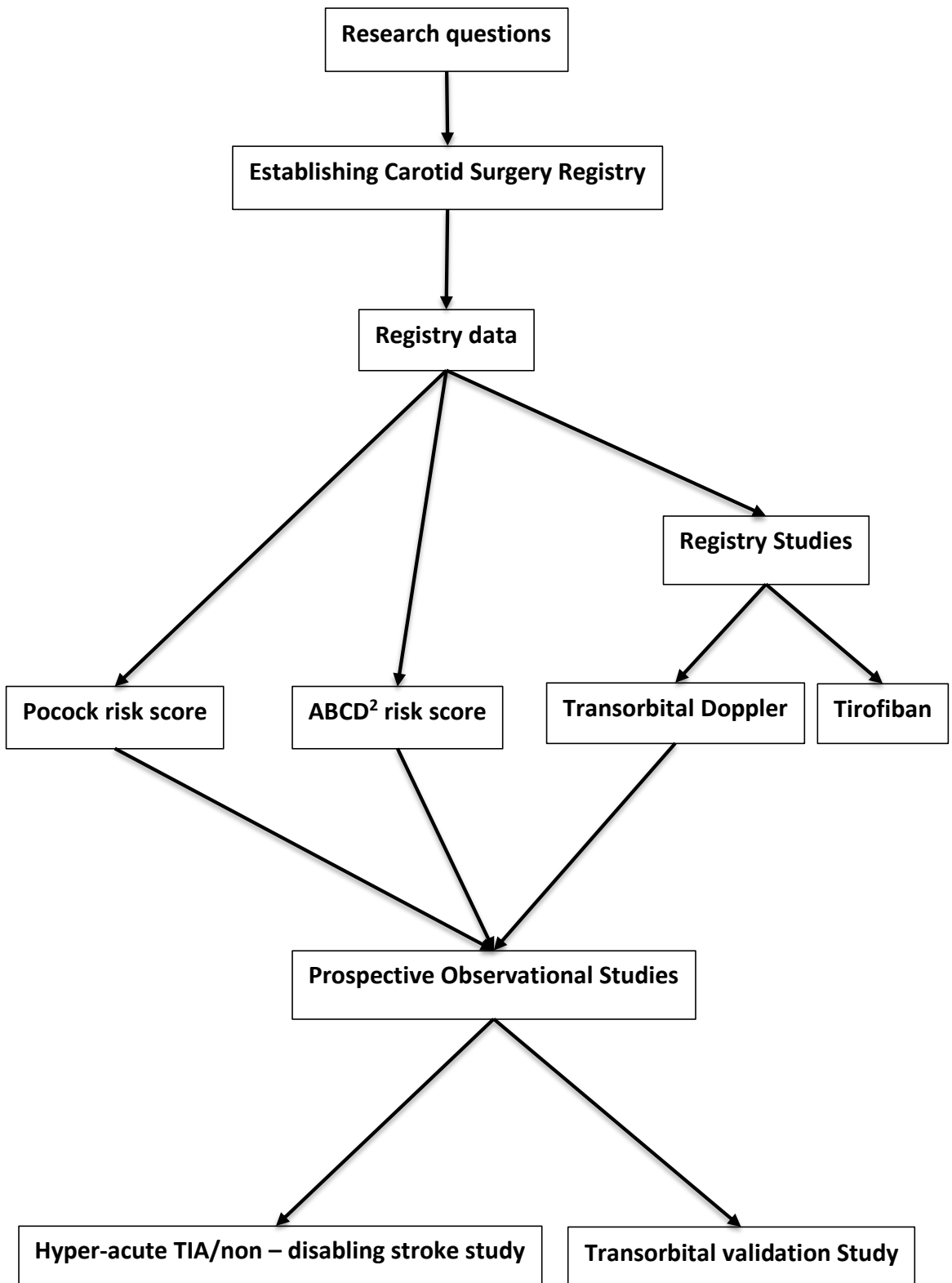
CHAPTER ONE

1 Background

1.1 Symptomatic Carotid Artery Disease

1.1.1 Risk and mechanism of recurrence stroke

Stroke is the third leading cause of death in developed countries and a leading cause of long term disability[3]. It is also the most frequent neurological diagnosis that requires hospitalisation[4]. Data from Northern America showed that approximately 88% of all strokes are ischaemic, 9% are intracerebral haemorrhages and 3% are subarachnoid haemorrhages[5-7]. It has been estimated that approximately 20% of all ischaemic strokes are preceded by transient ischaemic attack or minor ischaemic stroke[8]. Approximately 46 000 people in the UK each year have first transient ischaemic attacks (TIA)[9]. The risk of recurrent stroke following first TIA or minor stroke is highest during the first 7 – 14 days[1]. It has been estimated that risk of recurrent ischaemic stroke was 8.0% (95% CI 2.3% to 13.7%) at seven days, 11.5% (95% CI 4.8% to 18.2%) at one month, and 17.3% (95% CI 9.3% to 25.3%) at three months after a TIA or minor stroke[10].

Extra-cranial cerebrovascular disorders affect the carotid and vertebral arteries which supply the cerebral tissue and is an important cause of stroke and TIA. The major cause of carotid artery stenosis results from atherosclerotic disease. However, other causes include dissection, arteritis, cystic medial necrosis, trauma, radiation and fibromuscular dysplasia. Atherosclerosis is a systemic disease and develops insidiously over a period of time. Stroke is unsurprisingly, the first clinical manifestation of symptomatic carotid artery disease. Symptomatic carotid artery disease patients are typically at high risk of developing other cardiovascular adverse events such as stroke, myocardial infarction and complications related to peripheral artery disease[11]. The pathophysiology of atherosclerosis in the carotid artery is similar to the atherosclerosis that affects other artery beds. The process starts with the initiation of the lipoprotein accumulation in the intima layer of the artery. This is followed by the oxidative and inflammatory processes[12]

aided by cytokines. This causes the migration of monocytes to the intimal area and release of additional cytokines, oxidants and matrix metalloproteinases which is then followed by the accumulation of lipid-laden macrophages and migration of smooth muscle from the media to the intima. Extracellular lipid then accumulates in the central core surrounded by a fibrous cap which is a layer of connective tissue. Some of these advanced plaques will be calcified and continue to grow causing carotid stenosis. These plaques often develop at the flow divider where there is turbulence and low shear stress such as at the carotid bifurcation area. Previous experimental study demonstrated that the carotid plaque is consistently found along the outer wall of the internal carotid artery, opposite the flow divider, which corresponds to an area of low shear stress[13]. Plaque disruption and the formation of subsequent thrombosis worsens the narrowing of the arterial lumen causing further stenosis or an acute occlusion which lead to the clinical events. This mechanism is similar to those proposed for the coronary arteries[14].

Atherosclerotic carotid artery stenosis is the most common underlying cause in patients who have had an earlier TIA[15]. The Cardiovascular Health Study evaluated the prevalence of extracranial carotid atherosclerosis assessed by carotid Duplex in men and women aged ≥ 65 years[16]. Overall, detectable carotid stenosis was present in 75% of men and 62% of women[16]. They also found that 7% of men and 5% of women had 50%-74% carotid stenosis and severe stenosis (>74%) was detected in 2.3% of men and 1.1% of women[16]. The findings are similar to the more recent Framingham Heart Study population which showed that the prevalence of >50% carotid stenosis was 7% in women and 9% in men ranging in age from 65 onwards[17]. Olin et al[18] reviewed the natural history and progression of 465 patients with asymptomatic moderate carotid artery stenosis (60% – 79%). They reported that the incidence of ipsilateral stroke were higher in cohort of patients who demonstrated a progression of carotid stenosis than those who did not progress (12.5% vs. 3.1%). A similar study by Rockman et al[19] also demonstrated that, in moderate stenosed carotid artery the occurrence of ipsilateral stroke is patients with increasing stenosed carotid lesion than static carotid lesion. More recent study by Nicolaides et al[20] reported that

those patients who demonstrated progressively increasing carotid stenosis were at twice the risk of ipsilateral stroke compared with those without stenosis progression.

Currently, clinically significant carotid artery stenosis contributed up to 20% of all ischaemic in comparison with other subgroups such as cardioembolic and small vessel stroke[15, 21, 22]. In carotid disease, the main mechanisms of ischaemic stroke and TIA may be attributed to several mechanisms such as thromboembolism originating from the unstable atherosclerotic plaque, embolism of cholesterol crystals or other atheromatous debris, acute thrombotic occlusion of the carotid resulting from plaque rupture, disintegration of the arterial wall following dissection or subintimal haematoma and reduced cerebral perfusion secondary to reduce blood flow. Caplan et al further suggested that the reduced blood flow at sites of narrowed arteries promotes formation of thrombi and thus increases the quantity of arterial thromboemboli[23].

In recent years, emphasis has dramatically changed to embolism as the predominant mechanism of brain infraction[23]. In carotid disease, thromboembolism is the predominant mechanism of recurrent stroke and was first established by Miller Fisher in the 1950s[24, 25]. Subsequently, further evidence supporting thromboembolism as the predominant mechanism emerged from different clinical sources. Microthromboemboli are often found at autopsy within an acute brain infarct[26]. Post-mortem and radiological studies have shown that thromboembolism appears to be the main mechanism of middle cerebral artery territory infarction in patients with carotid artery disease[27, 28]. Diffusion-weighted magnetic resonance imaging based studies[29, 30] have demonstrated that carotid artery originated microemboli cause the development of an ischaemic area in the deep layers of the cortex in the distribution of the middle cerebral artery. Direct evidence for the role of embolisation has been provided by transcranial Doppler detection of circulating microemboli. Transcranial Doppler ultrasound studies show that the presence of microemboli in symptomatic carotid artery disease is associated with increased risk of stroke[31-33]. These cerebral microemboli predict risk of future ischemic stroke events in patients with symptomatic or asymptomatic carotid stenosis, after controlling for other cardiovascular risk factors, degree of stenosis, symptoms, and use of aspirin or anticoagulant[34]. They can be used reliably as a biomarker of short

term stroke risk both following acute symptomatic carotid artery stenosis and carotid endarterectomy[35, 36]. Randomised controlled trial have shown that antiplatelet treatment, which suppresses microemboli, also reduces stroke risk[37] in carotid artery disease patients.

The severity of the stenotic lesion of the carotid artery remains the major prognostic factor in symptomatic patients[38] that indicates the likelihood of future development of ischaemic stroke and therefore influences the decision regarding carotid revascularisation. Other potential markers of carotid plaque vulnerability and its stroke risk association have been explored. The Framingham Heart Study showed fewer than half of the stroke events which affected the cerebral hemisphere ipsilateral to the bruit and carotid stenosis[6]. Whilst carotid bruit is clearly an indicator of increased stroke risk and a general sign of advanced atherosclerotic disease, it is not necessarily an indicator of carotid stenosis preceding cerebral infarction. Carotid plaque vulnerability characteristics such as ulceration, echolucency, intraplaque haemorrhage and high lipid content, and its association with stroke risk in symptomatic patients, has been studied[39, 40]. Park et al[41] evaluated the correlation between carotid plaque characteristics and patients presenting symptoms by examining carotid plaque intraoperatively during carotid surgery. Plaque ulceration correlated with symptomatic carotid stenosis compared with patients operated on for asymptomatic disease[41]. The presence of intraplaque haemorrhage was associated with more advanced stenosis of the internal carotid artery. Further histological analysis by Fisher et al demonstrated that microscopic morphology of plaque ulceration is more common in symptomatic patients regardless of the side of carotid symptoms, whereas thrombus is associated with ipsilateral symptoms and plaque ulceration[40]. Contrast-enhanced magnetic resonance imaging and computed tomography has been used to study the characteristics of unstable carotid plaque. Preliminary data demonstrated an association between fibrous caps, intraplaque haemorrhage, lipid rich or necrotic core with stroke risk of asymptomatic patients with more than 50% carotid stenosis[42]. The development of carotid plaque in symptomatic patients has been associated with an inflammatory process[14]. The increased uptake of ^{18}F-fluorodeoxyglucose, demonstrated by positron emission tomography (PET) imaging is believed to reflect the inflammation process[43].

Biomarkers indicating carotid plaque instability such as C-reactive protein[44] have also been investigated. In a small cohort of patients, Alvarez et al[45] found that metalloproteinase-2 and metalloproteinases-9 was associated with carotid plaque instability with higher serum levels in symptomatic carotid patients than asymptomatic patients. The reliability of these biomarkers to predict high risk symptomatic carotid patients has not been established.

Despite extensive research studies on other modalities to predict carotid plaque instability, the degree of stenosis defined by ultrasound Duplex remains the main determinant of disease severity and the basis for clinical decision making. The higher degree of stenosis reflects the greater volume of carotid which may embolise, causing thrombotic ischaemic stroke[46]. Data from North American Symptomatic Carotid Endarterectomy Trial (NASCET)[47] and the European Carotid Surgery Trial (ECST)[38] have confirmed that plaque size and degree of lumen obstruction represent an important risk of stroke associated with carotid artery stenosis. Furthermore, Streifler et al[48] and Norris et al[49, 50] found increasing cumulative rates of TIA or stroke in relation to increasing degree of arterial stenosis, and the incidence of ischaemic events also correlated with progression of stenosis. The pooled analysis of the major randomized carotid endarterectomy (CEA) trials showed that a stenosis exceeding 70 % was detected in 21 % of patients who experienced TIA or stroke, and a moderate stenosis (50 - 69%) in 25 % of patients[51]. The prevalence rates of clinically significant carotid artery stenosis in populations above 65 years of age have been 5-7 % in women and 7-9 % in men, and for the age group of 70 years or more, the prevalence rates were 12.5 % for men and 6.9 % for women[16, 17, 52].

Symptomatic carotid artery disease has a high recurrent risk of stroke and this cohort of patient would achieve maximum benefit from carotid revascularisation within 2 weeks after the last cerebrovascular symptoms[53]. Therefore, guidelines recommend that CEA should be performed within a time frame of 2 weeks[54].

Table 1 below shows the summaries of the evidence of recurrent stroke rate within 14 days following recent symptomatic critical carotid artery stenosis (≥ 50% lumen narrowing) for the last 10 years. As an example, I performed a literature search which only included prospective studies

focussing only on symptomatic haemodynamically significant ≥ 50% carotid stenosis. Stroke recurrence was defined as new developing focal neurological deficit lasting > 24 hour following the initial TIA or minor stroke.

Table 1 Risk of recurrent stroke in symptomatic carotid disease

Year	Authors	Research Method	No. of patient	Risk of recurrent stroke rate within 14 days
2013	Al-Khaled et al[55]	Registry	597	5%
2013	Johansson et al[56]	Prospective	230	11.2%
2011	Marnane et al[57]	Prospective	36	8.3%
2009	Ois A et al[58]	Prospective	163	25%
2006	Kastrup A et al	Prospective	131	12%
2005	Fairhead JF et al[59]	Registry	120	21%

A population-based study[59] focusing on patients with symptomatic carotid artery stenosis (≥ 50%) reported the risk of stroke prior to carotid endarterectomy was 21% at 2 weeks and 32% at 12 weeks, in half of which strokes were disabling or fatal. A more recent study showed that patients presenting with symptomatic carotid artery stenosis (≥ 50%) incurred a 17% risk of recurrent stroke at 72 hour, 22% at 7 days and 25% at 14 days[58]. Johansson et al found that despite being on optimal best medical therapy, the recurrent stroke risk in this cohort was high ~ 11% within 14 days of the last cerebrovascular symptoms.

Transcranial Doppler detected microemboli has provided direct evidence of thromboembolism as the main mechanism of carotid disease. Cerebral microemboli has also been established as a surrogate biomarker for short term stroke risk[60]. In my thesis, I attempted to clinically predict the presence of microemboli. This in turn could potentially be used as a clinical tool to identify cerebral microemboli in 2 clinical: following acute symptomatic carotid artery disease patients and acutely post carotid endarterectomy.

1.1.2 Cardiovascular risk factors in carotid disease

Patients with symptomatic atherosclerotic carotid disease typically have clusters of classical cardiovascular risk factors[61]. Patients with carotid artery disease frequently have atherosclerosis

in other arterial beds such as aorta, coronary and peripheral areas[62]. Several classical and non-classical cardiovascular risk factors have been associated with the development and severity of carotid atherosclerotic disease[63]. Recognition of these systemic risk factors is important because the long term management of symptomatic carotid disease is the control of these cardiovascular risk factors.

Population-based studies have consistently demonstrated that increasing age and male gender remain the main risk factors for carotid artery disease[16, 64-66]; even with advancing age, carotid artery disease is more common in males than females[64]. In a large population study, Joakimsen et al showed that until the age of 50 years, a higher proportion of males have carotid artery disease and thereafter it declines so that the prevalence of atherosclerosis is similar in elderly men and women[67]. This suggests that events related to the menopause may promote the development of atherosclerosis in elderly women.

Both active and passive smoking are associated with the severity of carotid artery stenosis[68]. Smoking has also been identified as a strong predictor of atherosclerosis in men[65] and elderly women[64]. The Framingham study demonstrated the development of carotid artery stenosis with the quantity of cigarettes smoked over time[69]. Increased exposure to smoking is significantly related to increased carotid artery intima media thickness and carotid artery stenosis[70-72]. The associations included thicker walls and higher degrees of stenosis from never-smokers to former smokers to current smokers[71]. Tell et al[71] found smaller degrees of carotid wall thickening in former smokers than current smokers. This may suggest that smoking cessation slows the progression of the atherosclerosis.

Epidemiology studies have established an association between hypertension and the development of carotid artery disease[69, 73]. Su et al demonstrated that hypertensive patients are five times more likely to develop critical artery stenoses in comparison to the normotensive cohort[74]. Whilst systolic hypertension has been closely associated with the severity of carotid artery disease in population-based studies[64, 65], diastolic blood pressure has a weak association with the severity of carotid disease[16]. In the Systolic Hypertension in the Elderly Program study[75],

systolic blood pressure of > or = 160 mmHg was the strongest predictor of carotid stenosis. In the longitudinal Framingham Heart Study[69], a twice greater risk of developing carotid artery stenosis was demonstrated with each increase of 20 mmHg in systolic blood pressure (odds ratio 2.11 (95% CI 1.51 - 2.97). Atherosclerosis may lead to arterial rigidity and cause a rise in systolic blood pressure. The strongly positive association between high systolic pressure and the development of carotid stenosis is a reminder of the importance of systolic pressure in determining the risk of cardiovascular disease.

The prevalence of critical carotid stenosis has been reported in up to 33% of patients in different high risk groups investigated for either ischaemic heart disease or peripheral arterial disease[76-78]. Patients with carotid artery disease have been shown to be at an increased risk of myocardial infarction and death related to ischaemic heart disease[11]. Many of them face a greater risk of death from myocardial infarction than stroke[11]. An autopsy study demonstrated that the prevalence of coronary atherosclerosis and myocardial infarction (MI) was significantly higher in patients with stroke than patients with other neurologic diseases[79]. The prevalence of coronary plaques, coronary stenosis, and MI was 79.0%, 42.9%, and 46.0%, respectively, in the presence of plaques in any segment of the extracranial and intracranial brain arteries, and 50.8%, 17.9%, and 23.9%, respectively, in the absence of plaques[79]. This showed that in stroke patients, the mortality related to the ischaemic heart disease is more frequent when atherosclerosis is present in the carotid and cerebral arteries.

The presence of diabetes mellitus increases the risk of atherosclerosis and its complications such as carotid artery disease[66, 80]. The Cardiovascular Health study reported that diabetes was associated with the development of increasing carotid intima-media thickness and the severity of carotid artery stenosis[16]. The Insulin Resistance Atherosclerosis Study (IRAS), an observational cohort study,[81] demonstrated that diabetes and high level of fasting glucose were associated with two fold higher progression of carotid artery disease than in non-diabetic patients. The Rotterdam study[82] demonstrated that diabetes predicted progression to severe carotid artery obstruction.

Some studies have found an independent association between hypercholesterolaemia and carotid atherosclerosis[66]. For 34 years, the Framingham study[69] observed the surviving members of participants of the original cohort of the Framingham Heart Study who had carotid ultrasound performed. These long term serial observations were thought to be better than a single contemporaneous measurement. They demonstrated that the risk ratio of carotid artery stenosis > 25% development was approximately 1.1 for every 10 mg/dL increase in total cholesterol[69]. The Multi-Ethnic Study of Atherosclerosis (MESA) cohort study showed that carotid plaque lipid core detected by MRI scan was strongly associated with total cholesterol level[83].

Carotid artery intima-media thickness has been shown to be a marker of systemic atherosclerosis[11] and is associated with cardiovascular risk factors. The measurement is obtained by an ultrasonographic technique. A positive association between carotid-artery intima–media thickness and the incidence of new myocardial infarction and stroke in adults 65 years of age or older who did not have a history of cardiovascular disease has been reported[84]. However, the use of carotid artery intima-media thickness to assess atherosclerotic risks has not yet become routine or part of the carotid artery disease assessment. Recent evidence suggests a prominent genetic influence for the presence of carotid artery disease[85-87]. Hunt et al found a statistically significant heritability for focal carotid artery plaque that may represent advanced stage of atherosclerosis after accounting for age and sex effects[86]. Systemic inflammation has been associated with atherosclerosis plaque formation and stroke risk[88,89]. Recently, there is an emerging interest in the diagnostic potential of biological markers of inflammation and thrombosis and their association with the presence and severity of atherosclerotic disease. A previous cross-sectional study by Selhub et al[90] showed high plasma homocysteine concentrations and low concentrations of folate and vitamin B6, through their role in homocysteine metabolism, are associated with an increased risk of extracranial carotid artery stenosis. The Rotterdam study[91] found that while C-reactive protein showed increased level in patient with carotid artery disease, the results were less consistent with inflammatory mediators compared with Interleukin-6 and soluble intracellular cell adhesion molecules such as sICAM-1.

Symptomatic atherosclerosis carotid disease patients typically present with clusters of systemic cardiovascular risk factors, hence the long term management of these patients relies on the control of all the cardiovascular risk factors. A number of composite risk scores have been developed to estimate the risk of patients developing cardiovascular disease, based on the presence of cardiovascular risk factors and established clinical vascular disease. The estimated cardiovascular disease (CVD) risk score has been widely advocated[92] as a tool to introduce medical intervention on patients such as prescribing antiplatelet, antihypertensive and lipid lowering agents. The reasons for estimating total cardiovascular disease risk based on the major risk factors are[93] : atherosclerotic disease is multifactorial and systemic in origin; risk factors tend to cluster; and co-existent risk factors tend to increase the risk even further and therefore it is important to take into account all the risk factors.

The three most commonly used cardiovascular risk scores in the UK are the Framingham risk score, the Joint British societies risk score and the ASSessing cardiovascular risk using SIGN (ASSIGN) score. The Framingham risk score was the first composite cardiovascular risk score, which was initially developed to estimate the 10-year risk of developing coronary heart disease based on the Framingham Heart Study[94]. It was subsequently updated to predict the 10-year cardiovascular disease (CVD) risk[95], which included the combined end point of coronary heart disease (fatal and non-fatal myocardial infarction and new angina) plus stroke (fatal and non-fatal stroke and cerebral haemorrhage) and transient cerebral ischaemia. The risk calculation algorithm used sex, age, systolic blood pressure, total cholesterol, HDL cholesterol, smoking status and presence of left ventricular hypertrophy as the equation variables. The risk score is an integer, which adds points for each cardiovascular risk factor. The score is sex-specific and the calculated points are categorised to the probability (expressed as percent chance) of developing CVD in 10 years. A risk score of more than 18, which is equivalent to 20% risk of developing CVD, is considered as high risk. However, a UK based multicentre cohort study has shown that the Framingham risk score significantly overestimates the risk assigned to individuals in the United Kingdom[96].

The Joint British Societies' Guidelines on Prevention of Cardiovascular Disease in Clinical Practice (JBS 2)[93] has been recommended for use in the UK population. It is an update from the previous version which only assessed the risk of coronary heart disease[93]. It was developed by the British Cardiac Society, British Hypertension Society, Diabetes UK, HEART UK, Primary Care Cardiovascular Society, and The Stroke Association. It is recommended as a screening tool for all adults from 40 years onwards, who have no history of CVD or diabetes, and who are not already on treatment for blood pressure or lipids. It uses the same risk calculation algorithm principles as the Framingham risk score, and a risk score resulting in 20% risk of developing CVD is considered as high risk and requires medical and lifestyle interventions. The ASSessing cardiovascular risk using SIGN (ASSIGN) score was developed in Scotland[97] to include the effect on cardiovascular risk of social deprivation. Studies from Scotland observed that classic cardiovascular risk factors inadequately explain social variation in disease and suggested that the Framingham risk score assigned preventive treatment that might lead to relative under-treatment of the socially deprived population[98]. The ASSIGN score is an evolution of the Framingham score, by incorporating social deprivation and family history and suitable for the Scotland population. It also used a 20% cut off to be considered as high risk. The Framingham, JBS 2 and ASSIGN risk scores were designed for healthy individuals at risk of developing symptomatic atherosclerotic disease[93, 95, 97]. Therefore, these risk scores are meant to help the decision making on initiation of the long term medical therapy in the context of CVD prevention in asymptomatic patients.

The Pocock score was developed to predict the 5-year risk of death from cardiovascular disease in a population with established cardiovascular risk factors. The score was derived from 47088 participants of 8 randomised controlled trials of antihypertensive treatment[99]. The risk score is an integer, with points added for each cardiovascular risk factor. The risk score was developed based on age, sex, systolic blood pressure, serum total cholesterol, height, creatinine, smoking, diabetes, left ventricular hypertrophy, history of stroke and history of myocardial infarction[99]. The Pocock score has the advantage of including weightings for the presence of clinical vascular disease. The 5-year risk of death from cardiovascular disease for scores of 10, 20, 30, 40, 50, and 60 was 0.1%,

0.3%, 0.8%, 2.3%, 6.1%, and 15.6% respectively[99]. The risk score was based on the large cohort of randomised controlled trials in different countries with reliable follow up. Therefore, the risk estimates are more precise than the other risk scores[99]. It is therefore a suitable CVD risk score with which to assess the cardiovascular risk factor burden of symptomatic carotid artery disease patients.

While recent evidence showed some promising approaches beyond the presence of classical cardiovascular risk factors in predicting risk of CVD, the clinical application of these approaches remains within research realms. Clinically, classical cardiovascular risk factors remain the only practical clinical tool to identify high risk carotid artery disease. There is no current evidence on whether composite cardiovascular risk scores are associated with the presence of microemboli. In this thesis, I attempted to evaluate individual cardiovascular risk factor and also the composite Pocock cardiovascular risk score to predict the presence of transcranial Doppler detected microemboli in symptomatic carotid artery stenosis patients.

1.1.3 ABCD^2 risk score in carotid disease

The ABCD^2 (an updated version of ABCD risk score[100]) risk prognostic scoring system [101] predicts who is likely to be at increased risk of suffering a recurrent early stroke following onset of the initial TIA[101], irrespective of the mechanism for TIA. The ABCD^2 stroke risk score[101] supports triaging of the priority with which patients with TIA or minor stroke are assessed. The ABCD^2 risk score uses clinical features and standard CVD risk factors such as history of diabetes, and blood pressure to determine a patient's short term risk of stroke. The ABCD^2 score ranges from $0 - 7$[101]. Age ≥60 years scores 1 point; blood pressure ≥140/90 mmHg scores 1 point; clinical features of arm/leg weakness scores 2 points, while speech disturbance scores 1 point; duration of symptoms score 2 points for ≥60 minutes and 1 point for 10-50 minutes; diabetes scores 1 point. An ABCD^2 risk score ≥ 4 predicts a 90-day stroke risk between 8% and 22%; ABCD^2 risk score <4 predicts a 90-day stroke risk of <3%[101].

The score was derived from prospective population-based studies of TIA with a high level of ascertainment and detailed assessment by neurologists[100]. It was designed to allow primary care clinicians, emergency department physicians and specialist physicians to triage patients suffering a suspected TIA or minor stroke, according to their predicted risk of suffering recurrent stroke[101]. Since the initial publication, the ABCD^2 risk score has been independently validated and has performed well in different clinical settings[102]. The strengths of the ABCD^2 risk score includes external generalisability and simplicity of the application for primary care physicians and specialists. The ABCD^2 risk score has now been recommended for triaging patients with suspected TIA/minor stroke by several major clinical guidelines[103-105]. The National Institute for Health and Care Excellence (NICE) guidelines[103] recommends that patients with an ABCD^2 risk score ≥4 should be assessed within 24 hours and those with an ABCD^2 risk score <4 should be evaluated within a week of the onset of symptoms.

There was concern that an ABCD^2 risk score <4 may miss high risk TIA patients[106-109]. Data from the SOS – TIA registry, which was based on a specialist neurology clinic which provided round-the-clock access (24 hours), which showed an ABCD^2 risk score <4 missed 1 in 5 TIA patients who were at a very high risk of recurrence stroke[106]. These included patients with symptomatic critical artery stenosis, atrial fibrillation, cardiac source of embolism and symptomatic intracranial artery disease. Amarenco et al recently demonstrated that in a high risk TIA cohort, ABCD^2 risk score <4 had a 90-day stroke risk similar to patients with ABCD^2 risk score ≥4, and recommended that additional investigations such as carotid ultrasound and electrocardiogram should be performed immediately, regardless of the ABCD^2 risk score[106, 107].

Currently, the association between ABCD^2 risk score and severity of structural carotid artery disease is unclear. This is an important issue to clarify as Ois et al[58] reported that patients presenting with symptomatic critical carotid artery stenosis incur a 17% risk of recurrent stroke at 72 hours, increasing to 22% at 7 days[58]. These patients are reported to benefit from carotid endarterectomy within 2 weeks[53].

Based on the same population studies that were used to derive the ABCD^2 risk score, Rothwell et al[110] reported that ABCD^2 risk scores appeared to be able to identify patients with ≥50% carotid stenosis requiring carotid endarterectomy or atrial fibrillation, which made them at high risk of recurrent stroke. Sheehan et al[111] also suggested that high ABCD^2 risk score may identify patients with significant carotid artery disease. This should be expected as many important elements of the score such as age, hypertension and diabetes are independent risk factors for carotid stenosis[16, 66, 74]. Weakness and speech disturbance are also related to the distribution of middle cerebral artery, which is the main branch of the internal carotid artery.

Amarenco et al[106] reported that an ABCD^2 risk score <4 would miss 9% (62/679) of patients with symptomatic critical carotid artery stenosis. Walker et al[112] found that the ABCD^2 risk score was not predictive of patients with symptomatic carotid stenosis requiring urgent carotid endarterectomy. Schrock et al[113] evaluated the ability of the ABCD^2 risk score to predict positive diagnostic test results in patients being acutely evaluated for TIA. They found that, even though an ABCD^2 risk score ≥4 was associated with an increased likelihood of carotid stenosis, 15% of patients with significant carotid artery stenosis would have been missed[113].

As both ABCD^2 risk score and cerebral microemboli predict short term stroke risk, I attempted to explore whether the ABCD^2 risk score predicted the likelihood of finding spontaneous cerebral microemboli using the data from the newly developed Carotid Registry and the hyper-acute TIA/minor stroke study.

1.1.4   Carotid duplex ultrasound – Measuring carotid stenosis

Conventional intra-arterial angiography has been established as the gold-standard for diagnosing and measuring carotid stenosis and was reference standard against which the non-invasive imaging studies have been compared. Both the North American Symptomatic Carotid Endarterectomy Trial (NASCET)[47] and the European Carotid Surgery Trial (ECST)[38] used intra-arterial angiography at the baseline. However, intra-arterial angiography has been associated with 1% - 2% procedure related neurological complications[114]. Stroke remains the most feared

complication and the periprocedural incidence is <1%, even in the hands of experienced

radiologists[115]. While angiography is not the first line of imaging to evaluate carotid stenosis, it is

increasingly used for planning the feasibility of deployment of a carotid stent.

Carotid duplex ultrasound modalities combines 2-dimensional real-time imaging (B-mode) with

Doppler waveform analysis in order to evaluate the anatomy of the artery and to assess blood

flow velocity. It has been evaluated and validated since the 1970s as a means of assessing carotid

stenosis and identifying patients at risk of stroke[116]. Since then, it has replaced intra-arterial

carotid angiography as the main imaging method for diagnosing carotid artery stenosis[117, 118] in

routine clinical practice. It has been shown that with a well-defined protocol, when the carotid

duplex ultrasound technique is performed by experienced practitioners, the results obtained

compare extremely well with intra-arterial angiography[118]. In comparison to angiography, the

sensitivities and specificities of carotid duplex ultrasound for detecting stenosis from 50% up to

near occlusion internal carotid artery stenosis ranging from 90 - 95%. Current trends show

increased dependence on carotid duplex scanning in recommending patients

for stroke prevention surgery. Carotid duplex scan was used in the landmark Asymptomatic

Carotid Surgery Trial (ACST) at baseline[119].

There are three methods of measurement of carotid artery stenosis on angiography: North

American Symptomatic Carotid Endarterectomy Trial (NASCET); European Carotid Surgery Trial

(ECST); and Common Carotid (CC) methods (Figure 1).

The NASCET method compared the diameter of the residual lumen of the stenosis to the diameter

of the distal internal carotid artery. This method underestimates stenosis in near occlusions with

collapse of the distal carotid artery. The ECST method compared the diameter of the stenosis to

the estimation of the diameter of the carotid bulb at the level of stenosis. The ECST method

typically leads to higher percentages of stenosis compared to the NASCET method, which could

produce an underestimation of percentage and cannot be applied in cases of near-occlusion. The

ECST and Common carotid methods had almost identical results. The common carotid method

compared the residual luminal diameter within the stenosis against the diameter of a normal

segment of proximal common carotid artery. Despite being easy to use, consistently the most

reproducible of the three methods (particularly for stenosis in the clinically important range of

50% to 90%), and to have lower intra- and inter observer variability than the NASCET and ECST

methods, is not commonly used[120].

Re – analysis of the ESCT trial data using the measurement used in the NASCET trial showed that a

50% NASCET stenosis was equivalent to 70% ECST, while a 70% NASCET stenosis equated to an

85% ECST[121]. In order to avoid confusion when grading carotid stenosis between centres, the

Vascular Society and Society of Vascular Technology of Great Britain and Ireland[118] recommended

criteria based on the NASCET method of grading carotid bulb disease.

Figure 1 Methods of measurement of carotid artery stenosis

A = narrowest point of ICA stenosis
B = normal-width artery distal to ICA stenosis
C = estimated original width at the narrowest point of stenosis
D = normal CCA proximal to the bulb

NASCET method: stenosis = (B-A)/B × 100 %
ECST method: stenosis = (C-A)/C × 100 %
Common Carotid method = (D-A)/D x 100 %.
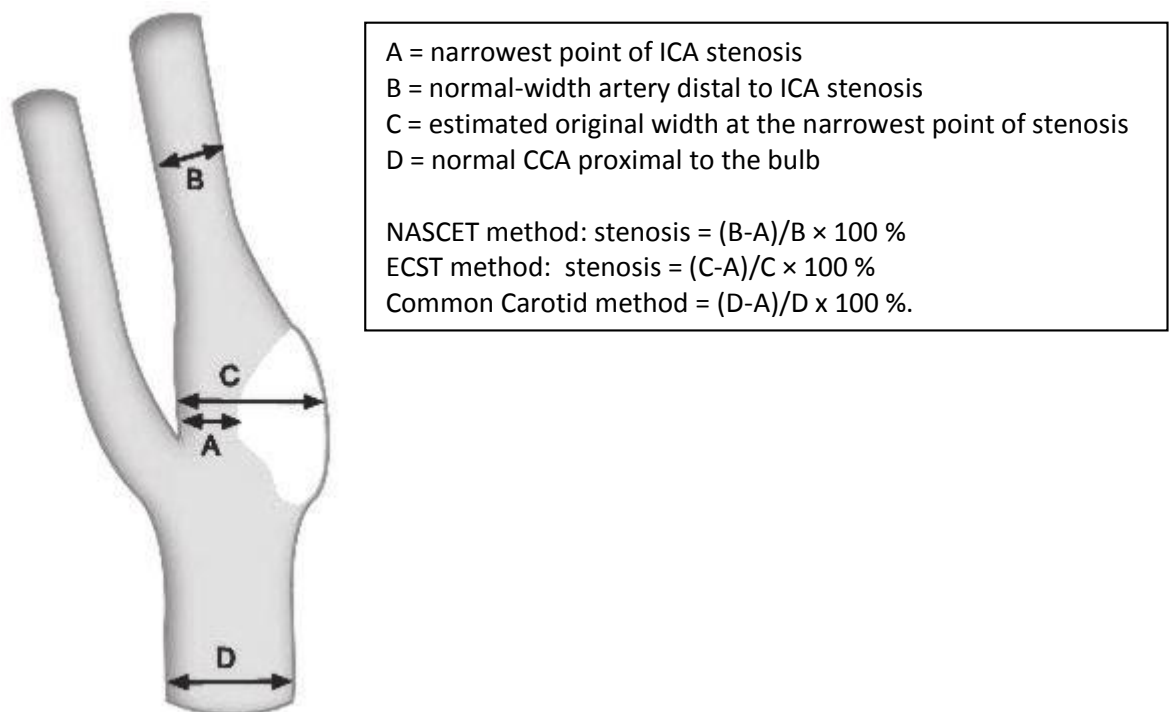
Carotid duplex ultrasound does not directly measure the stenosis of the artery; instead, increasing

blood flow velocity is used as an indicator of increasing degrees of stenosis. Based on the early

intra-arterial angiography studies[122-125], angiographic estimates of the carotid stenosis were used

as reference standard to generate numerous carotid duplex ultrasound parameters to diagnose and measure carotid stenosis. Most laboratories rely on one or more of the three following measurements: Peak systolic velocity (PSV), End diastolic velocity (EDV), and ratio of the PSV in the internal carotid artery (ICA) to the ipsilateral distal common carotid artery (CCA) (PSV_{ICA}/PSV_{CCA}). Duplex criteria for carotid stenosis evaluation were standardised in 1987 at the University of Washington[126]. Based on duplex criteria, the percentage of stenosis could be reliably predicted as 0-15%, 16-49%, 50-79%, 80-99% or complete occlusion.

Table 2 shows the recent consensus of the ultrasound criteria for diagnosing carotid artery stenosis, which can be applied in most situations[127, 128].

Table 2 Society of Radiologist in Ultrasound Criteria for the diagnosis of internal carotid artery stenosis[127]

Degree of Stenosis (%)	Primary Parameters		Additional Parameters	
	ICA PSV (cm/s)	Plaque estimate (%)[a]	ICA/CCA Ratio	ICA EDV (cm/s)
Normal	<125	None	<2.0	<40
<50	<125	<50	<2.0	<40
50 – 69	125 – 230	≥50	2.0 – 4.0	40 – 100
≥70 but less than near occlusion	230	≥50	>4.0	>100
Near occlusion	low, or undetectable	Visible	Variable	Variable
Total occlusion	Undetectable	Visible, no detectable lumen	Not applicable	Not applicable

[a] Plaque estimate (diameter reduction) with grey-scale and colour Doppler ultrasound

The disadvantage of this velocity based estimation of carotid artery is in women where the blood flow velocities is higher than men and abnormally elevated blood flow velocities in the presence of contralateral carotid artery occlusion[129, 130]. Severe calcification, arterial tortuosity, high carotid bifurcation level and obesity may also reduce the accuracy of Duplex ultrasound.

Even though duplex ultrasound shown to be accurate and reliable imaging for assessing degree of stenosis, it is not reliably sensitive to differentiate near occlusion and total occlusion of the diseased carotid artery. Differentiation between near occlusion and total occlusions is clinically

important. Patients with near occlusion remain at high risk for embolic stroke and are surgically

treated, while those with an occluded ICA are at minimal risk for embolic phenomena and are

treated medically for hypoperfusion. In both North American Symptomatic Carotid

Endarterectomy Trial (NASCET)[47] and the European Carotid Surgery Trial (ECST)[38], these patients

had low risk stroke on medical treatment. This is more likely to be due to the presence of a good

circulation. The benefit from surgery in near occlusion based on NASCET data[131] had been

minimal. ECST data[121] suggested no benefit of surgery in this group in terms of preventing stroke.

Due to its lack of sensitivity of duplex ultrasound, Magnetic Resonance (MR) Angiography or

Computed Tomography (CT) Angiography should be used to confirm its presence.

CT angiography provides an alternative carotid artery disease imaging technique. It is the only

imaging modality which provides direct imaging of the arterial lumen suitable for evaluation of

stenosis[132]. It is less user dependant and has been shown to accurately detect large intracranial

and extracranial arterial stenosis and occlusion[132]. It compared favourably with conventional

intra-arterial angiography with agreement of 96% (95% CI, 90 to 99%) vessels. The sensitivity of

CTA was 100%, with a specificity of 63% (95% CI, 25 to 88%), and 100% negative predictive value

in patients with <70% carotid stenosis[133]. Even though it tends to underestimate arterial stenosis,

it does provide multiple viewing angles and three-dimensional reconstruction that allows us to

identify calcium deposits separately from vessel lumen[134]. The requirement of a large dose of

iodinated intravenous contrast to outline the arterial lumen restricts its use in patients with

inadequate renal function.

MR angiography without contrast relies on the radiofrequency signal characteristics of flowing

blood flow which amplify the relative signal intensity of flowing blood allowing more detailed

evaluation of the arteries. It is a safe and non-invasive alternative for carotid stenosis imaging.

However, it tends to have problems with flow voids at the point of stenosis and overestimates the

degree of stenosis[135]. The image quality and reproducibility of measurement of stenosis are

significantly improved with contrast-enhanced MR angiography[135]. Compared with intra-arterial

angiography, the sensitivity of MRA ranges from 97% to 100%, while specificity ranges from 82%

to 96%[136]. The main disadvantages of MRA are overestimation of the stenosis and inability to discriminate between subtotal and complete arterial occlusion. The technique is also restricted in patients with metal devices and claustrophobia.

Using from both published and unpublished research and audit studies in the United Kingdom, Chappell et al[137] evaluated the accuracy of non-invasive imaging; duplex ultrasound, CT angiography, MR angiography and contrast-enhanced MR angiography against intra-arterial angiography as the reference standard. They found that contrast-enhanced MR angiography had the highest sensitivity and specificity for 70% - 99% carotid stenosis, followed closely by duplex ultrasound[137] and data for CT angiography were particularly lacking for analysis. All the non-invasive imaging modalities performed poorly for 50% - 69% carotid stenosis[137].

Every non-invasive vascular imaging modality has its limitations as to when it can be accurately and reliably used to measure carotid stenosis. With a well-defined protocol, duplex ultrasound is a reliable diagnostic tool that has an important role in the cost-effective diagnosis and grading of carotid disease[118]. It remains the most widely used imaging, both as a screening tool and as a stand-alone modality, to make a decision to proceed to surgery.

1.1.5 Carotid endarterectomy for prevention of recurrent stroke

The primary management of all carotid artery disease is aggressive medical therapy with cardiovascular risk factors modification. In symptomatic carotid artery stenosis, the effective management of stroke prevention includes best medical therapy and urgent carotid endarterectomy for those with a haemodynamically significant carotid stenosis ≥50%. Best medical therapy (BMT) utilises medications to optimise the control of cardiovascular risk factors that contribute to atheroma development, as well as antiplatelet therapy, to reduce thromboembolic risk. In carotid endarterectomy, the removal of the atherosclerotic lesion allows the correction of reduced blood flow and also eliminates the source of emboli.

The benefit of urgent treatment of TIA/minor stroke aimed at preventing recurrent stroke has been demonstrated in the prospective, population-based SOS-TIA Study[138] and the Early Use of

Existing Preventive Strategies for Stroke (EXPRESS)[139] studies. In the EXPRESS study, urgent treatment of TIA and minor stroke was associated with a significant 80% reduction in the risk of early recurrent stroke compared with standard treatment in primary care[139]. Similar findings were demonstrated in the SOS-TIA study[138] which was based in Paris. Following the initiation of 24-hour a day emergency TIA clinics in Paris, the prognosis of TIA or minor stroke patients has improved. The recurrence of stroke within 7 days and three months follow-up were 0.3% and 1.9% respectively[138].

There is increasing evidence that aggressive use of antiplatelet therapy is effective in preventing recurrent stroke. In MATCH (Management of ATherothrombosis with Clopidogrel in High-risk patients)[140], CARESS (Clopidogrel and Aspirin for Reduction of Emboli in Symptomatic Carotid Stenosis)[37] and FASTER (Fast assessment of stroke and transient ischaemic attack to prevent early recurrence)[141] randomised controlled trials, patients with recent TIA or symptomatic critical artery stenosis were randomised to a combination of aspirin and clopidogrel versus aspirin alone. These studies, suggest that a dual-antiplatelet therapy was better than monotherapy in reducing the risk of early recurrent stroke.

The landmark level 1 evidence for intervening in patients with symptomatic extra-cranial carotid artery disease came from the North American Symptomatic Carotid Endarterectomy Trial (NASCET)[47], European Carotid Surgery Trial (ECST)[38] and The Veteran Affairs Trial[142]. Veteran Affairs Trial was prematurely stopped after interim results of ECST were published[142].

Both the European Society for Vascular Surgery (ESVS) and the Society for Vascular Surgery (SVS) issued updated guidelines on the management of carotid disease[143] (Figure 2). These take into consideration the current literature and their recommendations are graded according to the levels of evidence.

Figure 2 Decision tree for carotid disease (NASCET criteria)[143, 144]
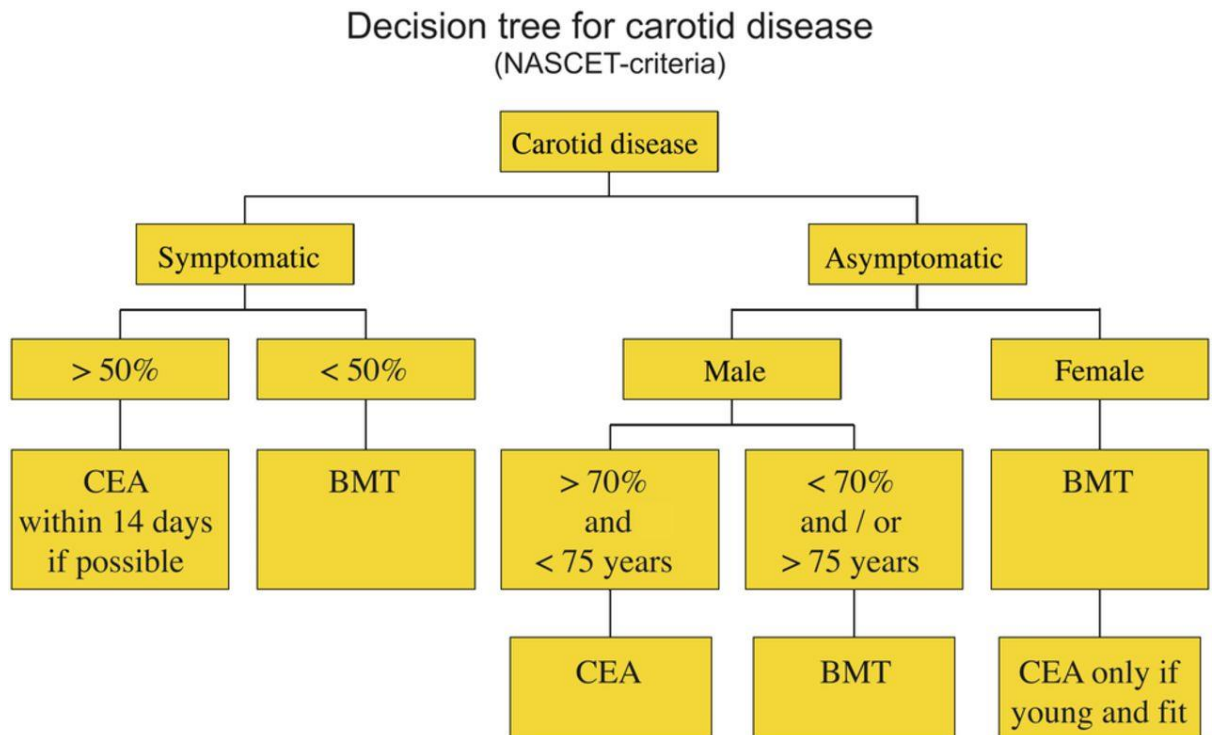
Decision tree for carotid disease
(NASCET-criteria)

North American Symptomatic Carotid Endarterectomy Trial (NASCET)[47] and the European Carotid

Surgery Trial (ECST)[38], have shown that carotid endarterectomy vs. BMT is highly effective in

reducing the risk of further strokes in moderate (5-year stroke risk; 15.7 vs 22.2% NASCET and

marginal difference in ECST) to severe (2-year stroke risk; 9 vs 26% NASCET and 6.8 vs 20.6% ECST)

symptomatic carotid artery stenosis. Surgery in carotid near-occlusion and stenosis less than 50%

conferred with no benefit at al[121]. Pooled analysis of data from the major randomised controlled

trials (ECST, NASCET and Veteran Affairs trials) of surgery for symptomatic carotid stenosis

showed surgery reduced the 5-year absolute risk of any stroke or death by 15% in patients with

70-99% stenosis, and by 7.8% in patients with 50-69% stenosis[51]. The analysis also showed the

benefit of surgery was greatest in men, elderly (age >75) and when performed within 2 weeks

after the last ischaemic event[51].

In order to maximise the benefit of carotid endarterectomy, it should be performed within 2

weeks after the last ischaemic event and at the same time minimise the operative stroke and

death complication rates. However, there has been a concern regarding the safety of the surgery

if performed too early. In general, the risk of endarterectomy for emergency surgery in patients with unstable symptomatic carotid disease is high[145, 146], especially in patients with crescendo transient ischaemic attacks and stroke-in-evolution[147]. Otherwise, there was no evidence of any increase in operative risk in neurologically stable patients operated within 2 weeks of the last ischaemic event based on the 2 major randomised controlled trials (Table 3)[53, 148] data. Table 3 showed there was no significant difference of operative risk when between surgery performed within 2 weeks of last ischaemic event and beyond 2 weeks.

Table 3 The operative risk (95% CI) due to trial surgery in relation to the time between the last symptomatic ischaemic event and surgery in the European Carotid Surgery Trial (ECST) and the North American Symptomatic Carotid Endarterectomy Trial (NASCET)[53, 148]

	Time between last symptomatic ischaemic event and surgery				
	<2 weeks	2-4 weeks	4-12 weeks	>12 weeks	P value
Any stroke or death within 30 days after trial surgery					
ECST	6.5% (1.0–12.0)	6.4% (3.4–9.5)	7.4% (5.5–9.3)	8.0% (5.9–10.0)	0.86
NASCET	7.1% (4.1–10.1)	5.0% (2.4–7.5)	6.5% (4.4–8.4)	7.4% (4.4–10.4)	0.64
Total	6.9% (4.5–10.1)	5.7% (3.9–8.0)	7.0% (5.7–8.5)	7.8% (6.2–9.7)	0.48
Disabling operative stroke or death within 30 days after trial surgery					
ECST	2.6% (0.3–9.1)	2.4% (0.9–5.2)	4.1% (2.8–5.8)	3.5% (2.2–5.2)	0.61
NASCET	3.2% (1.5–6.0)	2.5% (1.0–5.1)	1.6% (0.7–3.1)	1.0% (0.2–2.9)	0.23
Total	3.1% (1.5–5.4)	2.5% (1.3–4.2)	3.1% (2.2–4.1)	2.7% (1.8–4.0)	0.89

Despite clear evidence of benefit if carotid endarterectomy is performed within 2 weeks of the last ischaemic event, it is still undermined in healthcare systems by delays in investigation and intervention[148]. For an example, an Oxfordshire Study found that the median time from transient ischaemic attack or stroke to imaging was over four weeks and that the median delay to surgery was 14 weeks[59]. In a recent national survey, only 20% of symptomatic patients had carotid endarterectomy within the two week target time set by the National Institute for Health and Clinical Excellence (NICE)[149].

Kerber et al[150] performed the first carotid artery angioplasty in a human in 1980. Since then, the development of the carotid stent has evolved rapidly and has emerged as a potential alternative to carotid endarterectomy. It has an advantage of being performed under local anaesthesia. It

reduces the risk of myocardial infarction and pulmonary embolism. It also avoids the effect of general anaesthetic agents and promotes a more rapid recovery, thereby potentially reducing length of hospital stay. Since the 1990s, investigators have compared the effectiveness of carotid endarterectomy and carotid stenting. The first clinical trial of carotid stenting was a single centre study based in Leicester[151]. Since then, 14 clinical trials[152] and 21 clinical registries[153] comparing surgery and carotid stent effectiveness have been reported. These produced conflicting results due to differences in patient population, trial design, outcome measures, endovascular devices used and operator skills[152]. The two most recent international multi-centre randomised controlled trials comparing carotid endarterectomy and carotid stenting in symptomatic carotid stenosis have been published[154-156]. The International Carotid Investigators Stenting Study (ICSS) which is based in Europe, showed that surgery is superior to carotid stenting in symptomatic patients, especially in terms of stroke risk reduction and death rate in patients >70 years of age[154, 155]. Further meta-analysis of individual patient data from three randomised controlled trials from the same ICSS investigators concluded that carotid stenting for symptomatic carotid stenosis should be avoided in older patients (age ≥70 years)[154]. The Carotid Revascularisation Endarterectomy vs. Stenting trial (CREST), based in North America, demonstrated similar major complications for surgery and stenting in symptomatic and asymptomatic patients[156]. Both the European Society for Vascular Surgery (ESVS) and the Society for Vascular Surgery (SVS) issued updated guidelines indicate that carotid endarterectomy remains the better option[144].

Whilst the definitive management of the symptomatic carotid stenosis has been the subject of good quality medical research, further research is needed to identify the clinical method that enable us to identify these high risk patients which will benefit from urgent carotid endarterectomy. One option is to use transcranial Doppler microembolic signals as a surrogate biomarker of short term stroke risk. In my thesis, I aimed to study the clinical vascular disease characteristic and radiological detection of the TCD-detected microembolic signals.

1.2   Carotid artery disease associated cerebral microemboli

1.2.1   Transcranial Doppler imaging and cerebral microemboli detection

The Doppler effect is the basis upon which ultrasound provides information on blood flow velocity. Pulsed wave ultrasound is reflected by moving red blood cells. The frequency shift between the transmitted and reflected ultrasound wave correlates directly with blood flow velocity. The first transcranial Doppler (TCD) ultrasound recordings were performed in 1981 in Germany[157] to assess the velocity of cerebral blood flow. Since then, TCD has been used to study cerebral arteries flow velocities, cerebral CO_2 reactivity and microembolic signals (MES).

The technique is highly operator dependent and relies on sufficient ultrasound transmission through the skull to allow the recording of intracerebral blood flow using a low frequency transducer. A number of different acoustic windows of the skull have been used to monitor different intracerebral arteries. The terminal internal carotid artery, middle cerebral artery, anterior and posterior cerebral arteries can be accessed via the temporal window which is the thinnest area of the skull[157]. The occipital window can be used to insonate the vertebral and basilar arteries. The orbital area can be used to insonate the distal internal carotid artery and ophthalmic artery.

Various flow velocity parameters can be used to identify cerebral artery stenosis. For example, high flow velocity parameters have been strongly associated with middle cerebral artery stenosis[158, 159]. The cerebral reactivity measures the change of blood flow velocity in in response to the inhalation of CO_2 or the administration of acetazolamide. Reduced cerebral reactivity suggests that the patient has little haemodynamically reserve which has been associated with cardiovascular risk factors and severe carotid disease[160]. Whilst blood flow velocity calculation relies on the frequency of the reflected ultrasound signal, the intensity of the reflected signal provides information on the material which reflects the ultrasound signal. The amount of ultrasound reflected is proportionate to the intensity of the received signal. The intensity of the reflected signal relies on the type of tissue through which the signal is passed. This is the basis of the transcranial Doppler detection of microemboli. The unique scattering of the reflected

ultrasound signal, compared with the homogenous background of red blood cell signals, allows

microemboli to be detected as they pass through the intracerebral arterial circulation. The

microembolic material can be both gaseous and solid within the intracerebral vessels. They

appear as a short duration high intensity signal within the Doppler arterial waveform (Figure 3).

The low frequency of transducer used (2MHz) for TCD increases the embolic-to-background blood

signal ratio, hence making them easier to detect[161]. Sources of microemboli include symptomatic

or asymptomatic carotid stenosis[32, 162], atrial fibrillation[163], prosthetic cardiac valves[164] and

cardiopulmonary bypass[165]. In carotid disease, TCD – detected microemboli were first described in

a study evaluating blood flow velocity in the middle cerebral artery of nineteen patients during

carotid endarterectomy[166]. At that time, the detected microemboli were thought to be harmless

air microbubbles[166]. A study by Spencer et al[161] published in 1990 provided the first evidence of

the potential of TCD-detected microemboli as a biomarker of early stroke. Spencer et al[161]

described the accumulation of thrombus on the endarterectomy site in patients who developed

immediate post-operative stroke. Since then, TCD – detected microemboli have been identified as

a potentially useful surrogate marker for selection of high risk carotid disease patients for

intervention[32, 162], perioperative monitoring[167] in carotid endarterectomy and assessing the

effectiveness of novel antiplatelet therapies[168].

Transcranial Doppler is a blind ultrasonic technique and microemboli detection requires a certain

level of expertise. Even though high inter-observer reproducibility of identifying microemboli has

been reported in multi-centre studies[32, 169], care needs to be taken in order to differentiate

clinically relevant solid microemboli from artefact. Sufficiently specific and sensitive automated

embolic signal detection is also needed for wider clinical application[170]. In order to avoid

discrediting these techniques, experts around the world in this field gathered in 1997 and set out

the recommendations of microembolic signal identification for appropriate use in clinical practice

and scientific investigations[171]. The recommendations are based on distinct ultrasonic and audio

characteristics of the relevant microembolic signals. They are characterised by: (1) high intensity

transient signal; (2) short duration range of 10 - 100ms; (3) detection threshold range between 3

to 9 Db for discriminating embolic signal from the background noise; (4) produce characteristic 'chirp' sound and (5) unidirectional signal occurs randomly within the cardiac cycle to discriminate embolic signal from artefact[171].

Experimental studies have demonstrated that TCD has a very high sensitivity and specificity for detecting solid microemboli including platelet aggregates, thrombus and atheromatous debris[172]. The application of TCD relies on the clinical context as current technology does not reliably differentiate between cholesterol emboli, thrombus and platelet microemboli.

In carotid disease, the ultrasound probe is placed on the acoustic temporal window to insonate the middle cerebral artery which is in continuation with the distal internal carotid artery. Carotid disease associated microemboli appear to be primarily platelet aggregates[60]. A recent study by Kinsella et al[173] has shown that patients with symptomatic carotid disease had higher platelet counts (potentially reflecting increased platelet production, mobilization or reduced clearance) and platelet activation status than asymptomatic patients. Microemboli were more frequently detected in recently symptomatic than asymptomatic cohorts[173]. Studies have shown that the microemboli detected in carotid disease were almost completely abolished by an inhibitor of platelet adhesion and aggregation[174] and various antiplatelet agents such as aspirin, clopidogrel and tirofiban[175] [37, 176]. In contrast, heparin has been shown to be less effective in abolishing the microemboli[177, 178]. These findings are consistent with trials which have shown that antiplatelet agents are more effective than anticoagulants in preventing stroke recurrence[179].

Figure 3 Middle cerebral artery Doppler waveform. The typical MES appearance can be seen as a spike within the arterial waveform.

Typical MES seen as a spike
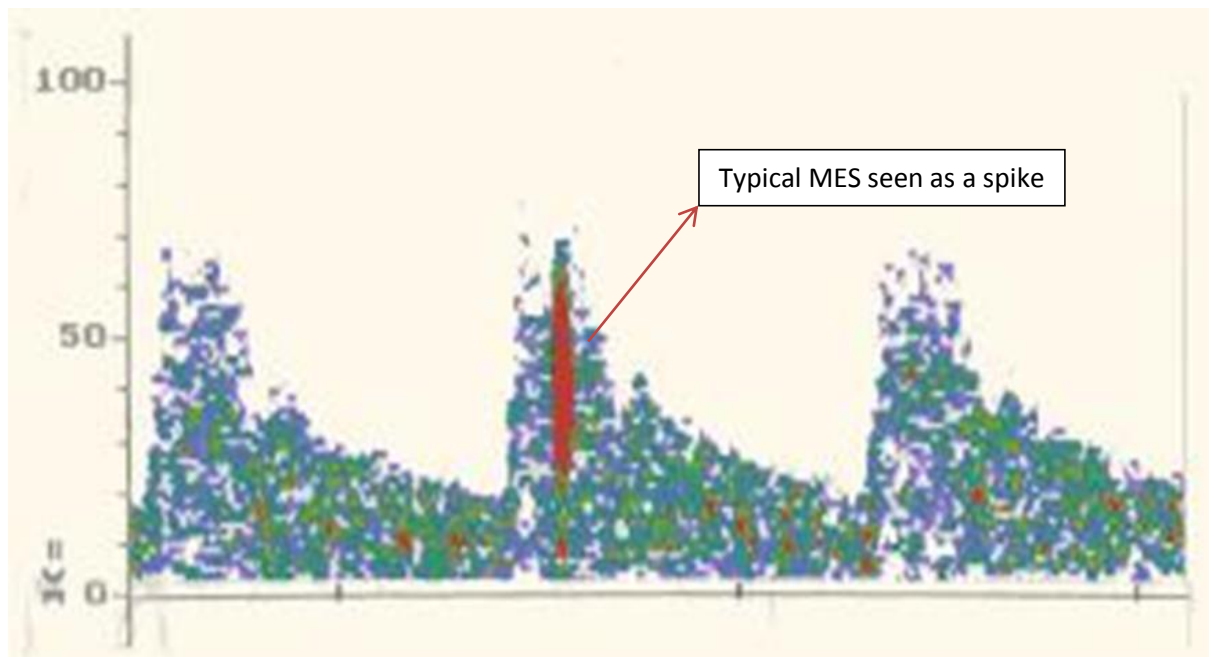
1.2.2   Prevalence and prognostic impact of cerebral microemboli

Microembolic signals following TIA/non-disabling stroke

Tanaka et al suggest a ratio of middle cerebral artery (MCA) to anterior cerebral artery (ACA) blood flow of approximately 75:25 from the internal carotid artery[180]. A recent in vitro study by Chung et al[181] showed that embolus trajectory favours the middle cerebral artery, especially large emboli. This preference of emboli to enter the MCA is consistent with the high ratio of MCA–ACA infarcts reported by the Harvard[182] and Lausanne[183] stroke registries. In carotid disease, cerebral infarcts occur more frequently in the territory of the middle, compared with that of the anterior, cerebral artery and occurrence of cerebral microembolism follows a similar distribution[184]. Microembolic signals (MES) have been detected in approximately 40% of symptomatic carotid artery stenosis patients[35, 185]. The presence of microembolic signal has been associated with recently symptomatic carotid stenosis, degree of stenosis[185], intraluminal thrombosis and unstable carotid plaque characteristics[162, 186-188].

Forteza et al[189] reported a higher occurrence of microembolic signals in patients presenting within 4 days of their index symptoms, as compared to those who presented with 12 days of their most recent symptoms. Valton et al[190] prospectively studied the association of microembolic signals in the ipsilateral middle cerebral artery and early ischaemic recurrence in 73 consecutive patients with anterior circulation, stroke or TIA in whom the TCD examination was performed within 7 days from the onset of symptoms. A further report has shown that patients with MES are at a nine-fold increased risk of stroke[190]. In the more recent Clopidogrel and Aspirin for Reduction of Emboli in Symptomatic Carotid Stenosis (CARESS trial)[37], the presence of MES was associated with a markedly increased risk of recurrent stroke and TIA at day 7 following the initial symptoms. This is also consistent with an epidemiological study demonstrating a high risk of early recurrent stroke or TIA at 7 days from the index symptom[10]. Ritter et al reported an increased prevalence of patients with MES with a higher degree of carotid stenosis[185]. Valton et al[187] demonstrated that microembolic signals were associated with the appearance of plaque ulceration on angiography. The analysis of endarterectomy specimens by Sitzer et al[186] demonstrated that microembolism before carotid surgery is closely related to surface thrombus and ulceration of the carotid plaque. These observations suggest that unstable ulcerated carotid plaque and surface thrombus are the source of the MES in acute symptomatic carotid disease patients. A more recent study by Van Lemmeren et al[188] showed that spontaneous MES were detected in a quarter of symptomatic carotid disease patients scheduled for CEA and were associated with unstable carotid plaque characteristics such as luminal thrombus.

Early work by Valton et al[190] on symptomatic carotid stroke and TIA presenting within 7 days of index symptoms showed patients with MES had significantly higher ischaemic recurrence than without MES (33% vs. 5%, P = 0.008). They demonstrated the daily incidence of ischaemic recurrence was 4.3% per 100 patients with MES and only 0.5% without MES.[190] A more recent prospective observational study by Mackinnon et al[36] provided more conclusive evidence that showed that MES predicts short-term ipsilateral stroke risk in symptomatic critical carotid artery stenosis patients (stenosis > 50%). In the same study Mackinnon et al[36] prospectively monitored

200 consecutive, recently symptomatic patients with 50 – 99% stenosis who underwent TCD evaluation. They found a significant association between microembolic signal (MES) and both recurrent stroke alone (P = 0.001) and the combined endpoint of stroke and TIA (P = 0.00001)[36]. The association between MES and stroke or stroke/TIA remained significant after taking into account the relevant clinical risk factors such as age, sex, smoking, hypertension, time from the last symptoms and degree of stenosis with an odds ratio of 4.67 (95% CI, 1.99 – 11.01, P < 0.0001)[36].

Ritter et al[185] report a systematic review investigating the prognostic impact of MES in symptomatic patients with carotid artery disease. They found that the daily risk of recurrent stroke or TIA was 1.3% in MES-positive patient, compared with 0.3% in MES-negative patients[185]. The overall 30-day risk of recurring stroke or TIA was 21% in symptomatic carotid artery disease patients[185]. Ritter et al[185] calculated from the pooled data that symptomatic carotid artery disease patients with MES have about a sevenfold increased risk of future events compared with patients without MES (OR 7.5, 95%CI 3.6 – 15.4, P < 0.0001). A more recent meta-analysis by King et al[35] confirmed that the presence of MES in symptomatic carotid artery stenosis patients predicted both stroke alone (OR 9.57, 95% CI 1.54 – 59.3, P = 0.02) and the combined end point of stroke or TIA (OR 6.36, 95% CI 2.9 – 13.96, P < 0.00001).

Mackinnon et al demonstrated that the presence or absence of microembolic signal rather than the rate of MES predicted the risk of recurrent stroke and TIA[191]. Using a higher rate or threshold of MES did not increase the predictive power of TCD. Ritter et al[185] also recommended that the stroke risk stratification in symptomatic carotid artery disease is dependent on the detection of one single MES. The predicted risk of recurrent ipsilateral TIA/stroke based on the presence or absence of MES was also used in a more recent prospective, multicentre, international study looking at the association between MES and risk of recurrent ipsilateral TIA/stroke in asymptomatic carotid disease patients[32].

Microembolic signals following carotid endarterectomy

Carotid endarterectomy reduces the risk of future stroke in patients with symptomatic critical artery stenosis[38, 47]. However, the procedure itself carries a significant peri-procedural risk of stroke[192]. Current national guidelines[103] recommend that carotid endarterectomy should be performed within 2 weeks of index TIA/minor stroke. Few studies have shown that early carotid endarterectomy confer higher risk of perioperative stroke[146, 193]. Reducing the risk of perioperative stroke improves the risk–to–benefit ratio for carotid endarterectomy.

The mechanisms of stroke following carotid endarterectomy are numerous. Early work on the pathogenesis of stroke from internal carotid artery occlusion showed that both haemodynamically induced and embolic infarction could be distinguished based on the anatomical distribution[194]. Thromboembolism generally give rise to infarcts within the territory of a main arterial branch, whereas low-flow states are associated with watershed infarcts[195]. Early work by Steed et al suggested thromboembolism as the principal mechanism of peri-operative stroke[196]. Morphological study of a cerebral infarct on CT scan following carotid endarterectomy strongly suggested thromboembolism as the principal mechanism of ischaemic perioperative stroke[195]. Subsequent work showed that thromboembolism from the thrombogenic endarterectomised media zone was the principal of mechanism of perioperative ischaemic stroke[195, 197, 198]. Recent clinical and radiological studies have confirmed that perioperative cerebral microembolisation originating from the endarterectomised zone is strongly associated with thrombotic stroke following carotid endarterectomy[199-201]. Excision of the carotid plaque removes the endothelium creating a thrombogenic surface on which platelet aggregation occurs. Human and experimental studies have shown that platelet deposition is increased at the site of carotid endarterectomy[202, 203], reaching a maximum within 20 - 60 minutes and potentially giving rise to microemboli causing thrombotic stroke. The platelet nature and origin of embolism in this group was further confirmed when the endarterectomised site was re-explored[31, 204, 205] and by its response to antiplatelet agents.

Microemboli detected during the dissection phase (before carotid arteriotomy) are believed to be associated with thrombotic stroke risk[35]. In the dissection phase, the fragile carotid plaque has not yet been removed. The exposure to the blood flow will potentially give rise to the emboli. Meta-analysis by Markus et al[35] has shown that the absence or presence of microemboli during dissection phase of carotid endarterectomy did not predict intraoperative stroke. However, a high rate of microembolic signals during dissection phase predicted stroke (Odds Ratio 14.79, 95%CI 5.15 – 42.47, P < 0.00001)[206].

Increasing high rate of detected microembolic signals acutely following carotid endarterectomy have been demonstrated to be associated with post-operative thrombotic stroke[31]. Approximately up to 5% of patients developed persistent high rate of postoperative microembolic signals[207] . Unfortunately, about 50% of these patients with high rate microembolic signals suffer a thromboembolic stroke[207]. A previous study has shown that the early microemboli were detected approximately 10 minutes following carotid artery flow restoration[46]. Work by Abbott et al showed that most of the patients who developed high microembolic signal rates, who were at risk of developing postoperative stroke, were identified during a 30 minute TCD study in the first post-operative hour[207]. More recent work by Sharpe et al[208] showed that a high risk cohort of patients who would go on to develop high rate embolization that require treatment with Dextran could be identified by measuring the magnitude and rate of embolization in the first 30 minutes after arriving in theatre recovery. The vast majority of patients (85%) require no further monitoring[208]. A meta-analysis by Markus et al[35] showed that the absence or presence of microemboli did not predict postoperative stroke. However, it did predict the combination of postoperative stroke and TIA (OR 3.56, 95%CI 1.37 – 9.22, P = 0.009). Further analysis has confirmed that high microembolic signals rate acutely after surgery predicted stroke alone and also stroke/TIA (OR 32.04, 95%CI 11.36 – 90.39, P < 0.00001)[35].

Currently, there is no consensus on the definitive threshold of MES rate which is associated with adverse neurological events following carotid endarterectomy due to variable outcomes between centres[31, 199, 200, 209]. The variation of the clinically significant threshold of MES rates could be

explained by the brain microcirculation which has considerable reserve capacity for microemboli. This capacity may be influenced by variables such as microembolus size, blood rheology, collateral flow and preoperative antiplatelet treatment[31]. Laman et al[200] evaluated the relationship between the rate of microemboli (MES/min) and stroke complications following carotid endarterectomy. They found that a rate of 0.9 MES/min was associated with fourfold increase in the rate of early post-operative stroke following carotid endarterectomy[200]. Based on a prospective study by Levi et al, [31] a threshold of MES rate > 50/hr (0.83 MES/min) has been shown to correlate with stroke occurrence within a time frame of approximately 60 minutes following their detection. Levi's group found that the positive predictive value for cerebral ischaemia of detecting MES rate > 50/hr was 0.71, and the negative predictive value of < 50/hr was 0.98[31]. Naylor et al[33] started Dextran therapy in patients with 25 or more emboli in any 10 minute period of monitoring (2.5 MES/min). Cantelmo et al[199] identified that MES rate of 0.33 MES/min was associated with clinically silent MRI ischaemic changes. Despite this variation of the MES rate threshold associated with thrombotic stroke between centres, a trend of increasing rates of embolisation carotid endarterectomy is strongly predictive of a high risk of progressing on to thrombotic stroke.

1.2.3 Treatment for cerebral microemboli

The evidence relating to the benefits and risks of antiplatelet treatment in symptomatic carotid artery stenosis is evolving. Historically, we relied on data extrapolated from global ischaemic stroke/TIA studies to guide us on the optimal antiplatelet treatment for carotid artery stenosis[210]. Combination therapy with dipyridamole or clopidogrel has greater efficacy but fails to prevent many stroke recurrences[211]. TCD-based studies[37, 212] have allowed us to use MES as a surrogate marker of short term stroke risk to assess the optimal antithrombotic and antiplatelet agents in both patients with symptomatic carotid artery disease and following carotid endarterectomy. Microemboli following acute stroke/TIA or carotid endarterectomy are much more common than the actual recurrent clinical event. The main advantage of this technique is to be able to evaluate antiplatelet, anticoagulant and antithrombotic effect of treatments in a much smaller number of patients than those required with the use of a clinical end point such as stroke[37]. The efficacy of

using TCD-detected microemboli as the surrogate marker to evaluate the effectiveness of

treatment pertinent to carotid artery disease has been reflected with the increasing published

data as summarised by the Table 4 below:

Table 4 Antiplatelet and antithrombotic agents evaluation based on TCD-based studies

Year	Authors	Research method	Clinical setting	Agents evaluated
2013	Saedon et al[176]	Registry	Carotid endarterectomy	Tirofiban vs. Dextran-40
2011	King et al[212]	RCT	Symptomatic carotid stenosis	Clopidogrel & aspirin vs. Dipyridamole & aspirin
2011	Markus et al[168]	RCT	Carotid endarterectomy	Von Willebrand Inhibitor ARC1779
2008	Vogten et al[213]	Prospective	Carotid endarterectomy	Clopidogrel vs. aspirin
2008	McMahon et al[178]	RCT	Carotid endarterectomy	Un-fractionated heparin vs. low molecular weight heparin
2008	Van Dellen et al[214]	Case series	Carotid endarterectomy	Tirofiban
2007	De Borst et al[215]	RCT	Carotid endarterectomy	Dipyridamole & aspirin vs. dipyridamole & aspirin & clopidogrel vs. Dipyridamole & aspirin & dextran
2005	Markus et al[37]	RCT	Symptomatic carotid stenosis	Clopidogrel & aspirin vs. aspirin
2005	Tytgat et al[216]	RCT	Carotid endarterectomy	Aspirin
2004	Payne et al[217]	RCT	Carotid endarterectomy	Clopidogrel & aspirin vs. aspirin
2003	Lennard et al[218]	Case series	Symptomatic carotid stenosis	Dextran-40
2001	Goertlet et al[219]	Prospective	Symptomatic carotid stenosis	Aspirin
2000	Hayes et al[220]	Prospective	Carotid endarterectomy	Dextran-40
1998	Molloy et al[221]	RCT	Carotid endarterectomy	S-Nitrosoglutathione

TCD – directed antithrombotic treatments have been shown to reduce the risk of thrombotic

stroke acutely in the context of carotid surgery[33]. Dextran was one of the earliest antithrombotic

agents evaluated and used to treat microemboli. It has been extensively studied in the context

carotid surgery, where it has been used to treat patients with high levels of postoperative

embolisation since the late 1990s[167, 222]. The previous studies suggested a number of potential

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modes of action involving platelets[223], von Willebrand factor[224], and the fibrinolytic system[225]. The most probable explanation comes from the Noorman et al study which showed that Dextran increases tissue – plasmin activator (t-PA) plasma concentration[225]. T-PA is a protein which activates fibrinolysis by converting plasminogen to plasmin. Noorman et al[225] demonstrated that in rats, dextran inhibits mannose receptor-mediated t-PA clearance. The inhibition of t-PA clearance during dextran infusion results in increased endogenous t-PA plasma concentrations. A recent laboratory study in Leicester exploring the potential modes of action of dextran involving platelets, von Willebrand factor and fibrinolytic system further supported the theory that dextran enhances fibrinolysis through blockade of the uptake of t-PA in vivo[226] . This study also clarified other potential mechanisms of dextran such as the breakdown effect of von Willebrand factor leading to a reduction of platelet adhesion to the vessel wall and also the dextran effect in causing thrombus instability. In a consecutive carotid endarterectomy series by Naylor et al[167], the use of perioperative dextran in 500 consecutive operations resulted in no postoperative strokes. More recent findings from a group of vascular surgeons based in New England found that dextran was not associated with a significant decrease in the incidence of postoperative ipsilateral stroke, all stroke, and stroke or death[227]. The use of dextran in their series was also associated with a significantly increased risk of myocardial infarction and congestive heart failure[227]. Hayes et al studied the association between microembolisation and platelet activity based on the hypothesis that the magnitude of postoperative embolisation reflected an enhanced state of platelet activation and aggregation in susceptible patients[228]. The platelet response to adenosine diphosphate was significantly higher in patients who developed high rates MES and these cohorts of patients may respond to the P2Y12 ADP receptor antagonist clopidogrel[228]. Further randomised controlled trials[37, 217] of combined platelet inhibition with aspirin plus clopidogrel vs. aspirin in both patients with symptomatic carotid stenosis and undergoing carotid endarterectomy showed a significant reduction of frequency of MES and stroke recurrence. The use of TCD technique has made it clinically practical and feasible for us to evaluate novel antiplatelet agents or multiple

antiplatelet treatments such as S-nitroso-glutathione [221], tirofiban[214], von Willebrand inhibitor [168], high dose aspirin[175] and a combination of aspirin, dipyridamole and clopidogrel [37, 215, 217]. Despite extensive studies, there is no consensus on which antiplatelet therapy or combination is most effective in abolishing TCD-detected microemboli, with both the aims of preventing post-carotid endarterectomy thrombotic stroke and recurrent stroke in acute symptomatic carotid artery disease patients.

1.3   Medical Registry – establishing Warwick Carotid Surgery Registry

1.3.1   Background

Medical registries have become increasingly significant in recent years[229] and are firmly established as part of the nation's public health system in measuring the delivery, effectiveness and efficiency of healthcare. Medical registry follows the principles of observational studies by focusing on the actual patient populations. The word registry came from the Latin word *registrum*, meaning 'list' or 'catalogue'. Brooke[230], writing for the World Health Organisation (WHO) in 1974, defined registry as "a file of documents containing uniform information about individual persons, collected in a systematic and comprehensive way, in order to serve a predetermined purpose". Solomon et al defined medical registry as "a systematic collection of a clearly defined set of health and demographic data for patients with specific health characteristics, for a predefined purpose[231]. A more recent definition is "an organised system that uses observational study methods to collect uniform data to evaluate specified outcomes for a population defined by a particular disease, conditions, interventions or exposure, and that serves one or more predetermined scientific, clinical, or policy purposes"[232]. Based on the recent document by the Agency for Healthcare Research and Quality[232], registry data criteria are the following:

1. The data are collected in a natural manner. The registry should not influence the standard clinical management of patients.

2. The purposes of the registry are defined before data collection and analysis process, so the data collection is purpose driven rather than the purpose being data driven.

3. Data collected have specific and consistent definitions.

4. Data are collected in a standard manner for every patient.

5. Data collected should reflect the clinical status of the patients; it is the type of data used by the clinicians for diagnosis and management of patients

6. At least one element of registry data collection is active or specifically for the purpose of the registry (usually collected from the patient and clinician).

Medical registries are powerful tools for improving the quality of patients' healthcare. The functions of medical registry can be looked at from 2 aspects of healthcare; clinical and funding body[229, 231-233].

From clinicians and patients' perspectives, the real-world data from registry are useful:

1. To understand the natural history of a disease.

2. To evaluate the existing treatments and outcomes.

3. To monitor the effectiveness and safety of any intervention delivered.

4. To audit the clinical practice and manage disease in accordance with regional/national guidelines or best available evidence.

5. For research – descriptive studies, improving research design, hypothesis testing

From the public health or funding body perspectives, registry data can potentially:

1. Public health surveillance

2. Plan the provision of health care

3. Monitor the impact of preventive measure

4. Evaluate the cost of the treatment

5. Assess different interventions in different populations.

6. Monitor the performance of new products or procedures.

Registry is often confused with clinical database. Whilst clinical database can be part of the data source for the registry, the reverse is not the case. Table 5 summarises the key differences between registry and database[234].

Table 5 Differences between registry and database

	Registry	Clinical database
Source of cases	Various to ensure completeness	Based on single cases treated in a single department. No requirement to identify all cases in any given population
Subjects	Predetermined inclusion and exclusion criteria	Not defined
Follow up data	Yes	Yes
Principal uses	Patient care, aetiological studies, needs assessment, health outcome assessment, surveillance	Patient care, health technology assessment, quality improvement
Advantages	Representative distribution of cases	Complete data on cases included "clinically-rich"
Disadvantages	Demanding to obtain data on all cases	Cases are selective

In terms of research methodology, there are clear distinctions between a registry and a clinical trial. In general, registry data reflect or observe the current standard medical management of patients. Whereas in a clinical trial, the data are focusing on the experimental or investigational aspects of homogenous groups. Both research methodologies require protocol-based data definition to ensure the quality of the data. Table 6 shows the important differences between a registry and a clinical trial[232]:

Table 6 Differences between registry and clinical trial

Registry	Clinical trial
Observational study design	Active/experiment intervention
Clinical decision is executed by the clinicians.	Protocol-based clinical decision
Broader definition of inclusion and exclusion criteria	Strict definition of inclusion and exclusion criteria
Current standard intervention	Protocol-specific interventions
Current standard follow up	Protocol specific follow up

The position of registry type studies in the traditional hierarchies of evidence is not entirely clear. Due to the data collection methods, a registry is traditionally placed below randomised control trials in the conventionally used hierarchies[232]. The limitations of a registry are often highlighted in comparison with a randomised control trial. Some of the limitations are selection bias, non-standardised follow-up, greater risk of missing data and less strict inclusion or exclusion criteria into the registry[233].

Reliability and quality of the data collected is the cornerstone of the robust registry, hence minimising the limitations of registry as a health quality improvement and research tool. Using the National Intensive Care Evaluation registry (Holland) as case study, Arts et al[235] described the various data errors that can occur at the various steps in the electronic and manual data collection process (Figure 4). Based on the Dutch case study and literature review, Arts et al[235] proposed a framework of procedures for the assurance of data quality which can be implemented in order to improve the data quality in medical registry (Table 7).

Figure 4 Data errors at the different steps in the data collection process

Automatic data collection   **Manual data collection**

Data errors:

Patient data → Data entry ← Incomplete: 4.0%

Electronic patient record (PDMS)

Inaccurate: 4.8%
Incomplete: 3.3%

Extraction ← Inaccurate: 1.7%
Incomplete: 1.4%

Inaccurate: 0.6%
(Corrected: 0.8%)
(Completed: 1.9%)

Local registry database

Patient data → Documentation → Paper patient record → Data collection → Case Record Form → Data entry → Local registry database

Data transfer ← Incomplete: 3.6%

Inaccurate: 0.9%
Incomplete: 0.6%

Data transfer

Central registry database

Automatically collected data	Manually collected data
Inaccurate: 2.0%	Inaccurate: 4.6%
Incomplete: 6.0%	Incomplete: 5.0%

Ref: Arts et al. J Am Med Inform Assoc. 2002; 9: 600 - 601

Table 7 Framework of procedures for the assurance of data quality

Central coordinating centre	Local sites
Prevention during set up and organisation of registry	
At the onset of the registry	**At the onset of participating in the registry**
Compose minimum set of necessary data items	Assign a contact person
Define data & data characteristics in data dictionary	Check developed software for data entry and for extraction
Draft a data collection protocol	Check reliability and completeness of extraction sources
Define pitfalls in data collection	Standardise correction of data items
Compose data checks	Continuously
Create user friendly case record forms	Train (new) data collectors
Create quality assurance plan	Motivate data collectors
In case of new participating sites	Make data definitions available
Perform site visit	Place date & initials on completed forms
Train new participants	Keep completed case record forms
Continuously	Data collection close to the source and as soon as possible
Motivate participants	Use the registry data for local purposes
Communicate with local sites	In case of changes (e.g., in data set)
In case of changes (e.g., in data set)	Adjust data dictionary, forms, software, etc.
Adjust forms, software, data dictionary, protocol, training material, etc.	Communicate with data collectors
Communicate with local sites	
Detection during data collection	
During import of data into the central database	Continuously
Perform automatic data checks	Visually inspect completed forms
Periodically and in case of new participants	Perform automatic data checks
Perform site visits for data quality audit (registry data <> source data) and review local data collection procedures	Check completeness of registration
Periodically	
Check inter- and intraobserver variability	
Perform analyses on the data	
Actions for quality improvement	
After data import and data checks	After receiving quality reports
Provide local sites with data quality reports	Check detected errors
Control local correction of data errors	Correct inaccurate data & fill in incomplete data
After data audit or variability test	Resolve causes of data errors
Give feedback of results and recommendations	After receiving feedback
Resolve causes of data errors	Implement recommended changes
	Communicate with personnel

Ref: Arts et al. J Am Med Inform Assoc. 2002; 9: 600 – 601

1.3.2   The Warwick Carotid Artery Registry

Warwick Carotid Artery Registry has been designed to study the clinical prediction, radiological detection and treatment of cerebral microemboli in acute carotid syndrome. I designed and initiated the Registry based on the The Swedish National Registry for Vascular Surgery (Swedvasc) model. SwedVasc started in 1987 initially as a local vascular registry in southern Sweden (VRISS)[236] covering a 1.9 million population. Since 1994, all Swedish hospitals have joined the SwedVasc. The VRISS was set up with predefined and clear objectives to quantify and evaluate the outcome of vascular procedures performed within a well defined area of Southern Sweden[236]. Below are the important learning points based on the Swedish vascular surgeons' experience in setting up the Registry which put the data collection process at the heart of an effective Registry[236]:

1. The standardised proforma should be a simple form that can be expanded in the future in line with increasing complexity of vascular procedures. The form also should be simple and user friendly in order to increase compliance and minimise missing data. A previous report has shown that a large amount of data required for registry leads to reduced compliance and increased missing data[237].

2. The data collection process must be conducted in systematic manner with designated persons.

3. A Registry must have a designated steering committee who meet regularly to highlight and review any data collection problems.

The long term aim of this newly established regional Carotid Registry is to evaluate risk factors and outcomes of surgical interventions for carotid artery disease. The registry is a collaborative project within the acute hospital trusts within the Coventry and Warwickshire region. The registry is hosted and managed by the University Hospitals Coventry and Warwickshire NHS Trust which serves a population of 950,000. It is the tertiary referral centre for neurovascular disease and provides a regional carotid surgery services for the Coventry and Warwickshire region that includes the George Eliot Hospital and the South Warwickshire NHS Foundation Trust.

The University Hospitals Coventry and Warwickshire NHS Trust has provided transcranial Doppler imaging as part of carotid artery disease imaging since 2000[218]. As part of the local vascular surgery department policy[209], all patients are required to undergo transcranial Doppler examination in 2 clinical circumstances; within 2 weeks of the last symptoms of acute symptomatic critical carotid artery stenosis and as part of monitoring tools for carotid endarterectomy.

Currently, the identification of the high risk critical symptomatic carotid artery disease requiring urgent carotid endarterectomy relies on carotid duplex. Therefore, it is dependent on the urgency of the patient's referral for the investigation by the clinicians. NICE guidelines recommend that carotid endarterectomy should be performed within 2 weeks of presenting symptoms. A recent national survey showed that this is not yet achievable[149].

The most important point in designing this registry was the key research questions related to the role of microemboli as the surrogate biomarker to predict and treat the high risk symptomatic carotid artery syndrome cohort.

In establishing this registry, I hypothesised that the understanding of cardiovascular risk factors, combination of the established $ABCD^2$ score[100] for predicting very early stroke risk after transient ischaemic attack[101], and improvement of microemboli detection and treatment, will potentially allow improved assessment of hyper-acute symptomatic carotid disease risk and help in decision-making about the need for more aggressive medical therapy[238] and the case for urgent carotid revascularisation. The details of the research issues that were considered prior to establishing the Carotid Surgery Registry were as follows:

- Our current knowledge is limited with regard both to causes of development and severity of microemboli in carotid artery disease and their association with cardiovascular risk factors.
- To assess whether $ABCD^2$ risk score predicts cerebral microemboli.

- 10-15% of patients have no acoustic temporal bone window for transcranial Doppler ultrasound insonation. Therefore, an alternative new method to detect microemboli will be required.

- Despite preoperative antiplatelet treatment, patients still develop microemboli[176], therefore monitoring microemboli and their response to treatment enables targeting of antiplatelet therapy in patients at high risk of recurrent stroke syndromes[36].

The expected benefits from setting up this Registry are:

1. The creation of a unique database which reflects the local population and geographical area.

2. The data from the Registry could be used as part of audit or quality improvement and research activities.

3. The Registry could be used as a source for future service planning.

In my thesis, I used the Registry as the source of information with which to test hypotheses about predicting risk from the disease, assessing imaging methods and response to treatment for carotid artery associated cerebral microemboli.

CHAPTER TWO

2 Hypothesis, Aims and Objectives

2.1 Hypothesis

The study of clinical prediction, radiological detection and treatment of cerebral microemboli will improve outcomes in patients with carotid disease and spontaneous embolisation.

2.2 Aims

1. To establish a Carotid Surgery Registry as a resource with which to test hypotheses about predicting risk from the disease, assessing imaging methods, and response to treatment for carotid artery associated cerebral microemboli.

2. From the Registry and prospective observational study, to assess whether information from classical cardiovascular risk factor burden predicts the presence of carotid artery disease associated cerebral microemboli.

3. To evaluate transorbital Doppler as an alternative to transcranial Doppler for detecting cerebral microemboli in patients with carotid disease without an acoustic temporal bone window.

4. From the registry, to compare effectiveness of agents used to suppress cerebral microemboli.

2.3 Objectives

1. To establish a new Registry of patients who have undergone carotid endarterectomy between January 2002 and December 2012 at the Regional vascular surgery centre, University Hospital Coventry and Warwickshire NHS trust. To establish a prospective cohort of patients who have had hyper-acute symptomatic carotid artery disease between February 2011 and May 2013.

2. From the Registry and the above prospective observational study, to explore whether the Pocock score predicts the likelihood of an increased prevalence of cerebral microemboli, and whether the ABCD^2 score predicts the likelihood of spontaneous embolisation in hyper-acute symptomatic carotid artery disease patients.

3. From the Registry, to assess the feasibility of using the transorbital Doppler in those patients with absence of a suitable temporal acoustic window for transcranial Doppler monitoring.

4. To validate prospectively the use of transorbital Doppler against transcranial Doppler to detect cerebral microemboli acutely following carotid endarterectomy.

5. To compare effects of the glycoprotein IIb/IIIa receptor antagonist tirofiban with the anti-thrombotic polysaccharide dextran-40 in patients with high microembolic rates acutely after carotid endarterectomy.

CHAPTER THREE

3 Methods

3.1 Establishing the new Carotid Surgery Registry

3.1.1 Background

A new Carotid Registry was set up for the purpose of my study. Prior to my work, there was no

previous database or a collection of clinical information regarding carotid surgery at the

University Hospital Coventry and Warwickshire NHS Trust. The Registry was set-up based on the

Solomon et al[231] description of setting up an effective Registry. Creating the Registry requires a

specific range of organisational and technical skills. These are summarised below:

1. An implementation plan

The committee was set up and met regularly every 4 months in order to discuss the development

of the Carotid Registry. The committee has agreed that this Registry would be a long-term project

hosted by the Department of Vascular Surgery in University Hospital Coventry and Warwickshire

NHS Trust. The long term aims of this Registry, set up at the initial meetings of the committee,

were:

- To permit review of medical record information contained within the Carotid
  Registry to identify patients who may be eligible for participation in future
  research studies.

- To create a source of information to initiate future observational research studies
  and audits on the management of carotid artery disease patients and outcomes
  of carotid endarterectomy.

2. Adequate documentation

The Registry team comprised of the vascular consultants in the Trust and the vascular technologists. The research fellow and vascular technologists assumed the responsibility for running the registry on a daily basis. They were responsible for identifying patients, collecting data, and entering data on the safe electronic database supervised by the research fellow. They undertook a short period of training and worked under close supervision to become familiar with the Registry data processing and data entry procedure. The Research fellow was responsible for implementing the strategies approved by the committee; for monitoring the data collection process; and for confirming data accuracy. The principle investigators arranged appropriate office space, administrative equipment and IT support.

3. Quality control procedure

The Registry committee oversaw the quality control procedure, which followed the principle of the above framework of procedures for the assurance of data quality[231] (Table 7).

4. Completeness of data

Since 2000, all patients who underwent carotid endarterectomy were required to undergo preoperative carotid Duplex and transcranial Doppler examination in the vascular laboratory[218]. Therefore, all the patients' details were obtained from the vascular laboratory prior to the data collection process. The list of the patients included in the registry was also cross-referenced with the theatre logbook to avoid missing patients.

5. Validity of the data

Prospective data were collected using a standardised proforma, which was approved by the Registry committee prior to the start of the data collection. Retrospective data were collected using the standardised proforma by 2 independent assessors and any discrepancy was re-examined by a separate assessor. This was to minimise inaccuracy of the retrospective data[239]. A

previous study has shown that by adopting a systematic approach, a high degree of reliability and validity of retrospective data could be abstracted from the medical records[239].

Double-entry from 2 independent persons was used to transfer the data from the proforma to the registry's electronic database. They entered both prospective and retrospective data which were cleaned in stages to identify invalid entries. Any invalid data were re-examined by a separate assessor. A trial of the data collection process was conducted prior to the initiation of the Registry[176, 240]. This initial data collection process trial allowed the evaluation of the accuracy, completeness and missing data.

6. Case definition

The inclusion criteria were all patients with evidence of carotid stenosis ≥ 50% on carotid duplex undergoing a carotid endarterectomy. Please see section 3.1.5 for details on data definition, coding and collection process.

7. Determination of the data elements

Only clinically relevant data were collected in this Registry. Validated clinical, laboratory and imaging measurements and scales for data collected were used. Data dictionaries were defined in accordance with the criteria and reporting standards set by the American Society for Vascular Surgery (http://www.vascularweb.org/practiceresources/Documents/Forms) and The Vascular Society Great Britain and Ireland (https://www.vsqip.org.uk/resource-documents/). The same data dictionaries were used for both the Carotid Registry and prospective observational studies conducted following data analysis from the Registry.

8. Data collection procedure

This Carotid Registry integrated data from various sources. All data were collected using a standardised proforma to ensure completeness and uniformity of the data collected. Each patient received an individual code to ensure anonymity. Then the data were transferred to the software

for further analysis. After completion of the data transfer, the proforma was stored in a secured

area after the data transferred completed.

Between January 2002 and December 2012, 670 patients were included in the Registry. Registry

data from 2002 to 2010 (n=581) were collected retrospectively based on the standardised

protocol. The clinical information were gathered from the medical records, nursing records,

preoperative assessment clinic records, laboratory results, diagnostic imaging reports and

previous anaesthetic charts . 15 patients from the retrospective cohort were excluded in the

analysis due to lack of data pertinent to the Registry.

Since January 2011, using a standard protocol, data (n=89) have been collected prospectively. The

prospective data collection procedure was divided into 3 stages: preoperative, intraoperative and

postoperative (Table 8) below shows the source of data and the details of data collected during

each stage. Any data uncertainty was cross-referenced with the patients themselves.

Table 8 Data collection process

Stages	Data source	Data collected
Preoperative	Preoperative assessment documentation Admission clerking Nursing Documentation IT system	Demographics $ABCD^2$ score Cardiovascular risk factors Indication for surgery Preoperative medications, blood pressure, blood results and imaging results
Intraoperative	IT system Medical notes	Details of the operation
Postoperative	Discharge letter Medical notes Follow up clinic	Postoperative outcomes

9. Data access policy

The principle investigators were responsible for ensuring that the confidentiality of the patients

included in this Registry was maintained. The proforma contained anonymised or pseudo

anonymised codes which represented each subject in the database. Once the data were gathered,

it was transferred to the spreadsheet. These were entered into coded fields designed for statistical analysis. The registry was stored in a secured password protected institutional machine. The access of the data has to be approved by the Registry committee.

10. A framework for dissemination of findings

Any data requested from an outside researcher had to be approved by the committee.

3.1.2 Subjects

This regional vascular surgery centre provides a carotid surgery service for a geographical area that includes a ~ 950 000 catchment population. Overall, 670 patients were included in this Registry. 581 patients were entered retrospectively from January 2002 to December 2010. Between January 2011 and December 2012, 89 patients were collected prospectively.

3.1.3 Inclusion criteria

The main inclusion criterium for this registry was to include all patients who had undergone carotid endarterectomy at University Hospital Coventry and Warwickshire NHS Trust from January 2002 and December 2012.

3.1.4 Ethics

This Registry followed the principles agreed with the Research & Development Department at University Hospital Coventry and Warwickshire NHS Trust, confirming that Research Ethics Committee review was not required under the harmonised GAfREC for research limited to use of previously collected information non-identifiable by researchers outside the usual care team. This exception also applies to research undertaken by staff within the care team using information previously collected in the course of care for the team's own patients, with the proviso that data

is anonymised or pseudonymised in conducting the research, as such research involves no breach

of the duty of confidentiality owed by care professionals.

(http://www.publichealth.hscni.net/sites/default/files/directorates/files/GAfREC_changes_Remit

_REC_2011_08.pdf)

3.1.5 The standard operating procedure (SOP) for data collection

The standard operating procedure for data collection described in this section was used for the

carotid registry and subsequent prospective observational studies. In general, the data collection

process was divided into 3 stages: preoperative, intraoperative and postoperative stages.

Preoperative stage

Patient demographic data, clinical data, cardiovascular risk factors and details prior to surgery

were available from the preoperative assessment documentation from the clinic, nursing

documentation and admission clerking. The cardiovascular blood results were identified from the

online Hospital IT system. Preoperative drugs including antiplatelet agent details were readily

available from the drug chart. Carotid Doppler results were obtained from the IT system and

clarified with our vascular technologists where appropriate.

Data collected

1. Patient demographics – Age, sex, gender, height and weight.

2. Validated $ABCD^2$ risk score - The $ABCD^2$ score ranges from $0 – 7$[101]. The score was obtained

   following patients' assessment in the fast-track TIA/minor stroke clinic by the neurologists

   and stroke physicians.

   - Age ≥ 60 years - 1 point.

   - Blood pressure ≥140/90mmHg - 1 point

- Limb weakness - 2 points; speech impairment – 1 point; duration of symptoms - 2 points for ≥ 60 minutes, and 1 for 10-59 minutes.

- Diabetes mellitus - 1 point.

3. Cardiovascular risk factors – Definition was based on the Joint British Societies' Guidelines on Prevention of Cardiovascular Disease in Clinical Practices[93].

   - Hypertension was defined as systolic blood pressure (BP) ≥ 140mmHg and diastolic BP ≥ 85 mmHg. Hypertension was coded as history of hypertension, treatment with antihypertensive drugs, or persistent high blood pressure during admission due to undiagnosed or untreated hypertension.

   - Smoking history (self-reported) was divided into non-smokers, current smokers (at least one cigarette per day) and ex-smokers. Details of history included pack/years history, when stopped and type of tobacco.

   - Family history of cardiovascular disease was based on patients' history and previous documentation in the medical notes.

   - Diabetes mellitus was defined as history of diabetes, including treatment with diet, oral medication and insulin, or new finding of diabetic range blood glucose (fasting blood glucose≥ 6 mmol/L).

   - Hypercholesterolaemia was defined as history of hyperlipidaemia, treatment with antihypercholesterolaemia drugs or new finding of high cholesterol level ≥ 4 mmol/L.

   - Peripheral vascular disease was defined as documented symptoms of claudication with evidence of reduced ankle-brachial pressure index (< 0.9).

4. Cardiovascular risk scores

Total cardiovascular disease (CVD) risk for an individual is estimated from several risk factors and slightly differs between individual cardiovascular risk score. CVD end point is

defined as combined end point of coronary heart disease (fatal and non-fatal myocardial infarction and new angina) plus stroke (fatal and non-fatal stroke and cerebral haemorrhage) and transient cerebral ischaemia[93]. The total CVD risk score is expressed as a probability (percentage chance) of developing CVD or death over a defined period of time. Framingham, Joint British Societies' Guidelines on Prevention of Cardiovascular Disease in Clinical Practice (JBS 2), Scottish ASSIGN scores estimate the CVD risk over 10 years where as Pocock score estimates the risk of CVD death over 5 years[93, 99].

Online cardiovascular risk scores calculator developed by University of Edinburgh (http://cvrisk.mvm.ed.ac.uk/) was used to calculate Framingham, JBS 2, Scottish ASSIGN scores. Pocock cardiovascular risk score was calculated using the online calculator (http://www.riskscore.org.uk/).

5. Indications for carotid endarterectomy

- TIA - Defined as any acute focal ischemic neurological deficit lasting at least 30 seconds but resolving completely within 24 hours[241]. Symptoms and signs resulting from the right carotid artery include left-sided weakness, paraesthesia, neglect, abnormal visual-spatial ability, and right monocular blindness or homonymous hemianopia. Symptoms and signs resulting from the left carotid artery include right-sided weakness, paraesthesia, aphasia, and left monocular blindness or homonymous hemianopia.

- Stroke - focal neurological deficit of abrupt onset persisting more than 24 hours and corresponding to a vascular territory.

- Amaurosis fugax – Often described as a shade drawn upward or downward over the field of view[242]. The symptom lasting at least 30 seconds but resolving completely within 24 hours.

- Asymptomatic with carotid artery critical stenosis - Asymptomatic carotid artery with stenosis ≥ 70% defined by ultrasound Duplex scan.

- Pre/post coronary artery bypass carotid endarterectomy.

6. Date of the last symptoms (for symptomatic carotid) – Defined as the date of the last symptoms prior to medical assessment.

7. Preoperative medications – This included antiplatelet agents or anticoagulant, antihypertensive medications and antihypercholesterolaemia.

8. Preoperative blood pressure – blood pressure measured according to British Hypertension Society (BHS) guidelines using validated devices. BP measured in both arms and the arm with the higher value used to assess BP control. Two measurements (1-2 minutes apart) were taken on each occasion. If large (>10 mmHg) difference between the first and subsequent readings, initial value discarded and further measurements made.

9. Preoperative blood results, ECG, echocardiogram, carotid Doppler and CT/MRI.

10. The number of microemboli detected by TCD.

Intraoperative stage

Operation details were obtained from the electronic copy or medical notes.

Data collected - Type of anaesthesia, details of arteriotomy closure and details of postoperative trancranial Doppler monitoring including microembolic rates.

Postoperative stage

Thirty days postoperative clinical events were obtained from the medical notes, discharge letter to the GP and follow up clinic. Postoperative outcomes coded include TIA/stroke (ischaemic or haemorrhagic), myocardial infarction, death, neck haematoma, cranial nerve injuries, thrombocytopenia.

3.1.6   Preoperative surgical preparation

Administration of antiplatelet agents

Aspirin 75mg oral or clopidogrel 75mg oral or, the combination of both antiplatelet agents in these doses was given before carotid surgery. The decision on preoperative antiplatelet treatment was decided by the individual surgeons.

Carotid duplex

All the patients who underwent carotid endarterectomy required preoperative carotid artery stenosis assessment. The Vascular Society and Society of Vascular Technology of Great Britain and Ireland[118] recommended criteria based on the NASCET method of grading carotid bulb disease were used to assess the stenosis of the carotid artery. See section 2.1.4 for further details of the carotid Duplex procedure.

3.1.7   Intraoperative - Imaging and surgical methods

Transcranial (temporal acoustic window) Doppler

In order to ensure accurate TCD monitoring, the examiner must have knowledge of the anatomy of the extra-cranial arteries and patients must be resting comfortably to avoid movement artefacts. The effective transmission of ultrasound through the cranium relies on the bone thickness. Ultrasound is transmitted more effectively through thin cranial bone as this reduces the power loss in the ultrasound beam. The conventional method relies on the temporal bone acoustic window (TAW), one of the thinnest cranial bones through which the ultrasound beam can be focused on the intracerebral arteries (Figure 5).

All patients with haemodynamically significant ≥ 50% carotid stenosis went on to have TCD examination which was performed by accredited vascular technologists[118]. TCD monitoring was performed (PC Dop 842, SciMed, Bristol, UK) with a 2 MHz probe focused on the middle cerebral artery (MCA) ipsilateral to the endarterectemised carotid artery (Figure 5). The probe was placed above the zygomatic arch and just in front of the ear. The identification of the MCA was based on

guidelines set out by Alexandrov et al[243] including spectral waveform, flow direction, blood flow velocity and flow pulsatility. Proof of traceability of the MCA was necessary for identification. The traceability referred to the fact that the MCA can be tracked from a shallow insonation depth (~ 35mm) to deeper depth (~55mm) without changes in the character of the flow profile and flow direction. An insonation depth of at least 45mm was used to identify proximal MCA flow signals and depths of 30 – 45mm for distal MCA flow signals. A standard axial sample volume of 5 mm and a low gain was used to provide a setting optimal from discriminating microemboli from the background arterial wave signals. A head-frame was used to secure a constant angle of insonation during TCD monitoring.

Figure 5 Transcranial Doppler apparatus

Figure 6 Illustration of MCA insonated by ultrasound probe[157]

Middle cerebral artery

Ultrasound probe insonating middle cerebral artery via temporal bone window
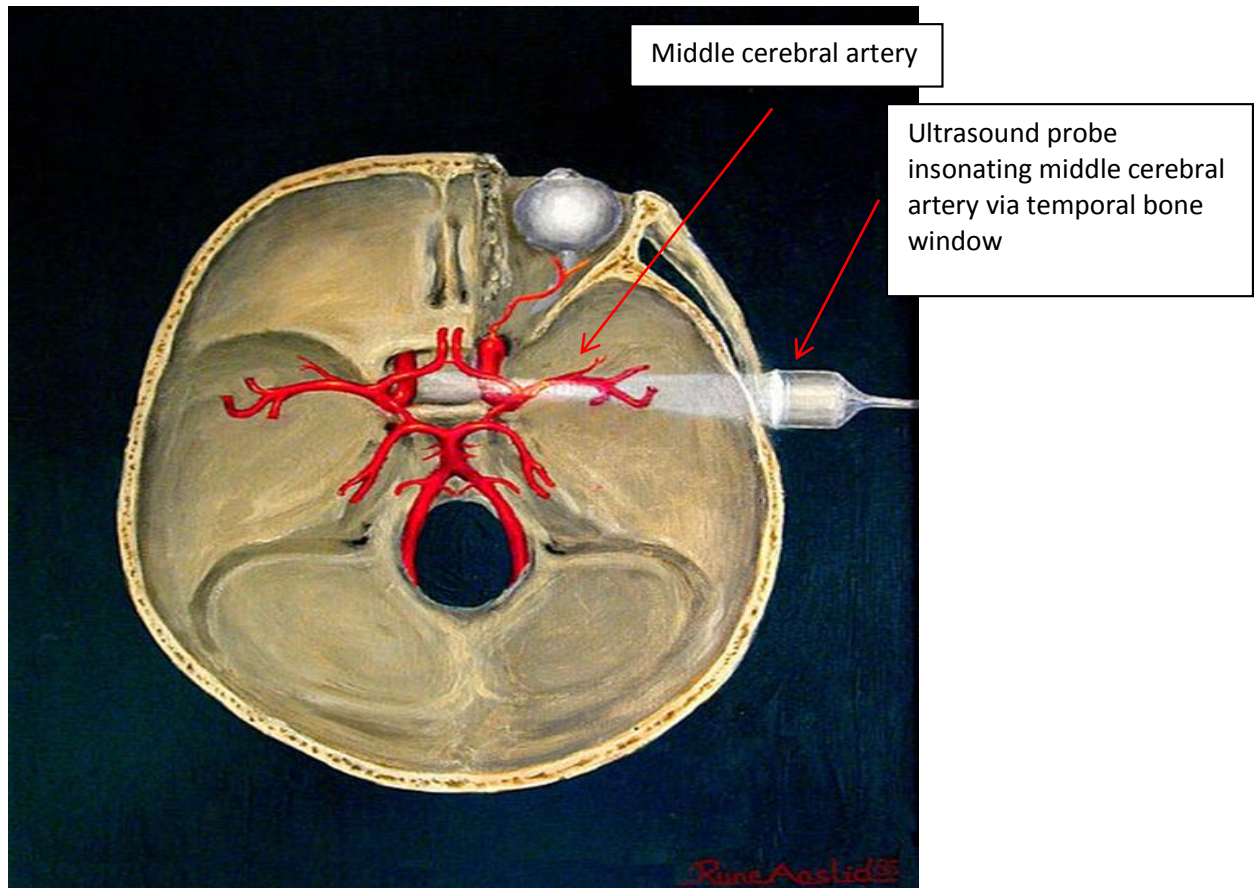
MES identification

Single gating system and a filter set to a low threshold to capture signals was used. Automated WinTCD v3.7 software (VIASYS Healthcare Inc., Conshohocken, PA, USA) was used to monitor and record the Doppler wave form and audio signals. Our Vascular Technologists have 90% agreement with software validated against a panel of international experts[244] and have participated in a multi-centre prospective study[168]. The recorded ultrasonographic images were then reassessed to exclude artefact and identify true microemboli based on the criteria of the International Consensus Group on microembolus detection[171]. MES were identified as unidirectional, short duration signals (range 10–100 ms) with intensity threshold above 6 dB, unidirectional, accompanied by characteristic audible clicks and occurring randomly throughout the cardiac cycle. In order to improve the accuracy of MES identification, our vascular technologists manually

recorded MES in the standard proforma. Any undecided MES signal recorded by the automated software can be cross-referenced with the manually recorded MES.

Carotid endarterectomy

Patients underwent conventional[167] CEA under either general (GA) or local anaesthesia (LA). The GA CEAs had both intra operative and postoperative TCD monitoring[245]. An incision was made anterior to the sternocleidomastoid muscle between the sternal notch and mastoid process. The incision was carried down through the platysma, and the sternocleidomastoid muscle was retracted laterally with self-retaining retractors, once the internal jugular vein was visualised, and the carotid sheath was opened along the anterior border of the vein. Dissection was continued until the common carotid artery and vagus nerve were identified. Careful attention was undertaken to avoid injuries to cranial nerves IX (glossopharyngeal nerve), X (vagus nerve), XI (accessory nerve), XII (hypoglossal nerve), the marginal mandibular branch of VII (facial nerve). The common carotid, internal carotid and external carotid arteries were mobilised and controlled. Heparin was administered intravenously, prior to clamping the carotid arteries. Arteriotomy was made once the internal carotid artery, the common carotid artery, and the external carotid artery were clamped.

Intraoperative hypotension is the main mechanism of cerebral ischaemia following the clamping of the carotid arteries prior to the endarterectomy stage[246]. In our unit, the intraoperative hypotension and neurological monitoring are performed using TCD in GA cases and awake patient monitoring in LA cases. Those who underwent surgery under GA will have a shunt to bypass the clamped vessel selectively, based on a greater than 50% drop in MCA time averaged mean velocity on clamping. Halsey et al identified that a fall in the middle cerebral artery velocity to less than 40% of its pre-clamp value caused mild cerebral ischaemia and a fall less than 15% of its pre-clamp velocity caused severe ischaemia[247]. A rigid metal head guard was used to protect the TCD probe. For those GA patients with no TCD window, shunting was routinely performed, but for those under LA selective shunting was used according to whether a neurological deficit occurred

on clamping. Both GA and LA CEA had postoperative TCD monitoring. After completion of the

endarterectomy, selective patching was performed according to surgeon preference[245], with

either a dacron (Fluoropassiv™ Vascutek Ltd, Inchinnan, Scotland) or polyester-urethane

(Microporus Patch, B.Braun Melsungen Germany) patch. The closure was performed once the

haemostasis had been achieved.

3.1.8 Post-operative - pharmacological management

Microembolic signal (MES) rate calculation post carotid endarterectomy:

Post-operative monitoring was started as soon as the patient was in Theatre recovery following

carotid endarterectomy. Monitoring was continued for at least 30 minutes post-operatively.

Monitoring was extended if any microembolic signals were detected. In our Unit, the rate of MES

was calculated based on the number of microemboli detected within 15 minute intervals. Rescue

antiplatelet therapy was initiated when the MES rate > 50/hr[31].

The protocol of postoperative administration of antiplatelet agents for MES rate > 50/hr[31] are

summarised below:

Tirofiban protocol

Tirofiban protocol recommended for use in acute coronary syndrome[248] were used. Tirofiban

(Aggrastat®; Merck Sharp & Dohme, Hoddeson, UK) was given intravenously as 0.4mcg/kg/min for

30 minutes then 0.1mcg/kg/min as a continuous infusion for 18 hours. Dosage had to be adapted

to renal function. Repeat TCD was performed and monitoring was continued until the MES rate

was < 4/hr. Tirofiban infusion was stopped once the MES ceased.

Dextran-40 protocol

Use of dextran-40 in reducing MES after CEA has been extensively investigated by the Leicester

Group[33, 209]. Our dextran-40 practice was based on Leicester Group work[33].

Patients were commenced on an intravenous infusion of dextran-40 (10% Gentran-40 [Baxter

Healthcare, Thetford, UK] in 5% glucose solution). An initial 20-ml bolus was given, and the

dextran-40 infusion continued at 20 ml/h.

If a patient continued to have TCD evidence of a persistent high rate of MES, the rate of the

dextran-40 infusion was increased at increments of 20 ml/h until the MES reduced. The dextran-

40 infusion was continued for 18 hours. Repeat TCD was performed and monitoring was

continued until the MES rate was < 4/hr. Dextran-40 infusion was stopped once the MES ceased.

3.1.9   Statistics

Statistical analyses were conducted using SPSS® version 21 (Chicago, Illinois, USA).

Parametric data are expressed as mean and standard error of the mean (SEM). Non-parametric

data are expressed as median and inter quartile range (IQR). Non-parametric unpaired data were

analysed using the Mann-Whitney U test for two group comparisons. Categorical variables were

analysed using the chi-squared test or Fisher's exact test. A P-value of less than 0.05 was

considered significant.

Framingham, JBS 2, ASSIGN scores considered 20% as the threshold where healthy patients

should be considered for pharmacology and lifestyle interventions. Pocock score was developed

based on data of patients who were already established as having cardiovascular disease[93, 99].

Therefore all the patients in my thesis are eligible for Pocock score estimation. The association

between microemboli and Pocock risk score was analysed using Receiver Operating Characteristic

Curves (ROC).

3.2　Hyper-acute symptomatic carotid artery stenosis study

Two hundred and six (age 70 ± 1 [SEM] years, male 149, Caucasian 196) patients were included in this study. This included 102 patients from our Carotid Registry retrospectively assessed between January 2002 and December 2010[176]. A further 104 consecutive patients were recruited between February 2011 and May 2013 within a new prospective study of hyper-acute symptomatic carotid artery stenosis. The prospective study was approved by the NHS Ethics Committee (MREC number 10/H1206/77) and Research & Development Department (R&D C1080610). The details of the clinical and imaging methods are discussed in chapter 5.

3.3　Prospective transorbital Doppler validation study

Two phases are described for studies to evaluate the transorbital Doppler technique in detecting cerebral microemboli. The 1st phase was to identify the proportion of patients who required transorbital monitoring due to a lack of an accessible temporal window for standard TCD after surgery from the Carotid Registry between 2005 and 2008. The 2nd phase was a prospective observational study to validate the transorbital technique against the standard transcranial Doppler using temporal window technique. One hundred consecutive patients fulfilling the inclusion and exclusion criteria who underwent elective CEA between February 2011 and May 2013 at the University Hospital Coventry and Warwickshire were included in the study. The prospective study was approved by the NHS Ethics Committee (MREC Number 10/H1211/51) and Research & Development Department (R&D Reference C1081810). The details of the clinical and imaging methods are discussed in chapter 6.

CHAPTER FOUR

4 Carotid surgery registry and the use of classical cardiovascular risk factors for predicting early post-operative microembolisation in patients undergoing carotid surgery

4.1 Introduction

This newly established regional carotid surgery Registry provides important information on the cardiovascular risk factor burden and clinical outcomes of surgery in patients with critical carotid artery disease. The Registry records precisely defined and standardised details on demography, cardiovascular risk factors, carotid imaging techniques, surgical technique and postoperative outcome at 30 days. The data for this Registry was gathered from unselected haemodynamically significant critical carotid artery disease patients who underwent carotid endarterectomy in everyday clinical practice. Therefore, it reflects the actual clinical practice and is invaluable for any quality improvement programme and research activities. The data available in this Registry has been used to evaluate novel carotid imaging techniques[240] and a novel antiplatelet agent[176] to treat transcranial Doppler detected cerebral microemboli to reduce thrombotic stroke risk and forms part of my thesis. Data between 2002 and 2012 were analysed for the completion of this thesis.

4.2 Subject and Methods

Please refer to section 3.1 for the details of methods for the Registry.

4.3 Results

4.3.1 Clinical features

Preoperative

Between January 2002 – December 2012, 670 patients were included in the Registry (Age 71 ± 0.4 [SEM] years, 474 [71%] male and 652 [97%] Caucasian) (Table 9). Registry data from 2002 to 2010

(n=581) were collected retrospectively based on the protocol. Since January 2011, using a

standard protocol, data (n=89) have been collected prospectively, including for perioperative

clinical and laboratory data, and for carotid imaging, including detection of microemboli.

Table 9 Demographic and clinical profile of the 670 patients who underwent carotid endarterectomy between January 2002 and December 2012

	n (%)
Age, mean ± SEM	71 ± 1
Male	474 (71)
Caucasian	652 (97)
South Asian	18 (3)
Hypertension	496 (74)
Never smoke	196 (29)
Current smoker	133 (20)
Ex-smoker	341 (51)
Ischaemic heart disease	219 (33)
Type 2 Diabetes mellitus	123 (18)
Hypercholesterolemia	327 (49)
Peripheral arterial disease	98 (15)
Cerebrovascular disease	144 (22)
Family history of cardiovascular disease	126 (19)

Carotid endarterectomy was undertaken in 474 (71%) patients for symptomatic carotid stenosis (

Table 10). Carotid endarterectomy was undertaken in 196 (24.8%) asymptomatic patients during

the same period. The proportion of asymptomatic patients who underwent CEA was significantly

less during the period 2011-2012 than 2002-2010 (10% vs. 33%, P < 0.001, X^2 test).

Of the 670 patients included in this thesis, 74% patients were hypertensive, 71% patients were

smokers and 49% patients had hypercholesterolaemia (Table 9). Mean systolic BP was 145±1

(SEM), diastolic BP was 74±1 mmHg, BMI was 27±0.2 kg/m^2 and total cholesterol was 4.6±0.1

mmol/L (Table 11).

Table 10 Indications of carotid endarterectomy

Indications	n (%)
TIA	251 (38)
Minor stroke	125 (19)
Amourosis fugax	76 (11)
Retinal artery occlusion	22 (3)
Asymptomatic with carotid artery critical stenosis	166 (25)
Pre/Post CABG	30 (5)

Table 11 Blood pressure and preoperative laboratory values of 670 patients who underwent carotid endarterectomy between January 2002 and December 2012

Laboratory variables	Mean ± SEM (n)
Systolic blood pressure	145±1 (645)
Diastolic blood pressure	74±1 (645)
BMI (kg/m^2)	27±1 (553)
Cholesterol (mmol/L)	4.6±0.1 (586)
Triglyceride (mmol/L)	1.7±0 (433)
HDL (mmol/L)	1.4±0 (479)
Creatinine (μmol/L)	104±1.3 (670)
Glucose (mmol/L)	5.8±0.1 (563)
Haemoglobin (g/L)	135±1 (670)
Platelet (x 10^9/L)	253±2.7 (670)
White cell count (x 10^9/L)	7.7±0.1 (670)
CRP (units)	6.3±1.1 (71)
HbA$_{1c}$ (%)	6.7±1.1 (119)

The proportion of patients with hypercholesterolaemia (44% vs. 50%, P = 0.035), previous history of stroke or TIA (13% vs. 23%, P = 0.029) and history of ischaemic heart disease (21% vs. 34%, P = 0.009) was reduced significantly during the period 2011-2012 than 2002-2010 (Table 12).

However, the preoperative level of systolic blood pressure (151±2 vs. 145±1mmHg, P = 0.003), glucose (6.5±0.4 vs. 5.7± 0.1, P = 0.023) and white cell count (8.2±0.2 vs. 7.6± 0.1, P = 0.024) were significantly increased during the period 2011-2012 when compared to 2002-2010 (Table 13).

Overall, 4 patients were on preoperative anticoagulant, 383 patients were on single antiplatelet agent preoperatively, 270 were on dual and 13 were on triple antiplatelet agents preoperatively (Figure 7). The proportion of patients who were on dual or triple antiplatelet agents prior to carotid endarterectomy were significantly higher during the period 2011-2012 when compared to 2002-2010 (Table 14) (Figure 7).

Table 12 Demographic and clinical profile of 581 patients (retrospective) between January 2002 and December 2010 and 89 patients (prospective) who underwent carotid endarterectomy between January 2011 and December 2012

	Retrospective, n =581(%)	Prospective, n = 89(%)	X^2 test, P value
Age, mean ± SEM	70±1	72±1	0.135
Male	417(72%)	57(64%)	0.087
Caucasian	565(97%)	87(98%)	**0.025**
Hypertension	430(74%)	66(74%)	0.546
Never smoke	165(28%)	31(35%)	0.104
Current smoker	111(19%)	22(25%)	
Ex-smoker	305(53%)	36(40%)	
Ischaemic heart disease	200(34%)	19(21%)	**0.009**
Type 2 Diabetes mellitus	101(17%)	22(25%)	0.231
Hypercholesterolemia	292(50%)	39(44%)	**0.035**
Peripheral arterial disease	88(15%)	10(11%)	0.212
Cerebrovascular disease	132(23%)	12(13%)	**0.029**
Family History of cardiovascular disease	115(20%)	11(12%)	0.059

Table 13 Blood pressure and preoperative laboratory values of 581 patients (retrospective) between January 2002 and December 2010 and 89 patients (prospective) who underwent carotid endarterectomy between January 2011 and December 2012

Laboratory variables	Retrospective, Mean ± SEM (n)	Prospective, Mean ± SEM (n)	T-test, P value
Systolic blood pressure	144.5± 1 (556)	151.3±2 (89)	**0.008**
Diastolic blood pressure	73.9± 1 (556)	75.6±1 (89)	0.225
BMI (kg/m^2)	26.8± 0.2 (469)	27.6±0.5 (84)	0.182
Cholesterol (mmol/L)	4.6±0.1 (519)	4.9±0.2 (67)	0.085
Triglyceride (mmol/L)	1.7±0.1 (398)	1.5±0.1 (35)	0.233
HDL (mmol/L)	1.4± 0.02 (414)	1.448± 0.1 (65)	0.942
Creatinine (μmol/L)	105.1± 1. (581)	95.2± 4.4 (89)	**0.01**
Glucose (mmol/L)	5.7± 0.1 (510)	6.5±0.4 (53)	**0.023**
Haemoglobin (g/L)	135±1 (581)	134± 2 (89)	0.578
Platelet (x 10^9/L)	252.1±2.9 (581)	262.7±7.5 (89)	0.187
White cell count (x 10^9/L)	7.6± 0.1 (581)	8.2±0.2 (89)	**0.024**
CRP (units)	7.0± 1.4 (53)	4.2±0.4 (18)	0.064
HbA$_{1c}$ (%)	6.6±0.1 (98)	6.8± 0.3 (21)	0.510

Table 14 Different antiplatelet therapy regime prior to carotid endarterectomy (n = 670)

	Retrospective, n (%)	Prospective, n (%)	X^2 test, P value
Anticoagulant	4 (1)	0	**< 0.001**
Single antiplatelet	352 (60)	31 (35)	
Dual antiplatelet	221 (38)	49 (55)	
Triple antiplatelet	4 (1)	9 (10)	

Figure 7 Different antiplatelet regime therapy prior to carotid endarterectomy (n = 670)
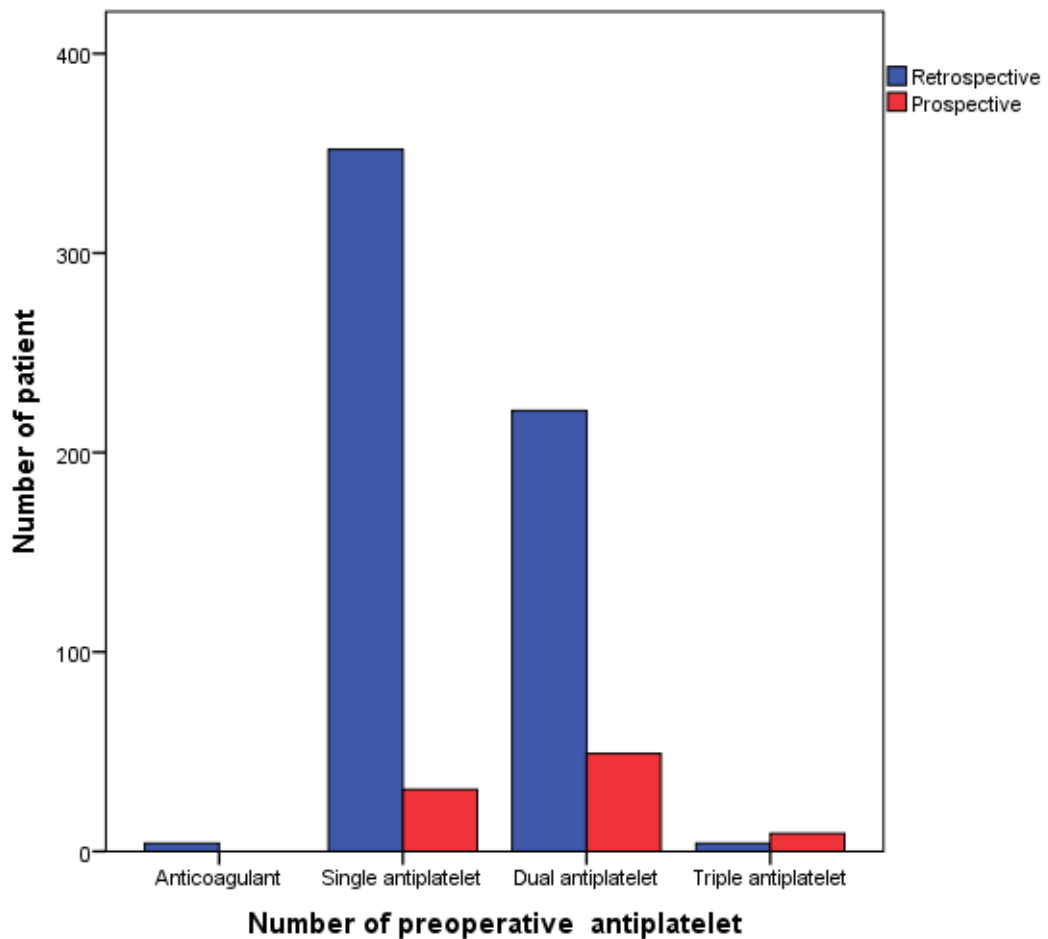
Intraoperative

465 patients underwent carotid endarterectomy under general anaesthesia and 205 patients were under sedation and local anaesthesia. Following endarterectomy of the carotid artery, 351 patients underwent prosthetic patch, 11 patients underwent vein patch and 302 patients underwent primary arteriotomy closures. A shunt was required in 168 patients while 493 patients did not require a shunt during endarterectomy.

Post-operative

Following carotid endarterectomy, 335 (53%) patients developed acute postoperative cerebral microemboli (≥ 1 emboli). The proportion of patients who developed acute postoperative cerebral

microemboli was significantly higher in the retrospective group (n = 327, 56%) than prospective group (n = 28, 32%), (Odds ratio 0.357 95%CI 0.221 – 0.574, P < 0.001).

Eighty six (13%) patients developed sustained high microembolic rate (MES > 50hr^{-1}) requiring additional antiplatelet therapy to reduce risk of postoperative stroke. The proportion of patients who developed sustained high microembolic rate (MES > 50hr^{-1}) were higher in the retrospective group (n = 80, 14%) than prospective group (n = 6, 7%), (Odds ratio 0.453 95%CI 0.191 – 1.07, P = 0.065).

The overall 30 day mortality was 13 out of 670 patients (1.9%). The 30 day morbidity was CVA or TIA in 16 (2.4%), myocardial infarction in 9 (1.3%), neck haematoma in 24 (3.6%) and transient cranial nerve injury in 34 (5.1%) patients (Table 15). Cumulative major event included perioperative stroke or TIA, myocardial infarction and death and cumulative minor event included neck haematoma and cranial nerve injuries. The cumulative 30 day major event rate was 4.3% (29) and minor event rate 8.4% (56) (Table 15).

Table 15 30 day complications of 670 patients who underwent carotid endarterectomy between January 2002 and December 2012

Complications	n (%±95%CI)
Cerebrovascular accident	16 (2.4 ± 1.24 – 3.56)
Myocardial infarction	9 (1.3 ± 0.44 – 2.16)
Death	13 (1.9 ± 0.87 – 2.93)
Neck haematoma	24 (3.6 ± 2.19 – 5.01)
Cranial nerve injury	34 (5.1 ± 3.43 – 6.77)
Cumulative major event (CVA,MI, Death)	29 (4.3 ± 2.76 – 5.84)
Cumulative minor event (neck haematoma, cranial nerve injuries)	56 (8.4 ± 6.3 - 10.5)

There was a trend of increasing cardiovascular risk score with 30-day mortality, but this was not statistically significant (Table 16). There was no difference in terms of cardiovascular risk score between patients who had a postoperative major event and those who did not have any postoperative major events (Table 17). Overall, there was a trend of increasing number of major

cumulative events with increasing cardiovascular risk scores, but this was not statistically

significant (Table 18).

Table 16 Cardiovascular risk score for 30-day mortality of 670 patients who underwent carotid endarterectomy between January 2002 and December 2012

Cardiovascular risk score	P – value (MW test)
Pocock	0.139
CVD (Cardiovascular Disease) Framingham	0.730
CVD-Death Framingham	0.145
JBS2	0.701
ASSIGN	0.552

Table 17 Cardiovascular risk score for 670 patients for any 30-day postoperative major event (n = 30) vs. non-major event (n = 640) between January 2002 and December 2012

Cardiovascular risk score	P – value (MW test)
Pocock	0.315
CVD Framingham	0.942
CVD-Death Framingham	0.386
JBS2	0.981
ASSIGN	0.656

Table 18 Cardiovascular risk score for 30-day major cumulative events of 670 patients who underwent carotid endarterectomy between January 2002 and December 2012 (1 event vs. 2 events vs. 3 events)

Cardiovascular risk score	P – value (Kruskal Wallis test)
Pocock	0.451
CVD Framingham	0.241
CVD-Death Framingham	0.425
JBS2	0.245
ASSIGN	0.736

4.3.2   Classical cardiovascular risk factor to predict for microemboli in patients acutely following carotid surgery

Overall

The proportion of patients who had Pocock risk score ≥ 0.8% was higher than patients who had Framingham, ASSIGN and JBS2 scores ≥ 20% (Table 19). The proportion of patients who had Framingham, ASSIGN scores ≥ 20% and Pocock risk score ≥ 2.3% were similar. The proportion of patients who developed microemboli (≥ 1 microemboli) among this cohort were similar between these 4 scores (Framingham≥ 20%: microemboli 53%, JBS 2: 55%, ASSIGN: 55%, Pocock ≥ 2.3%: microemboli 55%).

Table 19 Distribution of microembolic patients based on risk score: 355 with postoperative microemboli vs. 315 without postoperative microemboli

Cardiovascular risk score, n	Non-embolising, n	Embolising, n	X^2 test P value
Pocock ≥ 0% (18)	10	8	0.370
Pocock ≥ 0.3% (37)	23	14	
Pocock ≥ 0.8% (135)	67	68	
Pocock ≥ 2.3% (218)	98	120	
Pocock ≥ 6.1% (191)	84	107	
Pocock ≥ 15.6% (71)	33	38	
CVD Framingham ≥20% (481)	223	258	0.607
CVD-Death Framingham ≥20% (134)	60	74	0.629
JBS2 ≥20% (357)	162	195	0.394
ASSIGN ≥20% (473)	212	261	0.089

Microembolic cohort vs. non-microembolic cohort

Patients with evidence of cerebral microemboli acutely following CEA had a significantly higher Pocock score at baseline (microemboli 4.6% [2.2 – 9.5] vs. non-microemboli 4.2% [1.7 – 7.9], P = 0.039, MW test) (Table 20).

Table 20 Cardiovascular risk score for 670 patients who underwent carotid endarterectomy between January 2002 and December 2012: 355 with postoperative microemboli vs. 315 without postoperative microemboli

Cardiovascular risk score	P – value (MW test)
Pocock	**0.039**
CVD Framingham	0.178
CVD-Death Framingham	0.291
JBS2	0.216
ASSIGN	0.067

Figure 8 Shows the difference of Pocock score between 355 with postoperative microemboli and 315 without postoperative microemboli who underwent carotid endarterectomy between January 2002 and December 2012
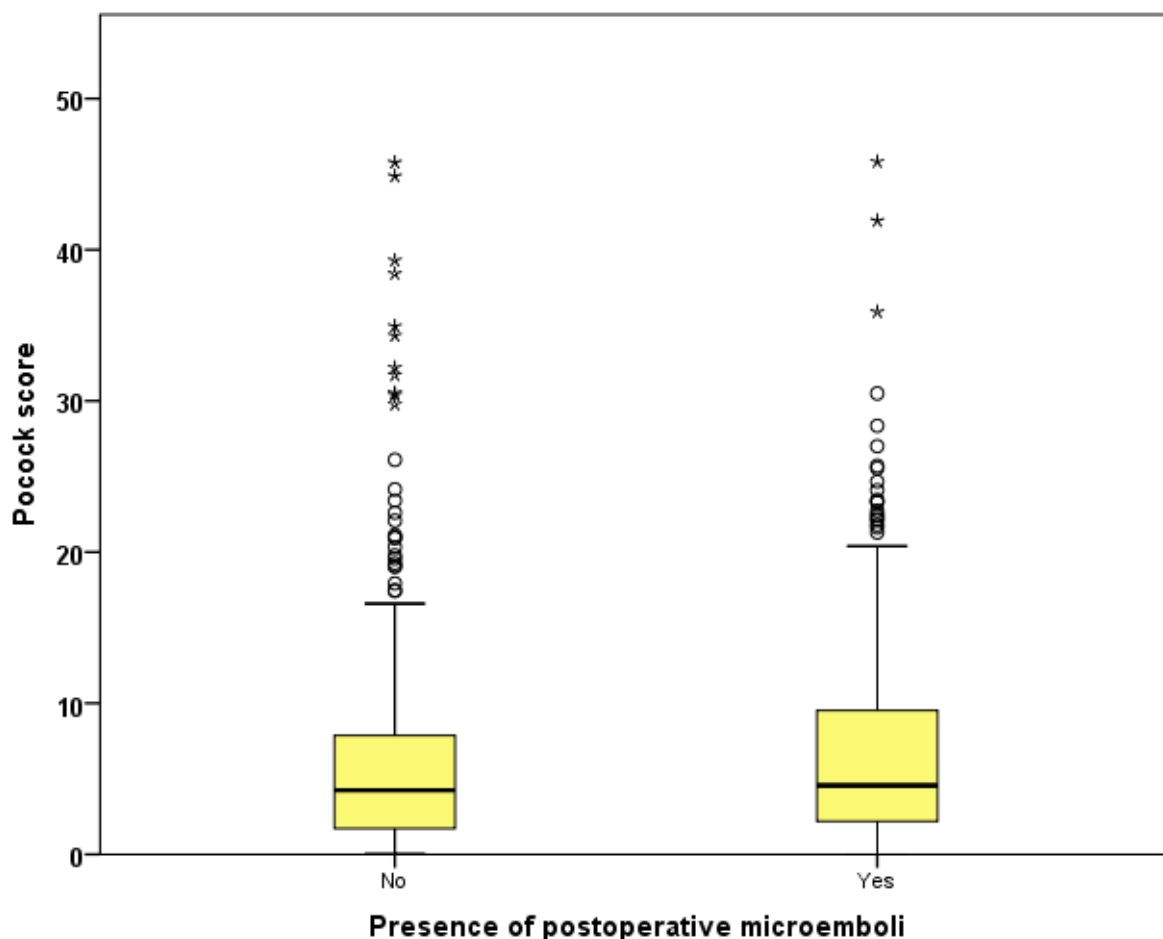
Cardiovascular risk score showed an increasing trend, but this was not statistically significant, to predict patients who developed sustained high MES rate > 50hr[1] acutely following carotid endarterectomy requiring additional antiplatelet therapy (Table 21).

Table 21 Cardiovascular risk score for 670 patients who underwent carotid endarterectomy between January 2002 and December 2012: 86 patients had a sustained high microembolic rate requiring additional antiplatelet therapy vs. 584 did not require additional antiplatelet therapy

Cardiovascular risk score	P – value (MW test)
Pocock	0.164
CVD Framingham	0.638
CVD-Death Framingham	0.503
JBS2	0.695
ASSIGN	0.196

Receiver Operating Characteristic

For further analyses, whether Pocock score was able to predict the presence of cerebral microemboli acutely post carotid endarterectomy was analysed in 4 stages; general, Pocock score ≥ 0.8%, Pocock score ≥ 2.3% and Pocock score ≥ 6.1% as a cut-off point. Patients who had a Pocock score ≥ 0.8%, 2.3% and 6.1% are 0.8%, 2.3% and 6.1% at risk of cardiovascular mortality in 5 years.

The Receiver Operating Characteristic score (ROC) for the Pocock score was statistically significant for predicting acute post-operative microemboli (**AUC 0.546 95% CI 0.502 – 0.590, P = 0.039**: Figure 9). The ROC for Pocock score showed no significant prediction of acute postoperative high microemboli rate (MES > 50hr^{-1}) requiring additional antiplatelet therapy. (AUC 0.546 95% CI 0.482 – 0.610, P = 0.164: Figure 10).

Figure 9. ROC for Pocock score against presence of microemboli acutely following carotid endarterectomy (n = 670) (AUC 0.546 95% CI 0.502 – 0.590, P = 0.039)
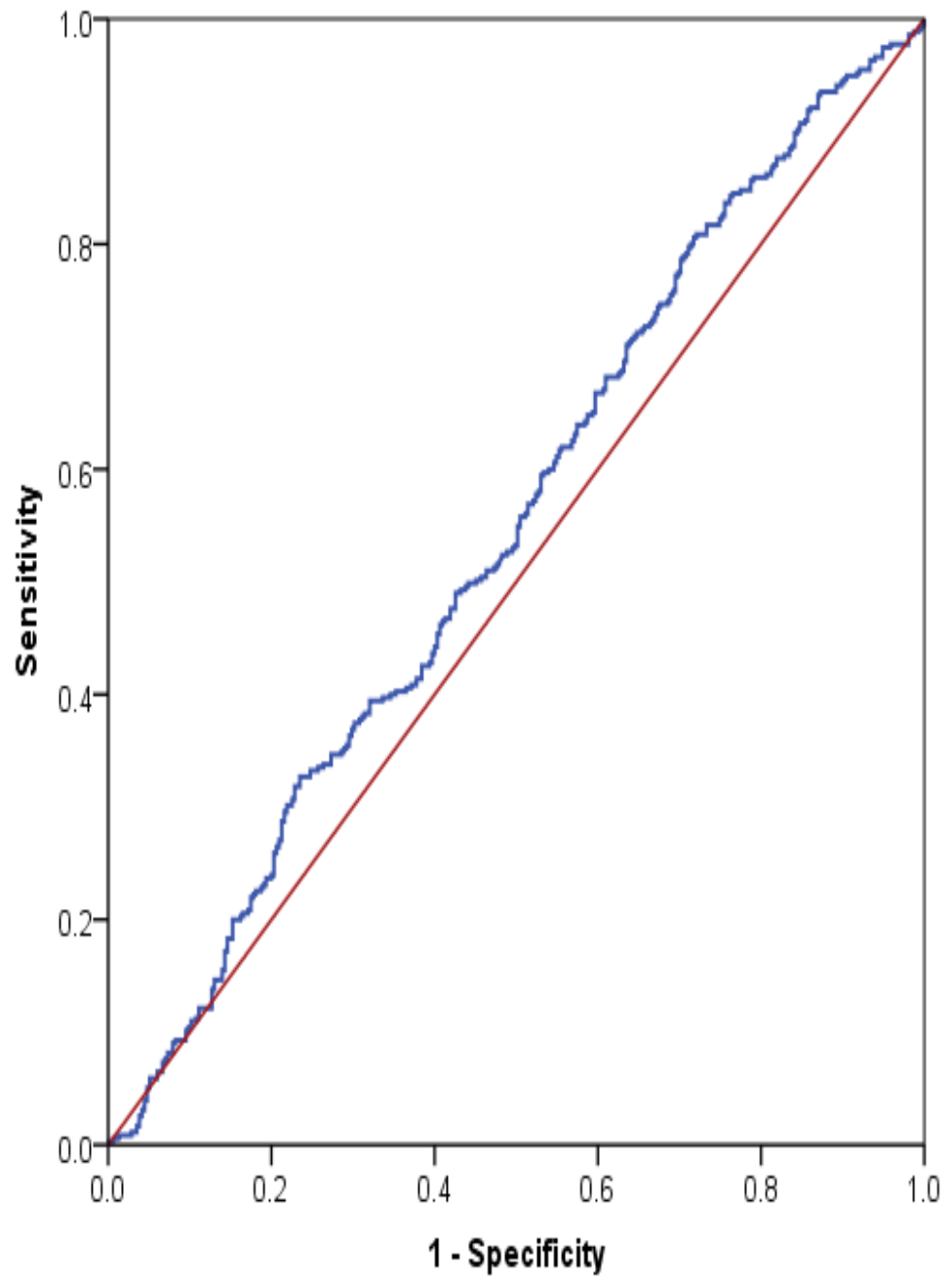
Figure 10. ROC for Pocock score against presence of high microemboli rate (MES > 50hr^{-1}) acutely following carotid endarterectomy (n = 670) (AUC 0.546 95% CI 0.482 – 0.610, P = 0.164)
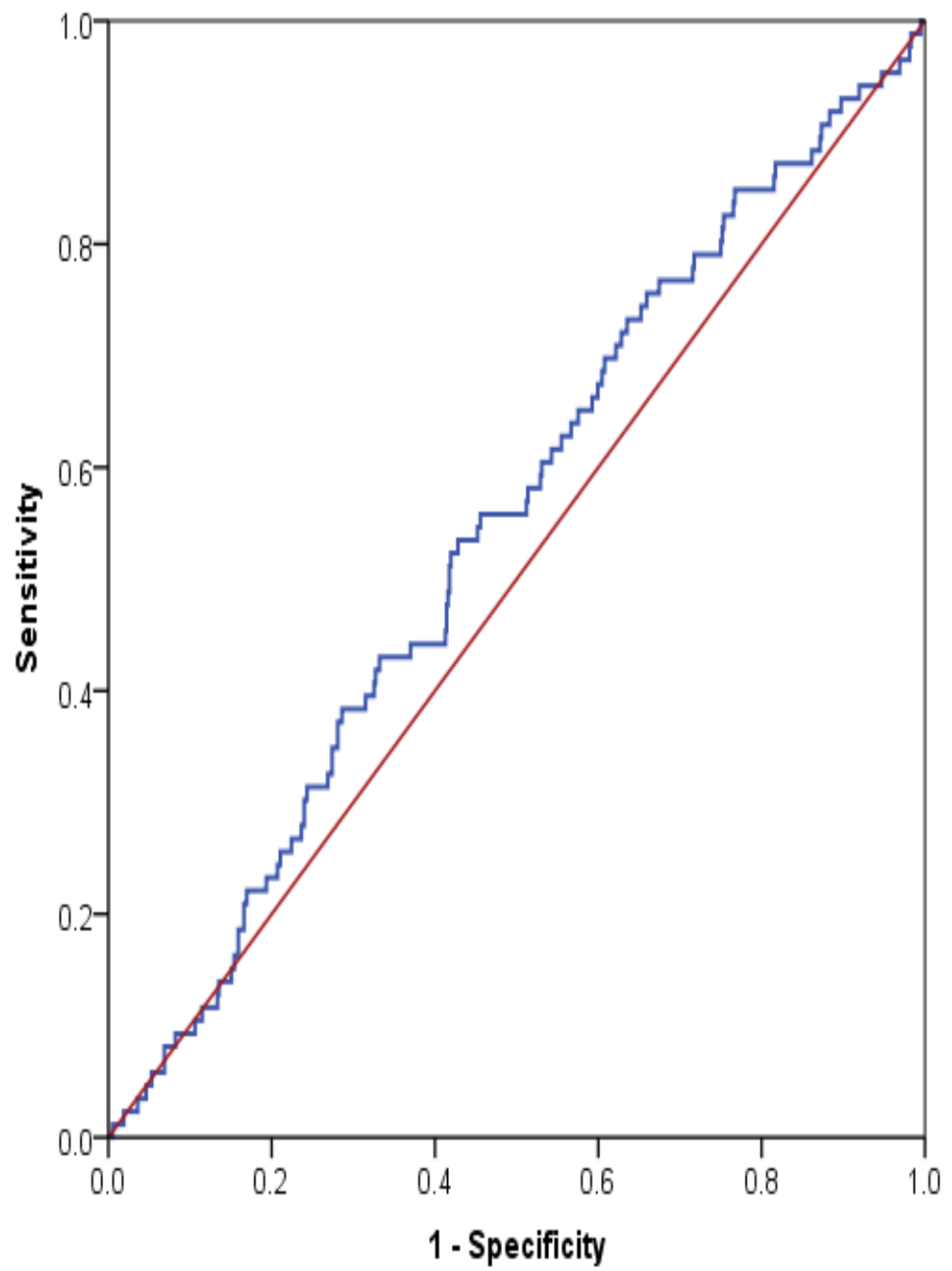
The ROC for Pocock's score ≥ 0.8% showed significant prediction of acute postoperative microemboli **(AUC 0.582 95% CI 0.507 – 0.658, P = 0.038)** (Figure 11). The ROC for Pocock's score ≥ 0.8% showed no significant prediction of acute postoperative high microemboli rate (MES > 50hr^{-1}) requiring additional antiplatelet therapy. (AUC O.514 95% CI 0.437 – 0.590, P = 0.371) (Figure 12).

Figure 11 ROC for Pocock score ≥ 0.8% against presence of microemboli acutely following carotid endarterectomy (n = 670) (AUC 0.582 95% CI 0.507 – 0.658, P = 0.038)
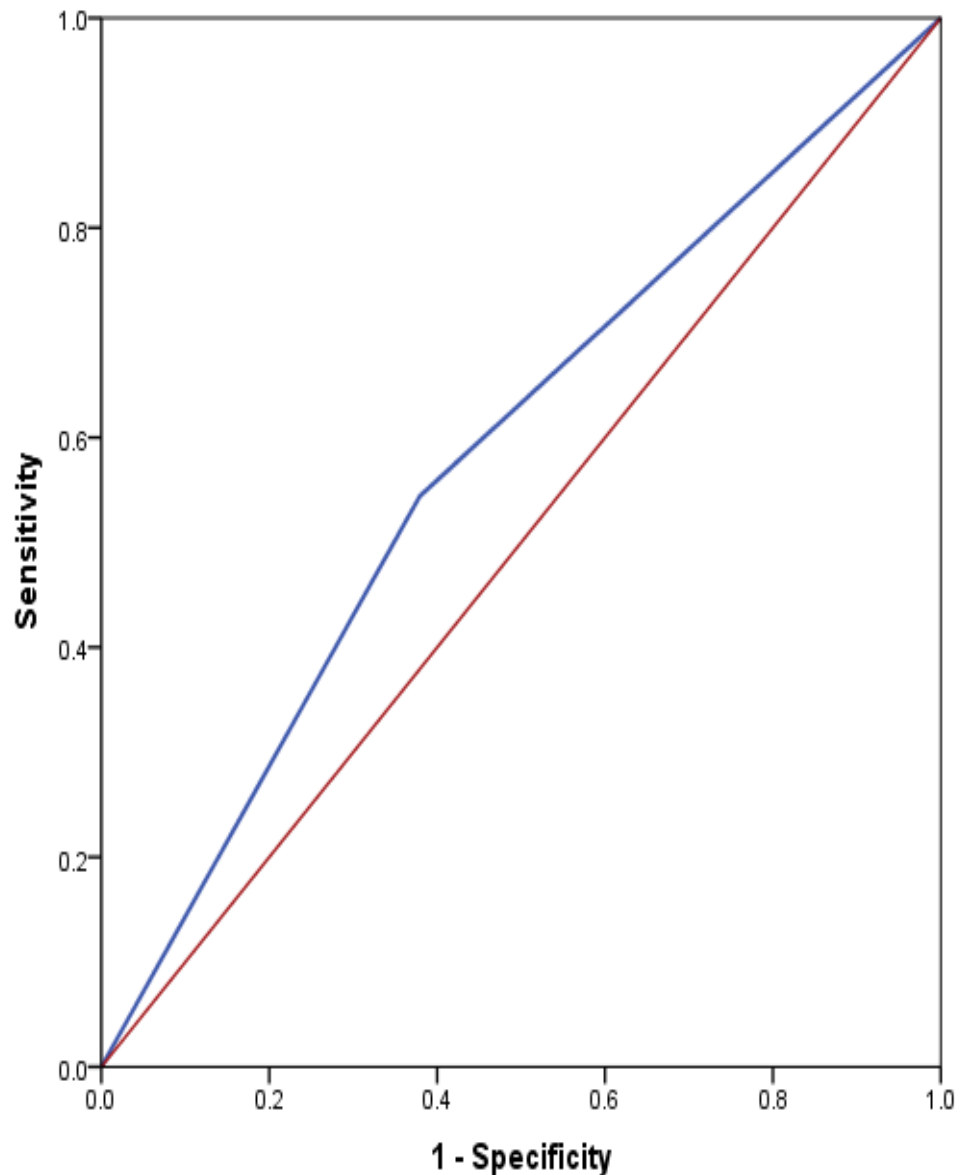
Figure 12 ROC for Pocock score ≥ 0.8% against presence of microemboli >50^{-1} acutely following carotid endarterectomy (n = 670) (AUC O.514 95% CI 0.437 – 0.590, P = 0.371)
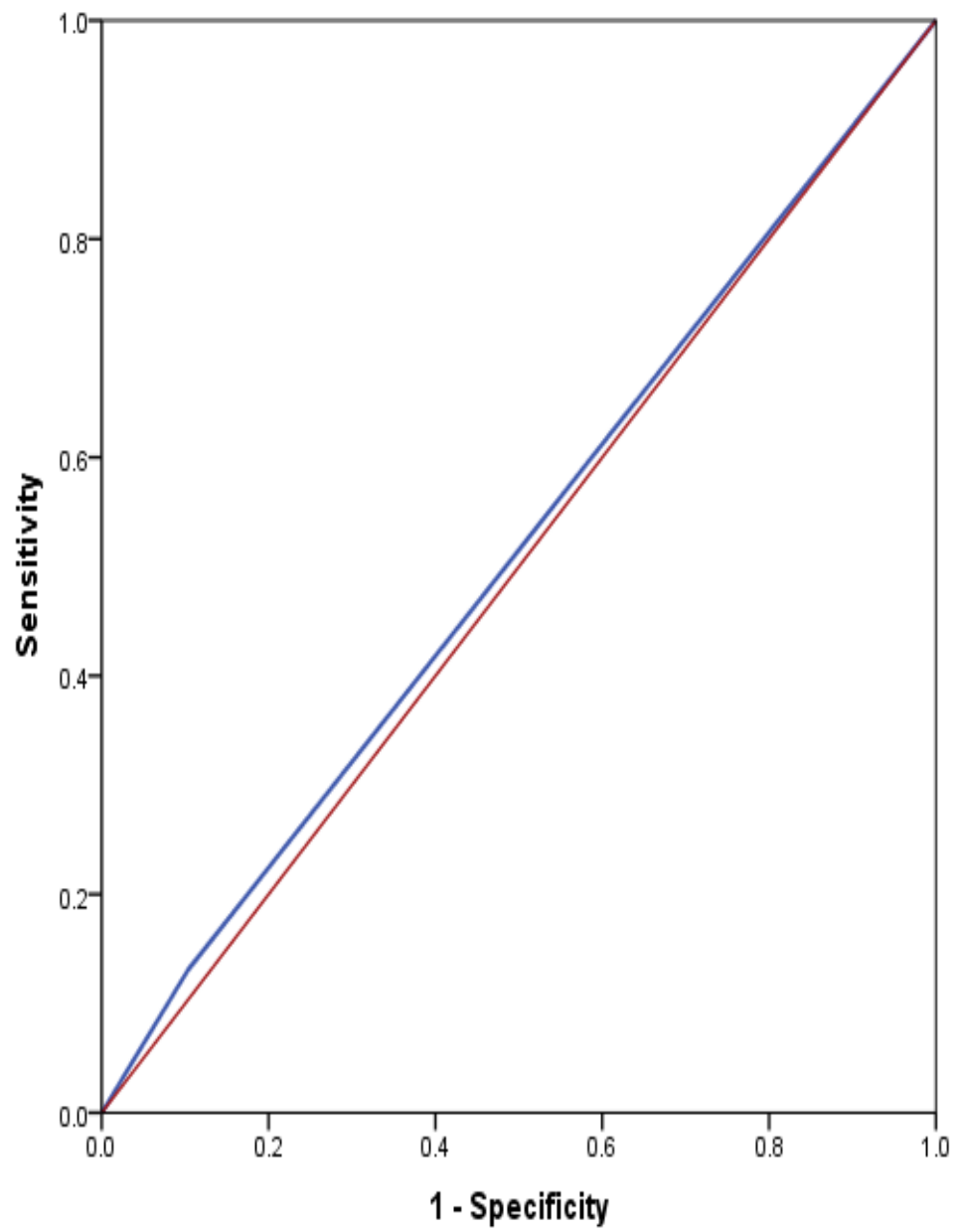
The ROC for Pocock score ≥ 2.3% showed no significant prediction of acute postoperative microemboli (AUC 0.531 95% CI 0.487 – 0.574, P = 0.172: Figure 13). The ROC for Pocock score ≥ 2.3% showed no significant prediction of acute postoperative high microemboli rate (MES > 50hr⁻¹) requiring additional antiplatelet therapy. (AUC 0.530 95% CI 0.466 – 0.594, P = 0.367: Figure 14).

Figure 13 ROC for Pocock score ≥ 2.3% against presence of microemboli acutely following carotid endarterectomy (n = 670) (AUC 0.531 95% CI 0.487 – 0.574, P = 0.172)
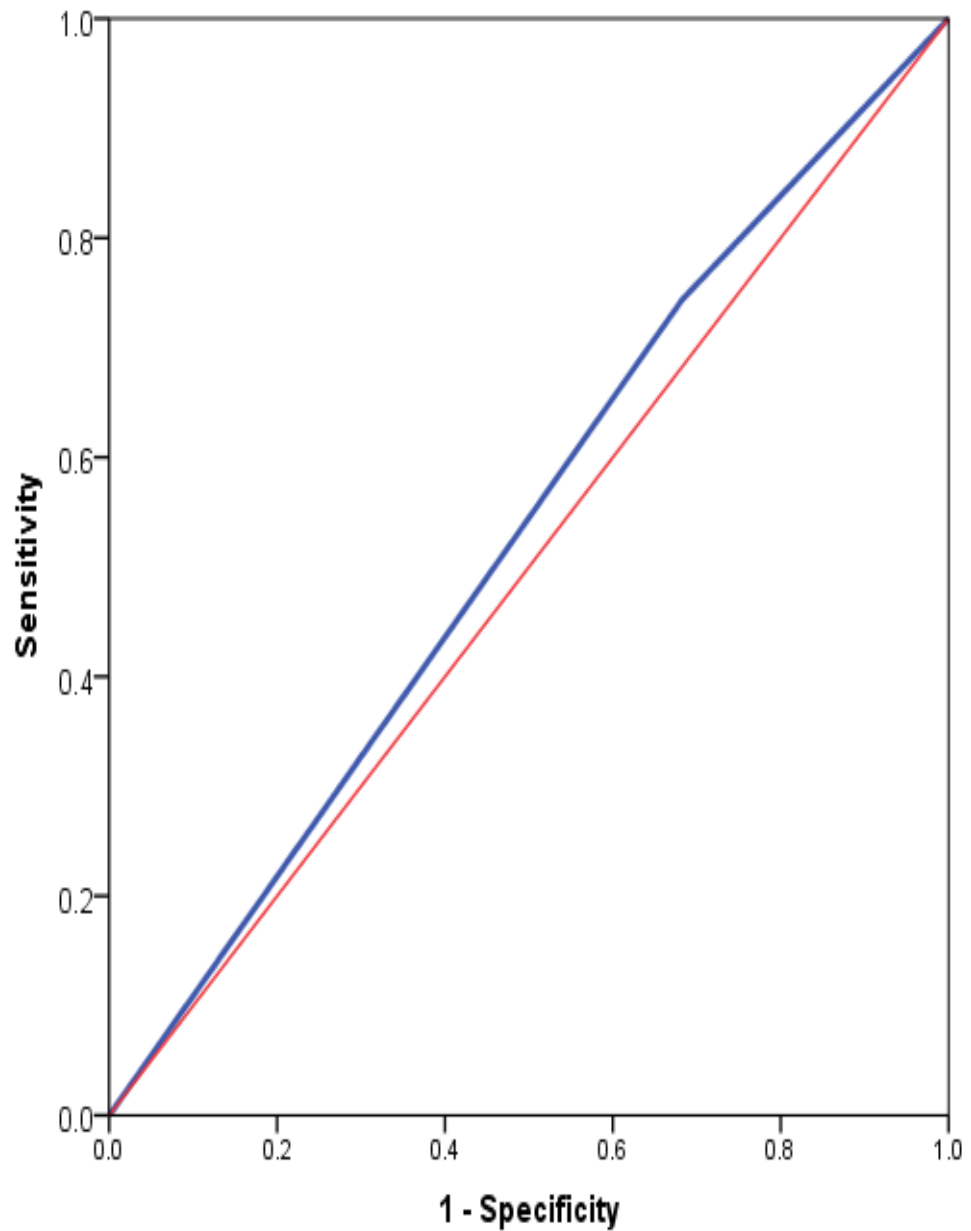
Figure 14. ROC for Pocock score ≥ 2.3% against presence of high microemboli rate (MES > 50hr^{-1}) acutely following carotid endarterectomy (n = 670) (AUC 0.530 95% CI 0.466 – 0.594, P = 0.367)
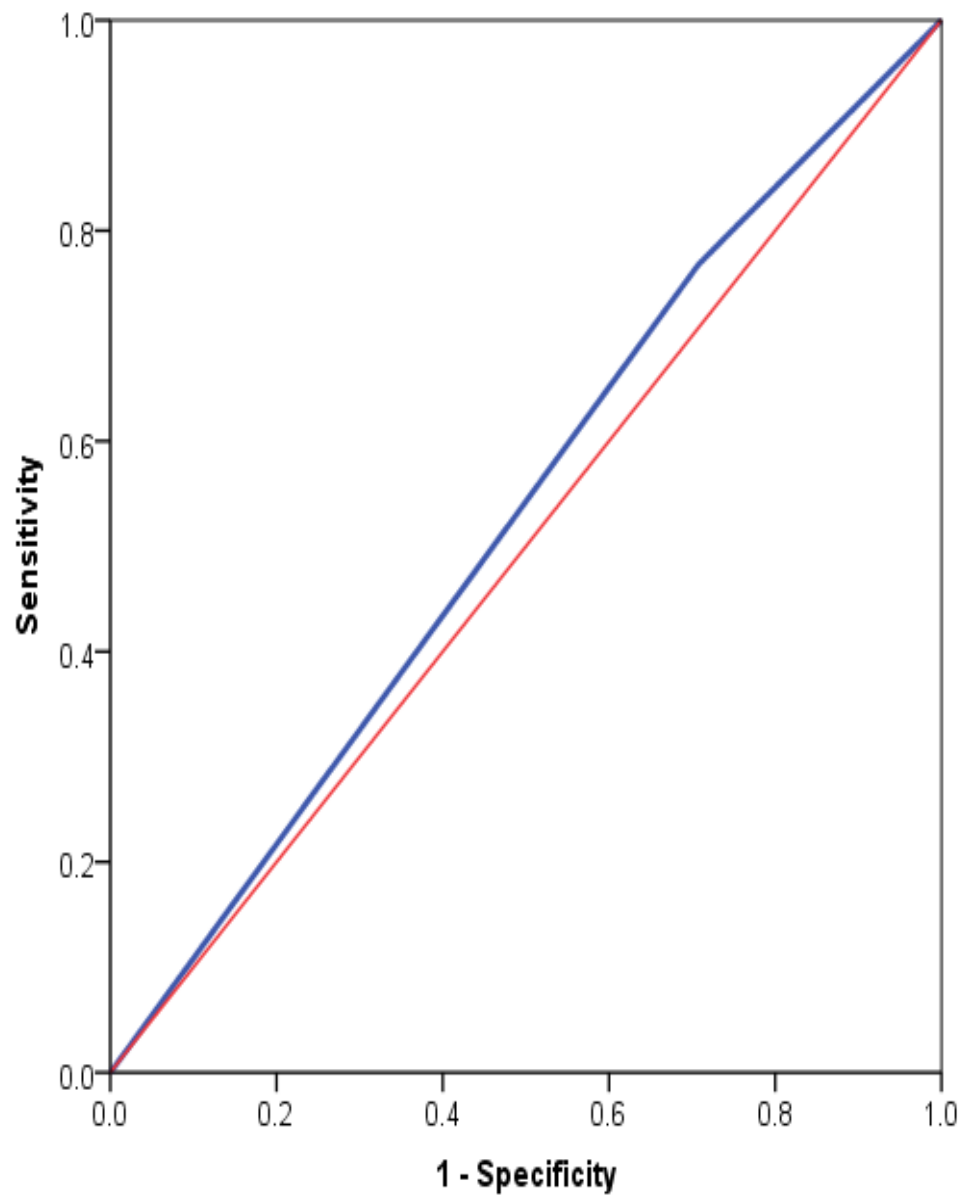
The ROC for Pocock score ≥ 6.1% showed no significant prediction of acute postoperative microemboli (AUC 0.519 95% CI 0.475 – 0.562, P = 0.408). The ROC for Pocock score ≥ 6.1% showed no significant prediction of acute postoperative high microemboli rate (MES > 50hr^{-1}) requiring additional antiplatelet therapy. (AUC 0.529 95% CI 0.463 – 0.595, P = 0.382).

Diagnostic accuracy

Pocock score ≥ 0.8% showed a high sensitivity for the presence of acute postoperative cerebral microemboli. It also showed high sensitivity and negative predictive value for patients who developed sustained high microembolic rate (MES > 50hr^{-1}) requiring additional antiplatelet therapy to reduce risk of postoperative stroke (Table 22, Table 23). Pocock score ≥ 2.3% showed similar diagnostic accuracy as Pocock score ≥ 0.8% (Table 24, Table 25). Pocock score ≥ 6.1% showed high specificity and negative predictive value for the presence of microemboli (Table 26, Table 27).

Table 22. Diagnostic accuracy Pocock's score ≥ 0.8% vs MES

Sensitivity	**93.80%**	**95% CI: 90.77 to 96.07**
Specificity	11.42%	95% CI: 15.47 to 8.13
Positive Predictive Value	54.41%	95% CI: 50.37 to 58.41
Negative Predictive Value	62.07%	95% CI: 48.37 to 74.48

Table 23. Diagnostic accuracy Pocock's score ≥ 0.8% vs MES rate > 50hr[1]

Sensitivity	**93.02%**	**95% CI: 85.42 to 97.38**
Specificity	8.90%	95% CI: 6.72 to 11.51
Positive Predictive Value	13.07%	95% CI: 10.50 to 16.00
Negative Predictive Value	89.66%	95% CI: 78.82 to 96.08

Table 24. Diagnostic accuracy Pocock score ≥ 2.3% vs MES

Sensitivity	74.37%	95% CI: 69.49 to 78.83
Specificity	31.75%	95% CI: 26.64 to 37.20
Positive Predictive Value	55.11%	95% CI: 50.54 to 59.63
Negative Predictive Value	52.36%	95% CI: 45.02 to 59.61

Table 25. Diagnostic accuracy Pocock score ≥ 2.3% vs Postoperative MES rate > 50hr[1]

Sensitivity	76.74%	95% CI: 66.39 to 85.17
Specificity	29.28%	95% CI: 25.62 to 33.16
Positive Predictive Value	13.78%	95% CI: 10.82 to 17.19
Negative Predictive Value	89.53%	95% CI: 84.29 to 93.49

Table 26. Diagnostic accuracy Pocock score ≥ 6.1% vs MES

Sensitivity	40.85%	95% CI: 35.69 to 46.16
Specificity	62.86%	95% CI: 57.26 to 68.21
Positive Predictive Value	55.34%	95% CI: 49.10 to 61.46
Negative Predictive Value	48.53%	95% CI: 43.58 to 53.50

Table 27. Diagnostic accuracy Pocock score ≥ 6.1% vs MES rate > 50hr[1]

Sensitivity	44.19%	95% CI: 33.48 to 55.30
Specificity	61.64%	95% CI: 57.56 to 65.61
Positive Predictive Value	14.50%	95% CI: 10.47 to 19.36
Negative Predictive Value	88.24%	95% CI: 84.71 to 91.20

4.4   Discussion

Our Registry data on carotid surgery demonstrated patients' baseline characteristics and perioperative outcomes are comparable with recent major randomised carotid intervention studies[155, 156]. Persistent high rates of transcranial Doppler detected – microemboli acutely following carotid endarterectomy have been associated with thrombotic stroke[31]. These high risk patients require additional anti-thrombotic agents to reduce stroke risk[176, 215, 217]. Based on the Registry data, the Pocock score has been shown to be associated with the presence of transcranial Doppler detected – microemboli acutely following carotid endarterectomy. Here I show that Pocock score ≥ 0.8% significantly predicted the presence of microemboli following surgery. I have shown that a Pocock score ≥ 0.8% has a high sensitivity and negative predictive value in detecting those cohort who required additional antiplatelet agent to treat persistent acute postoperative microemboli. These findings suggest that the Pocock score could potentially be used as a clinical tool to identify patients who are at increased risk of developing acute postoperative microemboli. The Registry demographic profiles such as age and sex were similar to the expected carotid disease characteristics and consistent with other recent major carotid intervention studies[155, 156]. The proportion of asymptomatic patients who underwent CEA was significantly less during the period of 2010 and 2012. This is expected as a previous randomised controlled trial has shown that carotid endarterectomy is beneficial in only a selective cohort of asymptomatic critical carotid artery stenosis patients[249]. The proportion of TIA, amaurosis fugax and retinal artery

occlusion patients who underwent carotid endarterectomy in the Registry were similar to the participants in a multi-centre European study on carotid intervention[155]. The proportion of ischaemic stroke patients who underwent carotid endarterectomy was half of those participating in the European carotid intervention study[155].

The baseline cardiovascular risk factors in our cohort were similar to the 2 most recent multi-centre randomised controlled trials on carotid intervention[155, 156]. There is a trend of decreasing incidence of hypercholesterolaemia, TIA or stroke and ischaemic heart disease in the Registry cohort. There is also an increasing trend of using multiple antiplatelet agents prior to carotid endarterectomy in order to reduce the risk of perioperative stroke. This can be explained with the general improvement of medical treatment for cardiovascular disease such as better antiplatelet therapy, widespread use of statins and decline of smoking habit[140, 250, 251]. Those who were on triple preoperative antiplatelet agents had the additional antiplatelet agent started in hospital. In the preoperative dual antiplatelet group, the number of patients who had the additional antiplatelet agent started cannot be determined reliably due to individual surgeon preference and the various sources of referral either from the community, TIA clinics in other hospitals or the Emergency Department in Coventry or the surrounding regions.

In terms of the operative technique, the proportion of patients who underwent carotid endarterectomy under general anaesthesia and requiring a shunt during the carotid clamp stage was significantly less than a recent multi-centre randomised controlled trial on carotid intervention[156]. This could be explained by the utilisation of TCD during carotid endarterectomy. A shunt was used selectively depending upon TCD evaluation of cerebral blood flow before and after carotid clamping.

The overall 30 day mortality and morbidity rate such as CVA/TIA, myocardial infarction and postoperative bleeding were similar to the most recent nationwide UK carotid endarterectomy audit[192]. Our postoperative cranial nerve injury (5.1%) was higher than the national audit.

The proportion of patients who developed microemboli acutely following surgery were similar to a previous published report[252]. There is a decreasing trend in the proportion of patients who

developed microemboli acutely following surgery over the years. The number of patients who developed a persistent high rate of microemboli requiring additional anti platelet therapy was also fewer in more recent years. These findings correlate with the increasing use of multiple anti platelet agents prior to carotid endarterectomy during the same period. The use of multiple antiplatelet agents has been shown to be more effective in reducing the rate of carotid associated microemboli[37, 217].

None of the cardiovascular composite risk scores were able to predict the presence of microemboli in these high risk patients. Only the Pocock score showed an association with the presence of transcranial Doppler detected – microemboli acutely following carotid endarterectomy. Further analysis confirmed that the Pocock score predicted the presence of transcranial Doppler detected – microemboli acutely following carotid endarterectomy. The association of the Pocock score with acute postoperative MES rate $> 50hr^1$ was unclear. These were the patients at high risk of developing postoperative thrombotic stroke and requiring additional antiplatelet therapy[176].

In this study, I found that a Pocock score $\geq 0.8\%$ was able to predict microemboli. It also has a high sensitivity and negative predictive value for those with a rate of $> 50hr^1$. A Pocock score $\geq 2.3\%$ has been shown to have high sensitivity for the presence of microemboli and negative predictive value for the presence of microembolic rate of $> 50hr^1$. When the level of Pocock score was $\geq 6.1\%$, a high negative predictive value for the presence of microemboli was noted. This suggests that the Pocock score could potentially be used as a clinical tool for identifying patients at very high risk of developing significant post-operative microembolisation.

There are several limitations to this Registry. Fifteen patients from the retrospective cohort were excluded for the final analysis due to lack of relevant data pertinent to the Registry. Therefore only 670 patient data were analysed in this chapter. This small number of missing patients is unlikely to influence the overall results. Furthermore, the baseline patient characteristics between the retrospective and prospective cohorts were similar and any relevant differences were consistent with the improvement of medical therapy and changes in clinical practice. Another

possible limitation of this Registry is the external data validation. The Registry demographic profiles and cardiovascular risk factor burden were similar to the expected carotid disease characteristics and consistent with other recent major European carotid intervention studies[155, 156]. It is possible to cross-match the Registry data against the UK National Vascular Registry[192]. However, the National Registry was only formed in 2013 by the amalgamation of the National Vascular Database and UK Carotid Interventions Audit projects[192]. The data also relies on individual surgeons self-reporting, so is subject to selection and information bias. In 2012, a new validated cardiovascular disease risk score (QRISK2) was published based on the routinely collected data from general practice in the UK[253]. It has incorporated similar data as Framingham, JBS2 and ASSIGN with additional information such as rheumatoid arthritis, atrial fibrillation and chronic kidney disease. It has a clear advantage of reflecting the cardiovascular disease state of the UK population and has been shown to be more accurate than the established Framingham score[254]. However, there are a few limitations that are worth noting. First, the data were based on the self-reporting by the general practitioners with potentially significant selection and information biases, so it unlikely to be as robust as data collected in the Framingham or Pocock risk score. Unlike the Framingham and Pocock risk scores, the cohort included in this risk score was not standardised and cardiovascular disease treatment naive with unclear natural history. Due to its limitation and the publication timing of the validated study, the QRISK2 risk score was not considered in my thesis.

In conclusion, a Registry is useful in demonstrating clinical population based data which can be used to review and improve clinical practice. In this chapter, I used the data to evaluate the association between composite cardiovascular score and the presence of microemboli acutely following carotid endarterectomy. The analysis of the data has shown that the Pocock score has the potential to identify patients who are at risk of developing microemboli following carotid endarterectomy.

CHAPTER FIVE

5   Classical cardiovascular risk factor burden and ABCD^2 stroke risk score to predict the presence of cerebral microemboli in patients with hyper-acute symptomatic critical carotid artery stenosis

5.1   Introduction

The risk of recurrent stroke following a first TIA or minor stroke is highest during the first 7 – 14 days[1]. About 15% of ischaemic strokes are associated with carotid artery stenosis, which confers a higher risk of early stroke recurrence compared with other stroke syndromes such as cardio-embolic or small vessel stroke[15]. Previous studies have shown that the benefit of surgery is greatest if it was performed within 2 weeks after the last ischaemic event[51]. United Kingdom National Institute for Health and Clinical Excellence (NICE) guidelines recommend that patients with symptomatic carotid artery stenosis undergo urgent endarterectomy within 2 weeks[103]. Transcranial Doppler (TCD) – detected cerebral microembolic signals (MES) predict short term stroke risk in patients with symptomatic carotid stenosis[34]. Cerebral microemboli have in particular been reported to be a reliable biomarker of short term ischaemic stroke risk[35, 36,32, 60]. The ABCD^2 risk prognostic scoring system[101] predicts who is likely to be at increased risk of suffering a recurrent early stroke following onset of the initial stroke/ TIA[101], irrespective of the mechanism for acute stroke/TIA. The ABCD^2 stroke risk score [101] can be used to triage the urgency for seeing recently symptomatic patients with a TIA or minor stroke in cerebrovascular clinics. National Institute for Health and Care Excellence (NICE) guidelines[103] recommend that patients with an ABCD^2 risk score ≥4 should be assessed within 24 hours and those with an ABCD^2 risk score <4 should be evaluated within a week of symptoms onset.

The ABCD^2 risk score is based on standard classical cardiovascular risk factors and clinical features for symptomatic carotid disease. One would expect symptomatic carotid artery disease patients

to have a high cardiovascular risk factor burden and high $ABCD^2$ risk score. TCD-detected microemboli have been associated with recent symptoms and vulnerable carotid plaque characteristics[188]. Both the $ABCD^2$ score and the presence of cerebral microemboli predict the risk of early recurrent stroke.

The association between $ABCD^2$ risk score and cerebral microemboli has not been studied. The aim of this study was to explore whether the $ABCD^2$ risk score predicts the presence of cerebral microemboli in patients with hyper-acute symptomatic critical carotid artery stenosis.

5.2   Subjects and Methods

University Hospitals Coventry and Warwickshire NHS Trust serves a population of 950,000. Our Neurovascular Fast-Track Clinic assesses approximately ~80 patients per month who are suspected of having suffered a TIA/minor stroke and who have been referred from the Community or by the Emergency Department. In the same visit, patients also undergo carotid duplex examination. Since 2002, it has been the policy in our Centre that patients who present within 2 weeks of index symptoms have an additional TCD examination to detect cerebral microemboli as this cohort of patients merits more aggressive treatment to prevent recurrent stroke[218]. In this thesis, I defined the hyper-acute period as within 2 weeks of stroke or TIA[255].

Two hundred and six (age 70 ± 1 [SEM] years, male 149, Caucasian 196) patients were included in this study. This included 102 patients from our Carotid Registry retrospectively assessed between January 2002 and December 2010[176]. In this chapter, the retrospectively cohort patient will be discussed in the Registry section. A further 104 consecutive patients were recruited between February 2011 and May 2013 within a new prospective study of hyper-acute symptomatic carotid artery stenosis.

Inclusion and exclusion criteria

Only patients who presented within 2 weeks of symptoms of acute non-disabling stroke (The Modified Rankin Scale [mRS] ≤ 2) or TIA were included. All had at least haemodynamically significant 50% carotid artery stenosis[121] measured by carotid duplex scan based on NASCET criteria as recommended by the Vascular Society and Society of Vascular Technology of Great Britain and Ireland[118]. All patients with haemodynamically significant ≥ 50% carotid stenosis went on to have TCD examination which was supervised by accredited vascular technologists[118].

Patients with atrial fibrillation, because this confers risk of an additional non-carotid source of emboli were excluded. In addition, patients with a prosthetic heart valve, because of the recognised effect of these valves to generate gaseous and other embolic signals[256] were also excluded. Patients who had a major stroke were excluded because the ABCD^2 risk score only applied to TIA or minor stroke[100]. Patients who presented more than 2 weeks of last symptoms of stroke or TIA were also excluded because this is not considered to be within the defined hyperacute period[255].

Assessment of the ABCD^2 risk score

It ranged from 0 - 7[101]. [Scale[9]: age ≥60 years - 1 point; blood pressure ≥140/90mmHg - 1 point; limb weakness - 2 points; speech impairment – 1 point; duration of symptoms - 2 points for ≥ 60 minutes, and 1 for 10-59 minutes; diabetes mellitus - 1 point]. The ABCD^2 score[9] was available in all patients recruited for the prospective observational study from 2011 to 2013. Seven patients from the Carotid Surgery Registry from the period of 2002 and 2005 were excluded from the analysis due to lack of data pertinent to the ABCD^2 risk score calculation.

Assessment of cardiovascular risk factors

Classical cardiovascular risk factors were assessed based on validated scoring systems[93]. Total cardiovascular disease (CVD) risk for an individual is estimated from several risk factors and

slightly differs between individual cardiovascular risk score. CVD end point is defined as combined end point of coronary heart disease (fatal and non-fatal myocardial infarction and new angina) plus stroke (fatal and non-fatal stroke and cerebral haemorrhage) and transient cerebral ischaemia[93]. The total CVD risk score is expressed as a probability (percentage chance) of developing CVD or death over a defined period of time. Framingham, Joint British Societies' Guidelines on Prevention of Cardiovascular Disease in Clinical Practice (JBS 2), Scottish ASSIGN scores estimate the CVD risk over 10 years where as Pocock score estimates the risk of CVD death over 5 years[93, 99].

Transcranial Doppler recording

TCD monitoring[171] was performed (PC Dop 842, SciMed, Bristol, UK) with a 2 MHz probe focused on the middle cerebral artery ipsilateral to the stenotic carotid artery. The TCD signal was assessed by an experienced vascular scientist. The MCA was identified by placing the probe above the zygomatic arch and just in in front of the ear. A head-frame was used to secure a constant angle of insonation during the TCD monitoring. Doppler signals were obtained within the depth range of 55 – 64 mm and time–averaged mean velocity in the region of 55 ± 12 cm/sec. Single channel was chosen and a filter set to a low threshold to capture all possible signals. The recorded ultrasonographic images were then assessed to differentiate artefact from microemboli based on criteria of the International Consensus Group on microembolus detection[171]. MES were identified as unidirectional, short duration signals (range 10–100 ms) with intensity threshold above 6 dB, accompanied by characteristic audible clicks and occurring randomly throughout the cardiac cycle [170, 171] Monitoring was performed for 60 minutes after carotid duplex assessment. TCD examination was performed by accredited vascular technologists[118].

Microembolic signals

In patients who had acute symptoms suggestive of TIA/non-disabling stroke with evidence of a haemodynamically significant 50% carotid stenosis, TCD monitoring was commenced for up to 60

minutes following the completion of the carotid duplex examination. Based on Markus et al[36], dichotomous outcome was used to classify patients with microembolic TIA or stroke, according to MES presence rather than rate or number of MES. This study showed that the presence of MES, but not the rate of MES, was associated with a high risk of recurrent stroke.[36] This policy has also been adopted in a recent prospective multi-centre study looking at the association between MES and stroke.[32]

Ethics

Registry

Data collection for the Registry was agreed with the Research & Development Department at University Hospital Coventry Warwickshire NHS Trust, which confirmed that Research Ethics Committee (REC) review was not required under the harmonised Governance Arrangement for REC (GAfREC) for research[257].

Prospective cohort study

The study was approved by the NHS Ethics Committee (MREC number 10/H1206/77) and Research & Development Department (R&D C1080610).

Statistical analyses

In view of differences among patients in antiplatelet treatment prior to their acute neurological events, the data were analysed in 2 stages. Initially, both microembolic and non-microembolic cohorts were studied together. Then, taking into account the influence of antiplatelet treatment on cerebral microemboli[37], the data were analysed separately for patients with or without pre–neurological event antiplatelet and statin treatment. Framingham, JBS 2, ASSIGN scores considered 20% as the threshold where patients should be considered for pharmacology and lifestyle interventions. The Pocock score was developed based on patients who were already

established as having cardiovascular disease[93, 99]. Therefore all the patients in this thesis were eligible for Pocock score estimation.

Statistical analyses were conducted using SPSS® version 21 (Chicago, Illinois, USA).

Parametric data are expressed as mean and standard error of the mean (SEM). Non-parametric data are expressed as median and inter quartile range (IQR). Non-parametric unpaired data were analysed using the Mann-Whitney U test for two group comparisons. Categorical variables were analysed using the chi-squared test or Fisher's exact test. The association between microemboli and $ABCD^2$ risk score was also analysed using Receiver Operating Characteristic Curves (ROC). A P-value of less than 0.05 was considered significant.

Sample size calculation:

We set an area under the curve (AUC) of 0.5 or below as the cut-off point for $ABCD^2$ score not predicting the presence of microemboli. For the whole group, the study had 80% power at the 5% level in a 2-tailed test to detect at least 0.69 as AUC for ROC (71 subjects per group).

5.3 Results

5.3.1 Clinical features

Two hundred and six patients (age 70 ± 1 year, 149 male, 196 Caucasians) were included in this study. Eighty six patients (age 70 ± 1, 58 male, 83 Caucasians) had evidence of one or more microemboli; 72 (84%) of these underwent carotid endarterectomy (CEA). A single patient had a TIA, one patient developed myocardial infarction and one embolising patient died due to renal failure 30 days after CEA. One hundred and twenty patients (age 72 ± 1 years, 91 male, 113 Caucasians) did not have microemboli detected; 102 (85%) of these underwent CEA. Thirty days complications after CEA were TIA in 2 patients, stroke in 3 patients, and death in 3 non-embolising patients (sepsis and haemorrhagic strokes). Antiplatelet treatment had been prescribed to 67 of

86 (78%) patients with microemboli, and to 82 of 120 (68%) patients without microemboli (P = 0.130, X^2).

Cardiovascular risk factor burden

A family history of vascular disease was more common in the non-embolising group (42 (35%)) than in those who had microemboli following a non-disabling stroke/TIA (19(22%); odds ratio 1.5 (95%CI 0.98-2.2) P = 0.045, Table 28). Peripheral arterial disease was also more common in the non-embolising group (21(18%)) compared with those who had microemboli associated with stroke or TIA (7(8%); odds ratio 1.8 (95%CI 0.95-3.6) P = 0.038, Table 28).

Detection of microemboli was not associated with significant differences in prevalence or severity of hypertension, diagnosed hyperlipidaemia, diabetes mellitus or ischaemic heart disease (Table 28, Table 29).

Laboratory and imaging investigations

The microembolic stroke/TIA cohort had a slightly higher level of total cholesterol (4.9 ± 0.2 mmol/L) than controls (4.5 ± 0.1 mmol/L; P = 0.01, MW; Table 2). There were no significant differences between these cohorts in renal function, blood glucose, HbA_{1c}, or in inflammatory markers (white blood count or C-reactive protein: Table 29). Carotid ultrasound imaging revealed no difference in degree of carotid artery stenosis between those with vs. those without microemboli (microemboli: 80 ± 1% vs. controls: 79 ± 1%; P = 0.845, MW test).

Table 28. Demographic and clinical profile in patients with hyper-acute symptomatic carotid stenosis: 120 without microemboli and 86 with microemboli

	Non-embolising group n (%)	Embolising group n (%)	X^2 test P value
Age, mean ± SEM	72 ± 1	70 ± 1	0.339
Male	91(76)	58(67)	0.184
Caucasian	113(94)	83(97)	0.290
South Asian	3(2.5)	(0)	
Hypertension	93(78)	65(76)	0.748
Never smoke	42(35)	28(33)	0.655
Current smoker	46(38)	30(34)	
Ex-smoker	32(27)	28(33)	
Ischaemic heart disease	28(23)	17(20)	0.157
Type 2 Diabetes mellitus	20(17)	16(19)	0.907
Hypercholesterolaemia	65(54)	51(59)	0.464
Peripheral arterial disease	22(18)	7(8)	**0.038**
Cerebrovascular disease	24(20)	20(23)	0.574
Family History	42(35)	19(22)	**0.045**

Table 29. Blood pressure and preoperative laboratory values in patients with hyper-acute symptomatic carotid stenosis: 120 without microemboli and 86 with microemboli

Laboratory variables	Non-embolising group Mean ± SEM (n)	Embolising group Mean ± SEM (n)	Mann-Whitney U test P value
Systolic blood pressure	151 ± 2 (120)	152 ± 3 (86)	0.88
Diastolic blood pressure	77 ± 1 (120)	82 ± 3 (86)	0.986
Cholesterol (mmol/L)	4.5 ± 0.1 (102)	4.9 ± 0.2 (66)	**0.041**
Triglyceride (mmol/L)	1.6 ± 0.1 (56)	1.95 ± 0.2 (41)	0.869
HDL (mmol/L)	1.37 ± 0.1 (95)	1.46 ± 0.1 (64)	0.697
Creatinine (μmol/L)	99 ± 3 (120)	104 ± 6 (51)	0.692
Glucose (mmol/L)	5.8 ± 0.2 (97)	5.8 ± 0.2 (56)	0.548
Haemoglobin (g/L)	137 ± 12 (119)	134 ± 02 (86)	0.133
Platelet (x 10^9/L)	254 ± 6 (119)	259 ± 8 (86)	0.762
White cell count (x 10^9/L)	8.2 ± 0.2 (119)	8.1 ± 0.3 (86)	0.418
CRP (units)	7.4 ± 2.0 (34)	7.8 ± 1.3 (57)	0.954
HbA$_{1c}$ (%)	6.3 ± 0.2 (19)	6.1 ± 0.1 (12)	0.764
Carotid stenosis (%)	79 ± 1 (86)	80 ± 1 (86)	0.845

5.3.2 Prediction of cardiovascular risk score for microemboli in 206 patients with hyper-acute symptomatic carotid artery stenosis

Table 30 shows the proportion of patients who developed microemboli among this high risk cohort were similar between these 4 scores.

Table 30. Distribution of microembolic patients based on risk score: 120 without microemboli and 86 with microemboli

Cardiovascular risk score, n	Non-embolising, n	Embolising, n	X^2 test P value
Pocock ≥ 0% (5)	2	3	0.875
Pocock ≥ 0.3% (7)	4	3	
Pocock ≥ 0.8% (29)	16	13	
Pocock ≥ 2.3% (53)	29	24	
Pocock ≥ 6.1% (86)	54	32	
Pocock ≥ 15.6% (26)	15	11	
CVD Framingham ≥20% (169)	102	67	0.203
CVD-Death Framingham ≥20% (61)	40	21	0.467
JBS2 ≥20% (148)	90	58	0.234
ASSIGN ≥20% (159)	98	61	0.07

Microembolic cohort vs. non-microembolic cohort

There was no significant difference in cardiovascular risk score between patients who had evidence of microemboli and no microemboli in patients with hyper-acute symptomatic carotid artery stenosis. Based on the Framingham score, there was a trend towards a higher risk of death from cardiovascular disease in patients with microemboli (P=0.055, MW test) (Table 31).

Table 31. Cardiovascular risk score in patients with hyper-acute symptomatic carotid stenosis: 120 without microemboli vs. 86 with microemboli

Cardiovascular risk score	P – value (MW test)
Pocock	0.11
CVD Framingham	0.172
CVD-Death Framingham	**0.55**
JBS2	0.121
ASSIGN	0.084

There was no significant difference in cardiovascular risk score between patients included from

Registry and prospective study cohorts (Table 32).

Table 32. Cardiovascular risk score in patients with hyper-acute symptomatic carotid stenosis: Prospective cohort (n = 104) vs. Registry cohort (n = 102)

Cardiovascular risk score	P – value (MW test)
Pocock	0.229
CVD Framingham	0.161
CVD-Death Framingham	0.222
JBS2	0.19
ASSIGN	0.259

Prospective cohort

There was no significant difference in cardiovascular burden score between patients who had

evidence of microemboli and no microemboli in patients with a hyper-acute symptomatic carotid

artery stenosis (Table 33).

Table 33. Cardiovascular risk score in prospective hyper-acute symptomatic carotid stenosis cohort: 53 without microemboli vs. 51 with microemboli

Cardiovascular risk score	P – value (MW test)
Pocock	0.375
CVD Framingham	0.793
CVD-Death Framingham	0.208
JBS2	0.485
ASSIGN	0.642

Registry cohort

There was no significant difference in calculated cardiovascular risk score between patients who

had evidence of microemboli and no microemboli in patients with hyper-acute symptomatic

carotid artery stenosis (

Table **34**).

Table 34. Cardiovascular risk score in Registry hyper-acute symptomatic carotid stenosis cohort: 67 without microemboli vs. 35 with microemboli

Cardiovascular risk score	P – value (MW test)
Pocock	0.104
CVD Framingham	0.063
CVD-Death Framingham	0.074

JBS2	0.064
ASSIGN	0.019

In the hyper-acute symptomatic carotid artery stenosis cohort, 57 patients were not on any antiplatelet agent or anticoagulant prior to the TIA or minor stroke events. Prior to the TIA or minor stroke events, 126 were already on single antiplatelet agent and 23 were on dual antiplatelet agents. Taking into account the influence of antiplatelet agents on microemboli, those who were not on antiplatelet agents and those on antiplatelet agents prior to the TIA or minor stroke events were analysed separately.

There was no significant difference in calculated cardiovascular risk score between patients who had evidence of microemboli and no microemboli in patients without antiplatelet treatment pre-neurological event (Table 35).

Table 35. Cardiovascular risk score in hyper-acute symptomatic carotid stenosis cohort without antiplatelet treatment pre-neurological event (n = 57): 38 without microemboli vs. 19 with microemboli

Cardiovascular risk score	P – value (MW test)
Pocock	0.660
CVD Framingham	0.919
CVD-Death Framingham	0.66
JBS2	0.986
ASSIGN	0.498

In patients already on antiplatelet treatment, the non-embolising symptomatic carotid artery disease cohort showed trend towards a higher cardiovascular risk factor burden (Pocock risk score, P = 0.055, MW test) and were at significantly higher long term risk of cardiovascular death (CVD-Death Framingham, P = 0.037, MW test) than the embolising cohort.

Table 36. Cardiovascular risk score in hyper-acute symptomatic carotid stenosis cohort with antiplatelet treatment pre-neurological event (n = 149): 82 without microemboli vs. 67 with microemboli

Cardiovascular risk score	P – value (MW test)
Pocock	**0.055**
CVD Framingham	0.142
CVD-Death Framingham	**0.037**
JBS2	0.077
ASSIGN	0.088

Taking into account the influence of statins on microemboli and atherosclerotic carotid disease, those who were not on statins and those on statins prior to the TIA or minor stroke events were analysed separately.

There was no significant difference in the cardiovascular burden score between patients who had evidence of microemboli and no microemboli in patients with hyper-acute symptomatic carotid artery irrespective of statins treatment (Table 37,

Table **38**).

Table 37. Cardiovascular risk score in hyper-acute symptomatic carotid stenosis cohort without statins pre-neurological event (n = 72): 41 without microemboli and 31 with microemboli

Cardiovascular risk score	P – value (MW test)
Pocock	0.293
CVD Framingham	0.193
CVD-Death Framingham	0.144
JBS2	0.185
ASSIGN	0.248

Table 38. Cardiovascular risk score in hyper-acute symptomatic carotid stenosis cohort with statins pre-neurological event (n = 134): 79 without microemboli and 55 with microemboli

Cardiovascular risk score	P – value (MW test)
Pocock	0.252
CVD Framingham	0.476
CVD-Death Framingham	0.203
JBS2	0.364
ASSIGN	0.200

Receiver Operating Characteristics

For further analyses, I examined whether the Pocock score was able to predict the presence of cerebral microemboli in hyper-acute symptomatic carotid artery disease cohort in 4 stages; general and using Pocock score ≥ 0.8%, 2.3% and 6.1% as a cut-off point.

The ROC for Pocock score showed no significant prediction for microemboli in patients with hyper-acute symptomatic carotid artery stenosis (AUC 0.435 95% CI 0.355 – 0.514, P = 0.11: Figure 15).

Figure 15. ROC for Pocock score against presence of microemboli in patients with hyper-acute symptomatic carotid artery stenosis (n = 206) (AUC 0.435 95% CI 0.355 – 0.514, P = 0.11)
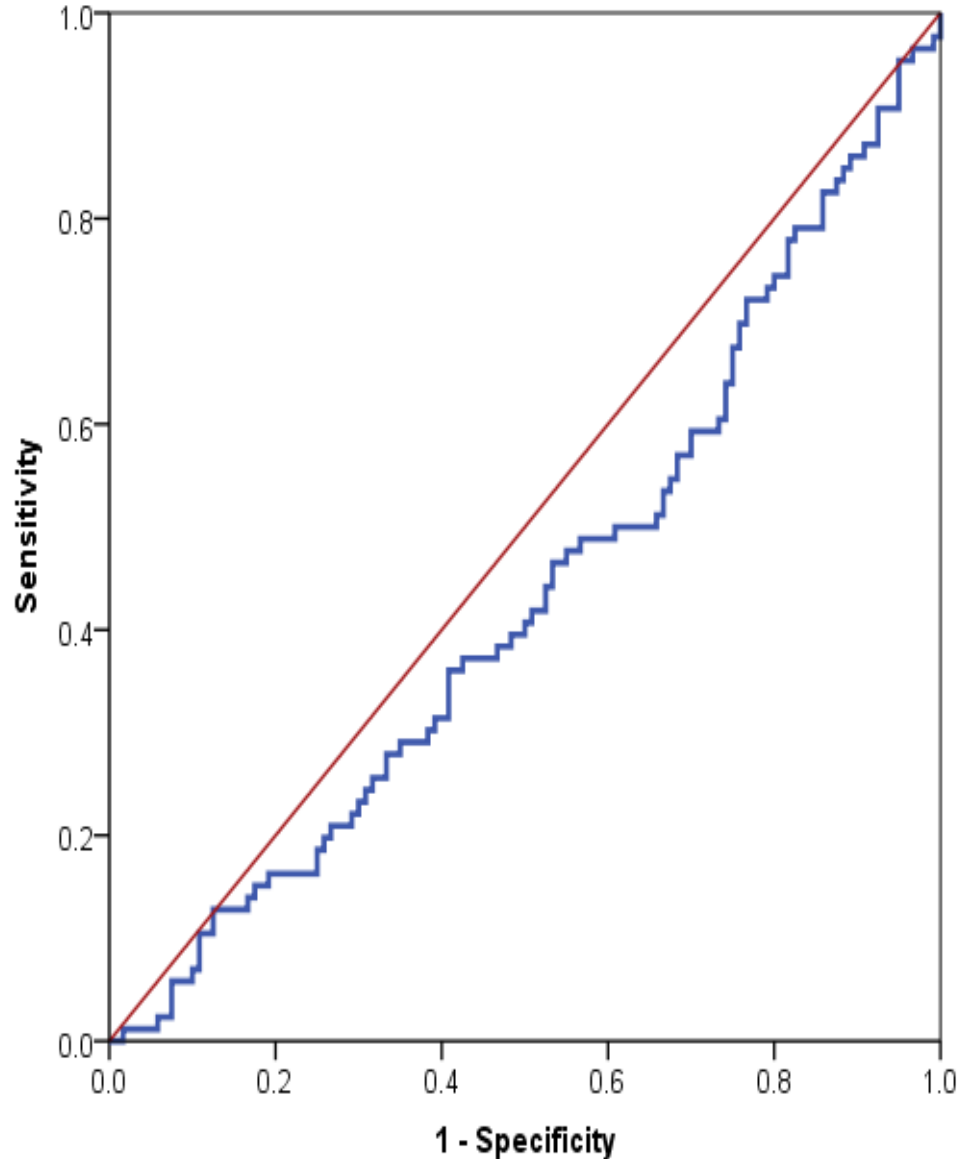
The ROC for a Pocock score ≥ 0.8% showed no significant prediction of microemboli following hyper-acute symptomatic carotid artery disease (AUC 0.509 95% CI 0.326 – 0.692, P = 0.922: Figure 16). The ROC for a Pocock score ≥ 2.3% showed no significant prediction of microemboli following hyper-acute symptomatic carotid artery disease (AUC 0.481 95% CI 0.401 – 0.562, P = 0.646). The ROC for a Pocock score ≥ 6.1% showed no significant prediction of acute postoperative microemboli (AUC 0.463 95% CI 0.383 – 0.542, P = 0.359).

Figure 16 ROC for Pocock score ≥ 0.8% against presence of microemboli following hyper-acute symptomatic carotid disease (n = 206) (AUC 0.509 95% CI 0.326 – 0.692, P = 0.922)
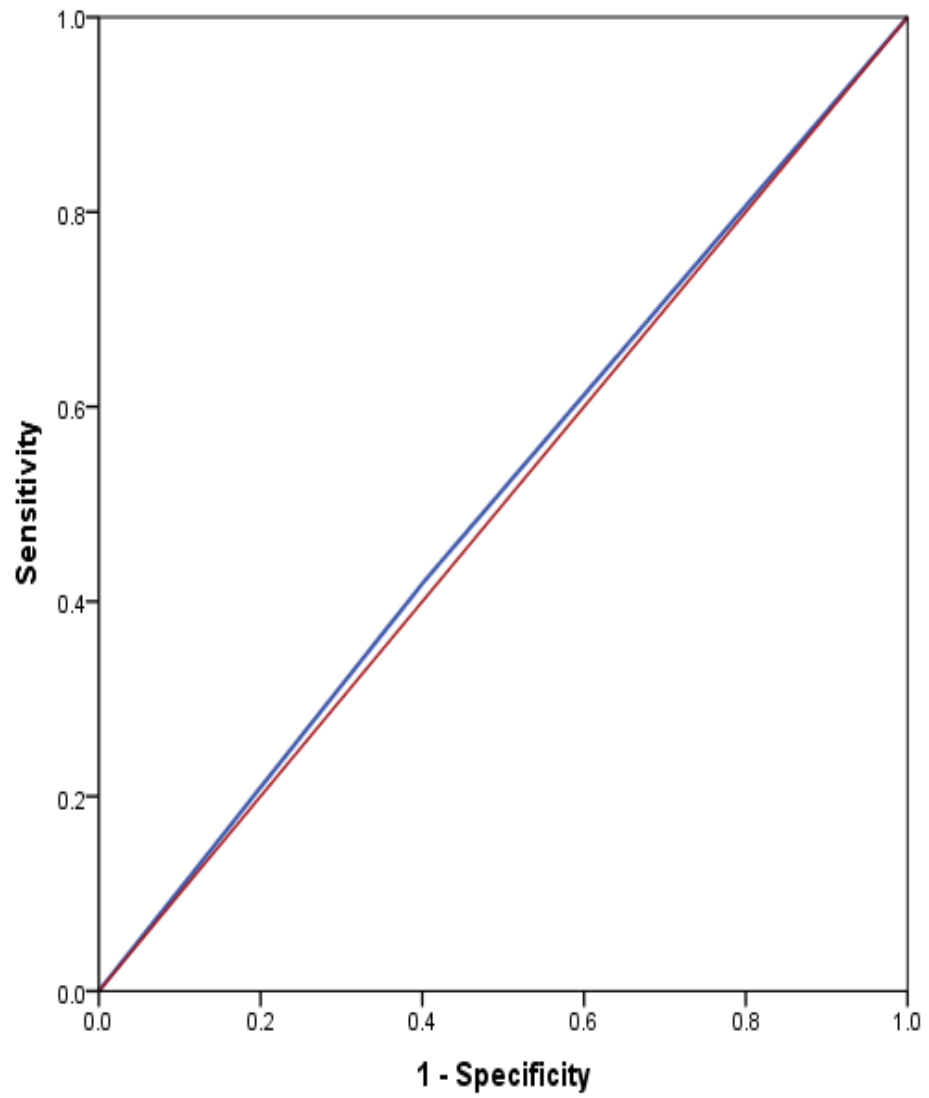
Diagnostic accuracy

A Pocock score ≥ 0.8% and 2.3% showed high sensitivity for the presence of microemboli

following acute symptomatic carotid disease (Table 39, Table 40). A Pocock score ≥ 6.1% showed

general poor diagnostic accuracy for the presence of microemboli (Table 41).

Table 39 Diagnostic accuracy Pocock's score ≥ 0.8% vs MES

Sensitivity	95.35%	95% CI: 88.51 to 98.69
Specificity	5.00%	95% CI: 1.87 to 10.57
Positive Predictive Value	41.84%	95% CI: 34.85 to 49.08
Negative Predictive Value	60.00%	95% CI: 26.37 to 87.60

Table 40. Diagnostic accuracy Pocock score ≥ 2.3% vs MES

Sensitivity	77.91%	95% CI: 67.67 to 86.14
Specificity	18.33%	95% CI: 11.86 to 26.43
Positive Predictive Value	40.61%	95% CI: 33.04 to 48.52
Negative Predictive Value	53.66%	95% CI: 37.45 to 69.34

Table 41. Diagnostic accuracy Pocock score ≥ 6.1%% vs MES

Sensitivity	38.39%	95% CI: 29.36 to 48.06
Specificity	54.26%	95% CI: 43.66 to 64.58
Positive Predictive Value	50.00%	95% CI: 39.02 to 60.98
Negative Predictive Value	42.50%	95% CI: 33.53 to 51.85

5.3.3 ABCD^2 risk score to predict presence of cerebral microemboli in hyper-acute symptomatic critical carotid artery stenosis patients

Table 42, figure 17 and figure 18 show the overall distribution of cerebral microemboli and their

associated ABCD^2 score in hyper-acute symptomatic critical carotid artery stenosis patients.

Table 42. Distribution of ABCD^2 score and microemboli based on degree of carotid stenosis

Carotid stenosis (%), n	No MES		MES	
	ABCD^2 score <4	ABCD^2 score ≥4	ABCD^2 score <4	ABCD^2 score ≥4
50 – 59 (15)	2	9	1	3
60 – 69 (25)	3	11	3	8
70 – 79 (47)	10	17	4	16
80 – 89 (48)	8	17	11	12
90 – 99 (71)	16	27	8	20

Figure 17. Distribution of microembolic patients based on carotid stenosis: 120 without microemboli and 86 with microemboli
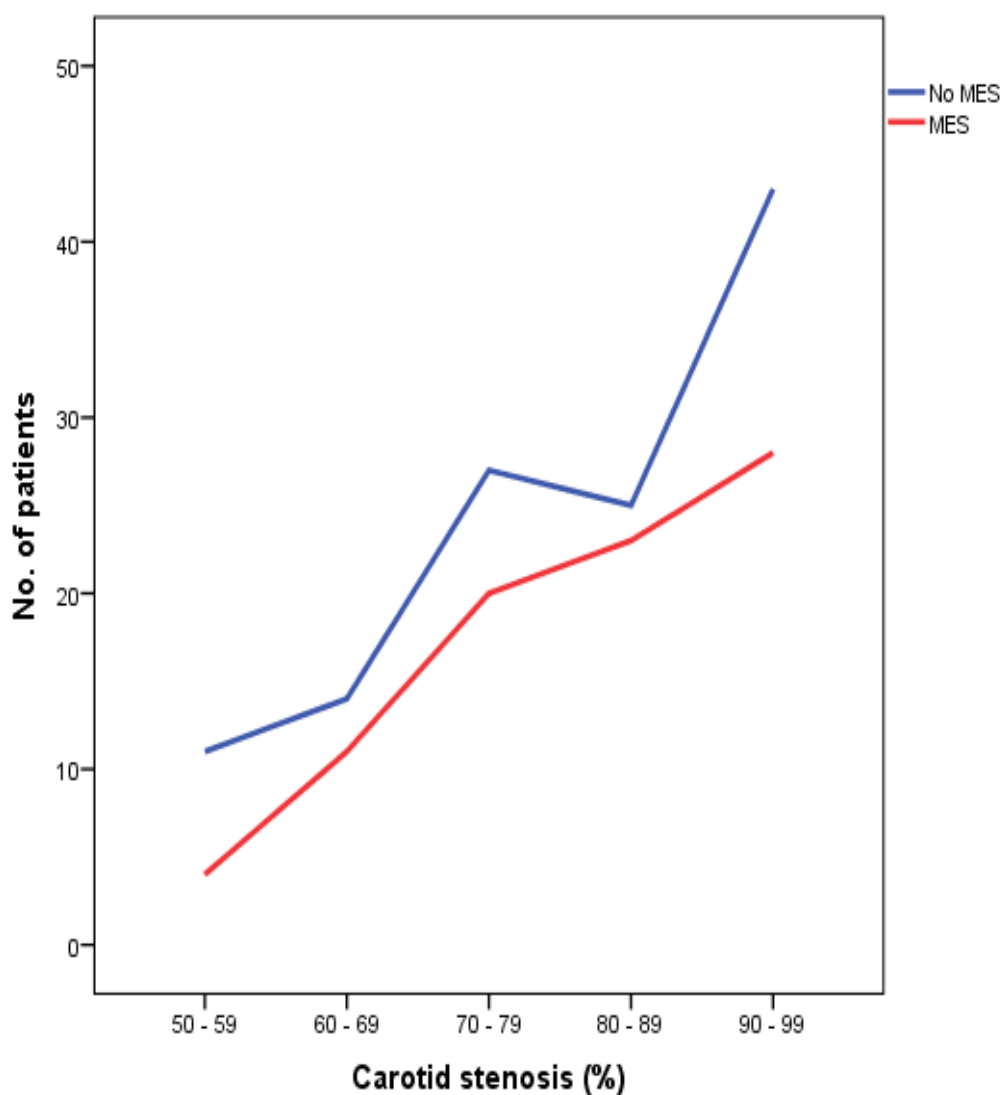
Figure 18. Distribution of ABCD^2 score based on carotid stenosis: 120 without microemboli and 86 with microemboli.
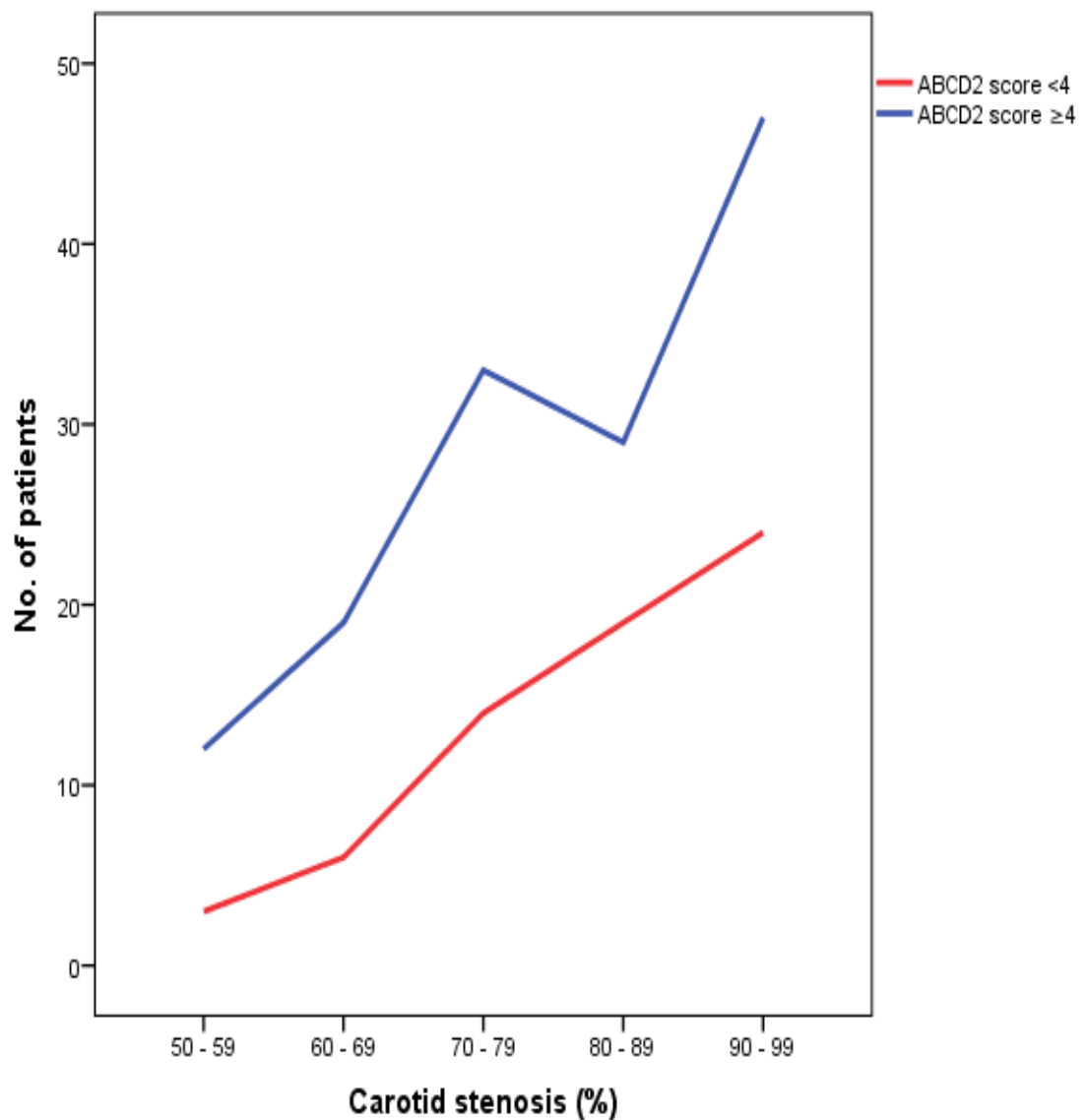
One hundred and forty of 206 (68%: 95%CI 62 – 74%) patients with hyper-acute symptomatic critical carotid stenosis had an ABCD^2 risk score ≥4. The ABCD^2 risk score was ≥4 in 59 of 86 (69%: 95%CI 59 – 79) embolising patients and in 81 of 120 (68%: 95%CI 60 – 76) without cerebral microemboli. There was no significant difference in the NICE criterion for early assessment (ABCD^2 risk score ≥4) for patients with cerebral microemboli vs. those without microemboli (59/86 vs 81/120 patients: odds ratio 1.05 (95% CI 0.58 - 1.90, P = 0.867).

Sixty six of 206 (32%: 95%CI 26 – 38) patients with hyper-acute symptomatic critical carotid stenosis had an ABCD^2 risk score <4. The ABCD^2 risk score was <4 in 27 of 86 (31%: 95%CI 21 – 41) embolising patients and in 39 of 120 (31%: 95%CI 23 – 39) without cerebral microemboli.

There was no significant ABCD^2 score difference between these 2 cohorts (non-embolising group, n = 120: ABCD^2 score 4(IQR 3 – 5) vs. embolising group, n = 86: 4(IQR 3 – 5), P = 0.855 MW test). There was no significant ABCD^2 score difference between Registry patients and prospective cohort study patients (Registry, n = 102 (ABCD^2 score 4(IQR 3 – 5) vs. prospective cohort study, n = 104: 4(IQR 3 – 5) P = 0.661 MW test).

Registry (n = 102)

Seventy-one of the 102 patients (70%: 95%CI 61.11 – 78.89) with acutely symptomatic critical carotid stenosis had an ABCD^2 risk score ≥4. The ABCD^2 risk score was ≥4 in 25 of 35 (71%: 95%CI 56 – 86%) embolising patients and in 46 of 67 (69%: 95%CI 58 – 80%) without cerebral microemboli. There was no significant difference in the NICE criterion for early assessment (ABCD^2 risk score ≥4) for patients with cerebral microemboli vs. those without microemboli (25/35 vs 46/67 patients: odds ratio 1.14 (95% CI 0.47 – 2.80, P = 0.773).

Twenty-one of the 67 patients (31%: 95%CI 20– 42%) with symptomatic critical carotid stenosis had an ABCD^2 risk score <4. The ABCD^2 risk score was <4 in 10 of 35 (29%: 95%CI 14 – 44%) embolising patients and in 21 of 67 (31%: 95%CI 20 – 42) without cerebral microemboli.

Prospective hyper-acute symptomatic carotid artery stenosis study (n = 104)

Sixty-nine of the 104 patients (66%: 95%CI 57 – 75) with symptomatic critical carotid stenosis had an ABCD^2 risk score ≥4. The ABCD^2 risk score was ≥4 in 34 of 51 (67%: 95%CI 54 – 80) embolising patients and in 35 of 53 (66%: 95%CI 53 – 79) without cerebral microemboli. There was no significant difference in the NICE criterion for early assessment (ABCD^2 risk score ≥4) for patients

with cerebral microemboli vs. those without microemboli (34/51 vs 35/53 patients: odds ratio

1.03 (95% CI 0.46 – 2.23, P = 0.946).

Thirty-five of the 104 patients (34%: 95%CI 25 – 43%) with symptomatic critical carotid stenosis

had an ABCD^2 risk score <4. The ABCD^2 risk score was <4 in 17 of 51 (33%: 95%CI 20 – 46)

embolising patients and in 18 of 53 (34%: 95%CI 21 – 47) without cerebral microemboli.

Receiver Operating Curve (ROC) for ABCD^2 score to predict the presence of microemboli

Two hundred and six patients were included into this analysis. One hundred and twenty patients

without and eighty six patients with microemboli. The ROC for ABCD^2 risk score showed no

significant prediction of microemboli (AUC 0.49 95% CI 0.41 – 0.57, P = 0.860: Figure 19).

Figure 19. ROC for ABCD^2 risk score against presence of microemboli in patients (n = 206) with hyper-acute
symptomatic carotid stenosis (AUC 0.49 95% CI 0.41 – 0.57, P = 0.860)
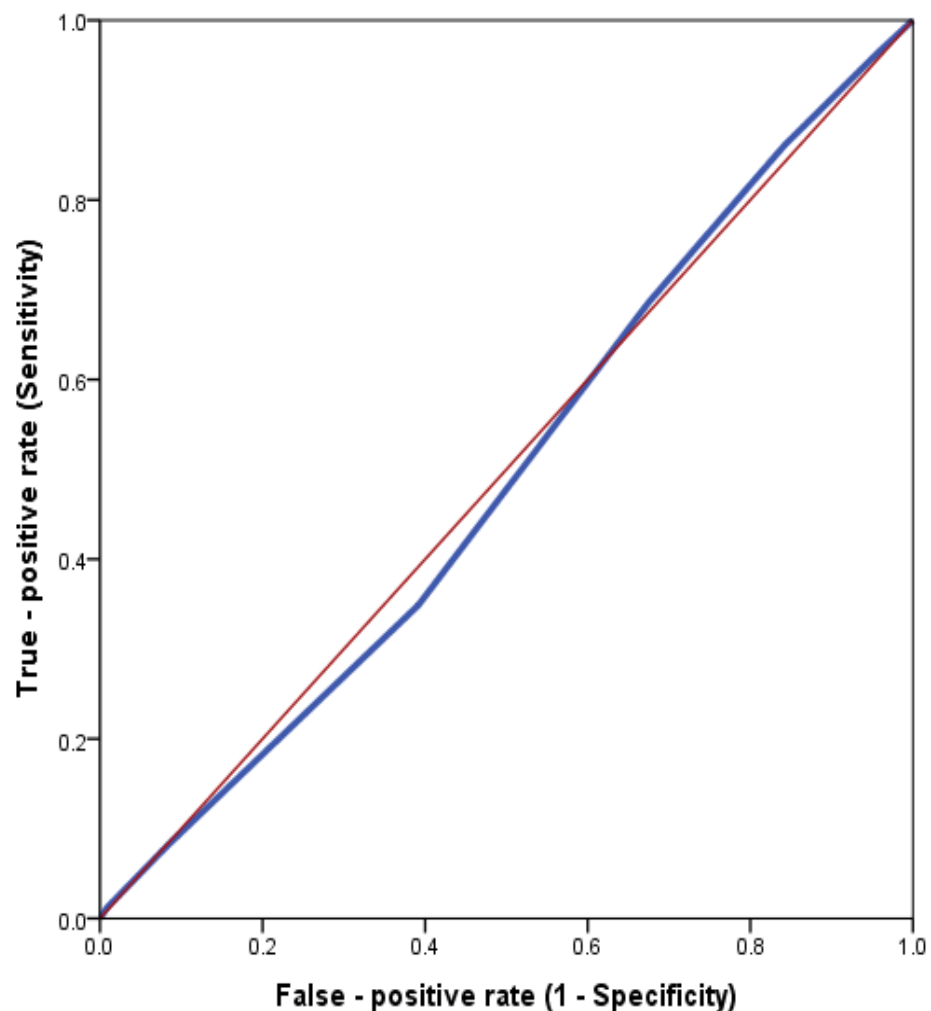
Patients without antiplatelet treatment before the neurological event:

Fifty seven patients were included in this analysis, where 19 of those patients had an evidence of

microemboli and 38 patients had no evidence of microemboli. The ROC for ABCD^2 risk score

showed no prediction of microemboli (AUC 0.45 (95% CI 0.30 − 0.60), P = 0.531: Figure 20).

Figure 20. ROC for ABCD^2 risk score against presence of microemboli in patients (n = 57) without
antiplatelet treatment pre-neurological event (AUC 0.45 (95% CI 0.30 − 0.60), P = 0.531)
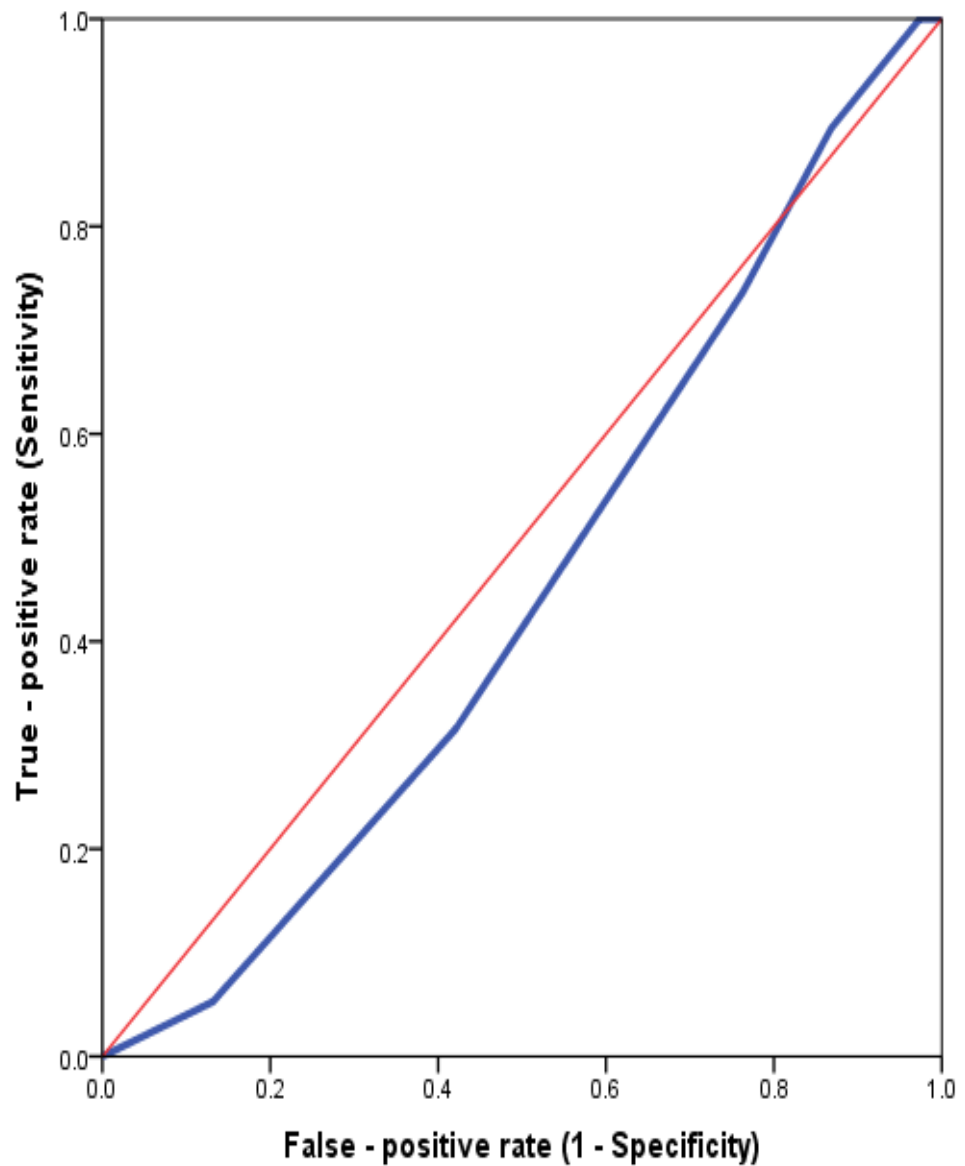
Patient with antiplatelet treatment before the neurological event:

One hundred and forty nine patients were included in this analysis. Sixty seven of these patients

had an evidence of microemboli and eighty two patients had no evidence of microemboli. The

ROC for ABCD^2 risk score showed no prediction of microemboli (AUC 0.51 (95% CI 0.42 – 0.60), P =

0.804: Figure 21)

Figure 21. ROC for ABCD^2 risk score against presence of microemboli in patients (n = 149) with antiplatelet treatment pre-neurological event (AUC 0.51 (95% CI 0.43 – 0.60), P = 0.531)
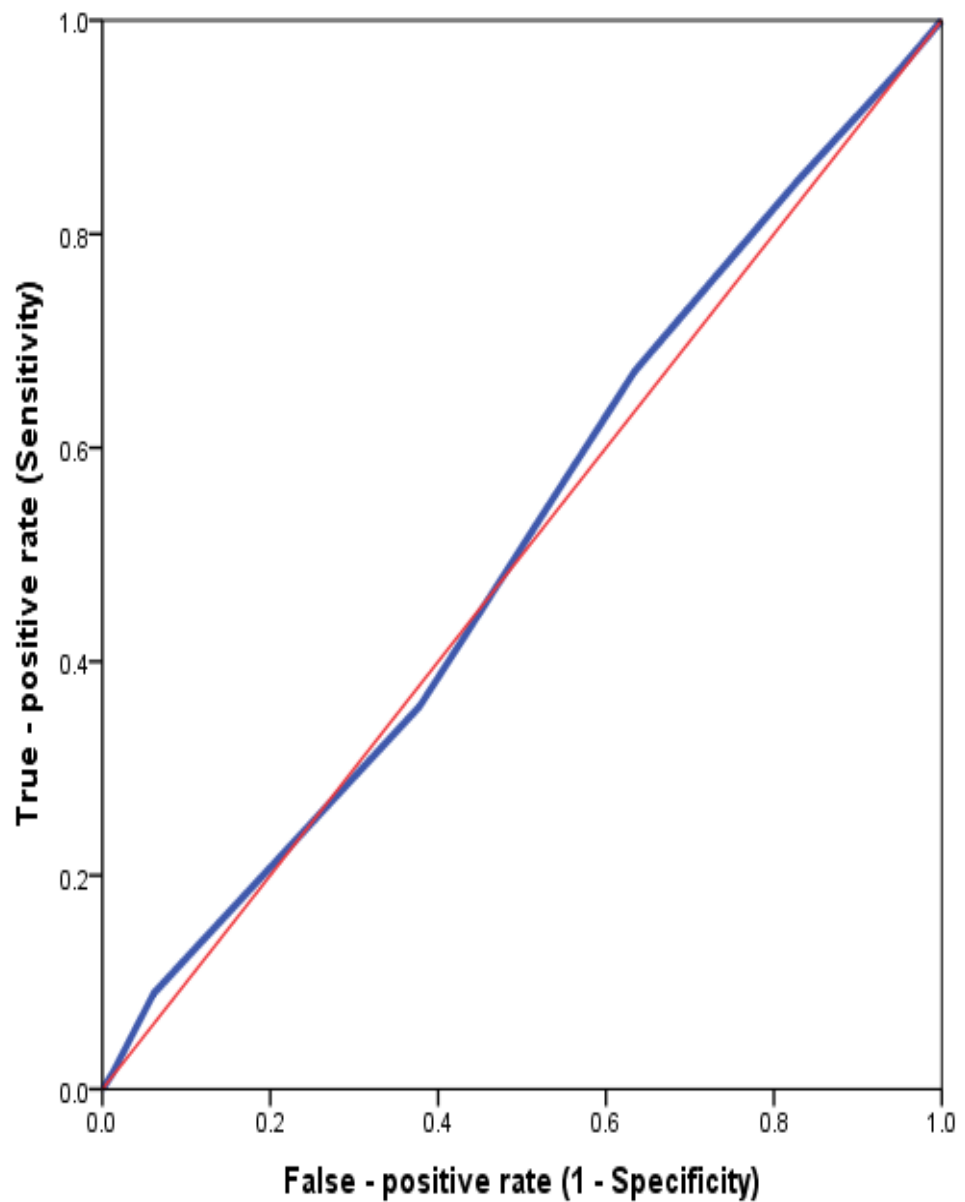
Patients without statin treatment before the neurological event:

Seventy two patients were included in this analysis, where 31 of those patients had an evidence of microemboli and 41 patients had no evidence of microemboli. The ROC for ABCD^2 risk score showed no prediction of microemboli (AUC 0.48 (95% CI 0.34 – 0.61), P = 0.716: Figure 22).

Figure 22. ROC for ABCD^2 risk score against presence of microemboli in patients (n = 72) without statin treatment pre-neurological event (AUC 0.48 (95% CI 0.34 – 0.61), P = 0.716)
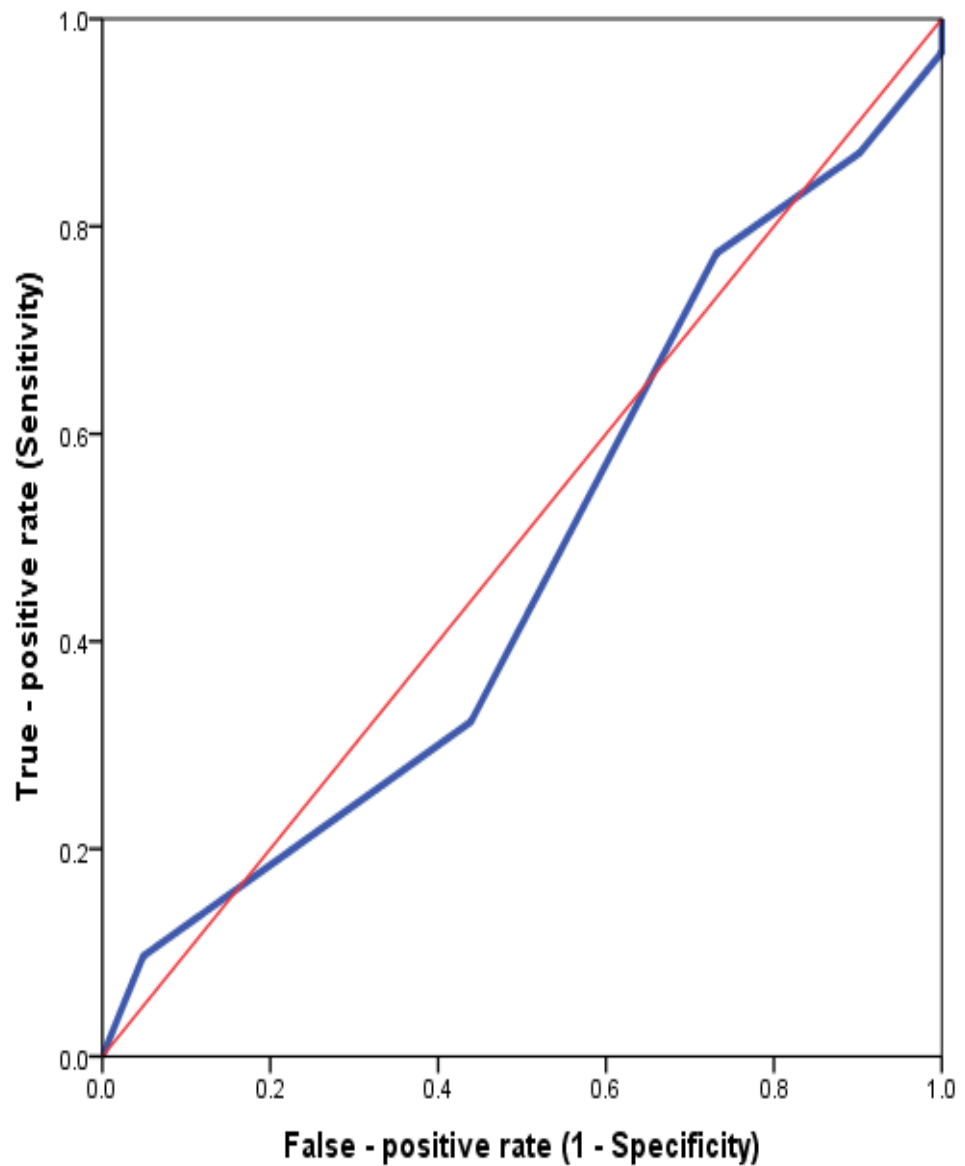
Patient with statin treatment before the neurological event:

One hundred and thirty four patients were included in this analysis, where 55 of those patients

had an evidence of microemboli and 79 patients had no evidence of microemboli. The ROC for

$ABCD^2$ risk score showed no prediction of microemboli (AUC 0.50 (95% CI 0.40 – 0.60), P = 0.980:

Figure 23).

Figure 23. ROC for $ABCD^2$ risk score against presence of microemboli in patients (n = 134) with statin
treatment pre-neurological event (AUC 0.50 (95% CI 0.40 – 0.60), P = 0.980)
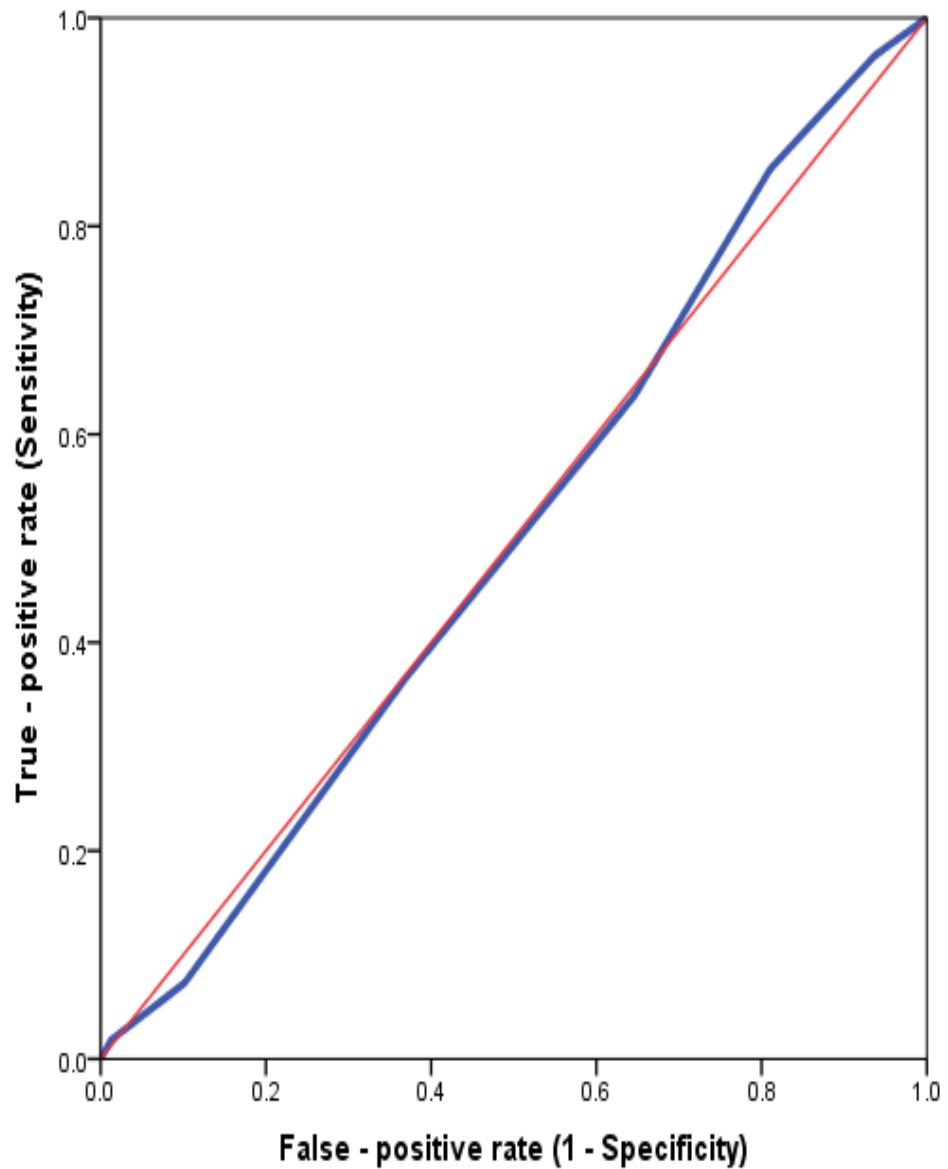
5.4   Discussion

In this study, the ABCD^2 risk score was found to be unable to identify patients with cerebral microemboli associated with symptomatic critical carotid artery stenosis. The study also suggests that 66 (32%) patients with hyper-acute symptomatic carotid disease with or without microemboli are unlikely to be triaged effectively for urgent management using the ABCD^2 risk score alone. Furthermore, 27 (31%) out of 86 patients with microemboli with an ABCD^2 risk score <4 would also not have been assessed within 24 hours if NICE guidelines had been followed. Both ABCD^2 risk score and cerebral microemboli predict short term risk of recurrent stroke. Therefore, the ABCD^2 risk score is expected to be associated with occurrence of cerebral microemboli. These patients are known to benefit from targeted medical treatment[37] to suppress microemboli prior to urgent carotid endarterectomy[218]. To my knowledge, this is the first study to explore the possible links between ABCD^2 risk score and cerebral microemboli in patients with hyper-acute symptomatic critical carotid artery stenosis. Taken together with previous reports[32, 34-36, 60], the study provides important evidence that the occurrence of cerebral microemboli, predictors of stroke risk, is dissociated from the results for the ABCD^2 risk score.

Of note, in this study, vascular complications were lower in patients with microemboli. This is likely to be due to reverse causality as in our centre patients with microemboli were assigned to receive more aggressive antiplatelet treatment compared to those in whom microemboli were not detected[214, 218].

Currently, the association between the ABCD^2 risk score and the severity of structural carotid artery disease is unclear. This is an important issue to clarify as Ois et al[58] reported that patients presenting with symptomatic critical carotid artery stenosis incur a 17% risk of recurrent stroke at 72 hours, increasing to 22% at 7 days[58]. These patients are reported to benefit from carotid endarterectomy within 2 weeks[53].

Whilst Amarenco et al[106] and Walker et al[112] did not find an association between ABCD^2 risk score and carotid artery disease, Schrock et al[113], Sheehan et al[111] and Koton et al[110] suggested that high ABCD^2 risk score may identify patients with significant carotid artery disease. However, Amarenco

et al[106] reported that an ABCD^2 risk score < 4 would miss 9% (62/679) of patients with symptomatic critical carotid artery stenosis.

Patients with symptomatic carotid artery disease are at high risk of developing stroke. I would expect to find a significantly higher cardiovascular risk factor burden in patients with cerebral microemboli. It appeared in this study that prevalence and severity of individual classical cardiovascular risk factors were similar or lower in the microembolic stroke cohort. Peripheral arterial disease and family history of vascular disease were more common in the non-microembolic stroke cohort. Of note, a moderate ~10% increase in total cholesterol might contribute to excess risk of microembioli.

The cardiovascular composite score including the Pocock score showed no significant difference between microembolic and non-microembolic cohorts. However, a high risk Pocock score (≥ 0.8% and 2.3%) showed high sensitivity for the presence of microemboli. None of the cardiovascular composite risk scores were able to predict the presence of microemboli in this high risk symptomatic carotid disease patients.

The proportion of patients on antiplatelet therapy prior to the stroke syndrome event was similar in patients with or without microemboli. Thus differences in prescribed antiplatelet treatment were not sufficient on their own to explain whether a patient would have a higher or lower prevalence of embolisation. Of note, in patients already on antiplatelet treatment, the non-embolising symptomatic carotid artery disease cohort showed a trend towards a higher cardiovascular risk factor burden and were at significantly higher long term risk of cardiovascular death than the embolising cohort. This may reflect the fact that the embolising cohort received more aggressive antiplatelet treatment[37].

Seven patients from the Registry were excluded in the analysis due to lack of relevant data pertinent to the ABCD^2 risk score calculation, therefore only 206 patients were included for the final analysis. This was the retrospective cohort between the period of 2002 and 2005. The clinical features and duration which comprise 4 of the 7 possible points were not able to abstract reliably from the medical notes. This small number of missing data is unlikely to influence the overall

results. Furthermore, the baseline patient characteristics and results analysis between the retrospective and prospective cohorts were similar (high convergent validity)[258] and any relevant differences were consistent with the improvement of medical therapy and changed of clinical practice. Overall, one quarter of patients in this study were not on antiplatelet agents prior to hospital admission with TIA or minor stroke despite well-established best medical treatment for cardiovascular disease. The reasons for this are unknown and beyond the scope of this study. It would be worthwhile to study the effectiveness and compliance of the best medical treatment in carotid disease patients.

This study also emphasises the need for further work to elucidate cardiovascular risk factors and causative mechanisms for microemboli associated with acute stroke syndromes, and to identify optimal treatment to reduce microemboli-attributable stroke risk.

In conclusion, the $ABCD^2$ risk score offers a low cost and rapid assessment to identify patients at high risk of recurrent stroke syndrome. However, the study showed that the $ABCD^2$ risk score does not appear to predict the presence of cerebral microemboli in hyper-acute symptomatic carotid stenosis cohort. Thus, based on $ABCD^2$ risk score alone, a significant minority of patients with hyper-acute symptomatic carotid stenosis with or without cerebral microemboli would be likely to be overlooked in triage for more urgent assessment and these exposed to delayed treatment for preventable recurrent stroke.

CHAPTER SIX

6 Transorbital Doppler as an alternative to transcranial Doppler for detecting cerebral microemboli in patients with carotid disease without an acoustic temporal bone window.

Two phases for studies to evaluate the transorbital Doppler technique in detecting cerebral microemboli in patients who lacked acoustic temporal window were conducted. The 1st phase was to identify the proportion of patients who underwent transorbital examination and its feasibility to monitor microembolic signal after surgery from the Carotid Registry. The 2nd phase was a prospective observational study to validate the transorbital technique against the standard transcranial Doppler using temporal window technique for detecting microemboli.

6.1 The feasibility of transorbital Doppler to detect carotid associated cerebral microemboli in patients without a temporal bone acoustic window

6.1.1 Introduction

Microemboli associated thrombotic stroke and cerebral hyperperfusion syndrome most commonly occurred acutely post carotid endarterectomy[167]. Transcranial Doppler (TCD) monitoring in the postoperative phase can be used to detect both cerebral hyperperfusion and transient cerebral microemboli. Persistent high levels of postoperative cerebral microembolisation are associated with early carotid thrombosis and based on the historical data, it was accounted for 2-3% of postoperative strokes [167].

Transcranial Doppler ultrasound is a non-invasive technique that can be used to detect changes in middle cerebral artery (MCA) velocity and circulating cerebral microemboli. It has been shown to reliably detect carotid circulation-associated microemboli[259]. The use of this 'through-the-skull'

ultrasonography relies on an 'acoustic window' through which the ultrasound beam focuses on the intracranial arterial tree. The temporal bone acoustic window (TAW) is said to be absent or unsatisfactory in 10-15% of subjects although a figure as high as 29.1% is reported in the literature[260, 261]. This technical limitation has reduced the general routine clinical applicability of TCD.

The transorbital Doppler approach has been described as an alternative to the standard TCD in monitoring blood flow velocity during carotid surgery[262, 263]. In the setting of post carotid surgery, those patients who lacked an acoustic temporal window, transorbital approach could be used to monitor the blood flow velocity for hyperperfusion syndrome. Its role in detecting cerebral microemboli has not been investigated before. In transorbital Doppler (TOD) monitoring the ultrasound beam is focused on the distal internal carotid, rather than the middle cerebral artery. It utilizes the thin orbital plate of the frontal bone, optic canal and superior orbital fissure for the penetration of ultrasonic waves. In transorbital Doppler (TOD) monitoring the ultrasound beam is focused on the distal internal carotid, rather than the middle cerebral artery (Figure 24).

Figure 24 Angiographic image of the carotid siphon. C5 (pre-cavernous/ganglionic segment), C4 (inferior carotid siphon), C3 (carotid knee), C2 (upper carotid siphon), and C1 (terminal ICA segment). Ref: Valaikiene et al. Am J Neuroradiol. 2008; 29:347-53.
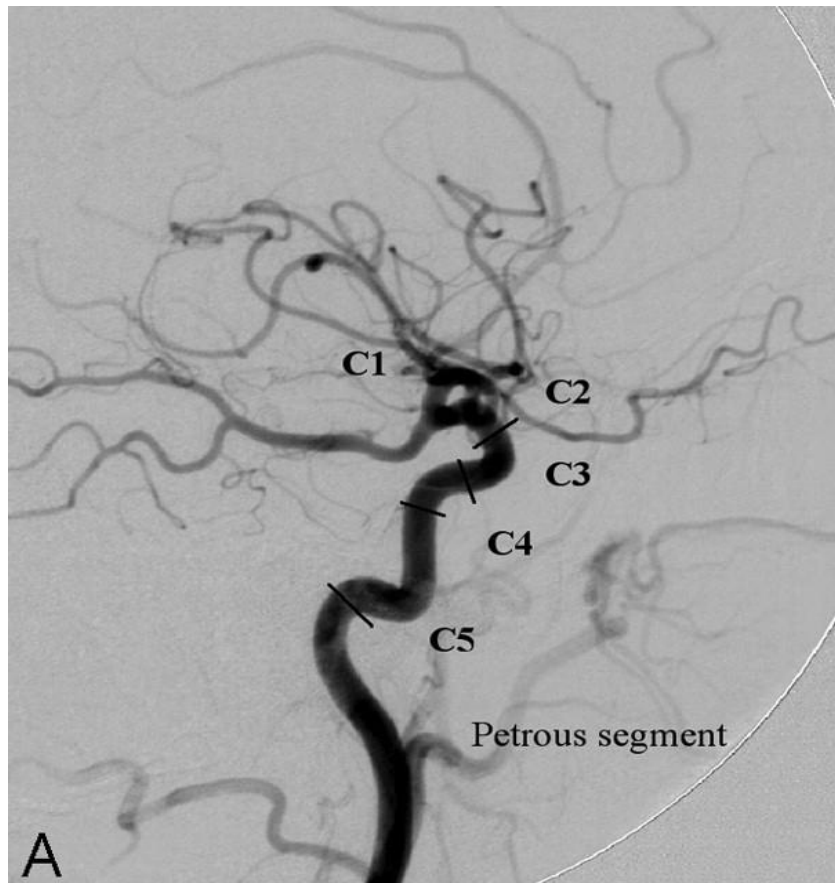
The aim of this study was to assess the feasibility of using the transorbital acoustic window in those patients with absence of a suitable temporal bone acoustic window to detect acute cerebral microemboli post carotid endarterectomy.

6.1.2   Subjects and Methods

A Registry review was undertaken of all CEA performed between 2002 and 2008 at the University Hospital Coventry and Warwickshire. All patients who underwent CEA between 2005- 2008 were assessed for a temporal acoustic window (TAW) for TCD examination prior to carotid surgery. Within this period, patients who were found to be unsuitable for standard TCD examination, were assessed for suitability via the transorbital approach.

The standard TCD examination has been described in chapter 3. All patients who underwent

postoperative TCD or TOD examination during this period had manually recorded data on the

presence of microemboli by the vascular technologists using a standardised proforma. The TCD

spectral wave was also recorded using the automated detection software for further reviewed.

Transorbital Doppler imaging

The equipment used for TOD was the same as for TCD – a PC Dop 842 (SCI Med, Bristol, UK) 2

MHz transducer probe. The probe was placed on the closed eye ipsilateral to the CEA, after

acoustic coupling gel had been applied to that eye (Figure 25). Care was taken to avoid exerting

undue pressure on the eye. An anterior-posterior orientiation was used to insonate the cavernous

sinus and supraclinoid portions of the internal carotid artery. Once the carotid siphon wave

spectrum had been identified (depth typically 65–70 mm), the acoustic amplitude was reduced to

10% (Thermal Index (TI) < 0.1) of its initial transmitting power[264]. This allowed, adequate

visualisation, while minimising the theoretical risk of orbital tissue heating[265].

Figure 25 Ultrasound probe is placed on the closed eyelid by a vascular scientist for the TOD technique. The candidate (MH Mahmud Saedon) placing a second Doppler probe on the temporal bone window

Ethics

University Hospitals Coventry Warwickshire (UHCW) research ethics department has advised that this study did not fall under the remit of the National Health Service Research Ethics Committee as it was audit/service evaluation. Therefore informed consent process has been waived by UHCW research ethics department.

6.1.3 Results

318 CEAs were performed between 2005 and 2008. Of this subgroup, 29 (9.1%) had no TAW. TOD windows were used in 25 (86%) of these 29 patients. 4 (1.2%) patients could not be monitored.

Twenty-five patients (19 women) were detected with TOD windows; their ages ranged between 59-86 years. Nineteen patients had symptomatic carotid disease with an average stenosis of 82% (range 70% to 99 %), and 21 were performed electively. The postoperative duration of monitoring was between 30 minutes to 70 minutes, while the number of emboli detected by TOD ultrasonography ranged from 1 to 28. None of the TOD monitored patients had postoperative detectable neurological deficits. A single patient required glycoprotein IIb/IIIa receptor antagonist (Tirofiban) infusion for 1 day postoperatively due to excessive carotid microembolisation[214].

6.1.4   Discussion

The key finding in this study is the feasibility of using transorbital approach in the majority of patients without a temporal acoustic window to detect microemboli were demonstrated. In one patient, intravenous glycoprotein IIb/IIIa receptor antagonist infusion to reduce the microembolic rate were monitored by the TOD technique. In that particular case, the transorbital approach was used on the basis of clinical judgement with intention to reduce postoperative stroke risk.

The rate of encountering an absence TAW rate in the vascular unit was comparable to the rate quoted in the literature[260]. Failure to identify TAW has been attributed to age, female gender and skull thickness[266]. Transcranial Doppler is also an operator dependant technique. All these factors explained the significant variation of absent TAW rate reported in literature[260, 266].

The potential hazard of thermal injury to the eye is a concern with the transorbital approach. During the study period, there were no complications associated with this approach. A sufficient amount of gel was applied on the closed eyelid to minimise any mechanical pressure. Spencer and Whisler[267] showed that transorbital Doppler examination is safe for the eye and reported no complaints or adverse effects. Subsequently, the safety of transorbital ultrasound examination has been discussed in the literature and believed to be safe for the eyes[268].

Recent evidence highlighted the importance of transcranial Doppler as a monitoring tool in carotid disease and antiplatelet infusion in reducing cerebral microemboli[176]. Wolf et al[28] found significant correlation between microembolic signals and ischaemic lesions as well as brain

infarction during dissection. Abbott et al[207] concluded that, postoperative detected TCD

microembolic signal are associated with 15 times higher risk of stroke. Sharpe et al has

demonstrated the high risk patients could be identified within 30 minutes of TCD examination

following skin closure[208]. Therefore, early postoperative TCD monitoring provides important

information relevant to perioperative carotid endarterectomy stroke prevention.

In this study, the transorbital approach has been shown to enable us to monitor the majority of

patients with an absent TAW post-carotid endarterectomy and to commence antiplatelet agent

infusion in patients with high microembolic loads. The technique is fairly challenging for any

untrained scientist and the patient undergoing the procedure. However, the evidence is

overwhelmingly supportive of the use of TCD as part of stroke prevention in carotid surgery. This

should offset the perceived challenging practicality of the transorbital approach.

In conclusion, using the carotid Registry data, I was able to demonstrate the technical feasibility of

transorbital approach in detecting microemboli in those patients with an absent temporal

acoustic window for standard TCD examination.

6.2 A prospective validation study of transorbital Doppler for the detection of cerebral microemboli using transtemporal Doppler imaging as the reference standard

6.2.1 Introduction

Transcranial Doppler (TCD) detected microemboli has provided direct evidence of

thromboembolism as the main mechanism of symptomatic carotid artery disease[269]. Transcranial

Doppler ultrasound studies show that the presence of microemboli in symptomatic carotid artery

disease are associated with an increased risk of stroke[31-33]. Microemboli have also been used as an

indirect biomarker to evaluate the efficacy and safety of using dual anti platelet or novel

antiplatelet therapies in symptomatic carotid artery disease[37, 168, 176, 217]. More recently, TCD has

been used to monitor and guide the antiplatelet treatment for cerebral microemboli during carotid stenting procedure to prevent stroke[270].

Transcranial Doppler is the only real-time clinical imaging modality for detecting microembolic signals associated with carotid artery disease. TCD relies on there being an acoustic temporal bone window through which the ultrasound beam can focus on the middle cerebral artery. The temporal bone acoustic window (TAW) is reported to be absent or unsatisfactory in around 15% of subjects, with an absent TAW in up to 29% of patients in some populations[240].

The transorbital Doppler (TOD) method has been suggested as an alternative option for patients who do not have a temporal bone window[240]. In TOD monitoring, the ultrasound beam is focused on the distal internal carotid artery, rather than the middle cerebral artery. In my earlier work, I have demonstrated the feasibility of using the transorbital approach to detect microemboli[240]. The next stage was to assess the validity and reliability of this technique to detect microemboli. The aim of this study was to validate the transorbital Doppler method for detecting carotid disease-associated transient cerebral microemboli, using the transcranial Doppler method as the reference standard.

6.2.2 Subjects and Methods

Subjects

For the validation of TOD, patients undergoing elective carotid endarterectomy (CEA) were studied prospectively. A previous study has shown that up to 60% of patients may develop MES acutely following carotid surgery[33]. So, carotid endarterectomy was used as the research setting for this validation study due to the higher frequency of encountering microemboli following surgery. Patients who had both temporal and orbital acoustic windows were identified before surgery. Patients who did not have temporal bone or orbital acoustic windows were excluded from the study. Patients with atrial fibrillation were excluded, because this confers a risk of an additional non-carotid source of emboli. Patients with a prosthetic heart valve were also excluded, because of the recognised effect of these valves to generate gaseous and other embolic

signals[256]. 105 patients were initially recruited for the study based on the presence of a temporal acoustic window. However 5 of those patients did not have a satisfactory TOD window for monitoring. Therefore, 100 consecutive patients fulfilling the above inclusion and exclusion criteria who underwent elective CEA between February 2011 and May 2013 at the University Hospital Coventry and Warwickshire were included in the study. All patients gave written informed consent to the validation study, which was approved by the Local Research Ethics Committee.

Prior to carotid endarterectomy, 35 patients were on single antiplatelet agent; 55 patients were on dual antiplatelet agents and 10 patients were on triple antiplatelet agents. Immediately after recovery from anaesthesia (typically less than 30 minutes after skin closure) patients underwent simultaneous TCD and TOD monitoring for 30 minutes by 2 vascular technologists accredited by the Society for Vascular Technology of Great Britain and Ireland[118]. The recorded signals were then re-examined by a 3rd accredited vascular technologist to confirm or refute the presence of microembolic signals.

Transcranial Doppler method

TCD monitoring was performed (PC Dop 842, SciMed, Bristol, UK) with a 2 MHz probe focused on the middle cerebral artery (MCA) ipsilateral to the endarterectemised carotid artery. The MCA was identified by placing the probe above the zygomatic arch and just in front of the ear. The identification of the MCA was based on guidelines set out by Alexandrov et al[243] including spectral waveform, flow direction, blood flow velocity and flow pulsatility. A head-frame was used to secure a constant angle of insonation during TCD monitoring. An insonation depth of at least 45mm was used to identify proximal MCA flow signals and depths of 30 – 45mm for distal MCA flow signals.

Transorbital Doppler Method

The equipment used for TOD was the same as for TCD – a PC Dop 842 (SCI Med, Bristol, UK) 2 MHz transducer probe. The probe was placed on the closed eye ipsilateral to the CEA, after acoustic coupling gel had been applied to that eye. Care was taken to avoid exerting undue pressure on the eye. An anterior-posterior orientation was used to insonate the cavernous sinus and supraclinoid portions of the internal carotid artery. Once the carotid siphon wave spectrum had been identified (depth typically 65–70 mm), the acoustic amplitude was reduced to 10% (Thermal Index (TI) < 0.1) of its initial transmitting power[264]. This allowed, adequate visualisation, while minimising thermal index to reduce theoretical risk of tissue heating[265].

MES identification and treatment

For both methods, a single gating system and a filter set to a low threshold to capture signals were used. Automated WinTCD v3.7 software (VIASYS Healthcare Inc., Conshohocken, PA, USA) were used to monitor and record the Doppler wave form and audio signals. The recorded ultrasonographic images were then reassessed to exclude artefact and identify true microemboli based on the criteria of the International Consensus Group on microembolus detection[171]. MES were identified as unidirectional, short duration signals (range 10–100 ms) with intensity threshold above 6 dB, unidirectional, accompanied by characteristic audible clicks and occurring randomly throughout the cardiac cycle. We added treatment with tirofiban (Aggrastat®; MSD, Hoddeson, UK) in patients with an MES rate >50/hr[176]. Tirofiban[248] was given intravenously at 0.4mcg/kg/min for 30 minutes then at 0.1mcg/kg/min for 18 hours.

Carotid endarterectomy

CEA was performed as described in chapter 3. Shunting was performed if the patient developed focal neurological signs when CEA was performed under local anaesthetic; or in those cases performed under general anaesthetic when mean velocity of the middle cerebral artery dropped

by more than 50%. All patients received intravenous heparin (40 units kg^{-1}) prior to the cross clamp phase of the carotid endarterectomy.

Ethics

This prospective observational study was approved by the Regional Coventry and Warwickshire Research Ethics Committee.

Statistical analyses

The diagnostic accuracy parameters of TOD in comparison to TCD were reported based on the STAndards for the Reporting of Diagnostic accuracy studies (STARD) statement recommendations[271]. TCD detected the clinically important circulating microemboli in the MCA (Reference standard), therefore the microemboli which ended up in the anterior circulating artery were unaccounted for. TOD detected microemboli at the distal end of the internal carotid artery. The same ultrasonography technique was used at different anatomical sites, so direct comparison between these two methods was not possible. We aimed to demonstrate that TOD is not clinically inferior to the TCD method. Therefore, for this experimental study, a false positive was defined as cerebral microemboli detected by means of TOD only as TCD remains the 'gold standard' imaging modality to identify microemboli [272, 273]. During this study, the decision to treat cerebral microemboli acutely post carotid surgery was based on the findings of TCD only as TOD is not a validated method. It was therefore not considered ethical to use TOD findings as the basis to initiate antiplatelet treatment to reduce the risk of stroke [273] for those who have had a sustained high microembolic signal rate on TOD alone during this experimental study.

Data are show as mean ± SEM. Categorical data were analysed by Chi – Squared test (X^2) and non-parametric data by Mann – Whitney U test (MW). Spearman's rank correlation coefficient was used to assess the strength of relationship between numbers of microemboli detected by TCD vs. TOD. A P-value of less than 0.05 was considered significant. Bland – Altman analysis, including was

used to compare absolute numbers of microemboli detected by TCD vs. TOD. Statistical analyses were conducted using SPSS® version 21 (Chicago, Illinois, USA).

Sample size

The sample size for the study was estimated by considering the confidence interval (CI) width for the ratio of microemboli detected by TCD vs. TOD, based on the ratio of blood flow and the distribution of microemboli from the internal carotid artery into the MCA[184]. For an estimated agreement rate of TCD vs. TOD of 83% based on pilot data, and a minimum acceptable agreement rate set at 70%[184], a sample size of 100 patients provided a power of 80% at the 5% significance level (2-tailed) for a confidence interval width of 18% (95% CI 74 – 92%) for microemboli detected by TCD vs. TOD.

6.2.3 Results

Background profiles

100 patients (age 72 ± 1 [SEM], male 65, Caucasian 97) were included in the study. Indications for CEA were: TIA 55, non-disabling stroke 19, asymptomatic critical artery stenosis 10, and amaurosis fugax 16. Carotid ultrasound imaging revealed the degree of carotid artery stenosis as 76 ± 1% (mean ± SEM).

MES detection

The depth for optimum ultrasound insonation for TCD was 53 ± 0.3 mm (mean ± SEM) and for TOD 69 ± 0.3 mm. The time - averaged mean velocity from TCD was higher than for TOD (40 ± 2 vs. 33 ± 1 cm s^{-1}, P = 0.002 MW).

TCD detected 426 microemboli in 30 minutes whereas TOD detected 415 microemboli in 30 minutes (P = 0.157, X^2 test) in total. There was no significant difference in MES detected in patients symptomatic before surgery vs. those asymptomatic before surgery (TCD: median 3, 1QR (1-12), X^2, P = 0.126; TOD: median 3, 1QR (1-10), X^2, P = 0.621).

Microemboli were detected by one or both methods in 40 patients: by TOD and TCD in 24 patients, by TOD alone in 10 patients; by TCD alone in 6 patients. The overall level of agreement between these 2 diagnostic methods was 86% (86/100). The diagnostic odds ratio for TOD vs. TCD was 24.0 [95% CI 1.8 – 314.3]. There was a strong positive association for MES detection between the two methods (r_s = 0.74 (2-tailed), P < 0.001 – Spearman's test; Figure 26).

Figure 26. Correlation plot for microembolus signal (MES) detection by the two techniques TCD and TOD

To assess further the level of agreement of MES detection between both techniques, a Bland and Altman analysis was performed. This revealed no significant bias [bias 0.11 microemboli (95% CI: -0.52 to 0.74), P = 0.81], with upper and lower limits of agreement of + 6 microemboli and – 6 microemboli. However, there was a systematic variation for the mean differences of the two measurements: at low MES rate (~ 2 – 4 MES/hour), both methods yielded false negative findings; at high MES rates (≥ 50 MES/hour), TOD has a higher sensitivity than TCD (Figure 27).

Figure 27. The Bland and Altman plot shows limits of agreement of -6 to +6 MES/30 mins with no significant bias [0.11 microemboli (95% CI: -0.52 to 0.74), P = 0.81] between the two diagnostic methods TCD and TOD
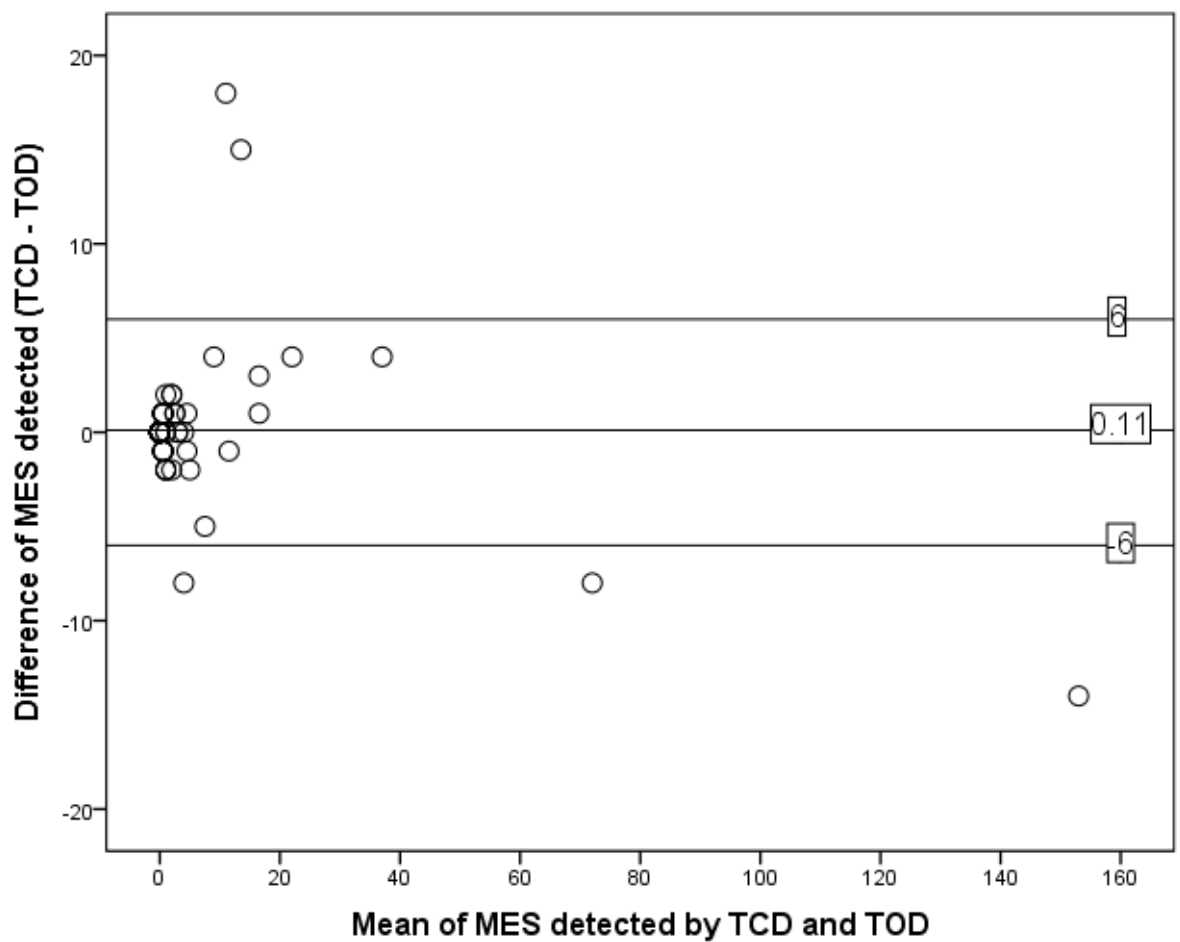
Table 43 shows the overall diagnostic accuracy parameters of TOD in comparison to TCD, reflecting the variation that may occur in clinical practice. TOD had better specificity and negative predictive value than sensitivity or positive predictive value for detecting cerebral microemboli.

Table 43. TOD diagnostic accuracy vs TCD

Sensitivity	80 %	95% CI: 61 % to 92 %
Specificity	86 %	95% CI: 76 % to 93 %
Positive Predictive Value	71 %	95% CI: 53 % to 85 %
Negative Predictive Value	91 %	95% CI: 82 % to 97 %

MES intervention

In this study, both methods simultaneously detected a sustained high MES rate (MES > 50hr^1) in 7 patients following carotid endarterectomy. Five out of 7 of those patients were treated with additional tirofiban[176] post-surgery to suppress the high microembolic rate. None of those patients developed post-operative thrombotic stroke.

6.2.4   Discussion

In this study, the same ultrasonography technique was used in different anatomical sites. The objective of this study was to compare TOD with an established TCD method with a view to demonstrating that it is not clinically inferior for detecting transient cerebral microemboli. This is the first prospective study comparing TOD with TCD for the detection of cerebral microemboli. On the basis of our results, TOD can be considered as an alternative to TCD in patients with an absent temporal acoustic window. Based on our study, TOD identified a high proportion, 8 out of 10 patients in whom microemboli were detected by the current reference standard, trans-temporal TCD, following carotid endarterectomy.

There are two clinical settings in which ability to detect cerebral microemboli is important for identifying patients at risk of thrombotic stroke, and in monitoring their suppression by rescue anti-platelet treatment. In acute symptomatic carotid disease, the presence of microemboli rather than the rate or number of microemboli[36] has been associated with recurrent stroke following TIA

or non-disabling stroke. In contrast, acutely following carotid endarterectomy, a sustained high MES rate has been associated with an increased risk of thrombotic stroke [33] and antiplatelet treatment can then be initiated to suppress these high rates of MES [272].

In this study, the sensitivity of detecting MES at low rates was similar between TOD and TCD methods. Thus either method appears clinically appropriate for detecting subsequent stroke risk in patients presenting with acute symptomatic carotid disease. However, TOD was more sensitive than TCD at high MES rates. Therefore, TOD appears an at least as good alternative method to TCD for identifying microemboli and directing antiplatelet treatment in these high risk carotid disease patients.

In this study, the source of microemboli was the denuded endothelial - intima area of the operated carotid artery following endarterectomy. One would expect any microemboli from the denuded carotid artery to travel past the carotid siphon before reaching the middle cerebral artery, the site insonated by the TCD method. As TOD insonates the carotid siphon which is closer to the endarterectomised area than the middle cerebral artery, TOD was expected to be more sensitive than TCD in detecting embolic signals. This may help to explain the increased microembolus detection frequency in our study for TOD vs. TCD at high microembolus rates.

There are several challenges to monitoring by TOD compared with TCD. Doppler images may be of lower quality with TOD for two major reasons. Unlike the TCD method, where the probe is held in place on the temporal bone using a head frame, for TOD the vascular technologist has to hold the ultrasound probe constantly throughout the monitoring in order to avoid exerting too much pressure on the eye. Despite having a probe on the orbital area for 30 minutes, none of our patients expressed concerns about the TOD examination nor did any express a preference for one imaging technique over the other.

Secondly, Doppler images are obtained using a small sample volume in the centre of an area of blood flow. Since the carotid siphon has a larger diameter than the middle cerebral artery, there is a risk of missing MES with the TOD method. It is unlikely that the lower sensitivity of TOD at low MES rates results from lack of expertise, or that emboli detected in TOD alone are likely to be

artefactual, as the vascular technologists in this study had at least 5 years of experience of TOD[274] and our observers have 90% agreement with software validated against a panel of international experts[244].

Thermal Indices (TI) of <0.1 through the orbit were maintained during the TOD insonation. A theoretical paper[265] has suggested that the real temperature increase in the orbit may be more than estimated by the TI, mainly due to poorer cooling than assumed during the calculation of the TI. Even so, at a TI of 0.1 transorbitally the real temperature increase would be unlikely to exceed 1^oC, at which level no significant adverse effects of ultrasound have been demonstrated.

Limitation

Further studies are required in additional cohorts of patients to validate our findings and to assess the diagnostic benefit from combining TCD with TOD following carotid surgery to identify patients who could benefit from additional antiplatelet treatment to suppress cerebral microemboli. In this study, a single-gated method was used for microembolus detection. Dual gated microembolus detection may have increased potential compared to single-gated methods to differentiate microemboli from artefacts. Future comparative studies between TCD and TOD should be performed to investigate the effect on sensitivity of microemboli detection if a dual gated method is used. However, the technical challenge will be to decide two different depths of TOD insonation of the carotid siphon. Previous study has shown that the sensitivity of microemboli detection with dual gated methods may be reduced due to the fact that microemboli may not be appear in both windows[275].

In conclusion, our study indicates that TOD appears a valid alternative to TCD for detecting transient cerebral microemboli. Further studies should address whether there is a difference in clinical end-points between microemboli detected by these two methods.

CHAPTER SEVEN

7   Kinetics of rescue antiplatelet treatment to abolish cerebral microemboli after carotid endarterectomy

7.1   Introduction

Antiplatelet treatment is well established as effective in reducing clinical vascular disease[210]. In patients with symptomatic carotid stenosis, developing a stroke is believed to result primarily from platelet-derived microemboli [32, 38]. Both a recent prospective, international study (ACES)[32] and a systematic meta-analysis[35] have established transcranial Doppler-detected cerebral microemboli as a biomarker of high risk of stroke syndromes both in patients with symptomatic carotid stenosis and after carotid endarterectomy (CEA).

Transcranial Doppler (TCD) is a simple non-invasive technique that both allows identification of high risk patients of stroke and evaluation of the efficacy of antiplatelet therapy as it provides real time monitoring of cerebral microemboli[35].

Randomised controlled trials have shown efficacy of antiplatelet agents in reducing the frequency of TCD-detected microemboli [37, 213, 215-217, 276]. Treatments assessed include dextran-40 [218], S-nitroso-glutathione [221], tirofiban[214], von Willebrand inhibitor [168], high dose aspirin[175] and a combination of aspirin and clopidogrel [37, 217]. There is no consensus on which antiplatelet therapy or combination is most effective in abolishing TCD-detected microemboli, with the aim of preventing postoperative thrombotic stroke.

Disease processes within the coronary artery and the internal carotid artery share much in common[60]. Antiplatelet treatment with the intravenous glycoprotein IIb/IIIa inhibitor tirofiban is effective in acute coronary syndromes [248]. Pharmacological agents effective in acute coronary syndromes could theoretically be beneficial in thromboembolic carotid diseases associated with transient ischaemic attacks (TIA) and strokes[60].

The aim of this study was to compare our experience of using the glycoprotein IIb/IIIa inhibitor tirofiban with response to dextran-40 in patients with high microembolic rates after carotid endarterectomy.

7.2   Subjects and methods

From the Carotid Registry, 128 out of 576 patients who had microembolic signals (MES) detected by TCD after elective CEA between August 2000 and December 2010 were identified. The Vascular Unit at The University Hospitals of Coventry and Warwickshire NHS Trust vascular performs approximately 60 CEA per annum.

Dextran-40 was our preferred choice[33, 222] as the rescue anti-thrombotic agents in patients with high microembolic rates after carotid endarterectomy until 2003. We noticed that a number of patients developed early stroke despite being on a dextran-40 infusion. We have since changed our department policy to tirofiban [214, 277]. Using the Registry data, a case controlled-study was used to explore the benefits of tirofiban over dextran-40 treatment. Patients who had tirofiban were compared with those who received dextran-40 and those who had MES rate < 50/hr which spontaneously resolved. The cardiovascular burdens between groups were similar.

Seventy four patients who had an MES rate ≥ 50/hr and were either given intravenous tirofiban (n=40) or intravenous dextran-40 (n=34) were reported. I also reported on 54 patients who had MES rate < 50/hr which spontaneously resolved on standard antiplatelet treatment.

All patients had oral antiplatelet treatment (aspirin, or aspirin and clopidogrel, or aspirin and dipyridamole) prior to carotid endarterectomy.

In the tirofiban group, 32 patients received single antiplatelet agent prior to the carotid surgery (aspirin, clopidogrel and dipyridamole) and 8 patients received dual antiplatelet agents. Two patients from the dextran-40 group and 18 from the spontaneous MES resolved group received dual antiplatelet agents.

Cardiovascular risk factors

Prevalence of hypertension, smoking (self-reported), ischaemic heart disease, hypercholesterolaemia, diabetes mellitus and previous medical history or family history of vascular diseases was recorded. Peripheral vascular disease was based on symptoms of claudication with evidence of reduced ankle-brachial pressure index.

Carotid endarterectomy

Carotid endarterectomy was performed as described in chapter 3. Shunting was performed if the patient developed focal neurological signs when CEA was performed under local anaesthetic; or in those cases performed under general anaesthetic when mean velocity of the middle cerebral artery dropped by > 50%. All patients received intravenous heparin (40 units kg^{-1}) prior to the cross clamp phase of the carotid endarterectomy.

TCD monitoring

TCD monitoring was used to assess half-life of MES decay, MES to resolution and cumulative number of microemboli detected.

TCD monitoring [274] was performed by an experienced vascular scientist (PC Dop 842, SciMed, Bristol, UK) with a 2 MHz probe focused on the middle cerebral artery ipsilateral to the operated carotid artery following recovery from anaesthesia. Monitoring was performed for at least 30 minutes if no MES was detected. In patients lacking an acoustic temporal bone window, TCD monitoring was performed via the transorbital window which focuses on the carotid siphon[274] . Microemboli were identified as recommended by the International Consensus Group on Microembolus Detection [171]. Doppler wave forms and audio signals were examined by a trained vascular scientist. MES were identified as unidirectional, short duration signals (range 10–100 ms) with intensity threshold above 6 dB, accompanied by characteristic audible clicks and occurring randomly throughout the cardiac cycle [170, 171] The rate of MES was calculated based on the number of microemboli detected within 15 minute intervals. Additional post-operative rescue

anti-thrombotic agents was initiated in patients with a MES rate > 50/hr based on the study by Levi et al [31]

Tirofiban protocol

The tirofiban protocol recommended for use in acute coronary syndrome[248] was used. Tirofiban (Aggrastat®; Merck Sharp & Dohme, Hoddeson, UK) was given intravenously as 0.4mcg/kg/min for 30 minutes then 0.1mcg/kg/min as a continuous infusion for 18 hours. TCD monitoring was continued until the MES rate was < 4/hr.

Dextran-40 protocol

Patients were commenced on an intravenous infusion of dextran-40 (10% Gentran-40 (Baxter Healthcare, Thetford, UK), in 5% glucose solution). An initial 20-ml bolus was given, and the dextran-40 infusion continued at 20 ml/h.

Based on Hayes's study[220], if a patient continued to have TCD evidence of a persistent high rate of MES, the rate of the dextran-40 infusion was increased at increments of 20 ml/h until the MES reduced. The dextran-40 infusion was continued for 18 hours.

Ethics

University Hospitals Coventry Warwickshire (UHCW) Research Ethics Department advised that this registry report did not fall under the remit of the National Health Service Research Ethics Committee.

Data and statistical analysis

Statistical analyses were conducted using SPSS® version 21 (Chicago, Illinois, USA).

Demographic variables are expressed as mean and standard error means (SEM). Data on MES rate are expressed as median and quartile range (IQR). Non-parametric unpaired data were analysed using the Kruskal-Wallace analysis of variance for multiple groups or Mann-Whitney U test for two

groups comparison. Categorical variables were analysed using the chi-squared test or Fisher's

exact test. Parametric data were assessed with one-way ANOVA. Survival data were evaluated by

Kaplan-Meier curves. A P-value of less than 0.05 was considered significant.

7.3   Results

The age, gender and ethnicity profiles of the 3 groups were comparable (Table 44). The

preoperative laboratory blood profiles were also similar between groups (Table 45).Eight (20%)

patients who went on to receive tirofiban were on dual antiplatelet agents preoperatively

compared with 2 (6%) patients who received dextran-40 after surgery, and 18 (33%) patients in

whom MES resolved (P = 0.199, X^2 test).

Table 44 Demographic and clinical profile of patients who had MES monitored after carotid endarterectomy

n (%)	Spontaneous resolution n = 54	Tirofiban n = 40	Dextran-40 n = 34	X^2 test P value
Age (mean ± SEM)	71 ± 1	74 ± 1	69 ± 2	0.199
Male	36 (67)	27 (66)	22 (65)	0.199
Caucasian	52 (96)	38 (95)	30 (88)	0.199
Asian	2 (4)	2 (5)	4 (12)	0.223
Hypertension	47 (87)	31 (78)	27 (79)	0.442
Never smoke	15 (28)	19 (47)	12 (38)	
Current smoker	12 (22)	13 (33)	9 (24)	0.143
Ex-smoker	27 (50)	8 (20)	13 (38)	
Ischaemic heart disease	24 (44)	14 (35)	14 (41)	0.611
Type 2 Diabetes mellitus	16 (30)	10 (25)	6 (18)	0.45
Hypercholesterolaemia	30 (56)	26 (65)	15 (44)	0.197
Peripheral vascular disease	8 (15)	8 (20)	10 (29)	0.253
Cerebrovascular disease	13 (24)	12 (30)	5 (15)	0.299
Family History	25 (46)	13 (33)	4 (12)	**0.04**

Table 45 Preoperative laboratory values of patients who had MES monitored after carotid endarterectomy

Laboratory variables	Spontaneous resolution Mean ± SEM (n)	Tirofiban Mean ± SEM (n)	Dextran-40 Mean ± SEM (n)	One-way ANOVA, P value
Cholesterol (mmol/L)	4.6 ± 0.1 (n=43)	4.6 ± 0.2 (n=32)	4.6 ± 0.5 (n=12)	0.97
Triglyceride (mmol/L)	1.6 ± 0.1 (n=27)	1.7 ± 0.2 (n=12)	1.8 ± 0.3 (n=12)	0.85
HDL (mmol/L)	1.6 ± 0.1 (n=20)	1.4 ± 0.1 (n=26)	1.2 ± 0.2 (n=5)	0.21
Creatinine (mmol/L)	106 ± 4 (n=53)	114 ± 10 (n=40)	96 ± 3 (n=40)	0.17
Glucose (mmol/L)	5.3 ± 0.2 (n=41)	5.3 ± 0.3 (n=30)	5.9 ± 0.5 (n=9)	0.571
Haemoglobin (g/dL)	13.5 ± 0.2 (n=53)	13.4 ± 0.2 (n=40)	12.8 ± 0.3 (n=34)	0.1
Platelet (x 10^9/L)	256 ± 8 (n=53)	282 ± 12 (n=40)	257 ± 16 (n=34)	0.21
White cell count (x 10^9/L)	7.8 ± 0.2 (n=53)	7.9 ± 0.3 (n=40)	7.8 ± 0.4 (n=34)	0.97

Microembolic signal kinetics after carotid endarterectomy

In view of differences in preoperative antiplatelet treatment between groups, data were analysed in 2 ways: An analysis of all subjects and separate analysis for those who were only on single antiplatelet treatment before surgery. There was no early postoperative stroke in the tirofiban treated group but 5 early strokes despite dextran-40 treatment. I also included an additional analysis excluding patients who had an early stroke despite dextran-40 treatment.

a) Comparison of the MES rate between tirofiban and dextran-40 treated groups

The initial MES rate was similar in dextran treated and tirofiban treated patients (P = 0.234) (Table 46).

Table 46 MES decay rate after carotid endarterectomy in patients who received rescue antiplatelet therapy (tirofiban or dextran-40 for MES ≥ 50/hr; for MES < 50/hr). After Mann – Whitney U test: ** Spontaneous resolution vs tirofiban, P < 0.001. * Spontaneous resolution vs tirofiban, P = 0.007. † Spontaneous resolution vs dextran-40 P < 0.001. ^ tirofiban vs dextran-40 P < 0.001

	Spontaneous resolution Median (IQR)	Tirofiban Median (IQR)	Dextran-40 Median (IQR)	Kruskal-Wallis test P value
Initial MES rate (MES/hr)	24 (16 – 36)**, †	88 (68 – 134)	102 (79 – 150)	**< 0.001**
Half-life of MES decay (minutes)	30 (22 – 38))**, †	23 (15 - 28)^	56 (43 – 83)	**< 0.001**
Time to MES resolution (minutes)	53 (49 - 68))*, †	68 (53 - 94)^	113 (79 – 146)	**< 0.001**

Table 47 Comparison of MES decay rate between tirofiban and dextran-40 in patients who received different antiplatelet therapy prior to carotid surgery

	Single antiplatelet therapy			Dual antiplatelet therapy		
	Tirofiban (n = 32) Median (IQR)	Dextran-40 (n=32) Median (IQR)	Mann – Whitney U test P value	Tirofiban (n = 8) Median (IQR)	Dextran-40 (n=2) Median (1st QR)	MW-U test P value
Initial MES rate (MES/hr)	96 (69 – 136)	104 (81 – 150)	0.268	81 (65 – 123)	77 (76)	0.6
Half-life of MES decay (minutes)	23 (15 – 30)	60 (40 – 83)	< 0.001	19 (15 – 23)	49 (45)	0.29
Time to MES resolution (minutes)	68 (53 – 98)	113 (71 – 154)	< 0.001	53 (53 – 79)	105 (83)	0.48

Figure 28 demonstrates the rapid initial reduction of MES rate in patients who received the tirofiban infusion in comparison to patients with no rescue antiplatelet treatment. This is despite almost 4 times higher initial MES rate after surgery (Tirofiban 88 minutes (IQR 68 – 134), spontaneous resolution 24 (16 – 36), p <0.001) (Table 46).

The time for 50% reduction in MES rate was shorter in patients who received tirofiban compared to dextran-40 (Tirofiban 23 (15 – 28), dextran-40 56 (43 – 83), P < 0.001;Table 46). Patients who received tirofiban had a significantly lower cumulative MES burden than dextran-40 treated patients (44 (28 – 65) vs. 91 (48 – 304), P < 0.001, MW test).

b) Results for subjects who had single antiplatelet treatment before surgery

Results for analysis of data on patients who received only single antiplatelet therapy prior to carotid endarterectomy were similar to those for the overall analysis; For tirofiban vs. dextran-40 treatment, the half-life for MES was shorter (23 minutes (15 – 28) vs. 60 (43 – 83), P < 0.001); the time to resolution was shorter (68 (53 – 98) vs. 113 (71 - 154), P < 0.001) (Table 47). Cumulative MES in tirofiban treated patients were lower at less than half that in dextran-40 treated patients: tirofiban 45 (32 – 75) vs. dextran-40 103 (51 – 308), P = 0.001, MW test (Figure 31, Figure 32).

Figure 28 Results of MES rate after carotid endarterectomy in all patients. ● dextran-40 infusion (n = 34) for MES ≥ 50/hr. ■ Tirofiban infusion (n = 40) for MES ≥ 50/hr. ▲ No additional antiplatelet treatment (n = 54) - MES < 50/hr. Initial MES rate for all groups: P < 0.001, KW test. Initial MES for tirofiban vs. dextran-40: P = 0.243, MW test. For half-life of MES decay rate: P < 0.001, KW test. Half-life of MES decay rate for tirofiban vs. dextran-40: P < 0.001, MW test. Time to MES resolution: P < 0.001, KW test. Time to resolution for tirofiban vs. dextran-40: P < 0.001, MW test
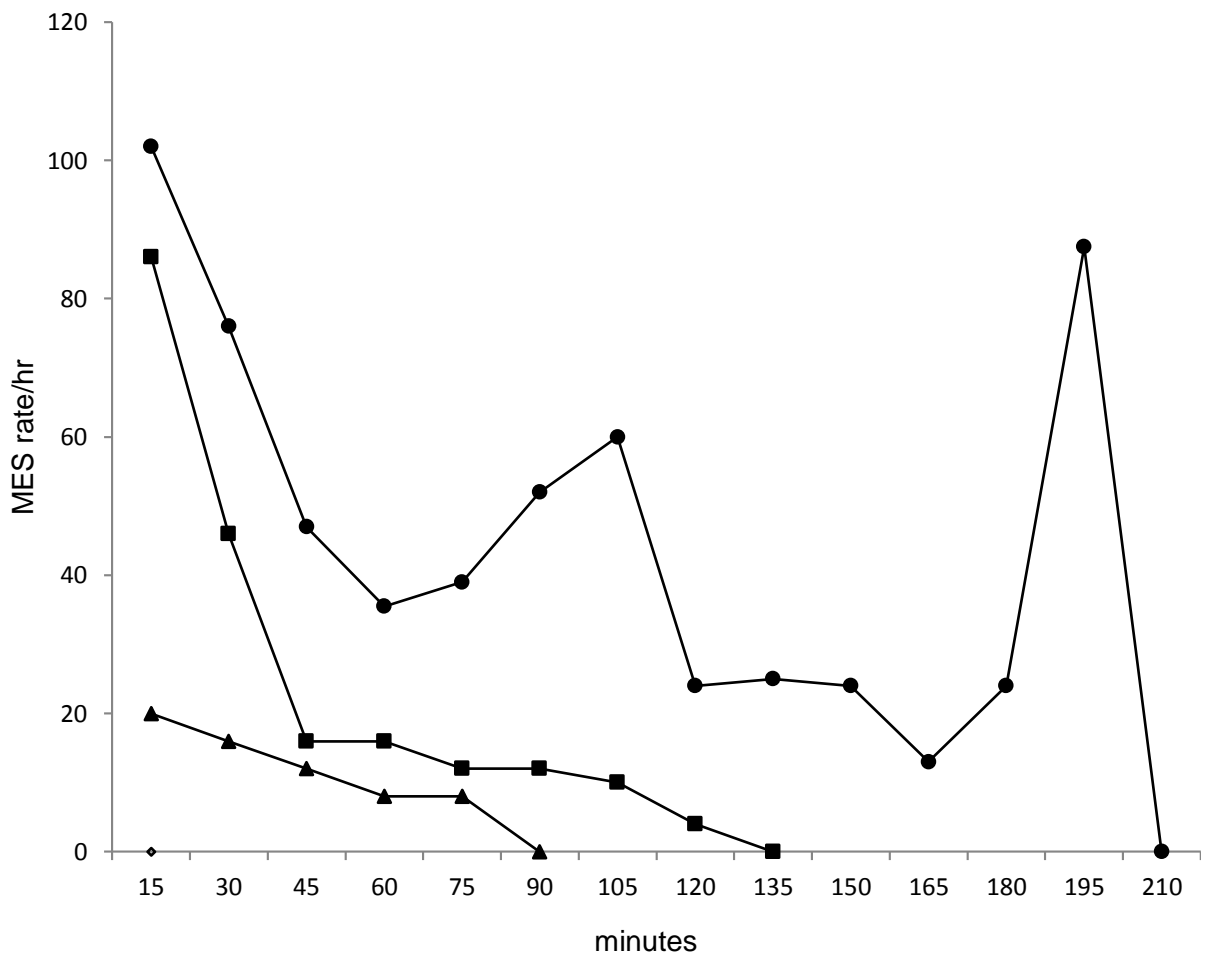
Figure 29 MES rate monitored following carotid endarterectomy. ● Dextran-40 infusion (n = 29) for MES ≥ 50/hr. ○ MES ≥ 50/hr patients (n = 5) who developed early stroke despite dextran-40 infusion
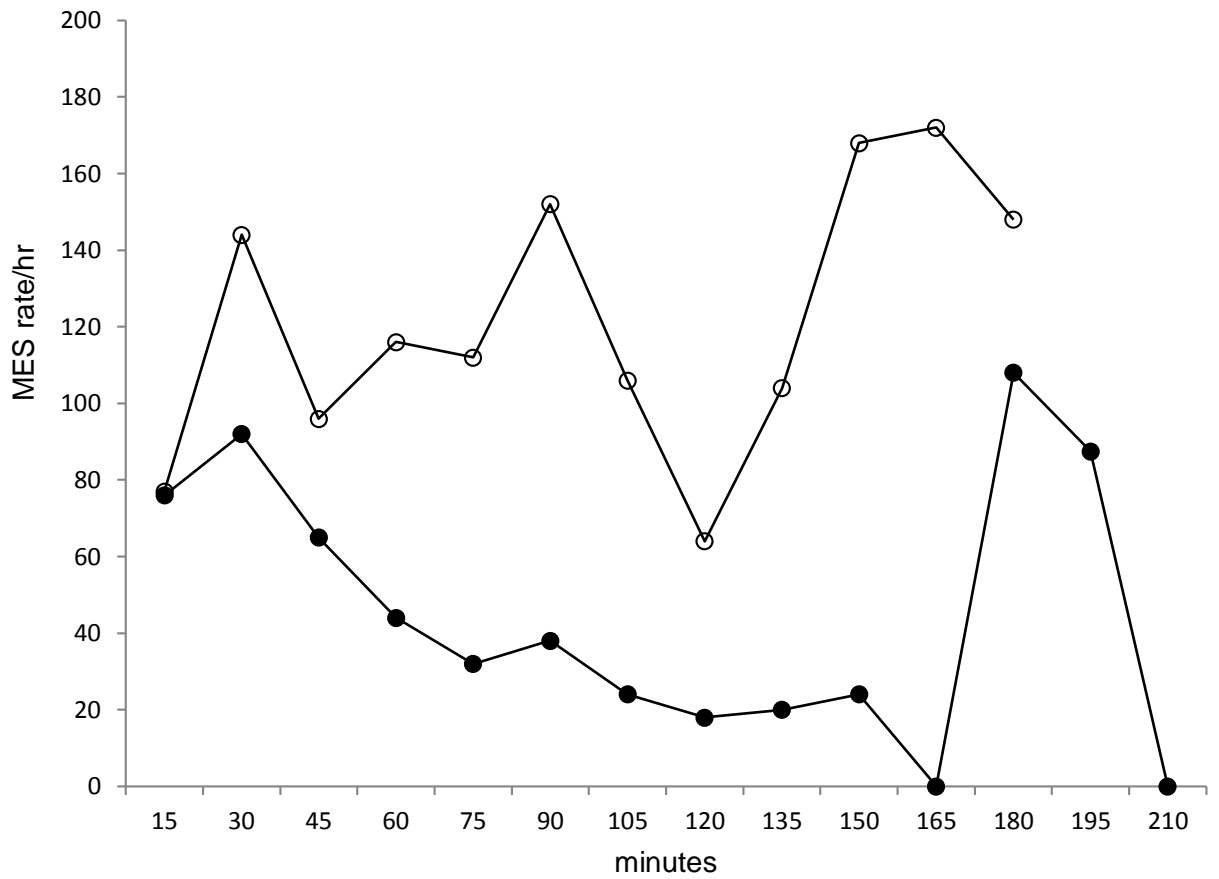
Figure 30 Results of MES rate after carotid endarterectomy in single antiplatelet therapy patients prior to surgery. ● dextran-40 infusion (n = 28) for MES ≥ 50/hr. ○ MES ≥ 50/hr patients (n = 4) who developed early stroke despite dextran-40 infusion. ■ Tirofiban infusion (n = 32) for MES ≥ 50/hr. ▲ No additional antiplatelet treatment (n = 36) - MES < 50/hr. Initial MES for tirofiban vs. dextran-40: P = 0.268, MW test. Half-life of MES decay rate for tirofiban vs. dextran-40: P < 0.001, MW test. Time to resolution for tirofiban vs. dextran-40: P < 0.001, MW test
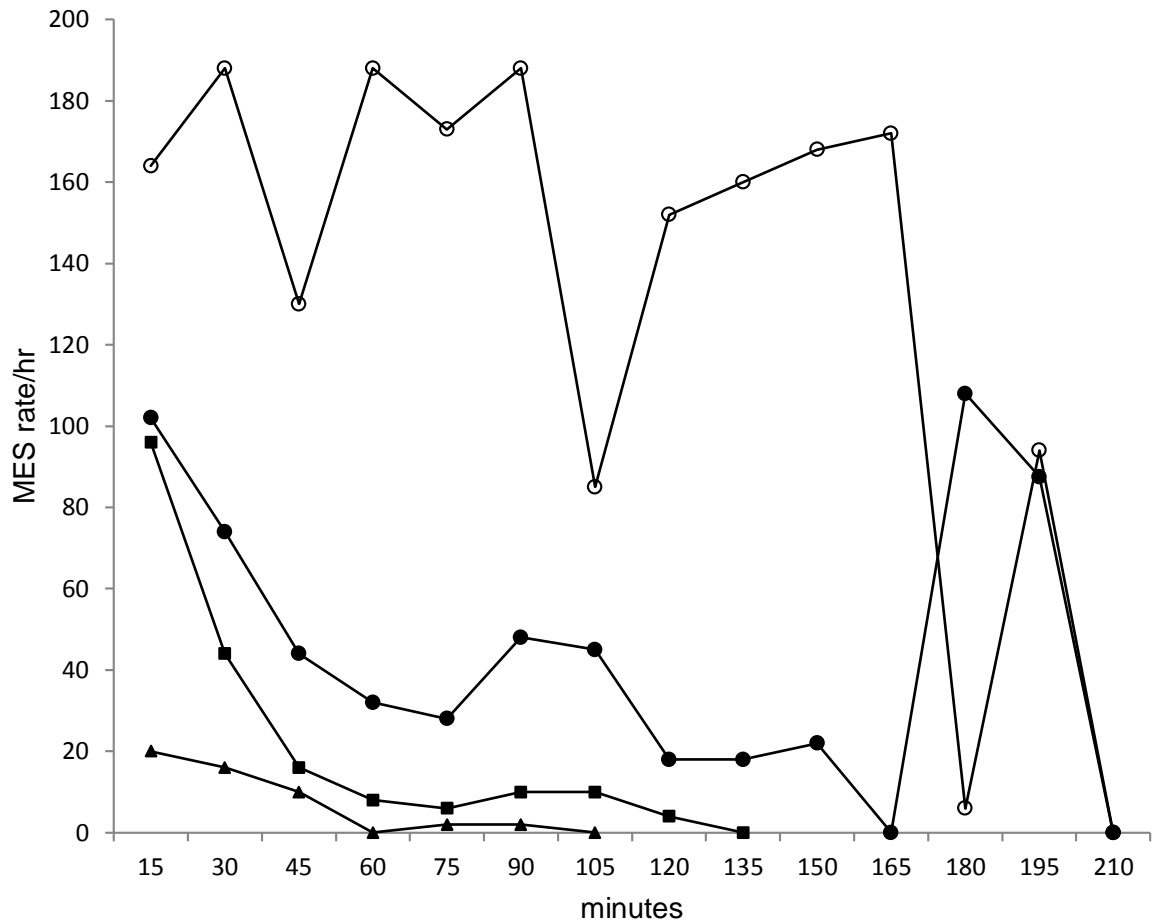
Figure 31 Cumulative MES following carotid endarterectomy in patients with single antiplatelet therapy prior to surgery. ● dextran-40 infusion (n = 28) for MES ≥ 50/hr. ○ MES ≥ 50/hr patients (n = 4) who developed early stroke despite dextran-40 infusion. ■ Tirofiban infusion (n = 32) for MES ≥ 50/hr. ▲ No additional antiplatelet treatment (n=36) - MES < 50/hr. Cumulative MES for all groups; spontaneous resolution 12 (7 – 20), tirofiban 45 (32 – 20), dextran 40 103 (51 – 308): P < 0.001, KW test. For tirofiban vs. dextran-40: P < 0.001, MW test
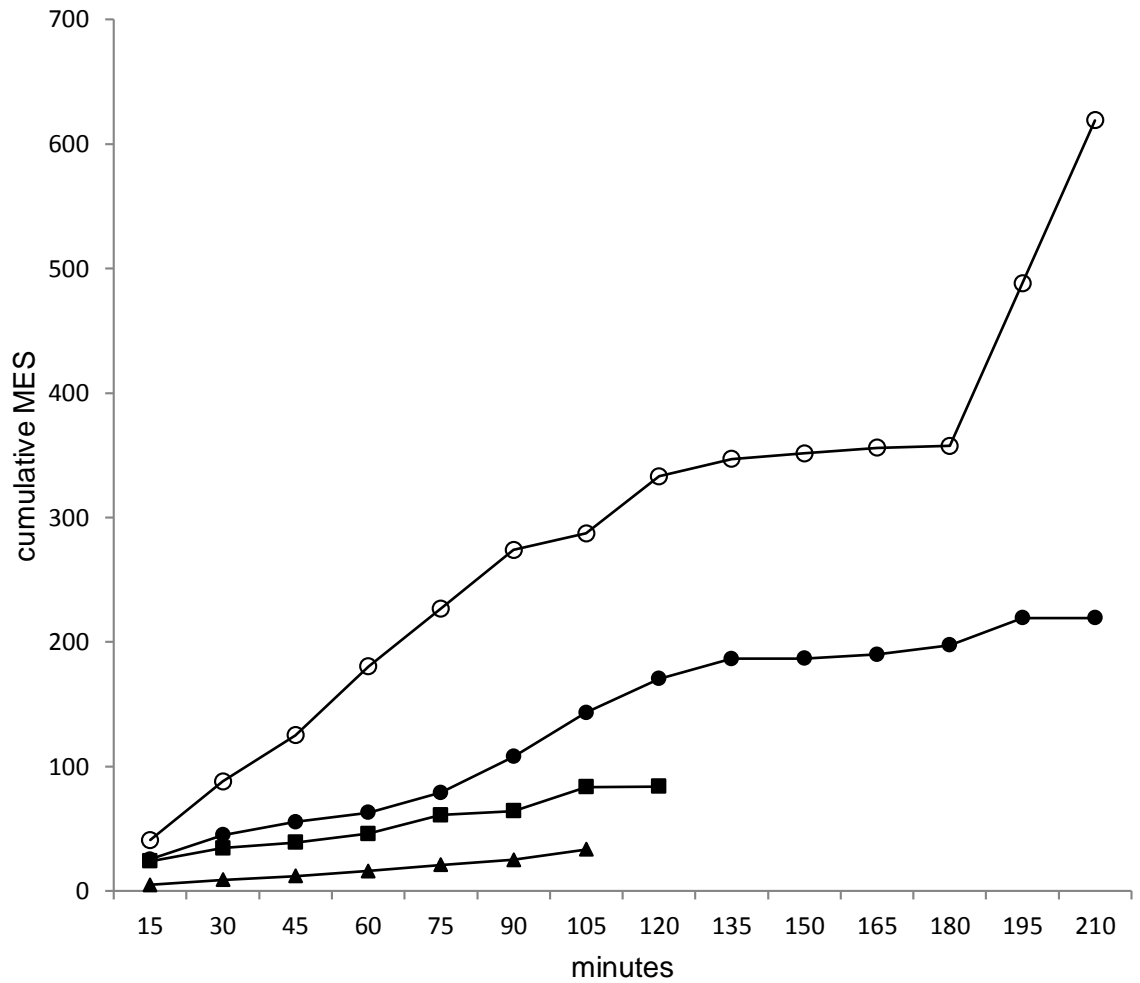
Figure 32 Cumulative MES following carotid endarterectomy in patients with dual antiplatelet therapy prior to surgery. ● dextran-40 infusion (n = 1) for MES ≥ 50/hr. ◯ MES ≥ 50/hr patients (n = 1) who developed early stroke despite dextran-40 infusion. ■ Tirofiban infusion (n = 8) for MES ≥ 50/hr. ▲ No additional antiplatelet treatment (n = 18) - MES < 50/hr. For Spontaneous resolution 11 (7 – 19) vs tirofiban 35 (25 – 54), P = 0.001, MW test
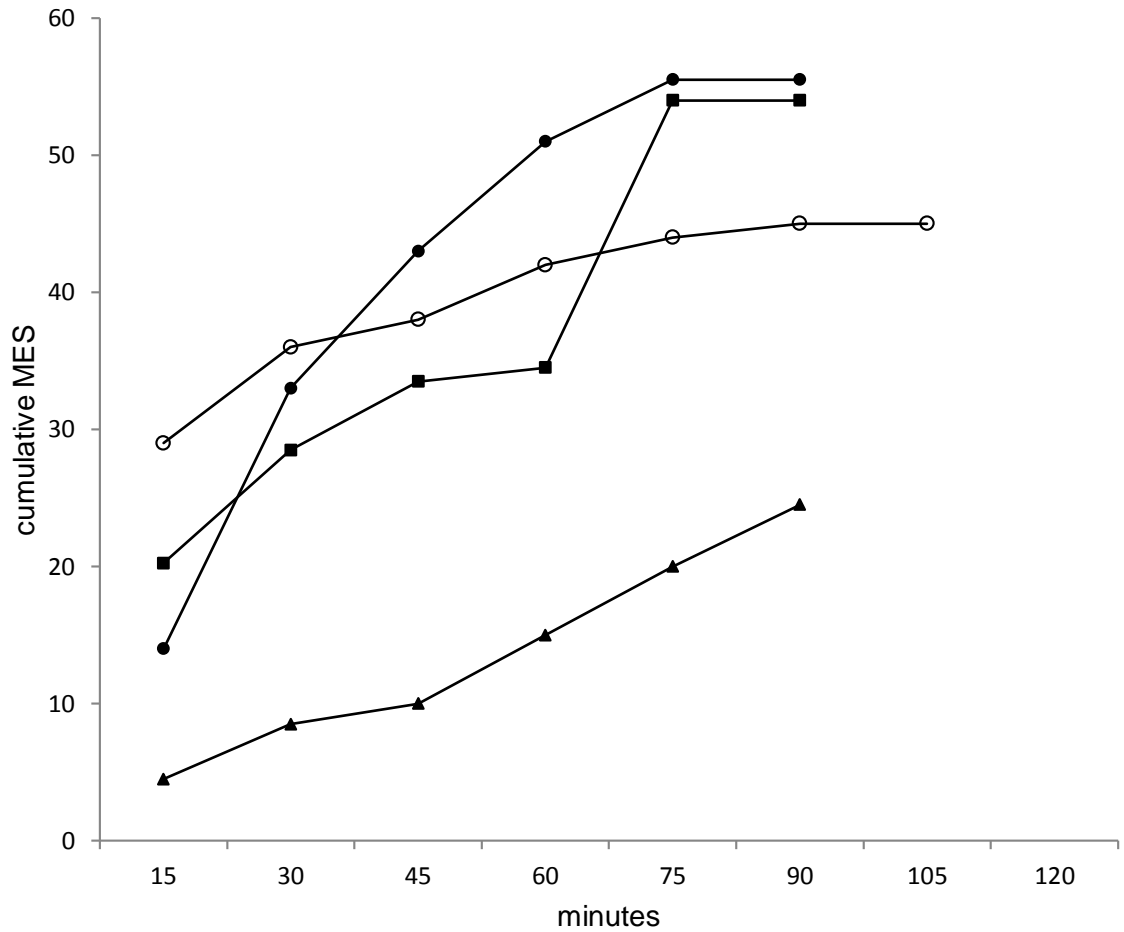
Time to resolution

The Kaplan – Meier plot shows that patients who received rescue tirofiban infusion achieved earlier complete MES resolution in comparison to dextran-40 treatment (Figure 33). This finding was similar when patients who were only on single preoperative antiplatelet therapy was analysed (Figure 34).

Figure 33 Kaplan – Meier plot comparing frequency of continued occurrence of MES acutely after carotid endarterectomy. Dotted & Dashed line: Patients (n = 5) who developed early stroke despite dextran-40 infusion. Dotted line: Dextran-40 infusion (n=34) for MES ≥ 50/hr. Bold line: Tirofiban infusion (n = 40) for MES ≥ 50/hr. Dashed line: No additional antiplatelet treatment (n = 54) as MES < 50/hr resolved spontaneously. Log Rank (Mantel-Cox), 4-way P < 0.001. Tirofiban vs dextran-40, Log Rank (Mantel-Cox), 2-way P < 0.001. Tirofiban vs early stroke dextran-40, Log Rank (Mantel-Cox), 2-way P = 0.001
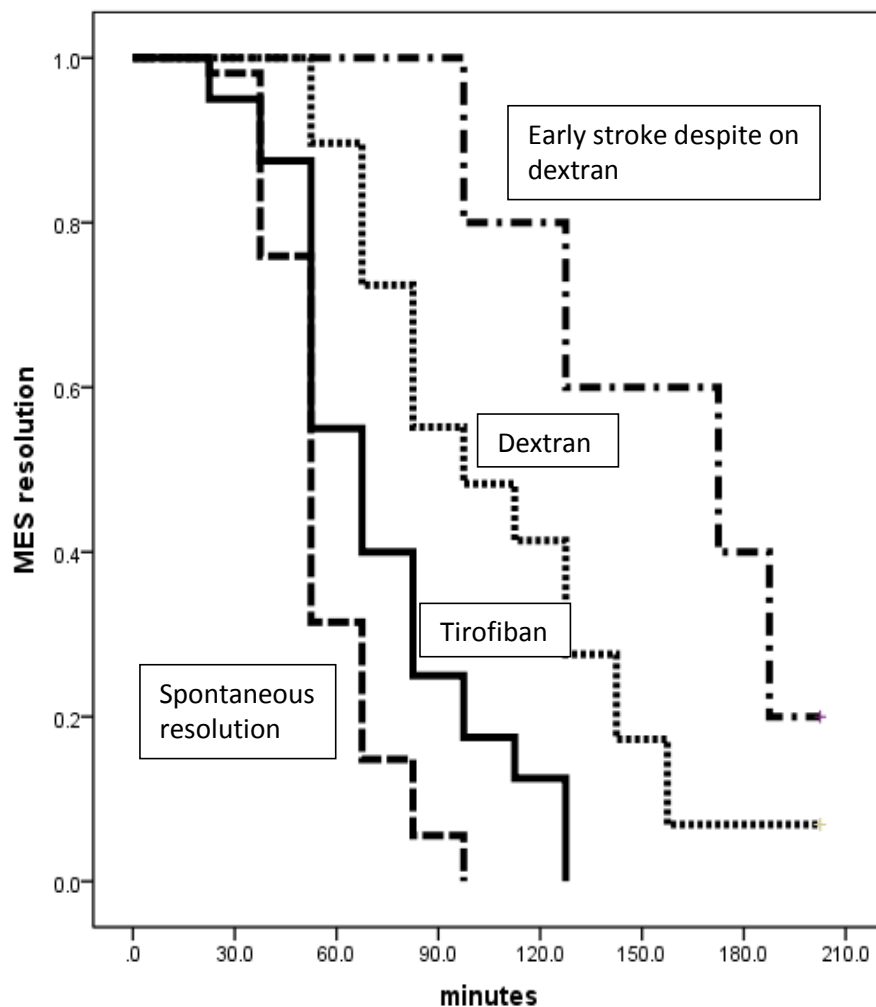
Figure 34 Kaplan – Meier plot comparing frequency of continued occurrence of MES acutely after carotid endarterectomy in patients with single antiplatelet therapy prior to surgery. Dotted & Dashed line: Patients (n = 4) who developed early stroke despite on dextran-40 infusion. Dotted line: Dextran-40 infusion (n = 28) for MES ≥ 50/hr. Bold line: Tirofiban infusion (n = 32) for MES ≥ 50/hr. Dashed line: No additional antiplatelet treatment (n = 36) as MES < 50/hr resolved spontaneously. Log Rank (Mantel-Cox), 4-way P < 0.001. Tirofiban vs dextran-40, Log Rank (Mantel-Cox), 2-way P = 0.001. Tirofiban vs early stroke dextran-40, Log Rank (Mantel-Cox), 2-way P = 0.002
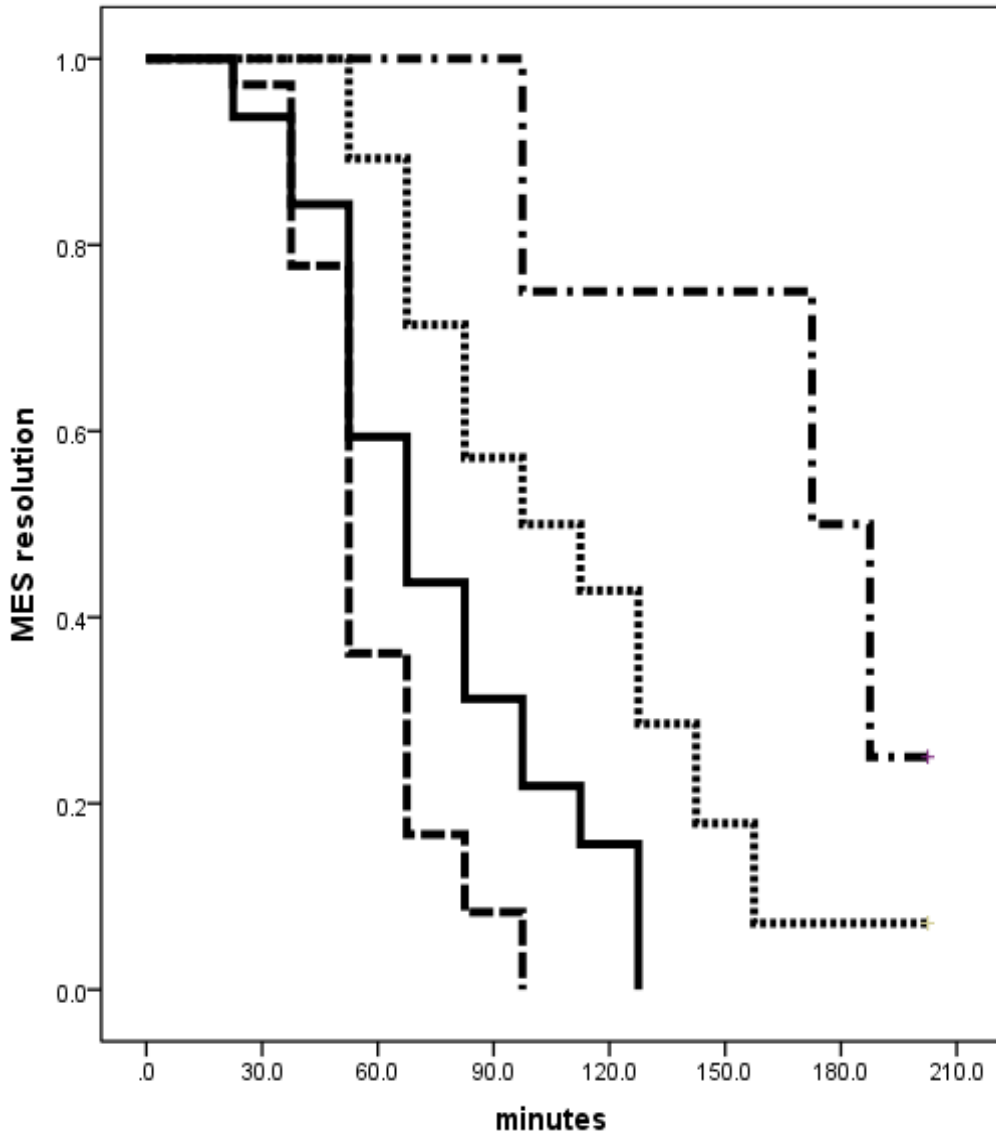
Complications

a) Early events – 24 hours post-surgery

5 patients developed stroke despite being on dextran-40 infusion for MES ≥ 50/hr following surgery. In the 5 patients, the MES rate was persistently higher than in patients (n=29) whom the MES rate resolved following dextran-40 infusion (Table 48).

b) Further events between 24 hours to 30 days post-surgery

One ischaemic stroke occurred in a tirofiban treated patients one week after the surgery. 2 fatal myocardial infarctions (MI) occurred in the dextran-40 treated patients and 1 fatal MI in both tirofiban treated patients and patients who did not receive rescue antiplatelet treatment. One patient who received postoperative tirofiban was readmitted from home and died from an intracerebral haemorrhage 4 days after surgery and 2 days post discharge. In the immediate postoperative phase, this patient had received an intravenous nitrate infusion for hypertension and a hyperperfusion syndrome but systolic blood pressure appeared to be controlled on discharge. The platelet count on discharge was normal.

c) Late events between 30 days and 1 year after surgery

One fatal myocardial infarction and one fatal intracerebral haemorrhage occurred in patients who did not receive rescue antiplatelet treatment (Table 48).

Table 48 Complications up to 30 days and between 30 days and 1 year after carotid endarterectomy in patients who received rescue antiplatelet therapy (tirofiban or dextran 40 for MES ≥ 50/hr) and no additional antiplatelet therapy (MES < 50/hr)

	30 days post carotid surgery			12 months post carotid surgery		
	Spontaneous resolution n = 54 (%)	Tirofiban n = 40 (%)	Dextran-40 n = 34 (%)	Spontaneous resolution n = 54 (%)	Tirofiban n = 40 (%)	Dextran-40 n = 34 (%)
Stroke (Ischaemic or haemorrhagic)		1(3)	5 (15)			
Myocardial Infarction	1(2)	1(3)	2 (6)	1(2)		
Death, cause: e.g. bleeding, thrombocytopenia etc.	1(2) MI	2(6) MI Intracerebral bleeding	3 (9) MIs, one case unknown	2(4) MI Intracerebral bleeding	1(3) Renal failure	

7.4 Discussion

Antiplatelet therapy prior to carotid endarterectomy is now standard practice to prevent post – surgical microemboli and early ischaemic stroke. However, despite this practice, patients may still have a high microembolic load acutely after carotid endarterectomy. This is of clinical concern because MES are known to lead to increased risk of postoperative thrombotic stroke[31, 33]. TCD-directed tirofiban therapy appears to be significantly more effective than dextran-40 in suppression of cerebral microembolisation in patients who were refractory to basal antiplatelet treatment. Despite having a similar initial MES rate to dextran-40 treated patients, tirofiban treatment resulted in a large ~ 60% reduction of the half-life of MES decay. Furthermore, earlier complete MES resolution was demonstrated in all patients treated with tirofiban than dextran-40. Similar effects were demonstrated in patients with single antiplatelet therapy prior to surgery.

None of the tirofiban patients had TIA or acute stroke while on tirofiban infusion. Some of the patients appeared to be resistant to dextran-40, both in terms of failure to suppress high MES rates and in that 5 out of 34 patients had adverse neurological outcomes.

Dextran-40 was one of the first and most common antithrombotic agent used to treat acute microemboli following surgery[167, 222, 278] to prevent stroke. Naylor et al showed that this strategy has worked well in abolishing postoperative stroke in Leicester, UK from 1995 to 2006[279]. It has been suggested that dextran-40 exerts a combined therapeutic effect, enhancing endogenous fibrinolysis, whilst also reducing platelet adhesion to von Willebrand factor and platelet activation by thrombin[226]. A study from Boston, US[227] found that dextran-40 used was not associated with lower peri-operative stroke but was associated with higher rates of myocardial infarction and congestive heart failure. In the study from Boston[227], the intraoperative dextran infusion was given based on the preference of the individual surgeon rather than based on the TCD monitoring. However, the documented dextran related complications were similar to our experience[214].

An experimental study by Junghans et al[277] has shown for the first time the effectiveness of tirofiban in reducing the frequency of cerebral microembolisation. The previous case-series from this unit anecdotally suggested that tirofiban could be used as an alternative to those patients who were resistant to dextran-40[214]. However, until this present report, it had not been clear whether tirofiban would have benefits over dextran-40 treatment. Using the kinetics of the microemboli, I was able to demonstrate the effectiveness of tirofiban in abolishing the microemboli and compared tirofiban and dextran-40 effectiveness in reducing the frequency of microemboli in more detail. A number of anti-thrombotic agents have been used to treat carotid surgery patients with microemboli, who are otherwise at a high risk of suffering stroke. These treatments include using dextran-40 [218], S-nitroso-glutathione [221], von Willebrand inhibitor [168] and a combination of aspirin and clopidogrel [37, 217]. Two randomised controlled trials which investigated TIA/minor stroke and post CEA TCD-detected MES cohorts have shown that a combination of aspirin and clopidogrel reduced the frequency of TCD-detected MES, but did not completely abolish the microembolisation[37, 217]. However, my study has shown that the use of selective platelet GPIIb/IIIa receptor inhibitor tirofiban results in complete suppression of MES in all patients including 8 of our patients who were on dual antiplatelet agents prior to CEA. In these cases, the medications were either not sufficient or not effective. The short acting selective

GPIIb/IIIa receptor antagonist provides an effective treatment option in patients resistance to aspirin and clopidogrel [280].

The commonly used antiplatelet agents such as aspirin and clopidogrel do not fully inhibit platelet aggregation activated through pathways different from thromboxane and adenosine phosphate[277]. Irrespective of the particular platelet activating pathway, final platelet aggregation is mediated by the GPIIb/IIIa receptor[277]. These fibrinogen receptors are exclusively expressed on platelets. Binding of GPIIb/IIIa receptor antagonist such as tirofiban results in highly selective inhibition of platelet aggregates independent from the respective platelet activating mechanism. Activated GPIIb/IIIa receptors bind fibrinogen molecules, which form bridges between adjacent platelets forming the platelet aggregates[248]. Tirofiban has a short half-life (2 hours) and platelet function fully recovers within hours after cessation of infusion [248]. Due to this unique pharmacodynamics, tirofiban could be used as the bridge treatment in acute symptomatic carotid artery disease who required urgent carotid endarterectomy.

Within the study, the high MES rate despite dextran-40 treatment was associated with an increased risk of stroke than with tirofiban. Bleeding complications were the most commonly reported events associated with tirofiban in previous clinical trials[248]. In the study, including rescue antiplatelet treatment, most of patients were on multiple antiplatelet agents perioperatively, yet the incidence of major bleeding was surprisingly low. The tirofiban bleeding complication rate was comparable to a recently published larger randomised controlled trial looking at the safety on tirofiban in the different setting of acute stroke[281]. The single episode of major bleeding was a cerebral haemorrhage occurring after discharge from hospital, 3 days after the end of the tirofiban infusion. Since the half-life of tirofiban is two hours, I believe this adverse outcome was unlikely to be directly related to the tirofiban therapy.

TCD-directed tirofiban therapy appears more effective than dextran-40 in rapid suppression of cerebral microembolisation in patients despite preoperative antiplatelet treatment. The report provides support for randomised controlled trials for the clinical and cost-effectiveness of

tirofiban and other antiplatelet agents in suppressing microemboli and reducing the incidence of

stroke in patients undergoing carotid endarterectomy.

CHAPTER EIGHT

8   Discussion and future development

8.1   Carotid Surgery Registry

The effectiveness of carotid endarterectomy in reducing the risk of stroke in symptomatic critical

carotid artery stenosis has been well investigated in randomised trials[38, 47, 155]. In general, concern

regarding randomised control trial (RCT) has been expressed that the results from the highly

internally and externally validated patient characteristics in RCT may not be transferable to the

real and routine clinical setting[282]. The population based Registry would make it possible to assess

the efficacy and effectiveness of the intervention based on these randomised trials in the real

population. The Registry data also allows an immediate feedback of data which could be used as

part of an ongoing clinical quality improvement programme or an assessment of new techniques

or treatment tools. The main drawbacks of the Registry are similar to any observational study. It

relies on robust data collection and there is a lack of internally validated characteristics of the

patients.

There are 2 key findings in establishing this carotid Registry. Firstly, I was able to evaluate the

current clinical practice, radiological technique and a pharmacological approach in evaluating and

treating microemboli. The baseline patient characteristics and perioperative outcomes of patients

who underwent carotid endarterectomy in Coventry and Warwickshire were reviewed against the

major randomised trials and national audits. The similarities of the data indirectly validated the

accuracy of the Registry data. The smaller number of carotid endarterectomies performed on

asymptomatic carotid disease reflected the changing practice following the completion of the

randomised controlled trial on long term outcome of carotid endarterectomy on asymptomatic

carotid stenosis patients[249]. Our knowledge of perioperative antiplatelet therapy in carotid

endarterectomy has improved over the last few years. This is reflected in the increasing use of

dual antiplatelet agents prior to carotid endarterectomy based on the Registry data. This, in turn,

is associated with a lower number of patients developing microemboli acutely following carotid surgery.

The second key finding in establishing the registry was being able to: conduct cohort studies evaluating the association between composite Pocock cardiovascular risk score and microemboli; determine the feasibility of using transorbital Doppler technique to detect microemboli; and perform a case-control study to evaluate the effectiveness of tirofiban in abolishing microemboli acutely following carotid surgery.

The Swedish National Registry for Vascular Surgery has provided an excellent template on developing an effective Registry. The key to the development of the Swedish Registry is the robust data collection which I replicated in this thesis. The Swedish National Registry for Vascular Surgery has undergone a long process of internal and external validation of its data to eliminate any potential bias[283]. This can be seen in the increasing number of publications over the last 20 years[284]. The UK National Registry in Vascular Surgery was only formed in 2013 by the amalgamation of the National Vascular Database and UK Carotid Interventions Audit projects[192]. However, the data collection and registration still relies on self-reporting by individual surgeons. This data collection process will subject the Registry to selection and information biases.

The future development of the carotid Registry will be to standardise and link the data collected with the National Registry. This will minimise the selection and information biases of the regional data submitted to the National Registry. It also will act as an external validation for the carotid Registry. The Registry can also be expanded beyond carotid endarterectomy and the current geographical area.

8.2   Clinical prediction of MES

Pocock risk score

A recent observational study in the Leicester and Northampton region,[2] which shares a similar demographic area as ourselves, demonstrated a 23% recurrence rate of stroke/TIA in acute

symptomatic carotid artery disease in patients who had an evidence of microemboli. In the hyper-acute symptoms cohort (i.e. index symptoms within 14 days of assessment), the overall recurrence rate of stroke/TIA in patients who had an evidence of microemboli was 17%. These findings were in the context of the modern era of best medical therapy and rapid access to the TIA clinic[255]. Spence et al[238] reported a significant 3-fold reduction of the proportion of microemboli and cardiovascular event with intensive medical therapy in carotid artery disease patients. This demonstrated that those patients with microemboli are at higher risk of recurrent stroke/TIA within the already high risk group of acute symptomatic patients. It also confirmed the role of microemboli as an independent biomarker for short-term risk of stroke.

One would expect these high risk patients to have a high cardiovascular risk factor burden. This cohort has been shown to face a greater risk of death from myocardial infarction than stroke[11]. To date, the association between classical cardiovascular risk factors and microemboli has not been studied. In my thesis, I attempted to evaluate the association between the Pocock risk score and presence of microemboli following acute symptomatic carotid artery disease. The Pocock score is the only risk score derived from the established cardiovascular disease cohort. Unsurprisingly, 98% of our hyper-acute symptomatic carotid artery disease cohort had a quantifiable score which corresponded to a 5-year risk of death. In general, the Pocock score was unable to predict the presence of microemboli in hyper-acute symptomatic patients. However, a Pocock score ≥ 0.8% showed a high sensitivity (95%) for the presence of microemboli in this cohort.

Stroke, especially in the immediate post-operative period, remains one of the most important complications following successful carotid endarterectomy. The main mechanisms leading to stroke include post-operative hypertension causing hyperperfusion syndrome and thromboembolism from the endarterectomised area or elsewhere. Hyperperfusion syndrome is uncommon and strict post-operative blood pressure control with the provision of written guidance has been shown to minimise this[285]. Multi-centre studies have confirmed that acutely persistent microembolisation are associated with short-term stroke risk[31, 167, 198-200, 205]. TCD remains the only proven method of predicting post carotid endarterectomy carotid thrombosis,

hence identifying those at risk of stroke/TIA[286]. Therefore, a clinical scoring system that might be able to the risk of post-operative micro-emboli would be useful if the outcome were either stroke/TIA prevention by increasing peri-operative treatment and/or by longer-term strategy to reduce the severity of factors within the Pocock score contributing to the increased stroke/TIA risk.

Clinically, it is unclear which patients will develop acutely persistent microembolisation post carotid surgery. Surgical technical error has been excluded as the cause of microembolisation[287]. Patients who underwent staged bilateral carotid endarterectomy have been shown to have similar rates of postoperative microembolisation[288]. A randomised clinical trial has shown that the incidence of high rate postoperative embolisation was unrelated to carotid patch type[289]. Observational studies[290, 291] have suggested that postoperative microembolisation is more common in women. To date, the most promising theory came from Hayes et al's[228] work which showed that with increasing concentrations of ADP, the magnitude of platelet aggregation is higher in patients with a high rate of microembolisation. This may reflect the physiological increased platelet reactivity to the ADP pathway. Therefore the cause of the postoperative microembolisation may be related to the inherent characteristics of the patient or the atherosclerotic disease process itself. To date, the association between classical cardiovascular risk factors and microemboli acutely following carotid surgery has not been studied. One of the key findings of my study is that Pocock score shows an association with the presence of postoperative microemboli. A Pocock score of ≥ 0.8% was able to predict the presence of acute microemboli following carotid endarterectomy. A Pocock score of ≥ 0.8% and 2.3% was shown to have a high sensitivity regarding the presence of microemboli and a high negative predictive value regarding a microembolic rate of > 50hr[1]. This cut off point could possibly be employed to guide post-operative transcranial Doppler monitoring where it is not routinely available and could help identify patients where a more aggressive strategy of cardiovascular risk factor reduction could be employed. However, the association of Pocock score with a high rate of post-operative microemboli requiring additional antiplatelet agents was unclear in our cohort, as the score was

not significantly associated with an MES rate of > 50hr[1] when ROC analysis was used. This could be

cofounded by the fact that the majority of patients in our cohort were already on dual antiplatelet

therapy (Aspirin and Clopidogrel). Naylor et al[217] group demonstrated that dual-antiplatelet

agents are effective in minimising the occurrence of post-operative microemboli. A more recent

audit[279] from the same group reported that with routine use of preoperative dual-antiplatelets an

evening before surgery, the prevalence of high rate MES was reduced to the extent that post-

operative TCD monitoring was abandoned in 2010. This finding needs to be validated in a multi-

centre study setting because in my study, a small proportion of patients with high rate

microembolisation were on dual-antiplatelet treatment more than 24 hours prior to surgery.

Taking account into our findings and the aforementioned data on dual-antiplatelet therapy, we

could argue that post-operative TCD can selectively be used in those with a Pocock score ≥ 0.8%,

given that they already receive dual-antiplatelet agents. A multi-centre prospective observational

study is obviously needed to prove and validate this hypothesis.

ABCD^2 risk score

Even though the use of the ABCD^2 risk score was not intended to replace clinical assessment of

individual patients, it has been well established in triaging patients with suspected stroke/TIA for

urgent investigation and treatment[102, 112] from the community to emergency departments and

rapid access TIA clinics[255]. The key elements of the score such as age, hypertension and diabetes

are standard classical cardiovascular risk factors for carotid stenosis[16, 66, 74]. Weakness and speech

disturbance are signs of middle cerebral artery ischaemic stroke. The association between ABCD^2

risk score and carotid associated microemboli has not been studied before. Since both ABCD^2 risk

score and microemboli predict short term stroke risk, I attempted to investigate whether ABCD^2

risk score predicts the presence of microemboli. The study's findings have major policy

implications as ABCD^2 risk score has been recommended by NICE as the key clinical tool in triaging

suspected stroke/TIA[103]. Our study has shown that the ABCD^2 risk score, a widely used and relied

on score to guide stroke management is seriously flawed in failing to detect many high risk carotid

disease patients with or without microemboli. The presence of microemboli is already a well–established predictor of stroke[35] and treatment to suppress microemboli has been shown to reduce the rate of recurrent stroke[37, 238]. The attractiveness of using the ABCD[2] risk score lies in its simplicity. Unfortunately, our findings demonstrated that using ABCD[2] risk score alone is not sufficient to identify the high risk embolising carotid artery disease patients. It would not have been ethical to deprive patients identified as having microemboli from early active treatment. TCD should form part of carotid imaging and this service should be urgently available to any suspected stroke/TIA patients.

The inadequacy of the ABCD[2] risk score alone for stratification of high risk of a recurrent event after a stroke or TIA has been recognised by Merwick et al[292] in an international multicentre observational study based on pooled data from Europe and North America. Merwick et al[292] expanded the standard ABCD[2] risk score to the ABCD[3]-I risk score by including presence of ≥2 TIA within 7 days, critical carotid artery stenosis and acute diffusion-weighted imaging hyperintensity lesion. The ABCD[3]-I risk score was found to be better at stratifying stroke risk than the ABCD[2] risk score in stroke/TIA patients. A recent Japanese validation study confirmed that the ABCD[3]-I risk score was found to be better at stratifying stroke risk than ABCD[2] risk score in stroke/TIA patients[293]. This finding should be expected as patients with recent symptomatic carotid stenosis and diffusion-weighted imaging were at least 3-fold higher risk of stroke[292]. However, diffusion-weighted imaging and carotid imaging are only available at the secondary care level, so this defeats the initial purpose of applying the ABCD[2] risk score for triage in the community. Additional carotid stenosis in the risk score means that carotid imaging should generally be performed in all TIA/minor ischemic stroke patients as soon as possible before the score could be completed. This is potentially problematic in clinical settings with limited access to carotid imaging (whether the patient is admitted or not). Another limitation of studies by Merwick et al[292] and Kiyohara et al[293] was that only 12% and 20% of patients respectively had carotid disease in their studies.

Overall, the relationship between the Pocock risk score, ABCD[2] risk score and carotid-associated microemboli remains unclear. The hypotheses tested in this thesis were investigated in already

high risk established cardiovascular risk factor patients. Using microemboli as the biomarker of higher risk patients among patients with an already established cardiovascular risk factor burden was probably looking at a small number of patients, of which precluded reliable analysis of Pocock risk score and ABCD^2 risk score in predicting the presence of microemboli. It may be that for a significant result to become clear, a greater number of patients is needed (i.e. there may be a type 2 error).

My work has highlighted the risk of overlooking embolising symptomatic carotid artery disease patients based on the ABCD^2 risk score alone. A multi-centre prospective observational study with a greater number of patients is needed to further evaluate the relationship between Pocock risk score, ABCD^2 risk score and carotid-associated microemboli.

8.3   Transorbital Imaging

Evidence has consistently demonstrated the importance of TCD as a clinical tool for short term stroke risk stratification. The use of TCD in a general clinical setting is restricted by the lack of acoustic temporal window in a small proportion of patients and reliable automated microembolic signal identification software. Currently, the gold standard of microembolic signal identification relies on the combination of trained human observer and automated detection software to identify its audio and visual characteristics[171]. Previous inter-observer reproducibility study has reported a high reproducibility among trained observers in the detection of microembolic signals[294]. However, the review process of the recorded data for the presence of microembolic signal is time consuming and may limit its applicability in daily routine clinical environment. Therefore, an improved automated detection software which is sensitive and specific to microembolic signals is needed to reduce the dependency on trained human observer.

A lack of temporal acoustic window remains the main technical limitation in undertaking TCD examination, even in the presence of a trained observer. The exclusion of patients due to a lack of temporal acoustic window can be found consistently in any TCD-based study including major

clinical trials[32, 37, 217]. The temporal bone acoustic window (TAW) is said to be absent or unsatisfactory in 10-15% of subjects although a figure as high as 29.1% is reported in the literature[260, 261]. The combination of the technical challenge of conducting the standard TCD examination and its limitations precluded its use as a routine part of carotid imaging in any vascular laboratory.

Prior to my research, the transorbital Doppler approach has only been used as an alternative to the standard TCD in monitoring blood flow velocity [37, 262, 263] and it has been used to identify carotid arterial siphon stenosis. In our unit, the main purposes of postoperative monitoring was to identify microemboli and hyperperfusion syndrome. In those patients who lacked an acoustic temporal window, the transorbital approach was being used to monitor for hyperperfusion syndrome postoperatively only. Our vascular technologists have extensive experience with the transorbital Doppler approach as they also provide the regional service for the sickle cell anaemia screening programme in which this approach is also being used[295].

I used the Registry data as a proof of concept to demonstrate the feasibility of using the transorbital approach in detecting circulating microemboli. I demonstrated that using the combination of standard TCD examination and the transorbital technique, approximately 99% of patients were able to be monitored for microemboli[240]. I further expanded my work by setting up a prospective observational study to validate TOD against the standard TCD examination for microemboli detection. In the validation study, I demonstrated that TOD has good diagnostic agreement to detect microemboli with the standard TCD approach[296]. It also has a high sensitivity and specificity in detecting microemboli. These findings have reduced the technical limitations of using TCD as a clinical tool for risk stratification especially in those who lack of suitable acoustic temporal window.

All previous studies of microemboli detection were based on the ipsilateral middle cerebral artery[160]. This is because the middle cerebral artery is the main branch of the carotid artery and corresponds to the most commonly affected area in ischaemic stroke[22, 181, 182]. In the TOD approach, the same ultrasonographic technique was used in a different anatomical site (i.e the

carotid siphon). TOD is not a direct replacement for standard TCD examination, but a valid

alternative technique in those patients who do not have an acoustic temporal window.

A future study should be another TOD validation study in a different centre to assess the inter-

observer agreement. The next stage would be to evaluate the difference in the clinical end point

between microembolic detection by these 2 methods.

8.4   Antiplatelet treatment

There are two general key findings resulting from the analysis of the Registry data. Despite a

general consensus that antiplatelet therapy is important in reducing perioperative cardiovascular

complications for carotid endarterectomy, little is known about the type and optimal dose of

antiplatelet agents required. The kinetics analysis of the microemboli detected in response to

antiplatelet agents are invaluable in assessing the efficacy and effectiveness of antiplatelet agents

in the non-randomised controlled trial setting.

A single dose of aspirin administered an evening before carotid surgery has been reported to

reduce postoperative stroke events[297]. Based on the NASCET data[47], the risk of perioperative

stroke and death was 1.8% for patients taking 650mg – 1300mg aspirin daily, compared with 6.9%

for patients taking 0 – 325mg daily. Following this observation, a randomised controlled trial was

set up to investigate the optimal dose of preoperative aspirin in patients undergoing carotid

endarterectomy[298]. Patients were divided into a 'low dose' cohort receiving 81mg or 325mg

aspirin daily, while those who received 650mg or 1300mg aspirin daily were considered the 'high

dose' cohort. Surprisingly, the perioperative stroke, myocardial infarction and death rates were

significantly lower in the 'low dose' cohort than the 'high dose' cohort. The data suggested that

low dose aspirin is required for optimum cardiovascular protection in patients undergoing carotid

endarterectomy. It has been suggested that the exposed non-endothelialised endarterectomy

zone would act as a strong stimulus for platelet aggregation[299]. With additional effect of individual

cardiovascular risk factor burden and insult to the arterial vessel and its surrounding tissue, one

would expect patients who undergo carotid endarterectomy to require a larger dose of

antiplatelet agents. This finding may suggest an unknown mechanism related to the patient selection and its correlation with aspirin. The CAPRIE study[300] demonstrated that the use of clopidogrel in patients with atherosclerotic vascular disease is more effective than aspirin in reducing the combined risk of ischaemic stroke, myocardial infarction, or vascular death. Subsequently, further randomised control trials have shown that the combination of clopidogrel and aspirin is superior to aspirin alone for reducing the risk of early recurrent stroke in high-risk patients with recent ischaemic stroke or transient ischaemic attack[140, 141, 301].

The emergence of TCD detected microemboli as a surrogate marker of short term stroke risk allows us to evaluate the efficacy of antiplatelet agents in randomised controlled trials. Tytgat et al[216] have shown that a single preoperative dose of 120mg aspirin did not significantly reduce the microembolic rate during and after CEA. Markus et al[37, 212] have demonstrated that the combination of clopidogrel and aspirin is superior to aspirin alone in reducing the frequency of microemboli and early recurrent stroke in symptomatic carotid artery disease. Payne et al[217] also demonstrated the superior effect of the combination of clopidogrel and aspirin to aspirin alone in reducing the frequency of microemboli after carotid endarterectomy. However, the additional clopidogrel significantly prolonged wound closure, which was considered an indirect marker of haemostasis. Both aspirin and clopidogrel have irreversible platelet-inhibitory effects, therefore the risk of bleeding needs to be considered in choosing antiplatelet regimes. Li et al[302] have shown that clopidogrel must be stopped for 10 days to achieve a normal platelet aggregatory response in comparison to 4 days for aspirin. The dose of platelets required to reverse the anti-aggregatory effect of aspirin is significantly lower than the dose required for clopidogrel.

In my study, tirofiban (a reversible GPIIb/IIIa receptor inhibitor) was shown to be superior to dextran-40 and dual antiplatelet regime in treating microemboli[176]. This is unsurprising because it is a highly selective GPIIb/IIIa receptor inhibitor which is the final pathway of platelet aggregates. Despite using additional tirofiban, the risk of complications including bleeding was similar to the non-tirofiban cohort. This could be explained by the unique pharmacodynamic properties of tirofiban having a short half-life of only 2 hours. The SaTIS trial[281] addressed the safety of tirofiban

especially bleeding risk in an open-label randomised controlled trial in acute ischaemic stroke patients. Tirofiban was found to be safe in the acute ischaemic stroke setting and the risk of bleeding was comparable to the placebo cohort.

Microembolic signals are much more common than clinical events. Previous studies have also confirmed that microemboli independently predict stroke in symptomatic carotid artery disease[36] and acutely following carotid surgery[31]. Therefore, the use of microemboli as a surrogate marker will reliably allow us to design a study with a smaller number of patients. This is reflected in previous TCD based studies which were designed to evaluate the efficacy of novel antiplatelet agents[168, 174]. Dawson et al[303] investigated the relationship between aspirin resistance defined by platelet function tests and the presence of microemboli in symptomatic carotid artery disease patients. It showed that aspirin resistance appeared more common in patients with carotid disease who had microemboli compared to those without. This potential relationship between the presence of microemboli and antiplatelet resistance could be utilised as a powerful research or clinical tool.

By using the Registry data on the kinetics of microemboli, I was able to demonstrate the efficacy of tirofiban compared to dextran-40 in a non-randomised trial setting. I was also able to assess the cessation duration of the microemboli in response to tirofiban and dextran-40. These data are very useful in designing future randomised controlled trials (appendix 1). The Registry data also demonstrated a small number of patients who despite already being on dual antiplatelet agents, developed a persistently high rate of microembolic signals requiring additional tirofiban. This information suggests the possibility of aspirin or clopidogrel resistance or different platelet aggregation activating pathways. Further studies are needed to evaluate the prevalence of aspirin/clopidogrel resistance in this cohort (appendix 2). It also emphasised the value of highly selective GPIIb/IIIa receptor inhibitors in symptomatic carotid artery disease patients who do not respond to conventional antiplatelet agents.

8.5   Conclusions

The Pococks risk score has shown an association and high sensitivity for predicting the presence of microemboli following carotid surgery. However, the relationship between the Pocock score, ABCD^2 score and cerebral microemboli in hyper-acute symptomatic carotid artery disease patients needs further evaluation. Transcranial Doppler should be part of the routine vascular laboratory examination to complement ABCD^2 risk score in order to identify the high risk acute symptomatic carotid artery disease. With the discovery of the transorbital approach as an alternative to the standard transcranial Doppler in detecting microemboli, most patients would be eligible for microemboli screening. My report provides support for randomised controlled trials into the clinical and cost-effectiveness of tirofiban (a GPIIb/IIIa receptor inhibitor) and other antiplatelet agents in suppressing microemboli and reducing the incidence of stroke in patients with acute carotid syndrome.

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Appendix 1 List of publications

<u>Publications related to the thesis</u>

1. **Saedon M**, Hutchinson CE, Lee RW, Imray CH, Singer DR. *The ABCD^2 risk score does not predict presence of cerebral microemboli in patients with hyper-acute symptomatic critical carotid artery stenosis: a cohort study*. (submitted)

2. **Saedon M**, Saratzis A, Lee RW, Hutchinson CE, Imray CH, Singer DR. *Registry report prediction by Pocock cardiovascular score of cerebral microemboli acutely following carotid endarterectomy*. (submitted)

3. **Saedon M**, Dilshad A, Tiivas C, Virdee D, Hutchinson CE, Singer DR, Imray CH. *Prospective validation study of transorbital Doppler ultrasound imaging for the detection of transient cerebral microemboli*. Br J Surg. 2014 Nov; 101(12):1551-5. doi: 10.1002/bjs.9634. PubMed PMID: 25224848.

4. Imray C, Buxton P, **Saedon M**, Hutchinson C, Singer D. *Regarding "Intraoperative use of dextran is associated with cardiac complications after carotid endarterectomy"*. J Vasc Surg. 2013 Oct; 58(4):1167. doi: 10.1016/j.jvs.2013.05.102. PubMed PMID: 24075115.

5. **Saedon M**, Singer DR, Pang R, Tiivas C, Hutchinson CE, Imray CH. *Registry report on kinetics of rescue antiplatelet treatment to abolish cerebral microemboli after carotid endarterectomy.* Stroke. 2013 Jan; 44(1):230-3. doi: 10.1161/STROKEAHA.112.676338. PubMed PMID: 23086672.

6. **Saedon M**, Imray CH, Singer DR. "Breakthrough pinpoints new stroke treatment". Daily Express. November 23, 2012

7. Jaipersad TS, **Saedon M**, Tiivas C, Marshall C, Higman DJ, Imray CH. *Perioperative transorbital Doppler flow imaging offers an alternative to transcranial Doppler monitoring in those patients without a temporal bone acoustic window*. Ultrasound Med Biol. 2011 May; 37(5):719-22. doi: 10.1016/j.ultrasmedbio.2011.01.018. PubMed PMID: 21458149.

Other publications during PhD studentship

1. **Saedon M**, Athanasios Saratzis, Ahmed Karim, Steve Goodyear. *Endovascular versus surgical revascularisation for the management of chronic mesenteric ischaemia*. Vascular and Endovascular Surgery. January 2015 (in press)

2. **Saedon M**, Mt-Isa S, Saratzis A, Leung E, Mahmood A. *Outcome of open versus endovascular abdominal aortic aneurysm repair in obese patients: a systemic review and meta-analysis*. Int Angiol. 2014 April. PMID: 24824839.

3. Goodyear SJ, Yow H, **Saedon M**, Shakespeare J, Hill CE, Watson D, Marshall C, Mahmood A, Higman D, Imray CH. *Risk stratification by pre-operative cardiopulmonary exercise testing improves outcomes following elective abdominal aortic aneurysm surgery: a cohort study.* Perioper Med (Lond). 2013 May 19; 2(1):10. doi: 10.1186/2047-0525-2-10. PubMed PMID: 24472159.

4. [*]Saratzis A, [*]**Saedon M (Joint 1st authors)**, Melas N, Kitas GD, Mahmood A. *Obesity as an Independent Predictor of Outcome after Endovascular Abdominal Aortic Aneurysm Repair*. Ann Vasc Surg. 2013 Dec 27. doi: 10.1016/j.avsg.2013.07.008. PubMed PMID: 24378248.

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7. Saedon H, Salleh S, Balakrishnan A, Imray CH, **Saedon M**. *The role of feedback in improving the effectiveness of workplace based assessments: a systematic review*. BMC Med Educ. 2012 May 2; 12:25. doi: 10.1186/1472-6920-12-25. PubMed PMID: 22551353.

8. Papettas T, Saedon H, **Saedon M**. *Opportunities for learning in the surgical workplace and how they can be exploited: a practical guide*. Br J Hosp Med (Lond). 2011 Dec; 72(12):707-10. PubMed PMID: 22241230.

Appendix 2 List of presentations

International and national presentations

1. **Saedon M**, Saratzis A, Tiivas C, Singer DR, Imray CH. A prospective validation study of transorbital Doppler for the detection of transient cerebral microemboli. International Surgical Congress, Association of Surgeons of Great Britain and Ireland (ASGBI), 30th April - 2nd May 2014. Abstract – Br J Surg 2015; 102 (Suppl. 1): 9-118

2. **Saedon M**, Dishad A, Tiivas C, Hutchinson C, Singer DR, Imray CH. A prospective validation study of transorbital Doppler for the detection of transient cerebral microemboli using transtemporal Doppler imaging as the reference standard. Vascular Society Annual General Meeting, 27 – 29 November 2013, Manchester. Abstract - British Journal of Surgery 2014; 101(S2): 7 – 14

3. **Saedon M**, Hutchinson CE, Imray CHE, Singer DRJ. Cardiovascular Risk factors for microemboli in patients with symptomatic carotid artery stenosis. XXIII European Stroke Conference, 28 – 31 May 2013, London. Cerebrovasc Dis 2013; 35 (Supl 3) 1-854

4. **Saedon M**, Hutchinson CE, Imray CHE, Singer DRJ. ABCD^2 risk score does not predict cerebral microemboli in patients with critical carotid stenosis. British Microcirculation Society – Early Career Investigator Symposium, 13 April 2013, Warwick. Abstract – pending

5. **Saedon M**, Tiivas C, Hutchinson C, Singer DRJ, Imray CHE. A prospective study comparing cerebral microembolisation detection via the trans-orbital and trans-temporal routes in carotid endarterectomy. International Surgical Congress, Association of Surgeons of Great

Britain and Ireland (ASGBI), 9 -11 May 2012, Liverpool. Abstract - British Journal of Surgery 2012; 99(S6): 1 – 82

6. **Saedon M**, Pang R, Tiivas C, Mahmood A, Hutchinson C, Singer DRJ, Imray CHE Registry Report on use of the intravenous Glycoprotein IIb/IIIa Inhibitor Tirofiban to suppress Cerebral Microemboli. British Pharmacology Society Annual meeting, December 14 – 16, 2011, London. Abstract - Br J Clin Pharmacol; 73(6): 983–1013

Regional presentations

1. **Saedon M**, Hutchinson CE, Imray CHE, Singer DRJ. ABCD^2 risk score does not predict cerebral microemboli in patients with critical carotid stenosis. West Midlands surgical society meeting, April, 2014, Birmingham

2. **Saedon M**, Singer DRJ, Pang R, Tiivas C, Hutchinson C, Imray CHE. Comparison of the tirofiban with the dextran-40 for Suppression of Cerebral Microemboli after elective carotid endarterectomy. West Midlands Physicians association meeting, May 16, 2012, Coventry

3. **Saedon M**, Goodyear S, Dilshad A, Higman D, Mahmood A, Marshall C, Tiivas C, Imray CHE. Comparison of transorbital technique and transcranial Doppler in microemboli detection. West Midlands surgical society meeting, November 18, 2011, Worcester

Poster Presentations

International and national presentations

1. **Saedon M**, Hutchinson C, Imray C, Singer DRJ. ABCD^2 risk score and cardiovascular risk factors do not predict cerebral microemboli in patients with acute symptomatic critical carotid artery stenosis. European Society of Cardiology Congress, 31st August – 4th September 2013, Armsterdam. Abstract – Eur Heart J (2013) 34 (suppl 1): 13-338.

2. **Saedon M**, Hutchinson C, Imray CH, Singer DRJ. ABCD^2 scoring is not an effective triage tool for predicting need for urgent treatment in patients with symptomatic cerebral microemboli or critical carotid artery stenosis. 11th Conference of the European Association for Clinical Pharmacology and Therapeutics (EACPT), 28TH – 31ST August 2013, Geneva. Abstract – Pending

3. **Saedon M**, Hutchinson CE, Imray CHE, Singer DRJ. Cardiovascular Risk factors for microemboli in patients with symptomatic carotid artery stenosis. International Surgical Congress, Association of Surgeons of Great Britain and Ireland (ASGBI), 1 – 3 May 2013. Abstract - British Journal of Surgery 2013; 100(S7): 115 – 225

4. **Saedon M**, Pang R, Tiivas C, Hutchinson C, Singer DRJ, Imray CHE. Comparison of the tirofiban with the dextran-40 for suppression of cerebral microemboli after elective carotid endarterectomy. UK Stroke Forum 2012, 4 – 6 December 2012, Harrogate. Abstract - Int J of Stroke (2012) 7 (sppl 2)

5. **Saedon M**, Hutchinson C, Singer DRJ, Imray CHE. Cardiovascular Risk factors for microemboli in patients with symptomatic carotid artery stenosis. Vascular Society Annual General Meeting, 28 – 30 November 2012, Manchester

6. **Saedon M**, Hutchinson C, Tiivas C, Singer DRJ, Imray C. A prospective study comparing cerebral microembolisation detection via the trans-orbital and trans-temporal routes in carotid endarterectomy. Awarded highly commended for Ansell poster competition at the Clinical Radiology Annual Scientific Meeting 2012, 10th – 12th September 2012, London. Abstract - Clin Radiol 67 2012 S1 – S10

7. **Saedon M**, Singer DRJ, Pang R, Tiivas C, Hutchinson C, Imray CHE. Comparison of the tirofiban with the dextran-40 for Suppression of Cerebral Microemboli after elective carotid endarterectomy. European Society of Cardiology Congress, 25th – 29th August 2012, Munich, Germany. Abstract - Eur Heart J (2012) 33 (suppl 1): 19-338.

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Appendix 3 Antiplatelet therapy after carotid endarterectomy – The ANTIPACE Trial

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Abstract

Sustained transcranial Doppler detected cerebral microembolisation post carotid surgery has

been shown to cause thrombotic stroke. Antiplatelet treatment has been shown to suppress

cerebral microembolisation after carotid surgery. However, it is unclear the best strategy to

suppress cerebral microemboli. Aspirin and clopidogrel are widely use, however there are

concerns about the resistance of these agents in a significant minority of patients. Registry

experience in our centre suggests that glycoprotein IIb/IIIa antagonist tirofiban is an effective

option. We therefore propose a randomised controlled trial to compare the efficacy of rescue

tirofiban against rescue clopidogrel in patients who have cerebral microembolisation after

endarterectomy despite background aspirin treatment

Background

The risk of recurrent stroke following first transient ischaemic attack (TIA) or stroke is highest in

the first few weeks [1]. About 15 – 20% of strokes are caused by carotid artery stenosis and this

has a high early risk of recurrent stroke compared with other subgroups such as cardioembolic

and small vessel stroke [1]. The degree of carotid stenosis is strongly associated with stroke risk in

symptomatic patients, and the mechanism of stroke is believed to be primarily embolic

[2].Randomised controlled trials[2, 3] have shown carotid endarterectomy (CEA) is highly effective

in reducing the risk of further strokes in moderate to severe carotid artery stenosis. However,

carotid endarterectomy is associated with up to 7.5% risk of stroke and death at 30 days [4] .

Thromboembolism from the endarterectomised zone has been shown as the principal of mechanism of ischaemic stroke following carotid endarterectomy [5-10]. Human and animal studies have shown that platelet deposition is increased at the site of carotid endarterectomy [11, 12]. The platelet nature and origin of embolism in this group was further confirmed when the endarterectomised site was re-explored [13-15] and by its response to antiplatelet agents. Despite quality control strategies to eliminate intraoperative stroke and minimise technical error, thromboembolism still causes 2-3 % of post carotid endarterectomy stroke [16].Strategies capable of reducing perioperative stroke will potentially improve the risk/benefit ratio and cost-effectiveness of the operation.

Transcranial Doppler (TCD) ultrasound is a non-invasive technique that can be used to detect platelet-based and thrombus microemboli [17-19]. Current aims of carotid imaging are assessment of luminal stenosis and classification of atherosclerotic plaque characteristics. Carotid duplex ultrasound has been the mainstay imaging in diagnosing carotid artery stenosis and the decision to proceed for carotid endarterectomy [20, 21]. Currently, Magnetic resonance imaging of the carotid plaque imaging is emerging as the treatment of choice in identification of unstable plaque features in patients with recent carotid thromboembolic symptoms [22].

The effectiveness of various antiplatelet agents in abolishing TCD detected cerebral microemboli following carotid endarterectomy have been evaluated in randomised controlled trials [23-26]. Unfortunately, there is no consensus on which antiplatelet agent is the most effective in reducing TCD detected cerebral microembolisation. With increasing prevalence of aspirin and clopidogrel resistance [27, 28], the search for the best antiplatelet agent or its combination to abolish microemboli is challenging. A combination of oral antiplatelet agents (aspirin and clopidogrel) appear to have reduced rather than abolished TCD detected cerebral microembolisation [25, 29]. Our pilot study [30] concurs with Junghans's [19] finding that intravenous GPIIb/IIIa receptor antagonist (Tirofiban) resulted in complete suppression of TCD detected cerebral microembolisation.

Preliminary work

We compared the effects on high rates of cerebral microemboli after carotid endarterectomy of the glycoprotein IIb/IIIa receptor antagonist tirofiban with the anti-thrombotic polysaccharide dextran-40.

Based on our carotid surgery registry, we identified 100 subjects refractory to basal single anti-platelet treatment given prior to surgery between August 2002 and December 2012.

Anti-thrombotic treatment was given for MES ≥ 50/hr (Tirofiban in 32 patients (age 74±2 (SEM), males 23, Caucasian 31); dextran-40 in 32 patients (age 67 ± 2, males 21, Caucasian 28)). In 36 patients with MES < 50/hr (age 71±1, male 23, Caucasian 34), MES were monitored during their spontaneous resolution (controls). Cardiovascular risk factor burden was similar between groups receiving tirofiban vs. dextran treatment. We assessed half-life of MES decay, time to resolution and cumulative MES. Data are median (IQR). Groups were compared by Mann-Whitney or Kruskal-Wallis tests as appropriate.

The initial MES rate was similar in dextran (median 104/hr (IQR: 81-150) compared to tirofiban treated patients (96(69-136), $P = 0.268$, MW). Times were shorter for tirofiban vs. dextran for 50% reduction in MES (tirofiban: 23 minutes (15-30); dextran: 60 (40 – 83), $P<0.001$, MW) and for complete MES resolution (Tirofiban: 68 minutes (53-98); dextran: 113 minutes (71 – 154), $P<0.001$, MW). Cumulative MES shows similar differences (Tirofiban 45 MES (32 – 75); dextran 103 MES (51 – 308); controls 12 MES (7 – 20), $P = 0.001$, KW). There were more early cardiovascular complications in patients who received dextran. Early event rates were low and similar for tirofiban treatment compared to no additional anti-platelet treatment.
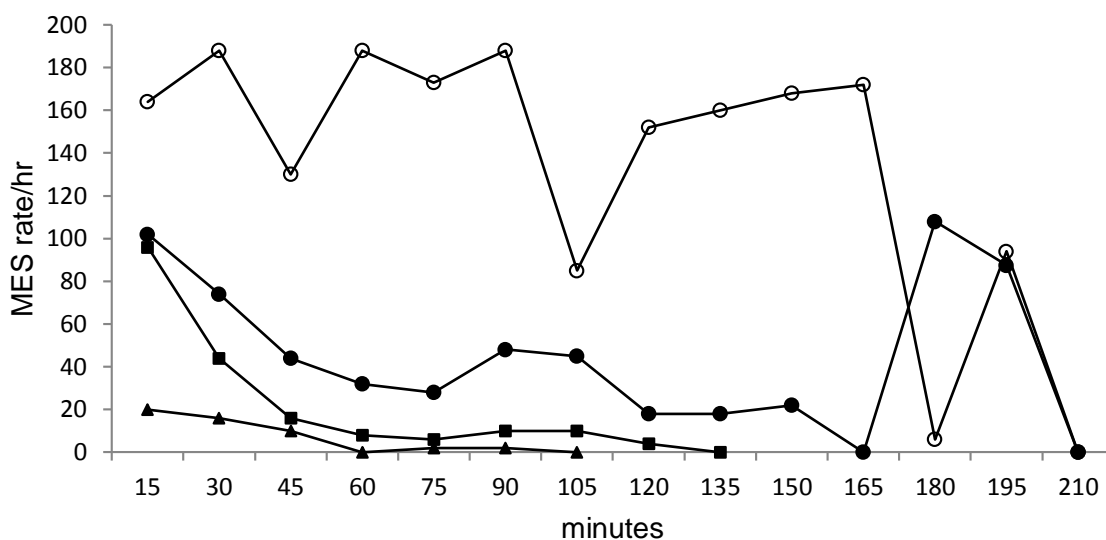
Results of MES rate after carotid endarterectomy in single antiplatelet therapy patients prior to surgery. ● dextran-40 infusion (n = 28) for MES ≥ 50/hr. ○ MES ≥ 50/hr patients (n = 4) who developed early stroke despite dextran-40 infusion. ■ Tirofiban infusion (n = 32) for MES ≥ 50/hr. ▲ No additional antiplatelet treatment (n = 36) - MES < 50/hr.

Analysis of our registry data suggests that TCD-directed tirofiban therapy is more effective than dextran-40 in rapid early suppression of cerebral microemboli after carotid surgery in patients refractory to basal antiplatelet treatment.

Hypothesis

Glycoprotein IIb/IIIa receptor is the final common step in platelet aggregation. There are several key genetic polymorphism influences which may make patients prone to the resistant pro drug clopidogrel. Our hypothesis is that tirofiban would lead to more rapid and lower suppression of cerebral microemboli after carotid surgery compared with clopidogrel.

Aim

In a double-blind, double placebo randomised controlled trial we aim to assess the relative efficacy of rescue tirofiban compare to clopidogrel in reducing cerebral microemboli after carotid endarterectomy in patients not responding to basal antiplatelet therapy.

Primary end points

1. Total number of microemboli to resolution within 3 hours monitoring period

2. Time for microemboli to cease

Secondary end points

1. Proportion of patients that require rescue anti platelet treatment

2. MRI scan evidence if cerebral ischaemic lesion

3. All vascular events prior to discharge from hospital, 30 days and 12 months after the procedure.

Objectives

1. Monitor HITS by TCD or TOD for 3 hours after CEA

2. Use MRI brain to monitor for evidence of cerebral emboli

3. Pre-operative MRI imaging of surgical CEA site

4. Assess genetic and functional markers of resistance to anti-platelet treatment

Study design

Double blind, double placebo randomised control trial

Subjects

Patients who undergo elective carotid endarterectomy. All recruited patients will be commenced on 75mg oral aspirin and other antiplatelet agents will be stopped 5 days prior to surgery.

Randomise process will be started when 2 or more microemboli identify in any 15 minutes period in the initial 3 hours after CEA.

Transcranial Doppler ultrasound

TCD monitoring [31] will be performed by an experienced vascular scientist (PC Dop 842, SciMed, Bristol, UK) with a 2 MHz probe focused on the middle cerebral artery ipsilateral to the operated carotid artery following recovery from anaesthesia. Microemboli will be identified as recommended by the International Consensus Group on Microembolus Detection [32]. Doppler wave forms and audio signals will be assessed by a trained vascular scientist. MES were identified as unidirectional, short duration signals (range 10–100 ms) with intensity threshold above 6 dB, accompanied by characteristic audible clicks and occurring randomly throughout the cardiac cycle [32, 33]

Magnetic Resonance Imaging (MRI) of neck and brain

The MRI of the neck will be using a standard neck coil. This will allow us to obtain sufficient resolution to image the plaque, to separate the fat from the surrounding tissues and to identify ulceration of the endothelial surface on these images. The brain will be imaged using both standard imaging and diffusion weighted imaging to best demonstrate the small areas of damage cause by the micro embolic events. Post-surgical imaging of the brain will be obtained to see if there is a significant difference in lesion characteristic following surgery [34].

Assessment of resistance to anti-platelet treatment

Assessment of platelet function is imperative in patients undergoing carotid endarterectomy as most of them are already on at least single antiplatelet agent. Those who on high on-treatment platelet reactivity will be at risk developing thrombotic stroke and low platelet reactivity will be at risk to develop bleeding intra or post carotid endarterectomy. Options available to assess platelet

functions are platelet aggregometry, Flow cytometry, Point of care platelet function assays (VerifyNow, Thrombelastogram)[35]

Statistics

a) Sample size estimation

A sample size of 22 subjects per group is expected to give at least 80% power to detect a difference to one standard deviation in the total number of cerebral microemboli after carotid surgery at the 5% level (2 tailed test) with expected 90% participation rate.

b) Statistical methods for data analysis

Significance between the groups are determine using t-tests or Mann-Whitney U test for continuous variables and chi squared tests for categorical data with a p-value of <

in 0.05 (2 – tailed) considered to represent significance.

Expected value of results

This randomised control trial will be an important step of establishing the efficacy of tirofiban in acute stroke syndrome. It allows us to compare the effectiveness of widely used antiplatelet agents such as aspirin and clopidogrel against tirofiban. This will enable us to form a more effective and focused strategy to abolish the cerebral microembolisation especially on those who have resistance to aspirin and clopiogrel. Whilst the point-of-care platelet function tests are not yet establish in clinical practice, the knowledge of effectiveness and safety of commonly used antiplatelet agents will be valuable. All of this will ultimately benefit patients with acute stroke syndrome in the UK, who will be given access to an effective antiplatelet treatment.

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Appendix 4 Indices of aspirin response in patients with acute stroke syndromes

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Professor Tim Warner, Platelet Biology & Vascular Inflammation

Background: Cerebral microemboli are established biomarkers of risk of recurrent stroke in patients with carotid disease who present with an acute stroke syndrome and following carotid artery surgery (1).

It is not clear what mechanisms determine which patients with carotid disease will develop microemboli. Patients with carotid disease may develop microemboli despite aspirin treatment. One explanation is poor adherence to treatment with aspirin previously prescribed to prevent the occurrence of clinical vascular events. Another reason may be failure to suppress platelet aggregation and activation despite aspirin treatment.

Aim: To assess biochemical indices of responsiveness to aspirin following an acute stroke syndrome

Subjects: Patients within 2 weeks of an acute stroke syndrome attending the vascular lab for studies of carotid structure, flow and presence or not of cerebral microemboli. Patients will have given written informed consent. Exclusions are causes of cardiac sources of emboli including: heart valve disease, recent acute coronary syndrome (may result in formation of cardiac mural thrombus) and/or presence of atrial fibrillation.

Design: Cohort study: sample size tbc

a) Patients with acute stroke syndrome with carotid disease and i) cerebral microemboli or ii) no cerebral microemboli;

b) Patients with acute stroke syndrome with no carotid disease;

c) Patients seen immediately before and after surgery for carotid endarterectomy

d) Controls: patients with asymptomatic carotid disease identified as the incidental finding of carotid bruit(s).

Methods:

1. Clinical assessment according to standard clinical guidelines

    a) Phenotyping for clinical and laboratory cardiovascular risk factors, including full blood count with platelet indices (with MPV…), lipid profile, glucose, renal function, liver function

    b) ECG to exclude atrial fibrillation and recent cardiac ischaemia

    a) Blood pressure and heart rate

    b) CT or (MRI) brain imaging

2. Carotid studies

a) Carotid imaging for plaque and flow on ipsilateral and contralateral side to symptoms and signs of presenting acute stroke syndrome.

b) Imaging for cerebral microemboli by temporal trans-cranial Doppler [or by transorbital Doppler if the temporal bone window is unsuitable (2)].

c) Platelet-related studies

a) Thromboxane assay in clotted whole blood. A 5ml blood sample will be collected into a glass tube and allowed to clot with the tube warmed to 37°C. Serum will then be removed and stored in a plain Eppendorf tube at -80°C until assay. Samples will be transferred on dry ice for assay in Professor Warner's lab at the William Harvey Institute, QMUL.

b) Thromboxane assay in urine. A 20ml sample of urine will be collected into a universal container and stored at -80°C until assay. Samples will be transferred on dry ice for assay in Professor Warner's lab at the William Harvey Institute, QMUL.

c) Light transmission aggregometry.

d) Response of platelets to aggregants. Platelet rich plasma (PRP) will be prepared by centrifugation.

i) PRP aliquots will be added to wells in a 96 well plate (to be supplied by Professor Warner) containing increasing concentrations of multiple selective stimuli for platelet aggregation (3). After 15 minutes on a plate warmer with mixing at 1200 rpm, responses will be assessed on a plate reader. Dose responses to the above agents will then be prepared.

ii) In selected patients, responses of PRP to standard stimuli will be assayed using a light transmission aggregometer.

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