Carotid Ultrasound for Stroke Prediction

Thesis submitted to Imperial College London for the degree of Doctor of Philosophy

Student: Ankur THAPAR

BSc, MBBS, MRCS

PhD candidate Imperial College October 2009 – October 2012

Academic Section of Vascular Surgery

Imperial College London

Academic supervisors: Professor Alun DAVIES, Professor Edward LEEN

Word count: (excluding bibliography and appendices): 40, 055

1.0   Abstract

Introduction

The aims of this thesis were to identify if carotid endarterectomy was cost-effective and affordable in the United Kingdom and secondly to explore the potential of contrast enhanced ultrasound and plaque texture analysis for risk stratification in asymptomatic patients with carotid atherosclerosis.

Methods

A cost-utility analysis based on results from the Asymptomatic Carotid Surgery Trial was performed using a Markov transition state model.

Three cross-sectional studies of symptomatic and asymptomatic individuals with 50-99% carotid stenosis were performed for late phase and dynamic phase contrast enhanced ultrasound, followed by plaque texture analysis.

Results

There was a high probability of surgical endarterectomy lying under the £20-30, 000 per quality adjusted life year National Institute for Health and Clinical Excellence acceptability threshold in the United Kingdom. In men under 75 years of age, the cost per quality adjusted life year gained was lower and in women there was improved effectiveness with reduced long-term costs.

Late phase contrast enhanced ultrasound imaging of carotid atherosclerosis suffered from a tissue suppression artefact which limited its ability to image microbubble retention. Quantification of plaque perfusion using low mechanical index imaging demonstrated a pseudoenhancement phenomenon from non-linear propagation, which artificially increased far wall intensity, again limiting its use for quantification of plaque perfusion. Semi-

quantitative grading of plaque perfusion revealed no significant difference in generalised plaque perfusion between symptomatic and asymptomatic individuals, however detection of ulceration using dynamic contrast enhanced ultrasound showed a trend towards an association with symptomatic status. Type II plaque showed a significant independent association with symptomatic status.

Conclusion

Carotid endarterectomy is likely to be cost-effective in those under 75 years of age, particularly women. However, without further selection, the upfront costs and high number needed to treat with endarterectomy limit its potential as a large scale strategy. Improvements in non-linear pulse sequencing are required before quantitative contrast enhanced ultrasound can reliably be used for functional imaging of carotid atherosclerosis. Qualitative assessment of plaque perfusion is unlikely to gain widespread use due to its high subjectivity. However assessment of plaque type and to a lesser extent imaging of ulceration using contrast enhanced ultrasound are promising and reproducible imaging biomarkers for further study. Validation of these markers with histology and then prospective study of individuals with these plaque phenotypes is proposed. In the future individuals with a recent transient ischaemic attack and moderate (50-69%) stenosis may prove to be an ideal group for risk stratification.

Table of Contents

Figures

Tables

1.1 Thesis structure

```
┌─────────────────────────┐
│   Cost-utility model of  │
│   asymptomatic carotid   │
│         surgery          │
└─────────────────────────┘
```

Review of risk stratification tools in asymptomatic carotid stenosis

Impact of testing high prevalence populations for asymptomatic carotid stenosis

Description of new sonographic artefacts using contrast enhanced ultrasound

Plaque perfusion and ulceration cross-sectional study

Plaque echolucency cross-sectional study

1.2   Abbreviations

ACAS – Asymptomatic Carotid Atherosclerosis Study

ACES – Asymptomatic Carotid Embolisation Study

ACSRS – Asymptomatic Carotid Stenosis and Risk of Stroke study

ACST – Asymptomatic Carotid Surgery Trial

ACST-2 - Asymptomatic Carotid Surgery Trial 2

AHA – American Heart Association

ALIU – arbitrary linear intensity units

BMT –best medical therapy

CAS – carotid artery stenting

CCA – common carotid artery

CEA – carotid endarterectomy

CEUS – contrast enhanced ultrasound

CI – confidence interval

CREST – Carotid Revascularisation Endarterectomy versus Stenting Trial

CT – computed tomography

CVA – cerebrovascular accident (stroke)

DCE-US – dynamic contrast enhanced ultrasound

DICOM – Digital Information Communication in Medivideo

ECA – external carotid artery

ECST – European Carotid Surgery Trial

ECST-2 – European Carotid Surgery Trial 2

GSM – grey scale median

HR – hazard ratio

ICA – internal carotid artery

ICER – incremental cost-effectiveness ratio

LP-CEUS – late phase contrast enhanced ultrasound

MI – mechanical index

MRA – magnetic resonance angiography

MRI – magnetic resonance imaging

NASCET – North American Symptomatic Carotid Endarterectomy Trial

NICE – National Institute for Health and Clinical Excellence

OR –odds ratio

PET – positron emission tomography

QALY – quality adjusted life year

ROI – region of interest

SD – standard deviation

SPACE-2 – Stent protected angioplasty in asymptomatic carotid stenosis (trial)

TIA – transient ischaemic attack

TCD – transcranial Doppler

US - ultrasound

USPIO – ultra-small paramagnetic particle of iron oxide

1.3   Declaration

This thesis was planned, executed and written by Ankur Thapar. However it would not have been possible without help from collaborators and supervisors who are experts in their field, whose contributions are detailed below. All ultrasound imaging was performed by Ankur Thapar, with the exception of scans performed specifically to test reproducibility.

This work was performed in accordance with the Declaration of Helsinki and Good Clinical Practice, supervised by Professors Alun H Davies and Edward Leen and performed at Imperial College Healthcare NHS Trust and Imperial College London. All patients gave informed consent to participate. Ethical approval was gained in advance for the entire project from the National Research Ethics Service ref 09/H0706/89. Figures and concepts from other authors are referenced with their permission. All co-authors of published manuscripts have agreed to work entering this thesis.

1.4   Acknowledgements

2.0   Introduction

2.1   Stroke epidemiology

Stroke is defined by the World Health Organisation as a focal neurological deficit lasting longer than 24 hours from a presumed vascular cause (7).   If symptoms resolve within 24 hours this is referred to as a transient ischaemic attack (TIA) (8).

Stroke is responsible for 11% of global mortality (9), with an incidence of 110-130 000/year in the United Kingdom (10). It is also the leading cause of serious disability in UK adults (11). The direct cost to the National Health Service is estimated at £2.8 billion per annum (12), but including the substantial costs of social care, this figure rises to £9 billion (13). This represents 3% of the total 2010-2011 Department of Health budget of £106 billion (14).

Seventy-eight percent of confirmed strokes are ischaemic in nature and 22% are due to intracerebral or subarachnoid haemorrhage (15). The causes of first ischaemic stroke can be broken down further as shown in Figure 1:
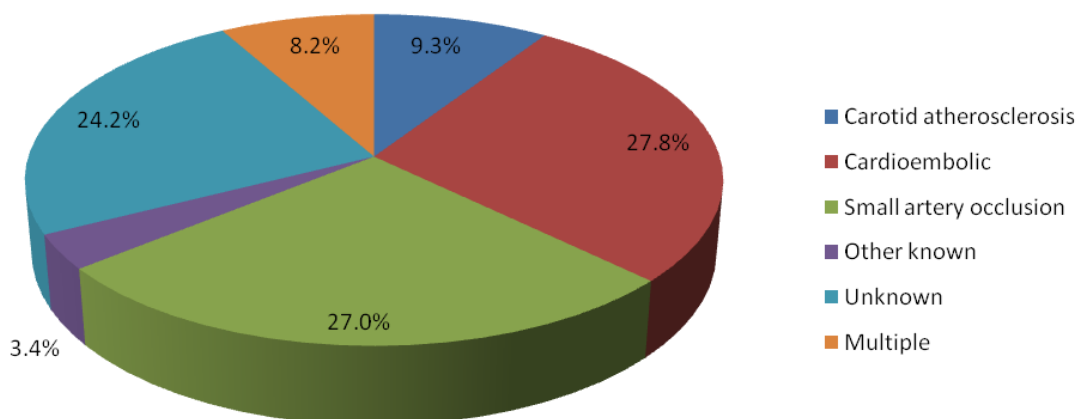
Figure 1: Subtypes of ischaemic stroke from the South London Stroke Registry. Source: Hajat et al. (16).

The South London Stroke Registry reported that 10% of ischaemic stroke was attributable to carotid atherosclerosis (16). This risk factor can be safely diagnosed using

ultrasound, with an 86-90% accuracy and a specificity of 87-94% against a conventional reference standard of two dimensional contrast arteriography (17, 18). Interestingly in Europe, a higher incidence (15%) of stroke attributable to carotid atherosclerosis is reported (19). It is noted that in South London both stroke prediction and asymptomatic carotid surgery trials have been active during the past 10 years (20) (5).

The prevalence of severe (>70%) carotid atherosclerotic narrowing (stenosis) in the population is 1-3% in persons over 70 (21) (Figure 2). The mean age of patients with carotid atherosclerotic stroke in Europe is 62 (SD ±8) years and over two-thirds of patients are male (22). One-third have previously experienced an acute coronary syndrome from advanced atherosclerosis in the coronary arteries (23). Approximately 100 000 people in the UK are estimated to have significant asymptomatic carotid stenosis (24), but its management is a source of contention.
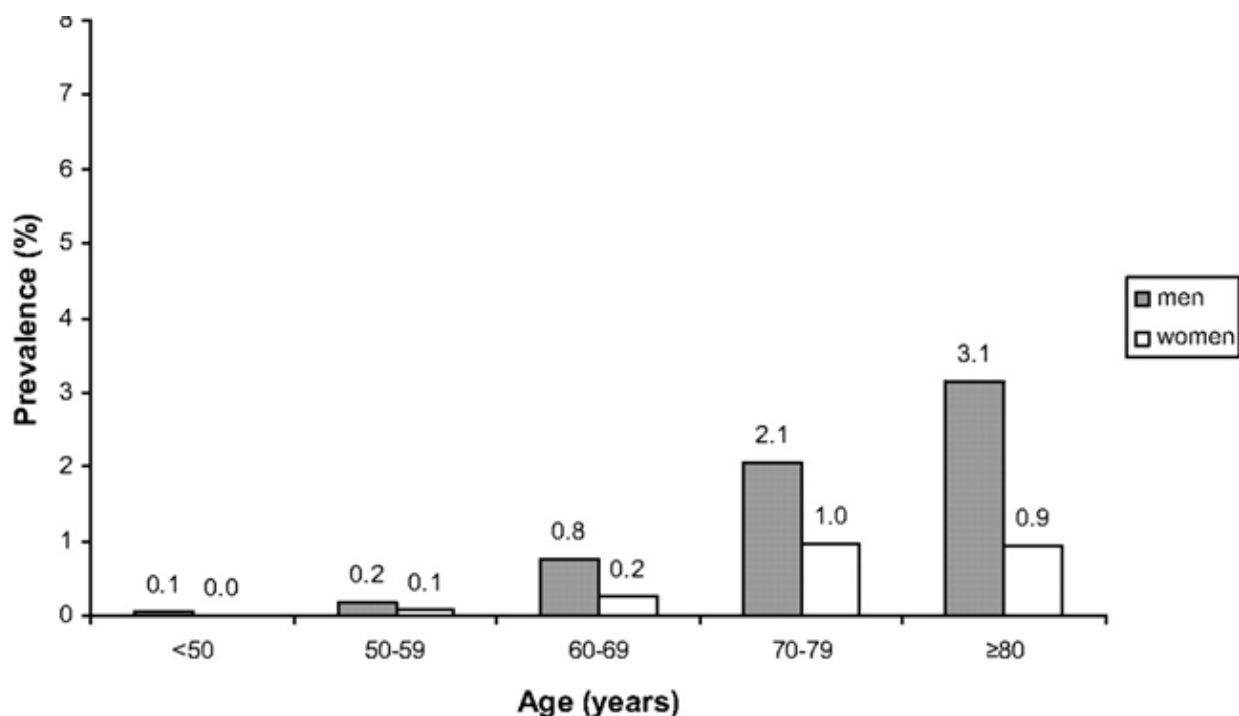
Figure 2: Prevalence of >70% carotid stenosis from 4 European cohort studies. With permission from de Weerd et al. 2010 (21).

2.2   Diagnosis of carotid atherosclerosis

For sonographers plaque is defined by the Mannheim consensus as "a focal structure that encroaches into the arterial lumen of at least 0.5mm or 50% of the surrounding intima-media thickness" (25).

Historically carotid atherosclerosis was assessed using arteriography which carried a 1% risk of stroke, the very disease clinicians were trying to prevent (26). Two dimensional arteriography measured luminal diameter reduction and therefore severity of stenosis was the initial measure of disease severity. This was validated as a risk marker in symptomatic disease in the landmark North American Symptomatic Carotid Endarterectomy Trial (NASCET) (23) and in the later European Carotid Surgery Trial (ECST) (22). However, for unknown reasons, stenosis did not provide the same degree of risk prediction in asymptomatic patients in the Asymptomatic Carotid Atherosclerosis Study (ACAS) (27) or the Asymptomatic Carotid Surgery Trial (ACST) (5). With the introduction of ultrasonic Doppler frequency shift to measure stenosis in 1979, a safer, non-invasive method of examining not only stenosis but the plaque itself became available (Figure 3) (28).
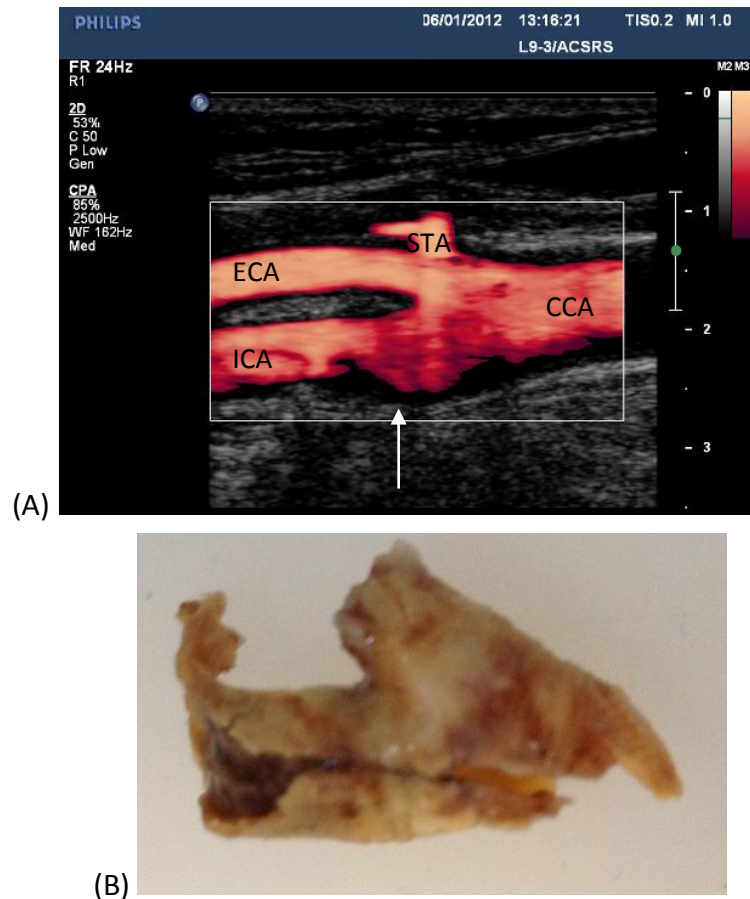
Figure 3: (A) Duplex ultrasound image of a carotid plaque. CCA=common carotid artery, ICA=internal carotid artery, ECA=external carotid artery, STA=superior thyroid artery. An echolucent ulcerated carotid plaque is seen on the far wall of the internal carotid (arrow) indenting the colour flow. (B) Endarterectomy specimen from the same patient in similar orientation. It is apparent that plaques are cylindrical in reality, however as two dimensional ultrasound is a uniplanar modality, they appear as separate near wall and far wall components (the latter is only visible in (A)). Source: Ankur Thapar (with permission from patient).

Use of Doppler velocity criteria to approximate angiographic stenosis has an accuracy of approximately ±7% (Table 1) (29). Ultrasound is now accepted as the most cost-effective first line imaging technique for carotid stenosis in the UK, with more complicated cases requiring a luminal contrast technique, such as magnetic resonance angiography (30).

NASCET equivalent stenosis (%)	Internal carotid stenosis peak systolic (cm/s)
<50	<125
50-69	125-229
70-89	230-399
90-99	>400
Near occlusion	Trickle flow*

Table 1: Conversion from peak systolic velocity using Doppler to NASCET angiographic measurements. Source: Oates et al. 2009 (31). These criteria are used by our vascular laboratory to grade stenosis.

However as investigators used ultrasound to characterise carotid atheroma, the following limitations became apparent: inability to measure the thin fibrous cap with ultrasound resolution at best around 0.2mm (axial resolution = ½ [pulse cycles x speed of sound / transducer frequency) (32), a sampling error as ultrasound is a uniplanar modality (33), an inability to discriminate lipid from other echolucent constituents such as intraplaque haemorrhage and a necrotic core (34) and an inability to image heavily calcified atheromas. Therefore, ultrasound to date has focussed on imaging the following characteristics of carotid atheromas:

- Echolucency which reflects lipoidic, haemorrhagic, or necrotic constituents (35, 36)

- Juxtaluminal echolucent area thought to represent a juxtaluminal necrotic core (37)

- Heterogeneity (38) (39)

- Surface irregularity and ulceration thought to represent cap rupture (40, 41)

- Plaque area or volume measurement (using a three dimensional transducer) reflecting local atherosclerotic burden (42) (43)

The evidence for these putative risk markers is considered in Chapter 5, however none has yet been incorporated into a UK guideline. The European Society for Vascular Surgery currently recommends that "plaque morphology should be assessed in all cases before invasive intervention" in their 2010 guideline without further qualification (44).

Unlike symptomatic individuals, in the asymptomatic population, luminal stenosis is a weak predictor of future ipsilateral stroke (Figure 4) (45). As such it imparts little risk stratification information and one could in fact question why it is used at all in asymptomatic patients. Flow limitation, although important in ischaemic heart disease, where the coronary arteries are functional end arteries, is compensated for in most individuals through both autoregulation (46) and collateral supply via the Circle of Willis, which is truly incomplete in <5% of healthy individuals (47) (48).
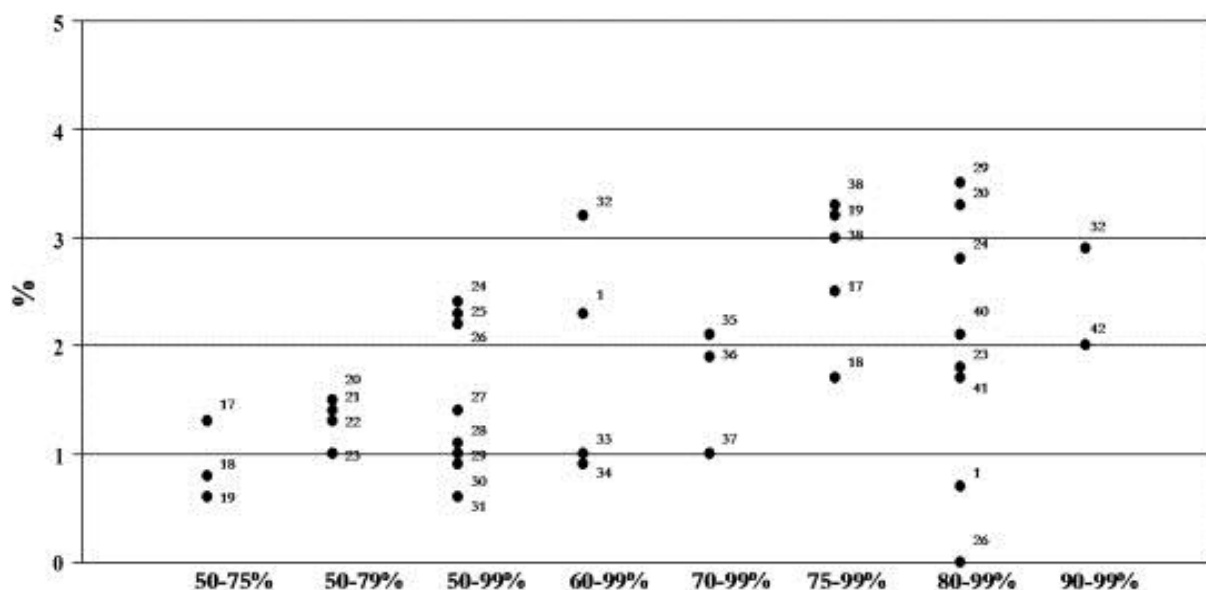
Figure 4: Annual ipsilateral stroke risk versus NASCET stenosis from 32 studies of asymptomatic patients with carotid atherosclerosis. This demonstrates a weak relationship between stenosis and future stroke risk. With permission from Naylor et al (45).

This lack of a meaningful relationship between stenosis and future stroke risk was a feature of the Asymptomatic Carotid Surgery Trial and the earlier Asymptomatic Carotid Atherosclerosis Study (Table 2).

NASCET Stenosis (%)	HR for CEA v BMT (ACST) Any territory stroke	HR for CEA v BMT (ACAS) Ipsilateral stroke
<70	0.58	0.45
70-79	0.48	0.67
80-89	0.49	0.45
90-99	0.65	

Table 2: Data from ACST (5) and ACAS (27) demonstrating no increase in benefit for surgery for increasing luminal stenosis. HR=hazard ratio for endarterectomy (CEA) versus best medical therapy (BMT). Luminal stenosis is described according to NASCET criteria.

Why stenosis is predictive of benefit in symptomatic disease but not asymptomatic disease is unknown. The uncertainty as to what constitutes a high risk asymptomatic stenosis is reflected in the variation in inclusion criteria for asymptomatic carotid interventional trials (Table 3).

	ACAS	ACST	CREST	SPACE-2	ACT-1	ACST-2
Stenosis range for randomisation	60-99%	No stenosis criteria	Varying depending on modality	70-99%	70-99%	No stenosis criteria

Table 3: Stenosis selection criteria vary in carotid interventional trials (NASCET equivalent measurements shown) (27) (5) (49) (50) (51) (52).

2.3   Mechanism of stroke secondary to carotid atherosclerosis

It was known historically that common carotid ligation could be performed without stroke in those with an intact Circle of Willis and normal cerebral autoregulation. Indeed this used to be routine practise to reduce the size of intracranial internal carotid artery aneurysms (53). This suggested that occlusion of the internal carotid artery, was not, unlike in the coronary circulation, the principal mechanism for downstream ischaemia. Indeed the carotid does not need to be occluded for acute stroke to occur and progression of a stenosis to occlusion was asymptomatic in 20/27 (74%) of patients in one cohort study (54). Therefore the patency of the extracranial internal carotid artery is not a reliable marker of symptomatology (55).

In 1954, it was noted that resection of a carotid bifurcation atheroma was associated with cessation of recurrent attacks of transient hemiplegia, today referred to as TIA (56). In 1970, a post-mortem study of patients with cerebrovascular disease delineated a characteristic pattern of thrombus adjacent to carotid atheroma in the internal carotid artery, with evidence of downstream emboli of different ages, in the anterior and middle cerebral arteries (57).   In 1979, an angiographic study revealed evidence of diffuse intracranial vascular occlusion in the ophthalmic and middle cerebral artery territory in patients with acute stroke and carotid atherosclerosis (58).
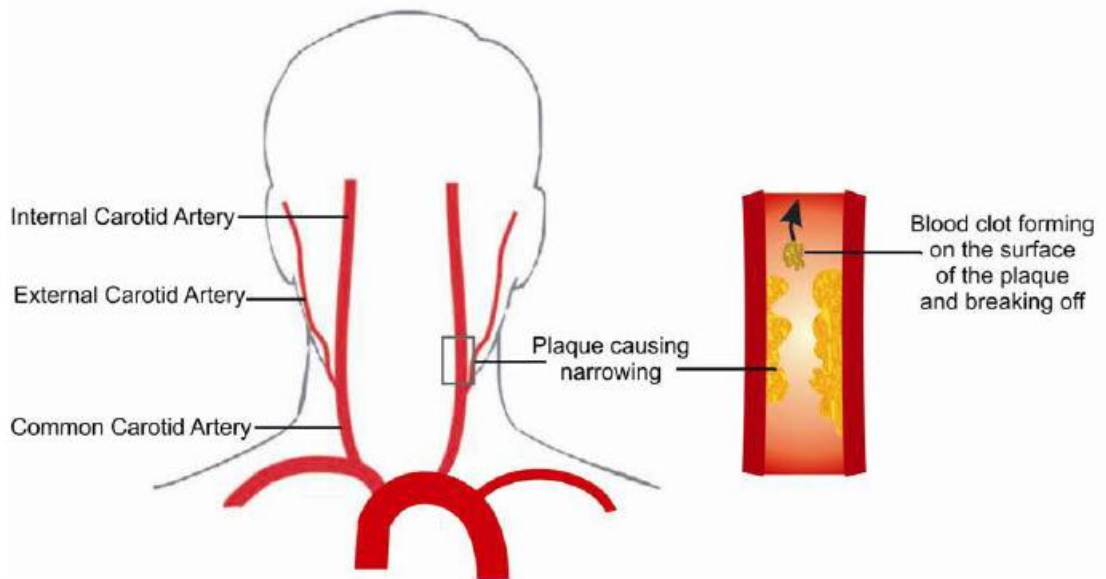
Figure 5: Accepted major mechanism for stroke secondary to carotid atherosclerosis involves carotid atheroma rupture, resulting in surface thrombosis and embolisation to the ophthalmic or anterior cerebral circulation. Diagram reproduced with permission from Gayani Jayasooriya .

In 1992 transcranial Doppler ultrasound was able to identify microembolic signals in the anterior cerebral circulation, supplied by the carotid in patients with recent stroke or TIA and carotid stenosis at a rate of 4/hour (59). Microembolic signals were absent in healthy controls. These findings constituted the evidence for an embolic mechanism for carotid atherosclerotic stroke that remains accepted to this day (Figure 5). It is interesting that emboli of differing ages are found and post-mortem in those succumbing to stroke associated with carotid atherosclerosis, suggesting that collateral circulation is important in determining whether symptoms result (57). Today, the sequelae of these embolic phenomena can be illustrated on diffusion weighted magnetic resonance cerebral imaging in acute stroke (Figure 6).
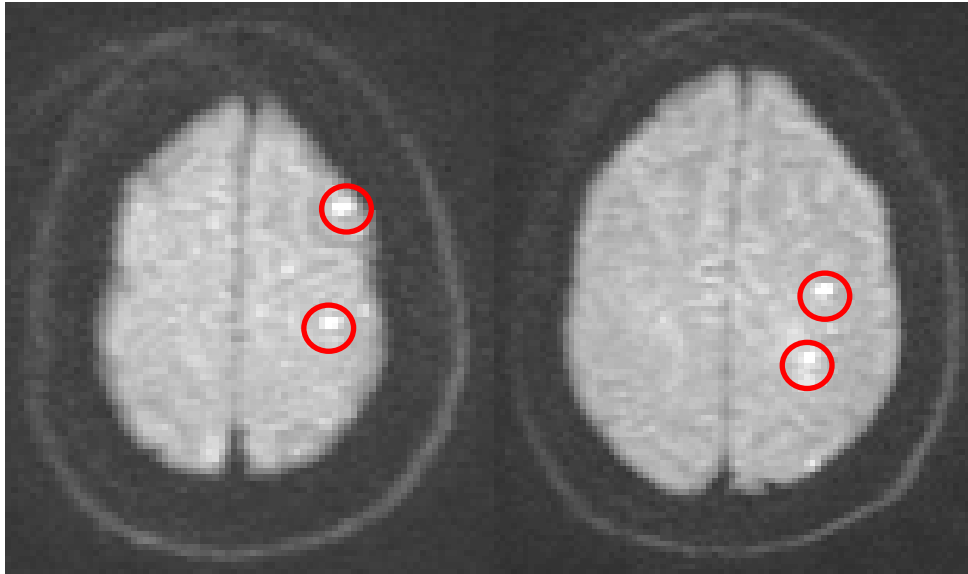
Figure 6: Diffusion weighted magnetic resonance image of a patient with acute left hemispheric symptoms and 70% left internal carotid stenosis, showing multiple embolic lesions in the left middle cerebral artery territory (ringed). Source: Ankur Thapar (with permission from patient).

It should be noted that alternative hypotheses are still suggested for stroke secondary to carotid atherosclerosis including interference with normal vasomotor tone through an effect on the nearby carotid sinus (60). However these hypotheses currently lack supporting evidence. Additionally it should be noted that patients with asymptomatic carotid stenosis can incur lacunar, cardioembolic or aortic arch related strokes in a similar fashion to the general population (61).

The embolic particle responsible is thought to be a mobile plaque surface thrombus, caused directly after plaque rupture, through exposure of a necrotic core, which in symptomatic patients is located close to a macrophage dense, thin fibrous cap of around 70µm thickness (62-64). It is noted that rupture is more common, but not exclusive to patients with ipsilateral symptoms (65, 66), in a similar manner to microembolic signals, but that the histological findings of erosion found in coronary plaques is uncommon (67). In the coronary circulation, the precursor lesion most closely resembling a ruptured atheroma is termed a thin cap fibroatheroma and delineation of this lesion non-invasively is the currently the focus of international efforts (64, 68). Conveniently carotid atheroma is easier to image using ultrasound and tissue is more easily available through endarterectomy

specimens, than coronary atherosclerosis. However preliminary recent evidence from the Athero-Express study suggests that identification of vulnerable carotid plaque may also help prognosticate for future ischaemic cardiac events (69).

The trigger for rupture is unclear, with some proposing an inside-out mechanism and others an outside-in mechanism. Proponents of the outside-in theory suggest that focal elevations in shear stress (through varying focal plaque motion during systole, or cap weakness) may cause microfractures in the plaque surface allowing entry of luminal blood (70, 71). Proponents of the inside-out rupture theory, suggest that rupture of thin walled intraplaque microvessels may be responsible for intraplaque haemorrhage and rapid expansion of the necrotic core through extravasation of erythrocytes and secondary accumulation of phagocytes (72, 73). Furthermore inflammatory cells in the cap may have a direct effect on the fibrous cap through production of proteolytic matrix-metalloproteinases (74-76).

2.4   The vulnerable plaque

To understand the potential targets for imaging, an appreciation of plaque (atheroma) histology is required. Plaque is defined by the Oxford English Dictionary as "a small, distinct, typically raised patch on or within the body, caused by local damage or deposition of material" (77). The American Heart Association (AHA) classify plaques into types IV (lipid rich with a thick fibrous cap), type V (fibroatheroma), type VIa (surface defect), type VIb (intraplaque haemorrhage), type VIc (luminal thrombus), type VII (calcified plaque), or type VIII (fibrous) (78, 79). Earlier types in the classification deal with pre-atheromatous lesions. Plaques are dynamic structures and can progress or regress, notably following intraplaque haemorrhage or statin therapy (43, 80). It is thus not essential for plaques to move linearly along the AHA classification as the nomenclature might suggest.

The term "vulnerable plaque" is used hereafter to describe a plaque that is prone to future thrombosis and embolism from plaque rupture (81). A vulnerable plaque phenotype has been described as a thin cap fibroatheroma in the coronary arteries (64). The term

"culprit plaque" is used to describe a plaque that has ruptured and caused symptoms (81). In the coronary circulation culprit plaques have a number of characteristic histological features (Table 4).

Major criteria for a culprit coronary plaque
Active inflammation (high macrophage activity)
Thin fibrous cap with large lipid core
Superficial thrombus
Fissured surface
>90% stenosis

Table 4: Histological features of culprit coronary plaques from histopathological studies. Source: Naghavi et al. 2003 (81).

Culprit carotid lesions may differ from culprit coronary lesions, as the main mechanism is thought to be embolic rather than in-situ occlusion. It should be noted that coronary arteries are around 2-4mm in diameter (82), whereas carotid arteries are around 4-7mm and therefore possibly less prone to occlusion (83). In 2000, Golledge et al. published a systematic review on the histological characteristics of culprit carotid lesions (63). This suggested that plaque rupture was the main macroscopic difference between symptomatic and asymptomatic patients with carotid atherosclerosis. Microscopically the culprit lesions had a higher macrophage and T-cell density and juxtaluminal necrotic cores with a thinner fibrous cap (Figure 7).
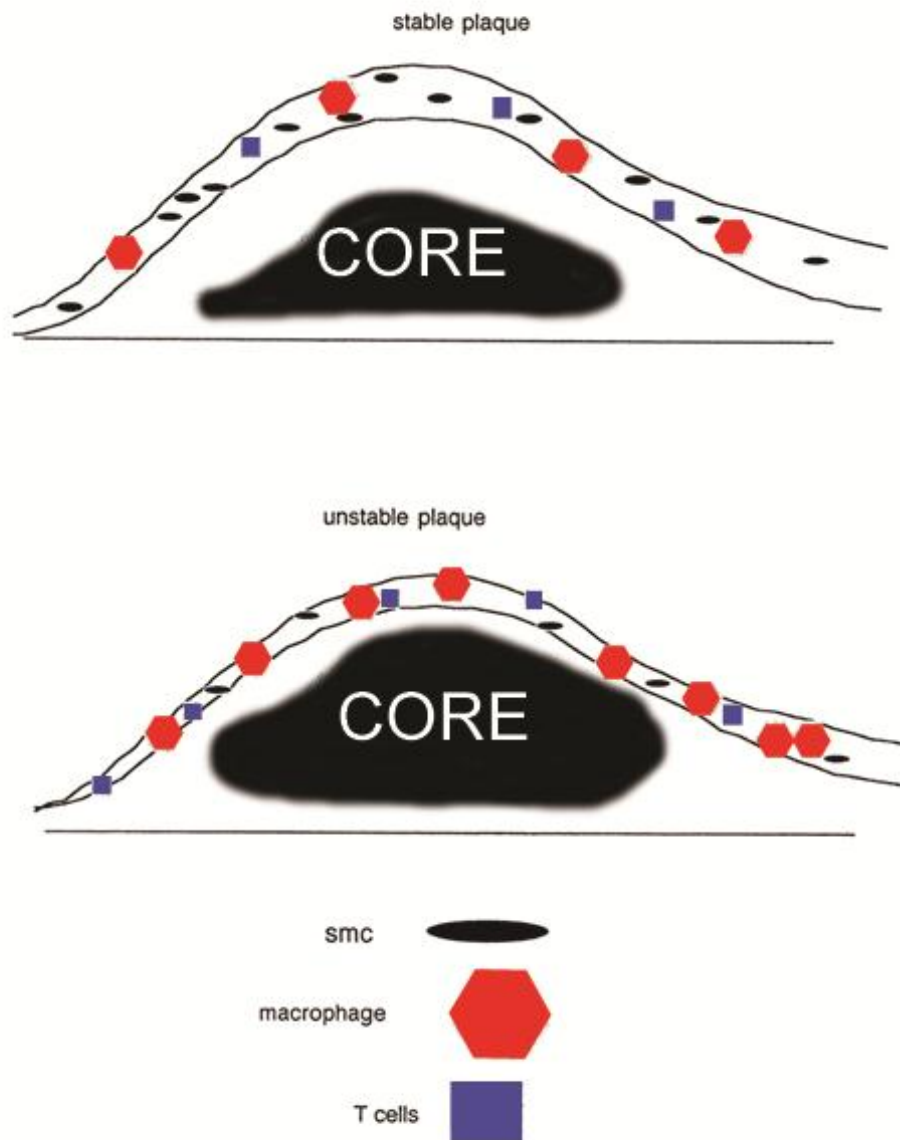
Figure 7: Uniplanar representation of the histological features of stable and unstable carotid atheromas that are targets for imaging. The unstable atheroma (bottom) demonstrates a thinner fibrous cap, with less smooth muscle and greater numbers of macrophages with a juxtaluminal necrotic core. Adapted with permission from Golledge et al. (63).

Recently, culprit carotid plaques have been demonstrated to have a significant increase in the density of intraplaque microvessels (84-86). In the 2010 Athero-Express

study, an increased density of plaque microvessels at carotid endarterectomy was associated with a hazard ratio of 1.5 (95% CI 1.1-2.1) for future acute cardiovascular events (69). Microvessels may have a threefold role in the development of atherosclerosis (72, 87):

1. To facilitate gas exchange and plaque growth when plaque thickness exceeds approximately 100μm.

2. The immature microvessel wall may facilitate the adhesion and extravasation of leucocytes into the plaque.

3. Microvessel rupture may lead to intraplaque haemorrhage.

An in-vivo method of identifying plaque vulnerability features with ultrasound would prove extremely useful as a low cost selection tool for endartectomy. Some features such as rupture are binary, however others such as microvessel density are better measured quantitatively as they are present in all plaques to differing degrees.

2.5   Medical management of asymptomatic carotid atherosclerosis

Medical management of the asymptomatic patient with carotid disease, of which there are estimated to be 100, 000 in the UK (24), is an important primary prevention issue. Preventative therapy has advanced, following the publication of landmark meta-analyses of intensive lipid and blood pressure lowering regimes, that were unheard of ten years ago (88, 89). In the 1993 Veterans' Affairs Trial, aspirin was the only prescribed medical therapy (90). In the 2010 Carotid Revascularisation Endarterectomy versus Stenting Trial (CREST), best medical therapy consisted of 1 out of 3 antiplatelet agents, statin therapy, multi-agent antihypertensive therapy and lifestyle advice (49).

These advances have led to consensus recommendations for primary prevention of stroke from European and North American bodies which are summarised in Table 5. It must be emphasised that the leading causes of death in the medical arms of endarterectomy trials are consistently ischaemic heart disease, cancer and then stroke (5, 23, 27, 90).

Therefore it is imperative that all patients, whether surgical candidates or not, receive optimal medical therapy, as carotid endarterectomy does not prevent death from ischaemic heart disease. Aspirin in particular has an important protective effect in coronary disease (91) and has now been demonstrated to have an, as yet, unexplained association with a reduced incidence of cancer (92). Importantly for the surgeon antiplatelet therapy, statins and control of hypertension improve the rates of stroke seen after carotid endarterectomy (46, 93, 94).

	ESVS 2010	SVS 2011
Guideline	Prevention in patients with carotid stenosis	Management of patients with extracranial carotid artery disease
Focus	Symptomatic and asymptomatic carotid atherosclerosis	Carotid/vertebral atherosclerosis
High-risk group definition	Carotid atherosclerosis Multiple cardiovascular risk factors	Atherosclerotic disease of the carotid or vertebral arteries
Target BP (mmHg)	<140/90 In diabetes or impaired renal function: <130/80 *Level A*	<140/90 *Level A*
Target LDL (mmol/L)	Statin for asymptomatic stenosis >50% LDL<2.6 or <1.8 if multiple risk factors *Level A*	All should receive a statin LDL<2.6 or 30% reduction from baseline *Level B* For diabetics, CAD or symptomatic atherosclerotic disease, or multiple risk factors* LDL<1.8 is suggested *Level B*
Antiplatelet therapy	Low dose aspirin for asymptomatic carotid stenosis >50% *Level of evidence C* Or if CHD risk >10% Periprocedural aspirin *Level A*	Aspirin 75-325mg to prevent MI and other cardiovascular events *Level A*
Adjunctive lipid lowering therapy	Fibrates, niacin, selective cholesterol inhibitors	Bile acid sequestrants, niacin *Level B*
BP algorithm	Unspecified Preference for calcium channel antagonist and ACE inhibitors	The 7th Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure
Target HbA1c (%)	Unspecified	7.0
Lifestyle measures	Smoking cessation *Level B* Reduce weight *Level C* Increase physical activity *Level C* Reduce alcohol *Level C*	Smoking cessation Diet and exercise for diabetics with atherosclerosis *Level B*

Table 5: Medical therapy recommendations for patients with asymptomatic carotid atherosclerosis from the European Society for Vascular Surgery (ESVS) (95) and the American Society for Vascular Surgery (SVS) (96). From collaborative work with Dr Kerry Davies (97).

A reduction in ipsilateral annual stroke rates has been identified in cohorts on contemporary medical therapy (Figure 8). In particular, three recent cohort studies have demonstrated that ipsilateral stroke rates <1% per year have been achieved in the UK (98), Canada (41) and the Netherlands (99). This has shifted emphasis away from carotid endarterectomy as a population strategy towards surgery targeted to those with a vulnerable plaque.

To identify these persons, as we shall see in Chapter 5, a whole host of new methods of plaque imaging are under development. In the population under consideration, conventional risk factors such as male gender and hypertension are found in 2/3 of persons (22) and are therefore not discriminatory enough to distinguish stable from unstable atheroma in longitudinal study. This represents a conceptual move towards, as Professor Spence, head of the Stroke Prevention and Atherosclerosis Research Centre in Ontario, Canada terms: "treating arteries instead of risk factors" – the concept that we can now obtain direct evidence of plaque structure and activity, rather than relying on surrogates, to guide therapy (43).
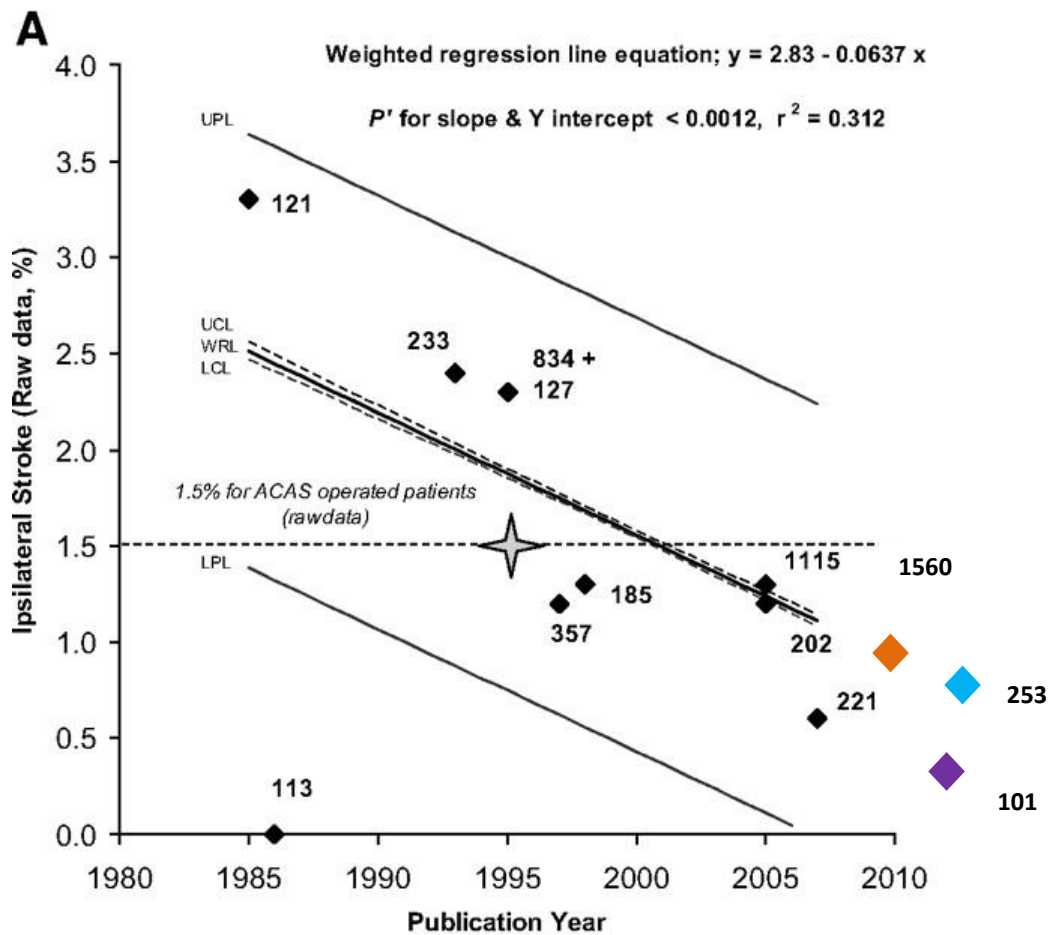
Figure 8: The reduction in average annual ipsilateral stroke rates in patients with asymptomatic 50-99% carotid stenosis over the last thirty years from cohort studies and the medical arms of randomised controlled trials. **Bold** numbers represents sample size. The results from three more recent studies have been added manually. WRL=weighted regression line, UCL=95% upper confidence limit for regression line, LCL=lower95% confidence limit for regression line, UPL=95% upper population limit, LPL=95% lower population limit, ACAS=Asymptomatic Carotid Atherosclerosis Study (adapted with permission from Abbott 2009) (100). Studies: **113** Chambers 1986 (101), **121** Johnson 1985 (102), **127** ECST 1995 (22), **185** Cardiovascular Health Study1998 (103), **202** Asymptomatic Emboli Detection Study 2005 (104), **221** Second Manifestations of Arterial Disease 2007 (99), **233** Hobson 1993 (90), **357** Asymptomatic Carotid Bruit Study 1997 (105), **834** Asymptomatic Carotid Atherosclerosis Study 1995 (27), **1115** Asymptomatic Carotid Stenosis and Risk of Stroke 1995 (106), **1560** Asymptomatic Carotid Surgery Trial 2010 (5), **101** OXVASC study 2010 (98), **253** Madani 2011 (41).

The effectiveness of contemporary medical therapy is being tested versus surgical and endovascular intervention for asymptomatic carotid atherosclerosis in contemporary trials such as SPACE II (Stent-Protected Angioplasty in asymptomatic Carotid artery stenosis vs. Endarterectomy trial II) (50). If the stroke rates in randomised trials of patients who are at equipoise for medical or surgical treatment substantiate the results from smaller cohort studies, it is unlikely that the asymptomatic population would need any form of risk stratification. Instead effort would be spent on intensively correcting traditional risk factors.

2.6   Surgical management of asymptomatic carotid atherosclerosis

Surgical treatment has become safer since the original carotid bifurcation resection and reconstruction performed for crescendo TIAs by Eastcott in 1954 at St Mary's Hospital, Paddington (56). Modern surgery is performed under general or regional anaesthesia, and conventionally involves endarterectomy (removal of the diseased intima-media complex) between clamps to prevent embolisation during the operation (Figure 9). Patch closure of the vessel prevents restenosis and has robust evidence of long-term lower stroke rates (107). On occasions, particularly when endarterectomy is challenging, e.g. post radiotherapy, another procedure such as bypass or interposition can be performed (108).

Cut in neck over
carotid artery

Narrowing (plaque)
removed by surgeon
through a cut in the neck

Cut in artery and
skin stitched
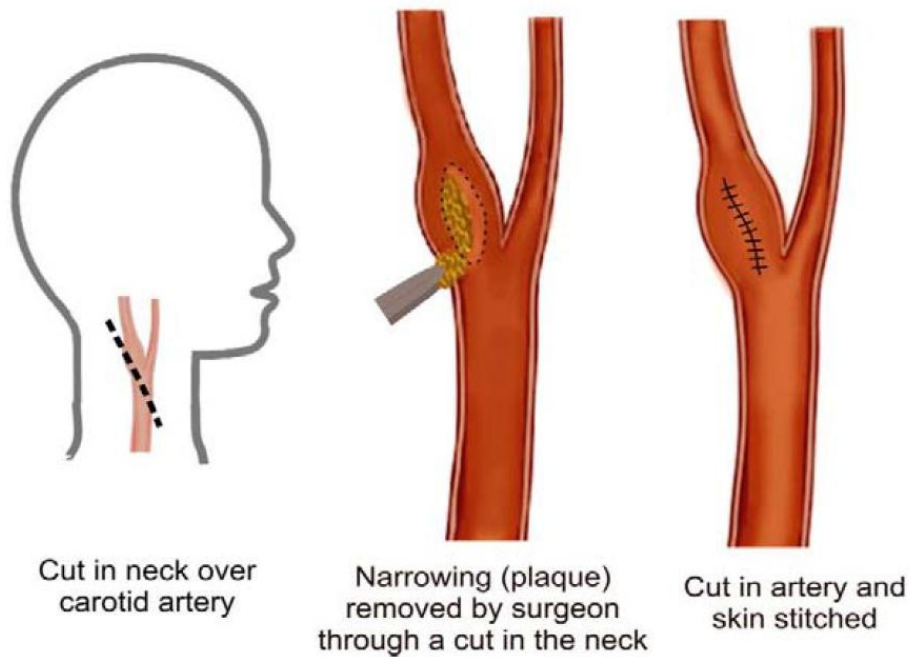
Figure 9: Illustration of the standard technique of carotid endarterectomy. In this operation, the patient is heparinised before clamps are applied above and below the carotid bifurcation. A longitudinal arteriotomy is made and the atheroma gently teased off the adventitial layer. In modern practise a patch is used to close the vessel to prevent stoke and restenosis. Reproduced with the permission of Dr Gayani Jayasooriya.

The 1993 Veterans' Affairs study reported a 4.7% 30 day stroke or death rate following endarterectomy for asymptomatic carotid atherosclerosis. With periprocedural use of low dose aspirin (109), the use of patch closure (107), duplex replacing arteriography and extensive audit of outcomes, perioperative stroke or death rates fell to 1.4% in the 2010 CREST study (110) and 3% in the 2011 UK carotid audit (111).

In the largest trial of medical versus surgical therapy in 2010, the Asymptomatic Carotid Surgery Trial (ACST) randomised 3120 patients with significant carotid stenosis (no lower limit defined) with clinical equipoise to a strategy of best medical therapy (BMT) with or without early carotid endarterectomy (CEA) (5). This trial followed patients for a median of 9 years and during the trial 80% of patients commenced statin therapy. The principle results are shown in Figure 10.

A Any stroke or perioperative death

Gain at
5 years: 4·1% (95% CI 2·0–6·2), p=0·0001
10 years: 4·6% (95% CI 1·2–7·9), p=0·009

17·9%

10·9%

13·4%

6·9%

Immediate
Deferred

Risk (%; 95% CI)

Years

Perioperative events/CEAs (%)+other events
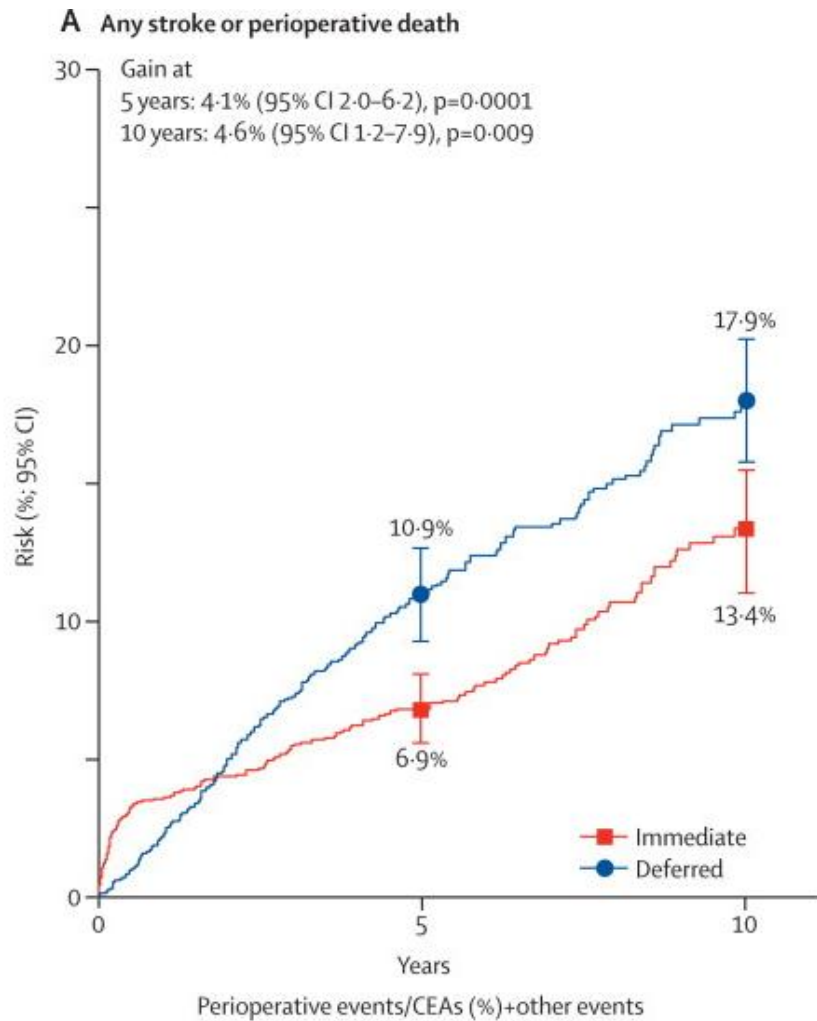
Figure 10: Kaplan-Meier life table analysis of 10 year results of immediate endarterectomy versus deferral. Source ACST 2010 (5). At ten years there was a 4.6% absolute risk reduction in any stroke or perioperative death in the surgical arm, equating to a number needed to treat of about 1 in 20.

ACST demonstrated an average 4.6% (95% CI 1.2-7.9) absolute risk reduction for BMT alone and BMT with CEA over 10 years. Patients incurred a perioperative risk of stroke or death of 3.0% (95% CI 2.4-3.9) for a long term stroke free survival advantage. The trial enrolled few patients over 75 and this subgroup had a high intercurrent mortality. Therefore ACST did not demonstrate a statistically significant long-term stroke reduction in patients over 75 (HR 0.81, 95% CI 0.43-1.51). The annual any territory stroke risk for those who were treated with medical therapy alone was 1.7% per year. For those taking statins there was a fall in the cumulative stroke rate from 24% to 13% at 10 years; however these were unmatched, non-randomised groups. This meant that although surgery was still

associated with a statistically significant stroke free survival advantage, for those on statins the absolute risk reduction was two-thirds only 0.5% per annum with statins versus 1.5% per annum without). Similarly in the first 5 years of ACST the annual stroke rate in the medical arm was 2.1%, whereas in the last five years it decreased to 1.5% per year, although it is recognised that this may also represent a survivor effect.

Following ACST, the introduction carotid endarterectomy for those under 75 years of age who are fit with significant carotid atherosclerosis has been challenged on the grounds of cost-effectiveness, affordability and a reduction in ipsilateral stroke risk on medical therapy alone (100).

2.7   Endovascular management of asymptomatic carotid atherosclerosis

In a manner analogous to the coronary circulation, angioplasty and stenting of carotid atheromas has also been trialled (Figure 11) (49). In symptomatic patients, stenting involves passage of a guide wire, balloon and stent across a lesion with an eroded cap with surface thrombus and has had disappointing results, despite the use of proximal or distal cerebral protection devices (112). However in elective asymptomatic lesions, stenting may be technically safer. However it is also logical to ask, do these lesions need to be treated at all?
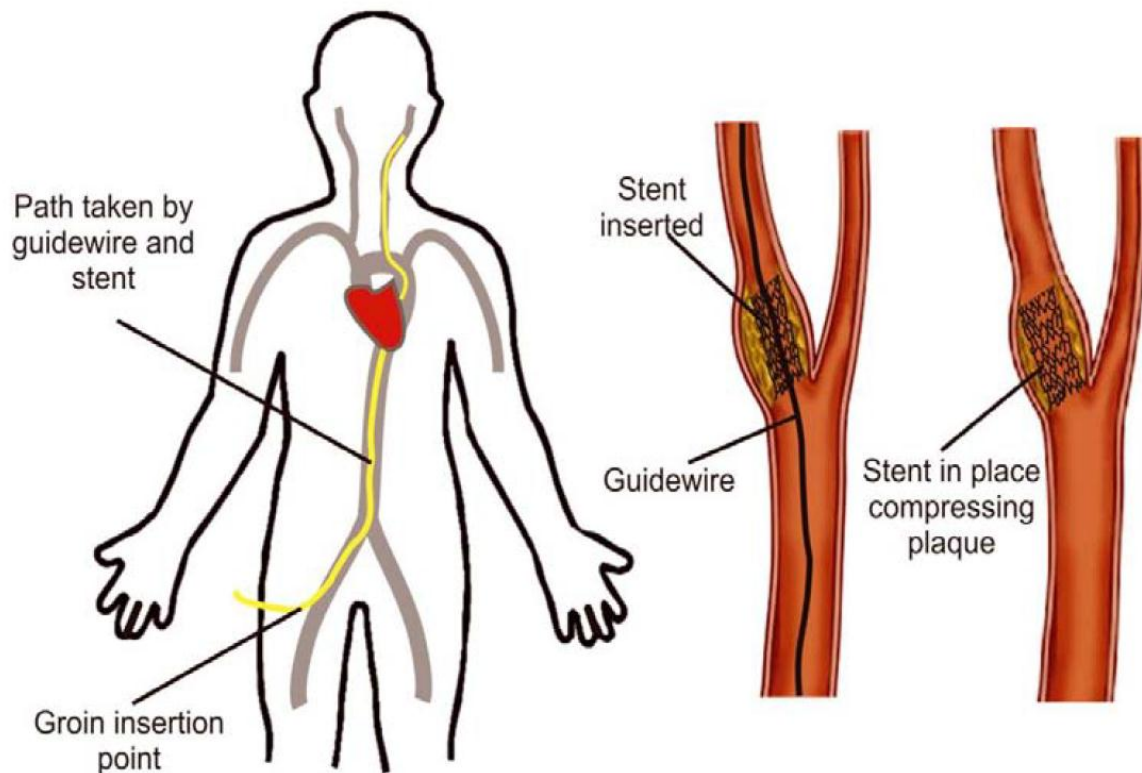
Figure 11: Diagram demonstrating the technique of carotid angioplasty and stenting. A transfemoral, transradial or even transcarotid approach can be undertaken. In modern practise a proximal (common carotid) or distal (internal carotid) cerebral protection device will be employed to arrest flow or serve as a filter respectively. Then angioplasty is performed, followed by deployment of a stent. To prevent atheromatous debris from exuding through the stent, the central portion is often covered, whilst leaving the periphery uncovered to prevent intimal hyperplasia. Pre and post-procedure cerebral angiography is performed. Reproduced with permission from Dr Gayani Jayasooriya .

Nevertheless stenting has been compared with surgery in three trials of asymptomatic patients, however, the number of endpoints is low, making any comparison at the present time uncertain (113). The data from the largest trial CREST, demonstrate a trend towards worse outcomes with stenting (Table 6).

	CAS (%)	CEA	HR (95% CI)
30 day stroke or death	2.5 (SE 0.6)	1.4 (SE 0.5)	1.88 (0.79-4.42)
30 day stroke, death or ipsilateral stroke to 4 years	4.5 (SE 0.9)	2.7 (SE 0.8)	1.86 (0.95-3.66)

Table 6: Periprocedural and 4 year outcomes for the 1181 asymptomatic patient subgroup in the 2010 CREST trial (49). CAS=carotid artery stenting, CEA=carotid endarterectomy, HR-hazard ratio, SE=standard error.

2.8   Physician management preferences

Professional opinion is split equally between medical treatment and revascularisation as first line therapy for severe asymptomatic carotid atherosclerosis (Figure 12) (114). The crux of this debate is the affordability of carotid endarterectomy (and in recent times carotid stenting) over medical therapy alone for the enormous asymptomatic population which could be treated (45).
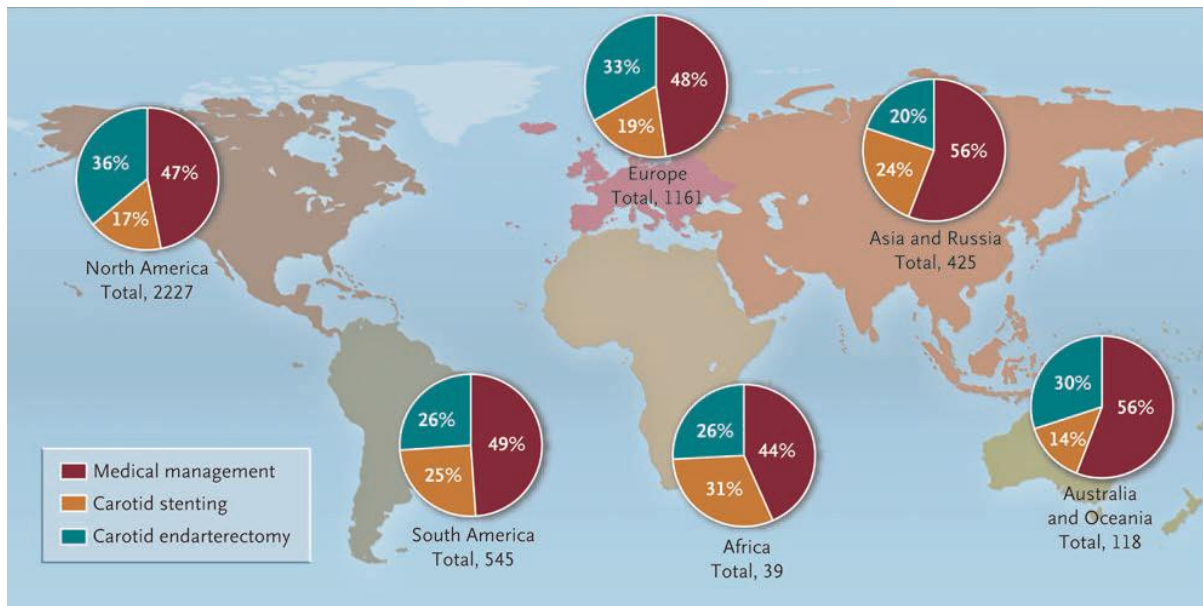
Figure 12: Results of a survey of 4669 healthcare professionals, considering the management of a 67 year old asymptomatic patient with a 70-80% stenosis of the internal carotid artery. Source Klein et al. 2008 (114).

2.9 Patient management preferences

The following hypothetical scenario was posed to 102 consecutive vascular outpatients at Imperial College Healthcare NHS Trust in 2011, before they had seen a surgeon:

"Imagine your doctor informs you that you have a narrowing of one of the internal carotid arteries picked up on a routine scan. The artery is 70% narrowed. The other arteries to the brain are working fine and you have never had symptoms of a stroke"

The risks of three treatment options were quoted from recent trials as follows:

- Best medical therapy (BMT): 11% risk of stroke at 5 years from ACST (5)

- Carotid endarterectomy (CEA): perioperative risk of 3% and 5 year stroke rate of 6% from ACST

- Carotid stenting (CAS): perioperative risk of 3-5% from stenting registries and the SAPPHIRE trial (115). The patients were informed that the procedure was less invasive but little was known about its long-term effects.

Patient treatment choices are shown in Figure 13.
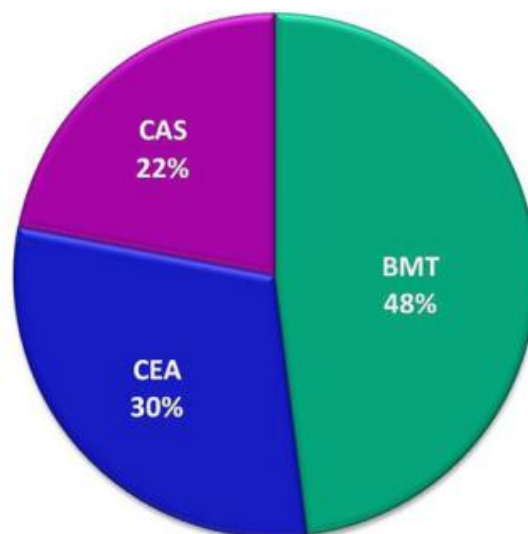
Figure 13: Results of an outpatient vascular clinic patient preference survey conducted at Charing Cross Hospital, in collaboration with the Academic Section of Vascular Surgery. BMT = best medical therapy, CEA = carotid endarterectomy, CAS = carotid artery stenting. From collaborative work performed at Imperial College with Dr Gayani Jayasooriya (55).

Patients were asked to give a reason for their preference. There were broadly two types of patient: those who gambled on a long-term benefit with endarterectomy and those who were averse to any short term risk (Figure 14).
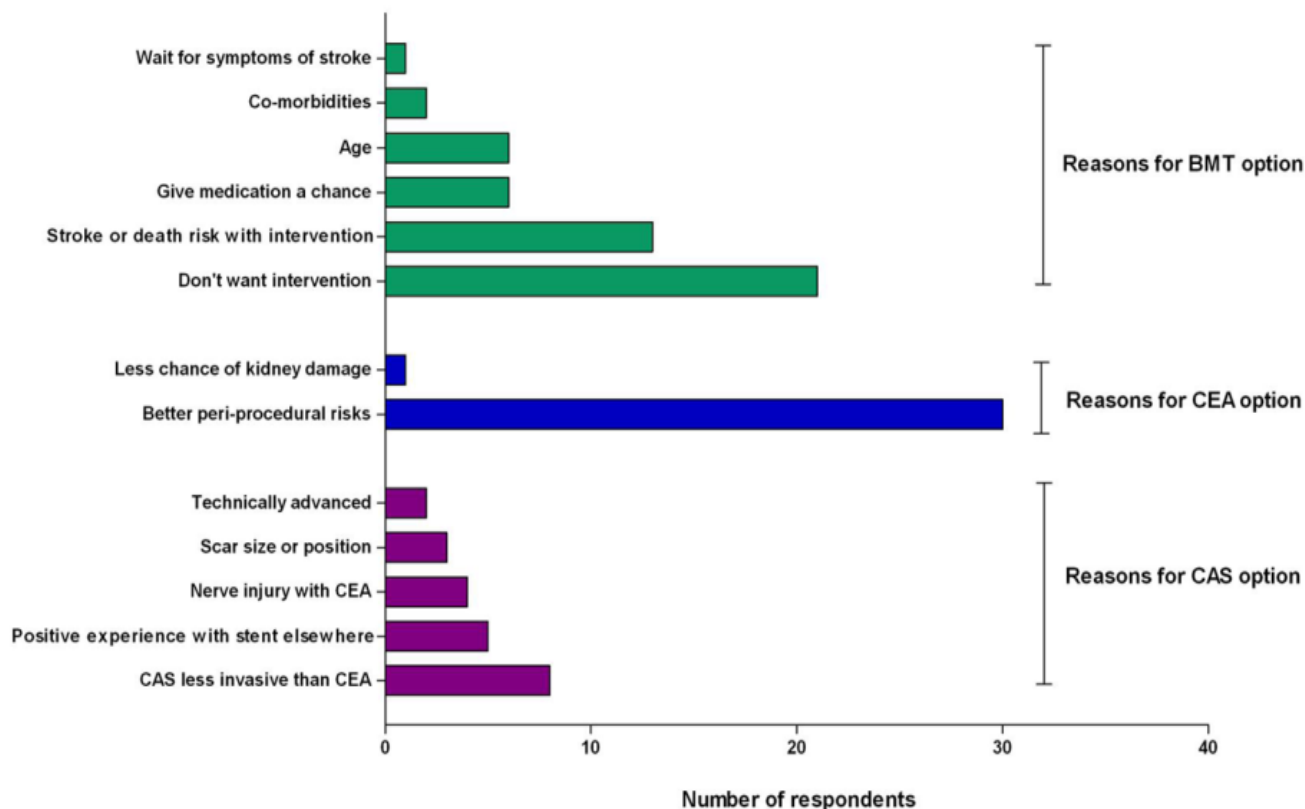
Figure 14: Reasons for the 102 survey patients for choosing either best medical therapy (BMT), carotid endarterectomy (CEA) or carotid artery stenting (CAS). From collaborative work performed at Imperial College with Dr Gayani Jayasooriya (55).

The survey performed at Charing Cross Hospital demonstrated that patients were also split equally between medical treatment and revascularisation. Half of patients accepted the risk of periprocedural stroke or death, whilst the other half felt this was unacceptable. This sequence of preferences was nearly identical to those of physicians. Yet in the UK asymptomatic carotid surgery is infrequent, whereas in the US it is common practice. This cannot be explained by trial results or patient or clinician preference. However, it has been pointed out that in countries where reimbursement is linked to numbers of procedures performed, surgeons naturally operate more (116). It would be useful to both patients and clinicians if an objective risk score for medium term stroke was available to help in making this decision, similar to the ABCD(2)I model for stroke after transient ischaemic attack, which has a c-statistic (analogous to predictive accuracy) of 78%

and is based on clinical symptoms and brain infarction (117). This latter score unfortunately has limited value in predicting recurrent events in patients with carotid stenosis (116). However other risk scores, using patient and angiographic characteristics derived from the European Carotid Surgery Trial are in use in patients with clinical symptoms (118). A similar score in asymptomatic patients would be useful to both patients and clinicians.

2.10   Aims of thesis

The aims of this thesis were:

1. To determine if asymptomatic carotid surgery could be considered cost-effective in the United Kingdom.

2. To understand the resource implications and impact of testing for asymptomatic carotid disease in the United Kingdom.

3. To review the evidence behind current imaging techniques for risk stratification in asymptomatic carotid atherosclerosis

4. To explore the potential for contrast enhanced ultrasound to identify unstable carotid atherosclerosis.

5. To explore the potential for quantitative measures of echolucency to identify unstable carotid atherosclerosis.

3.0 Cost-effectiveness analysis of asymptomatic carotid surgery

3.1 Introduction

Health economic analysis represents a transparent, utilitarian approach to approving surgical technologies for clinical use in the National Health Service. Analyses can be purely monetary (cost-benefit analysis) or an estimate of cost per unit of health gain (cost-effectiveness analysis). Cost-benefit analysis requires clinical events, e.g. death and disability to be expressed monetarily, which is somewhat subjective. Cost-effectiveness analysis is a more sophisticated approach where there is objective quantitative evidence of how much a designated comparator (life years, recurrence) changes with the implementation of several treatment strategies. Cost-utility analysis is a subset of cost-effectiveness analysis in which the comparator is health, measured in quality adjusted life years (QALYs). Cost-utility analysis uses the base currency of QALYs, which allow comparison of diseases with different measures of effect. This is important for those recommending certain treatments and for commissioners purchasing it. Cost-utility in the UK is performed according to guidance issued by the National Institute of Health and Clinical Excellence (NICE), allowing comparison between analyses of disparate conditions (119). There are several key considerations: accurate costs, accurate estimation of health benefit, time horizon and perspective of the analysis.

The total costs of a new intervention generally comprise the actual (direct) costs of treatment, indirect costs (e.g. hospital cleaning costs), overheads (e.g. salaries) and subsequent formal care, informal care costs and lost productivity years. Costs can be estimated from local sources (micro-costing) or from reimbursement tariffs based on average reported spending. In the UK average resource use per procedure is calculated by the Department of Health and procedure costs listed within Healthcare Resource Group (HRG) Tariffs. These were created as part of the government's payment by results programme in 2002, which aimed to reimburse trusts according to their activity and case

mix (120). If trusts spend substantially more than the HRG tariff, the activity generally becomes unsustainable. Although micro-costing figures may be more accurate for an individual institution, macro-costing figures are more generalizable across a nation.

Costs of living with disability comprise direct costs, e.g. care costs, loss of earnings and intangible costs, e.g. costs of depression. Cost savings occurring now and many years in the future are not considered equivalent, because patients are less likely to be alive to experience their benefits. This is particularly relevant for patients with asymptomatic carotid atherosclerosis, where approximately half of patients over 75 are dead within ten years (5). This is taken into account by discounting, i.e. considering £100 of money this year, equivalent to £96.5 of next year's money, using NICE guidance. Thus early cost savings are generally weighted in preference to those many years in the future. Positive inflation can have the opposite effect and make £100 today, equal to £110 next year. The NHS hospital and community health service inflation rate specifically reflects this effect in UK healthcare.

Health benefits are conventionally quantified across disparate interventions using a generic measure such as Quality Adjusted Life Years (QALYs). A QALY represents the projected number of life years gained multiplied by the quality of life per year (rated from 0=dead or comatose to 1=full health) (121). QALYs are an estimate of how many healthy life years can be gained as a result of an intervention. QALY changes are commonly derived from trials or longitudinal questionnaire studies, for example those using the EuroQoL EQ5D quality of life tool (122). An example of the use of QALYs is given below in Figure 15.

Scenario: A new policy of resuscitating patients who have suffered cardiac arrest > 2 hours previously is introduced. These patients on average live for an extra 2 years but in a persistent vegetative state (health score 0). The QALY gains for each strategy are shown below:

- Option 1 (do nothing): Life year gain = 0, Health score = 0, QALY gain = 0 x 0 = 0

- Option 2 (resuscitate): Life year gain = 2 years, Health score = 0, QALY gain = 2 x 0 = 0

Figure 15: An example of the use of quality adjusted life years (QALYs) as the outcome measure for a clinical scenario.

With a measure of costs and QALYs gained, the cost-utility of a healthcare intervention can be expressed as an absolute figure or that relative to standard care (more useful, if one exists). To do this an incremental cost-effectiveness ratio (ICER) is calculated, which in effect is a ratio of differences in cost and health benefit over and above standard care:

$$ICER = \frac{\Delta Costs}{\Delta QALYs}$$

ICERs allow a direct comparison of different treatment strategies by converting their healthcare effects into common units. Treatment strategies can then be plotted on a cost-effectiveness plane (Figure 16).

Δ Costs

More costly

Less effective

✗

More costly

More effective

✓✓

Δ QALYs

Less costly

Less effective

✓

Less costly

More effective

✓✓✓
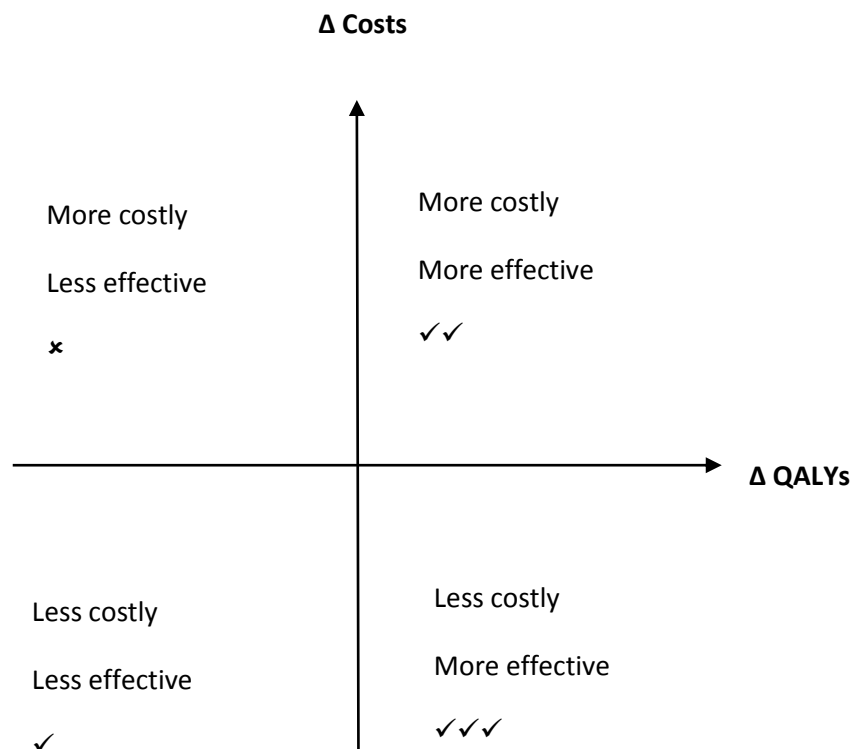
Figure 16: Schematic of a cost-effectiveness plane. A dominant strategy is one in the bottom right sector, i.e. more effective and less costly and should be adopted. A dominated strategy is one on the top left sector, i.e. less effective and more costly and should be avoided. The other two areas of the grid represent strategies that rely on the resources available in a healthcare system. For example during rationing, a less costly, less effective treatment may be the only one affordable (bottom left sector), whereas in a private healthcare system some may be able to afford more costly treatments with additional QALY benefits (top right sector).

In the UK, NICE sets a ceiling of £20 – 30, 000 per QALY gain for new surgical interventions (123).   Treatments which are less effective are excluded *a priori.*   The remaining ICERs are then compared, as described in the schematic in Figure 9. If any of the treatments are more effective and less costly than conventional care, they are said to dominate and become the strategy of choice. However, more likely is the scenario whereby additional health benefits require additional expenditure. In this case choosing a treatment strategy takes into account the number of people expected to receive the treatment, the healthcare budget and strategic health priorities. A strategy which is slightly more effective may be affordable if the incidence is low and the healthcare budget is large. In this case the upfront (immediate) direct costs are deemed affordable to the healthcare service. However, if the same intervention is required for ten times the number of patients per year, with the same budget, cost-utility remains the same but affordability becomes a limiting step. In this scenario better selection of patients is required to maintain affordability. In addition a less costly, less effective scenario may be an alternative if society is not willing to pay for the added health gain, e.g. during a recession.

The perspective of the analysis varies from provider costs, to the National Health Service (NHS) and social care (also known as Personal Social Services or PSS) costs. The latter is important because a saving at a local level (e.g. early discharge from hospital) may cause increased national costs (readmission to a distant unit). Another important factor is the time horizon, which should reflect the effects of the intervention. For example the time horizon of wound healing interventions may be months whereas for interventions on the unborn child may be a lifetime.

The management of severe asymptomatic carotid atherosclerosis has considerable economic implications, because it is prevalent in those over 60 years of age (21) and the strokes that result and operative treatment are both costly (124). To estimate the health economic implications of asymptomatic carotid surgery in the UK, this chapter modelled data from the Asymptomatic Carotid Surgery Trial (ACST), a 10 year, European, randomised controlled trial of best medical therapy with and without a strategy of early endarterectomy (5). The ACST had several advantages in that it was a pragmatic trial reflecting real world practice, outcomes were stratified by age and gender, it was the most recent trial to have a

medical arm, it was the largest trial with the longest follow up period of any asymptomatic carotid trial and a large proportion of the patients were British.

The ACST demonstrated that individuals under 75 years of age gained a 5.5-5.8% absolute risk reduction for any stroke following early endarterectomy (endarterectomy within 1 year of randomisation). For patients above 75 years of age, or in those with less than 70% carotid stenosis, there was no significant benefit for early endarterectomy. ACST demonstrated the maximum treatment effect was achieved by five years and persisted for at least a further five years, sufficient for most of the patients in the trial. The aim of this chapter was firstly to model the cost-utility of asymptomatic carotid surgery. The next chapter models its affordability.

3.3   Methods

Markov Model

ACST did not collect patient level costs, therefore a method of estimating costs for the events incurred in ACST was required. A Markov transition state model was used to estimate the lifetime costs and QALYs of early endarterectomy compared with medical therapy (more accurately termed deferral of endarterectomy, as 34.1% of patients eventually crossed over to endarterectomy). The model represented a probability tree, based on event rates reported in the ACST for each arm. The model was adapted for the United Kingdom from an earlier Swedish version (125). Patients entered the model after they had been randomised to early endarterectomy or deferral and started in the event-free state. The model comprised the following health states: no event, minor stroke, disabling stroke, and death (Figure 17). All patients faced a competing risk of intercurrent mortality (death from other causes) dependent on age and gender. The cycle length was one year. Those assigned to medical therapy were at a background risk of non-perioperative stroke (minor, major or fatal). Patients had reduced health related quality of life, higher costs and an increased mortality risk following stroke, which was assumed independent of previous treatment. Those assigned to surgery had a reduced long-term risk of non-perioperative

stroke (the treatment effect), but had a short-term risk of perioperative stroke or death as per ACST, i.e. 2.9% in the early endarterectomy arm and 3.6% for crossovers to surgery from the deferral arm. The perioperative stroke or death rate was higher in the crossover group, because some procedures were performed urgently following the development of symptoms.
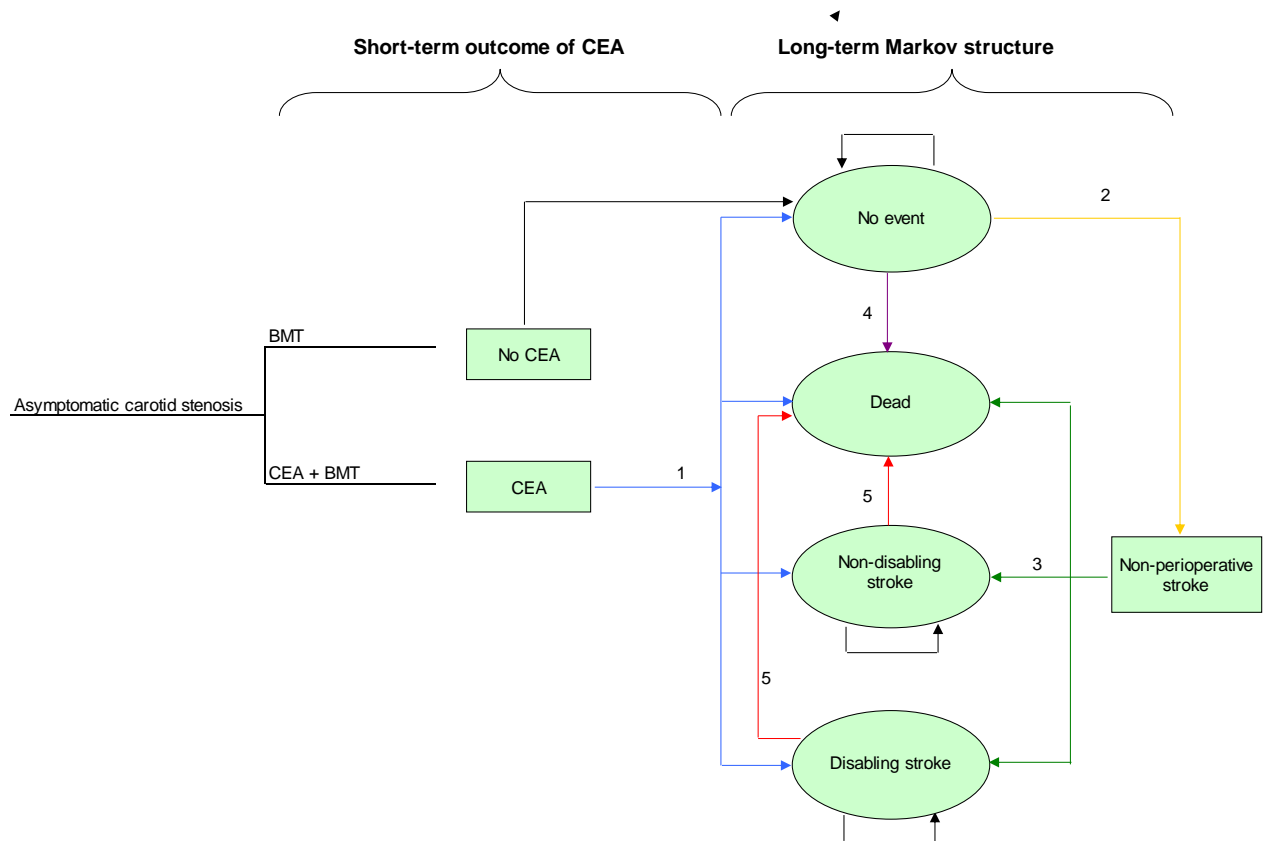
Figure 17: Structure of model (reproduced with permission from Henriksson (125)).

The outcome of the model was the cost per additional quality of life year (incremental cost-effectiveness ratio or ICER) for a strategy of early endarterectomy versus medical therapy alone. The perspective of the analysis included the National Health Service and Personal Social Services as recommended by the National Institute for Health and Clinical Excellence (119). This included the formal costs of supporting physically disabled persons.

Clinical effectiveness data

The main source of effectiveness data for the primary (base case) analysis was the ACST. The base case scenario was constructed to compare early endarterectomy versus medical therapy on average for the patients in the ACST. Table 7 shows the rates of perioperative stroke, non-perioperative stroke and treatment effects at 10 years from this trial. The base case analysis used separate hazard ratios from 0-5 years and 5-10 years from the ACST. Treatments were defined on an intention to treat basis. It was assumed that after 10 years there was no further treatment effect, that is, the absolute difference in the rates of stroke between the treatments was maintained after 10 years but did not continue to diverge.

	Early endarterectomy			Deferral of endarterectomy			
Non perioperative stroke or death (intention to treat)	Events (n)	Patient years at risk	Annual event rate (%)	Events (n)	Patient years at risk	Annual event rate (%)	HR (95% CI)
0-5 year (a)	56	6540	0.9	140	6553	2.14	0.43 (0.29 to 0.62)
5-10 year(a)	43	3042	1.4	48	3003	1.59	0.89 (0.52 to 1.52)
Average 0-10 year (b)	99	9582	1.03	188	9556	1.97	0.54 (0.43 to 0.68).

Table 7: Clinical inputs to the model. Rates of stroke related events and size of treatment effect. Source: ACST 10 Year Data Supplement (5). HR = hazard ratio, CI = confidence interval. (a) Inputs used in base case analysis (b) input used in sensitivity analysis

Quality of life after stroke

Health related quality of life after disabling and non-disabling stroke was taken from cohort studies by Haacke et al. (126) and Duncan et al. (127). The mean decrement in health related quality of life for stroke in comparison to the general population was 0 for non-disabling stroke and 0.35 for disabling stroke.

Unit Costs

The costs of preventative medical therapy were assumed to be identical in both arms. The reimbursement tariff for carotid endarterectomy (code QZ04Z, £3345) was obtained from the NHS 2010-2011 healthcare resource group (HRG). This tariff is calculated by the Department of Health as a national average unit cost for the direct, indirect and overhead costs related to an admission for this HRG in England over the previous year (128). As a sensitivity analysis, a higher UK micro-costing estimate was used (£4978) (13). Note that the derivation of this figure was unclear and it likely included symptomatic patients, with a higher level of resource use, however is a reasonable higher estimate. Post-stroke costs were obtained from published UK sources and included hospital, community, social services and informal care (129) (130) (131). All costs were standardised to January 2010 prices by use of the National Health Service hospital and community health service inflation index (132) (Table 8). A discount rate of 3.5% per year was used for costs and QALYs as per NICE guidance (119).

Item	Unit	NHS Tariff 2010-2011	Source
Carotid endarterectomy (HRG tariff)	Per procedure	£3345	HRG tariff 2010 – 2011 (133)
Carotid endarterectomy (micro-costing)	Per procedure	£4978	Saka et al. 2009 (13)
Disabling stroke	Per event, first year	£29,312	Wardlaw et al. 2006 (30)
Disabling stroke	Per subsequent year	£14,846	Sandercock et al. 2002 (134)
Non-disabling stroke	Per event, first year	£4938	Wardlaw et al. 2006 (30)
Non disabling stroke	Per subsequent year	£1152	Sandercock et al. 2002 (134)
Social services, community care and informal care	Per year after disabling stroke only	£3500	Patel et al. 2004 (131)

Table 8: Unit costs for stroke related procedures and events with data sources.

Intercurrent and stroke related mortality

The rate of intercurrent mortality for men and women at each age in the general population was estimated from national sources, by deducting stroke related mortality from all-cause mortality rates (135). National rates of stroke mortality were derived from ICD 10 codes I60 - I64 and I69 (cerebrovascular disease and its sequelae, excluding asymptomatic disease and unrelated diseases) (136).

Intercurrent mortality was greater in the ACST than the general population. This was represented in the model by a mortality ratio, calibrated to match survival at 10 years in the ACST.   The increase in the mortality rate post stroke (compared with asymptomatic

patients) was estimated from the Oxfordshire Community Stroke Project (137) which was large, British and consistent with a more recent study from Perth, Australia (138).

Base case and subgroup analysis

A base case scenario considered the average patient in the ACST: a person of age 68 years with a mean systolic blood pressure of 153mmHg and total cholesterol of 5.8mmol/L (Table 9). Published subgroups of men and women above and below 75 years of age in ACST were also considered (Table 9, Figure 19).

Sensitivity analyses

A series of sensitivity analyses were performed to test the robustness of the model predictions (Table 9). Probabilistic sensitivity analysis expresses the uncertainty of the ICER based on a range of plausible values for key cost-effectiveness parameters. Deterministic sensitivity analysis replaces one value with an alternative plausible value, e.g. for variables which have no established range. Parameters varied included: hazard ratio (treatment effect), mortality rate post-stroke and increased costs of surgery (micro-costing figure).

Probabilistic sensitivity analysis was performed using Monte Carlo simulation to reflect the sampling uncertainty in the model inputs (Figure 18). Monte Carlo simulation is a method of decision analysis using a probability distribution for each decision made in the Markov Model. A simulation of 1000 iterations was run using an Excel macro. Confidence intervals were taken from the ACST data supplement for the trial overall: hazard ratio for early endarterectomy (95% CI of 0.43-0.68) and the probability of perioperative stroke (2.1-3.8% early group and 2.2-5.7% deferral group). HRG costs and non-stroke mortality rates were derived from national data and were therefore not subject to sampling uncertainty.

Exploratory analysis 1: Restriction of crossovers for patient or physician preference

In ACST 11.7% of patients allocated to medical therapy required endarterectomy for new symptoms. However an additional 22.4% chose endarterectomy for other reasons. Similarly 10.2% of patients allocated early endarterectomy did not receive it by one year. These crossovers might be expected to reduce the treatment effect. An exploratory analysis was performed in which crossovers for patient or physician preference were excluded, illustrating what could be termed a per-protocol (or endarterectomy for new symptoms) scenario.

Exploratory analysis 2: Contemporary perioperative stroke rates

Improved perioperative safety has been reported since ACST. The perioperative stroke or death rate was 2.9% in ACST, 2.7% in the asymptomatic subgroup in the 2008 GALA trial (139), 1.6% from 2005-2009 US registry data (140), 1.4% in the 2010 US CREST trial (141). The 2011 UK carotid audit reported a 30 day stroke or death rate of 3%, however this data is hard to interpret as it was not stratified by symptom status (142). The range of 1.4-2.7% could thus be considered to reflect a plausible range of contemporary perioperative stroke or death rates. Similarly, recent any territory stroke rates from non-surgical, cohort studies vary between 0.8-2.3% per annum (20, 41, 98, 99). The effect of alternative perioperative stroke or death and background stroke rates is reported graphically in a two-way sensitivity analysis (Figure 20).

Statistical analysis

Data was analysed using Excel 2007 (Microsoft, California, USA). WinBUGS v1.4 (Medical Research Council, Cambridge, UK) was used to simulate per protocol event rates (Appendix 1) and figures created using Prism v5 (GraphPad, California, USA).

3.4   Results

Base case analysis

Results are shown as model predictions of the difference in lifetime discounted costs and quality adjusted life years (Table 9). For the base-case patient, the additional lifetime cost of a strategy of early endarterectomy compared with deferral of endarterectomy was £641 and the difference in lifetime QALYs was 1 additional month of healthy life. The predicted ICER was £7584 per QALY.

Model output	Immediate CEA	Deferral of CEA	Δ
10- year probability of any stroke or perioperative death	0.14	0.20	0.05
10- year probability of any non-perioperative stroke	0.12	0.19	0.07
Life time cost (£)	8496	7855	641
Life years	9.63	9.54	0.09
Quality adjusted life years (QALY)	7.25	7.17	0.09
ICER (incremental cost per QALY ratio)			7584

Table 9: Base case analysis of a 68 year old hypertensive, hypercholesterolaemic individual. Cost effectiveness of endarterectomy in all asymptomatic patients following intention to treat analysis. ICER = incremental cost-effectiveness ratio, QALY=quality adjusted life year, CEA=carotid endarterectomy, Δ=difference. The cost per additional QALY gained was £7584. Please note figures in this table are presented with two decimal places, however exact values have been used for calculation.

Sensitivity analyses

Deterministic sensitivity analysis was used to explore the effect of increasing the costs of endarterectomy in real terms by 50%. The increased ICER was £18, 677 per QALY for early endarterectomy.

The results of Monte Carlo simulation determined the mean difference in costs to be £942 (95% CI -73 to 1936) and the mean difference in QALYs to be 0.081 (95% CI -0.004 to 0.160) (Table 6). The negative sign indicates that there was a small chance of endarterectomy being cheaper or less effective than best medical therapy. At a threshold of £20, 000 per QALY there was a 74% chance of early endarterectomy being cost-effective, which rose to 84% at a threshold of £30, 000 per QALY (Figure 18).
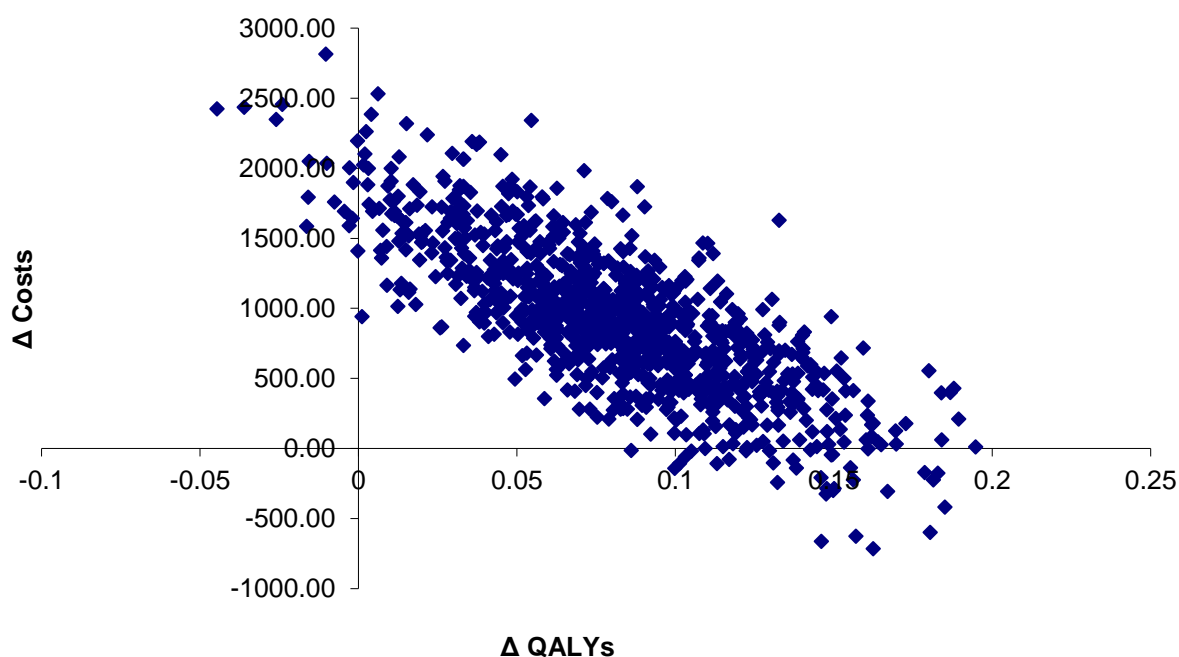
Figure 18: Cost-effectiveness plane of 1000 iterations for the base case patient in ACST, calculated through Monte Carlo simulation, varying the main clinical parameters. The iterations clustered in the upper right corner of the cost-effectiveness plane, representing increased quality of life at extra cost. There was a 74% chance of the strategy of early endarterectomy falling under a £20, 000 / QALY threshold and an 84% chance of early endarterectomy falling under a £30, 000 / QALY threshold.

Subgroup and exploratory analyses

For men under 75 years of age in ACST, the ICER for early endarterectomy was £3254 per QALY, and for women under 75, early endarterectomy was less costly and more effective (i.e. a dominant strategy). For men over 75 the ICER was £71, 699 per QALY. For women over 75, early endarterectomy was more costly and less effective than medical therapy (Figure 19), i.e. medical therapy was dominant.

Restricting crossovers to those with new symptoms slightly increased the ICER to £10 149 per QALY for early endarterectomy versus per-protocol medical therapy (Table 10).

Using lower perioperative stroke rates of 2.7% as per the 2008 GALA trial, the ICER for early endarterectomy crossed £20 000 per QALY at any territory stroke rates below 1.5% per annum. If perioperative stroke rates fell to 1.4%, as per CREST, the ICER for early endarterectomy crossed the £20 000 per QALY threshold at any territory stroke rates below 1% per annum (Figure 20).
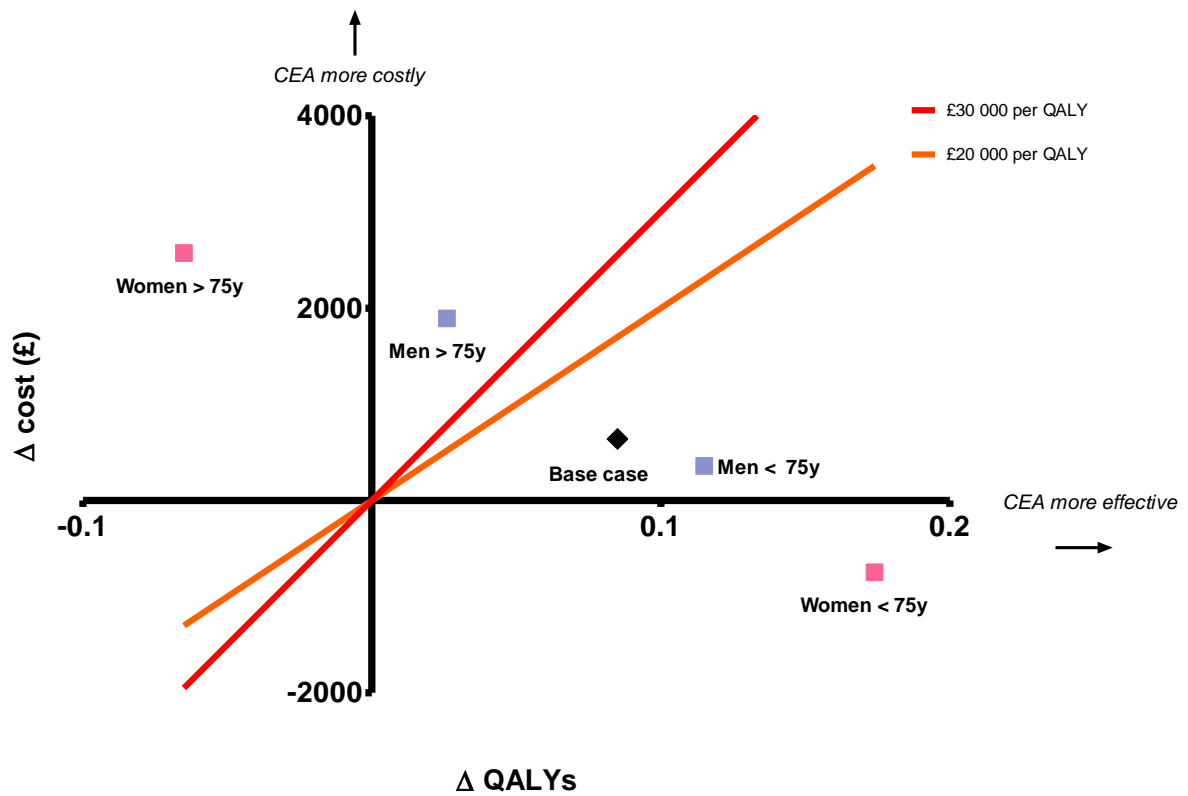
Figure 19: Cost-effectiveness plane for a strategy of early endarterectomy versus medical therapy. To be considered effective, an intervention should lie to the right of the y-axis. To be considered less costly an intervention should lie beneath the x-axis. The NICE £20-£30 000 thresholds are represented by orange and red lines respectively. To be considered cost-effective, an interventional should lie beneath the respective threshold line. QALYS = quality adjusted life years.
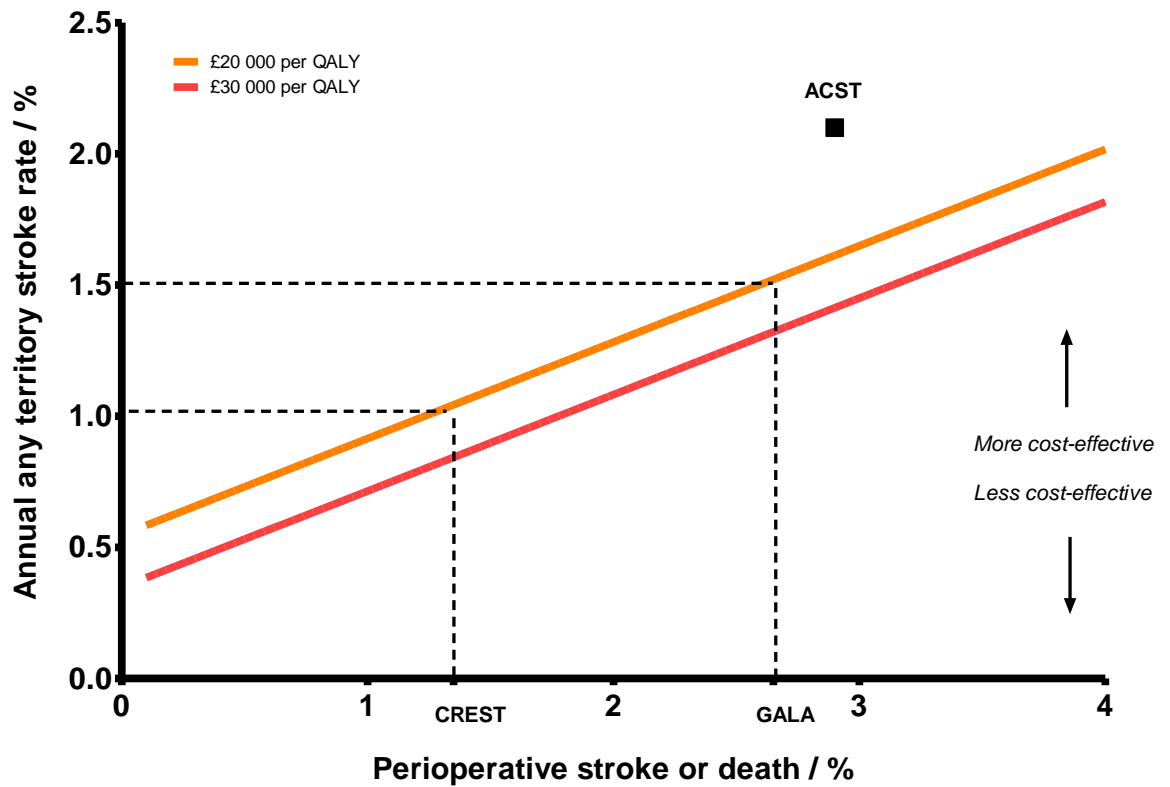
Figure 20: Two way sensitivity analysis of the incremental cost-effectiveness ratio versus perioperative stroke and death and non-perioperative stroke rates. The perioperative stroke or death rates from ACST (2.9%), GALA (2.7%) and CREST (1.4%) are plotted. The dotted lines indicate the any territory stroke rates below which a strategy of early endarterectomy crosses the £20, 000 NICE per QALY threshold (orange line) or the £30, 000 per QALY threshold (red line). To remain cost-effective at low background stroke rates, perioperative stroke rates should intercept them above the respective line.

Alternative scenario	Cost difference (£)	Difference in quality adjusted life years	Incremental cost-effectiveness ratio (£/QALY)	Interpretation of results
Men younger than 75 years	360	0.12	3254	Early endarterectomy is cost effective compared with deferral
Women younger than 75 years	-746	0.17	NC	Early endarterectomy is the dominant strategy
Men older than 75 years	1894	0.03	71, 699	Early endarterectomy is not cost effective compared with deferral
Women older than 75 years	2574	-0.07	NC	Deferral of endarterectomy is the dominant strategy
Restriction of crossovers for symptoms	1326	0.13	10, 149	Early endarterectomy is cost-effective compared with deferral

Table 10: Results of subgroup and exploratory analyses. NC: Not calculated (a negative cost per QALY ratio). Note figures in this table have been rounded to two decimal places, however exact figures were used for calculation.

3.5   Discussion

The principal finding of this analysis was that the ICER for early endarterectomy for the average patient at the end of the ACST was predicted to fall under the £20-30 000

threshold set by the UK National Institute for Health and Clinical Excellence. This was clear for individuals under 75 years of age, who had a larger, more certain treatment effect and lived longer. Interestingly, it was no less cost-effective to offer early endarterectomy to women compared with men less than 75 years, a new finding based on the 10 year data. The model predictions appeared robust if the cost of procedure rose by 50% or if a per-protocol scenario was simulated but were sensitive to background stroke rates and operative hazards.

Restricting crossovers for patient preference increased the ICER for early surgery. This was because the extra 22% of crossovers to surgery in asymptomatic patients occurred late. The benefits of surgery declined with age, making late crossovers to surgery less effective than early surgery and more expensive than medical treatment.

In contemporary practice there is evidence for improved operative safety and reduced background stroke rates. With a safety profile similar to the 2008 GALA trial which was UK based and included trainee operators, early endarterectomy crossed the £20 000 per QALY threshold at background, any territory stroke rates of 1.5% per annum, similar to those seen in the final years of ACST (Figure 20). However, if background, any territory stroke rates fell below 1% per year, endarterectomy would cease to become cost-effective, even if operative safety improved to the levels seen in CREST (Figure 20). It will be interesting to see whether these very low stroke rates on medical therapy are replicated in the on-going randomised interventional trial SPACE-2, which is due to report shortly (50). Perioperative disabling stroke or death rates of 1% have recently been reported by the ACST-2 trial, suggesting perioperative safety has also improved in Europe (143).

This is the first UK cost-utility analysis for asymptomatic carotid surgery based on long-term, European, randomised controlled trial data. Data was taken from the more recent, European ACST rather than ACAS, for several reasons (27). Firstly, in the earlier ACAS the incidence of stroke on medical therapy alone was substantially higher (ACAS medical arm 5 year any stroke or perioperative death rate 17·5% versus 10·9% in ACST), suggesting the medical therapy was suboptimal. Secondly the follow up period for the ACST was a median of 9 years in comparison with 2·7 years in ACAS, which is important because the long-term benefit of endarterectomy was examined in the ACST. Thirdly, the ACST was a

larger trial of 3120 patients, with a large British population (144). Finally the ACST used the primary endpoint of all stroke or perioperative death, which accounted for the effects on other stroke subtypes following carotid revascularisation, which are sometimes ignored, e.g. contralateral events.

This study has limitations. Firstly, resource use for individual patients was based on averaged national reimbursement tariffs. Some author shave questioned the accuracy of these tariffs, in particular in relation to length of stay (145). Secondly, ACST did not include transient ischaemic attack (TIA) as an endpoint, which is associated with cost of £1339 (HRG code AA29Z), but no lasting change in quality of life (126, 127). ACAS demonstrated a reduction in TIA after endarterectomy and therefore excluding TIA may be regarded as a conservative assumption (27). Thirdly, loss of earnings was not included in this analysis, as they were not reimbursed by Personal Social Services (PSS). However, as the mean age of patients in ACST was 68 years, only a minority of patients were, in fact, working. In these patients there would be additional productivity savings in the surgical arm. Fourthly, this analysis looked at cost-per-QALY over a lifetime, but did not estimate the affordability of offering surgery to the population at risk. This will be explored in the next chapter. Finally, this analysis focussed on the cost-effectiveness of surgery over medical therapy for established carotid stenosis, not versus a non-investigative approach. This approach is difficult to model, because to establish event rates the condition must first be diagnosed. There are many centres which do not test for asymptomatic carotid atherosclerosis and their patient outcomes remain unknown. In the ACST, the costs of case finding were of pre-requisite in both arms and would thus cancel for ICER calculation purposes.

These findings are in line with an earlier intention to treat analysis from ACAS which calculated the average ICER for early endarterectomy to be $8000 (approximately £5000) (146). The present study estimated lower ICERs for men and women below 75 years of age than an earlier Swedish cost-utility analysis (125). The main differences between the studies relate to the choice of inputs. The Swedish study used the 5 year ACST data which showed little benefit for women, used higher costs for surgery, and excluded the extra costs and perioperative deaths in the crossovers from medical therapy to surgery. In a systematic review of health economic studies of symptomatic patients undergoing carotid surgery

reported ICERs were \$434-4100 (approximately £280-2600) per QALY (147). Asymptomatic carotid-surgery is thus less cost-effective than symptomatic carotid surgery by at least a factor of three.

Several factors need to be taken into account when allocating resources: strategic priorities, burden of disease, healthcare budgets and cost-effectiveness. The results of this analysis suggest that in 2010, it was likely that a strategy of early endarterectomy was cost-effective in comparison to a strategy of medical therapy for long-term stroke prevention in individuals under 75 years of age with established asymptomatic carotid stenosis in the UK. This finding was sensitive to background stroke rates and perioperative hazards, factors which change with time. If any territory stroke rates on contemporary medical therapy fall substantially below that seen in the ACST, surgery would cease to be cost-effective. For this reason, development of low-cost, practical risk stratification tools, specialist surgical training and audit of operative safety are required. Further information will be gained from individual patient level costing studies in the future.

4.0 Testing for asymptomatic carotid atherosclerosis

4.1 Who is the target population?

The previous chapter examined the cost-effectiveness of surgical treatment for asymptomatic carotid atherosclerosis. The main findings were that in 2010, patients less than 75 years of age demonstrated an acceptable cost per quality of life year gain for a strategy of early surgery versus deferral of surgery. However this was noted to be conditional on background stroke rates and perioperative safety. Both an estimate of the impact of testing and its affordability are important to understand before adopting such a strategy.

It was previously observed that in European men aged 60-79 years, moderate to severe carotid atherosclerosis (50-99% using NASCET measurements) is found in 2.3-6.0%, a similar prevalence to aortic aneurysmal disease (4.9%) (148) for which a UK National Screening Programme has recently been introduced for men aged 65. This chapter considers the costs and benefits of testing high-risk subsets of the population for asymptomatic carotid atherosclerosis.

Testing the elderly

Neither the United States of America nor the United Kingdom has a national carotid screening programme. A landmark US cohort study examined the effect of screening the elderly population in 1998. Importantly this study was performed in the pre-statin era, where stroke rates were typically higher and therefore the benefits of case finding were predicted to be greater.

The 1998 Cardiovascular Health Study recruited 5888 American Medicare patients 65 years or over from 4 states (103). These patients had a mean age of 73 years, 41% were male and they were predominantly Caucasian. The prevalence of those with a peak systolic

velocity of 150cm/s (≈50% NASCET equivalent stenosis) was 3.4% and those above 70% stenosis (≈250cm/s) was 0.5%. In the latter group, the 5 year risk of ipsilateral stroke was only 5%, half the rate in the Asymptomatic Carotid Atherosclerosis Study (ACAS) (27).

These findings effectively halted proposals for screening of elderly patients in the USA. It had several messages, firstly that screening the general elderly population was unlikely to be detect many candidates for surgery and secondly that background stroke rates were 50% lower than expected. One possible explanation for this discrepancy was that patients in ACAS may have been tested because of symptomatic disease elsewhere and were more likely to have a vulnerable plaque. Perhaps these persons with symptomatic arterial elsewhere should be tested instead.

Testing patients with symptomatic arterial disease elsewhere

In patients with symptomatic arterial disease elsewhere, conveniently already within a vascular service, the prevalence of asymptomatic carotid atherosclerosis is much higher (Table 11). Opinion is divided as to whether these individuals should be tested with ultrasound. The American Society for Neuroimaging and the US Society for Vascular Surgery strongly recommend testing in high prevalence populations, such as those with symptomatic peripheral arterial disease or those aged ≥65 years with multiple risk factors (96, 149). Those with risk factors alone are generally managed in primary care. Therefore a logical place to start would be those patients already in the vascular service.

Mean prevalence of 70-99% asymptomatic carotid atherosclerosis in European populations (%)	
Peripheral arterial disease	15 (4)
Abdominal aortic aneurysmal disease	8 (6)
Contralateral carotid atherosclerotic stroke	<6 (150)

Table 11: Mean prevalence of 70-99% asymptomatic carotid atherosclerosis in arterial patients seen in a vascular outpatient clinic. It is interesting to note that the prevalence of asymptomatic carotid atherosclerosis is not highest in those with a contralateral carotid atherosclerotic stroke.

Unlike the United States, the UK Royal College of Physicians draft 4th National Clinical Guideline for Stroke does not recommend testing at all (151). A special case is patients awaiting coronary artery bypass grafting, where further observational data are awaited (152). The published data so far, excluding patients with occlusion and those with recent symptoms, suggest the risk of ipsilateral stroke during cardiac surgery with a unilateral asymptomatic 50-99% stenosis is 2% and but that strokes after cardiac surgery do not typically fit the territory of the internal carotid artery (153, 154).

Those with contralateral carotid atherosclerotic symptoms already receive ultrasound assessment of both carotid arteries and thus there are no case finding costs for these individuals. However around one-quarter of these individuals will be severely disabled from a major stroke and thus may stand to gain little from surgery (155).

Claudicants (those with mild symptomatic peripheral arterial disease) have the highest prevalence of carotid atherosclerosis amongst vascular patients and are generally treated medically as 75% of them will not progress with conservative measures (4, 156). Hence they are recommended by the American Society of Neuroimaging, the Society for Vascular Surgery and the US Joint Societies consensus guideline as candidates for testing (96, 149, 157).

4.2   The cost of preventing one ipsilateral stroke

The cost of preventing one ipsilateral stroke was calculated as shown in Figure 21. For the purposes of this estimate the following assumptions were made: the European surgical intervention threshold of 70% NASCET stenosis (44), a prevalence of 70-99% asymptomatic carotid stenosis of approximately 15% in those with symptomatic peripheral arterial disease (4), a number needed to treat of 1 in 20 to prevent one stroke at 10 years (5) and costs equivalent to NHS reimbursement tariffs 2011-2012, reflecting average national resource use (3).   The results of this analysis are shown in Figure 21 and approximate £69, 000 for those with contralateral carotid stroke, £76, 000 for claudicants and £82, 000 for those with aortic aneurysmal disease.

One can compare this with the approximate cost per stroke saved with warfarin for atrial fibrillation of $28 to $68, 000 (£17, 823 - £44 000) (158).   The figure for carotid atherosclerosis is about double primarily because 20 endarterectomies are required to prevent one stroke.

Those with contralateral carotid stenosis are picked up at the time of their stroke, therefore should we proceed with testing claudicants in the vascular service?

- Prevalence of ≥70% NASCET stenosis in persons with symptomatic peripheral arterial disease ≈ 15% (4)

- Number of patients with ≥70% stenosis that require surgery to prevent one stroke = 1 in 20 , allowing for perioperative stroke or death (5)

- Number of claudicants screened to prevent one stroke = 6.7 x 20 = 133 persons

- **Cost of preventing one stroke in claudicants** = (UK reimbursement tariff for carotid ultrasound code RA23Z   x 133 patients scanned) + (UK reimbursement tariff for carotid endarterectomy code QZ04Z   x 20 patients undergoing operation) = (£49 x 133) + (£3473 x 20) = £6533 + £69, 460 **= £75, 993 ($116, 908)** (3)

- Prevalence of ≥70% NASCET stenosis in aortic aneurysmal disease = 8% (6)

- Number of patients with aortic aneurysmal disease screened to prevent one stroke = 12.5 x 20 = 250 persons

- **Cost of preventing one stroke in aortic aneurysmal disease** = (UK reimbursement tariff for carotid ultrasound code RA23Z   x 250 patients scanned) + (UK reimbursement tariff for carotid endarterectomy code QZ04Z   x 20 patients undergoing operation) = (£49 x 250) + (£3473 x 20) = £12, 250 + £69, 469 **= £81, 719 ($131, 490)**

- **Cost of preventing one stroke in those with contralateral carotid stroke** = UK reimbursement tariff for carotid endarterectomy code QZ04Z   x 20 patients undergoing operation = £3473 x 20 = **£69, 460 ($111, 880)**

Figure 21: The approximate cost per stroke saved through a policy of testing and operating on high risk groups within the vascular service. The main cost is the resultant surgery, not the testing itself.

4.3 Large scale testing

There are approximately 3 million claudicants in the UK. To minimise costs and make such a programme practical a narrow age window for testing is desirable. From the European Carotid Surgery Trial, the mean age for presentation with carotid territory symptoms was established as 62 years of age (22). Hence to save at least half of potential strokes testing should occur beforehand, say at around age 60.

If this strategy was adopted, the number of claudicants that would be eligible in England and Wales, along with approximate costs and sonographer time required is shown in Figure 22. The approximate costs would be £17.5m to prevent 231 strokes, by performing 4613 carotid endarterectomies per annum. This would mean a six fold increase in the number of carotid endarterectomies performed in the UK in asymptomatic patients (774 carotid endarterectomies in 2011) (111). The total number of strokes saved would only be approximately 0.2% of the total number of annual strokes quoted by the National Audit Office in 2010 (approximately 110 000 strokes per annum) (10).

- Prevalence of intermittent claudication in the UK = 4.6% (1)

- General population of England and Wales aged 60-64 years in 2010 = 3, 343, 000 persons (2)

- Estimate of number of persons in England and Wales aged 60 years = 3, 343, 000 / 5 = 668, 600 persons

- Estimated number of claudicants in England and Wales aged 60 years = 0.046 x 668, 600 = 30, 756 persons

- Estimated cost of carotid ultrasound = 30, 756 x £49 = £1, 507, 024 (3)

- Estimated time requirement for carotid ultrasound (assuming 15 minutes per scan) = (30, 756 x 0.25 hours) / 24 = 320 days

- Claudicants with 70-99% stenosis identified = 4613 endarterectomy candidates

- Estimated cost of 4613 endarterectomies performed = 4613 x 3473 =£16, 020, 949 (3)

- **Estimated number of strokes prevented at 10 years = 231 strokes**

- **Estimated total cost = £17, 527, 973 ($26, 956, 826)**

Figure 22: Estimated costs of testing and operating on claudicants aged 60 with 70-99% asymptomatic carotid atherosclerosis in England and Wales.

4.4   Discussion

Should we spend such a sum of money on performing such a large number of unnecessary endarterectomies and preventing so few strokes? There are several points to note. Firstly the main cost driver is not ultrasound investigation but resultant surgery. A better surgical selection tool would make the cost per stroke saved more acceptable. Secondly, bearing in mind 122 claudicants and 250 patients with aortic aneurysmal disease

need to be tested to prevent one stroke, it is unlikely to make any meaningful difference to an individual practitioner. It therefore necessitates a co-ordinated national programme, for any meaningful impact. Thirdly, there is an inherent prevention paradox, in that more strokes from carotid atherosclerosis will occur outside of the population selected for testing. These individuals can only be reached through active risk factor modification.

Some argue that we should restrict asymptomatic surgery to the 6% of persons found to have an incidental contralateral stenosis when they present with a cerebrovascular event (150), to minimise the effort and expense associated with extra case finding. However recent UK data from the OXVASC study demonstrated the annual stroke rate associated with a 50-99% NASCET stenosis in the territory of the contralateral carotid artery to the presenting symptom, was only 0.3% (98). This is lower than the 1.9% contralateral stroke rate found in the ECST trial (22). Other international cohorts on intensive medical regimes, have also demonstrated ipsilateral stroke rates of <1% per annum and suggest there is less rationale for testing this group of patients than previously thought (41, 99).

In the two best scenarios, preventing one stroke requires scanning 133 sixty year old claudicants and operating on 20 of them at a cost of approximately £76, 000 per stroke saved, or operating on 20 contralateral carotid stenoses at a cost of approximately £69, 000 per stroke saved. The main cost driver is the resultant surgery required to prevent one stroke. Some argue that the money would be better spent on monitoring compliance with medical therapy (159). However, others argue risk stratification for surgery is still a worthwhile endeavour (160). The latter is explored in the next chapter.

5.0 Review of carotid specific imaging biomarkers

5.1 Introduction

In patients with a recent cerebrovascular event, the degree of luminal stenosis is a powerful predictor of benefit with carotid endarterectomy to 8 years (161). Asymptomatic carotid atherosclerosis faces a problem because the link between high-grade stenosis and future stroke does not, for unknown reasons, provide the strong basis for intervention that it does for symptomatic disease (45). As a result, the number needed to treat to prevent one stroke at 5 years has been estimated at between 18-32 in asymptomatic patients , three times that in symptomatic patients with a similar degree of stenosis (5, 44, 61). A 2005 Cochrane Review suggested that patients who are high-risk for stroke secondary to carotid atherosclerosis need to be identified to justify endarterectomy in the future (162).

Current recommendations for intervention focus on extrapolating the results of randomised controlled trials (157). The approach of extrapolating trial subgroups to individuals has limitations (163). Firstly, data does not exist for real-life situations, e.g. patients with concurrent atrial fibrillation or post-radiotherapy stenosis. Secondly, ipsilateral stroke rates on medical therapy have reduced over the last 20 years (100). ACAS reported a 2.3% annual ipsilateral stroke rate (27), however in ACST this figure was only 0.96% (5). It is clear that we will not be able to extrapolate the benefits of CEA versus historical medical controls forever. Thirdly, although no certain benefit exists for endarterectomy in asymptomatic individuals over 75 years of age (5), stroke incidence, secondary to large artery atherosclerosis, rise markedly with age, especially over 65 years of age (19) . As the longevity of older individuals increases, we should think carefully whether we wish to exclude the entire population over 75 on the grounds of age alone, if they are likely to become disabled by stroke.

An alternative approach is to model risk. Such a model already exists for symptomatic disease (118) based on patient and atheroma characteristics and efforts are

underway to validate one for asymptomatic disease (164). Carotid-specific risk factors are important because most patients with carotid atherosclerosis possess conventional cardiovascular risk factors. For example in the European Carotid Surgery Trial (22), half the patients were hypertensive or smokers. These generic cardiovascular risk factors are not specific enough to select out the ≈1% of individuals who currently suffer an ipsilateral stroke each year from plaque rupture (165). Carotid-specific biomarkers are particularly attractive as they are not confounded by atherosclerosis elsewhere, unlike serum biomarkers. Development of low cost, practical, non-ionising imaging biomarkers would afford the following benefits:

1. A reduction in the number needed to treat with surgery or stenting

2. Personalised risk estimates for patients

3. Personalised therapy

4. Reduced surgical costs and complications by reducing unnecessary procedures

5. Identification of high risk carotid lesions in patients with another embolic source

6. A means of monitoring the response to plaque-stabilising therapies

7. New eligibility criteria for interventional trials

8. A move away from a binary symptomatic or asymptomatic classification towards a continuum of risk, which is closer to reality

The aim of this review was to outline recent developments in structural and functional imaging biomarkers for asymptomatic carotid atherosclerosis.

5.2 Cohort studies examining the risk of future ipsilateral stroke

Microembolic signals

A recent meta-analysis by the Asymptomatic Carotid Emboli Study investigators demonstrated a hazard ratio of 7.46 (95% CI 2.24 – 24.89, p=0.001) with low heterogeneity (I^2=29%) for the presence of microembolic signals in the ipsilateral middle cerebral artery and ipsilateral stroke over six cohort studies with follow up ranging from 9 months to 2 years with a range of 21-467 patients with 50-99% stenosis (20). The number of ipsilateral strokes during follow up was 12/118 (10%) in patients with microemboli versus 8/559 (1%) in those without, giving an approximate sensitivity of 60%, specificity of 84%, positive predictive value (PPV) of 10% and negative predictive value (NPV) of 99%.

Microemboli detection was for 40-60 minutes, with 5/6 studies following protocols described in a 1998 consensus paper (Figure 23). Inter-reader agreement was reported in one of these studies with a k=0.93 (166). This provides the strongest evidence (level 2a) to date for any risk stratification modality.

A single centre reported reduced microemboli prevalence in a cohort of patients on intensive medical therapy since 2003 which was associated with reduced stroke rates over the same period (167). It is unknown, without a control group, whether this reflects co-incidence or the effects of a new treatment regime. However transcranial Doppler has not translated into clinical practice for several reasons (168), including the need for a skilled operator, a one hour time period for the examination, a temporal sampling error (*i.e.* a negative result may be a result of inadequate examination time), lack of assessment of the ophthalmic, anterior cerebral or posterior communicating artery which also originate from the intracranial internal carotid artery, and lack of carotid specificity . Prior selection of patients for TCD, miniaturization and automation allowing overnight monitoring would help, as embolic signals are rare and the agreement for positivity over two consecutive one hour recordings is only fair (κ=0.49) (169). However in acute stroke research, where emboli counts are higher, microemboli detection has proved a valuable surrogate endpoint for proof of concept studies (170).
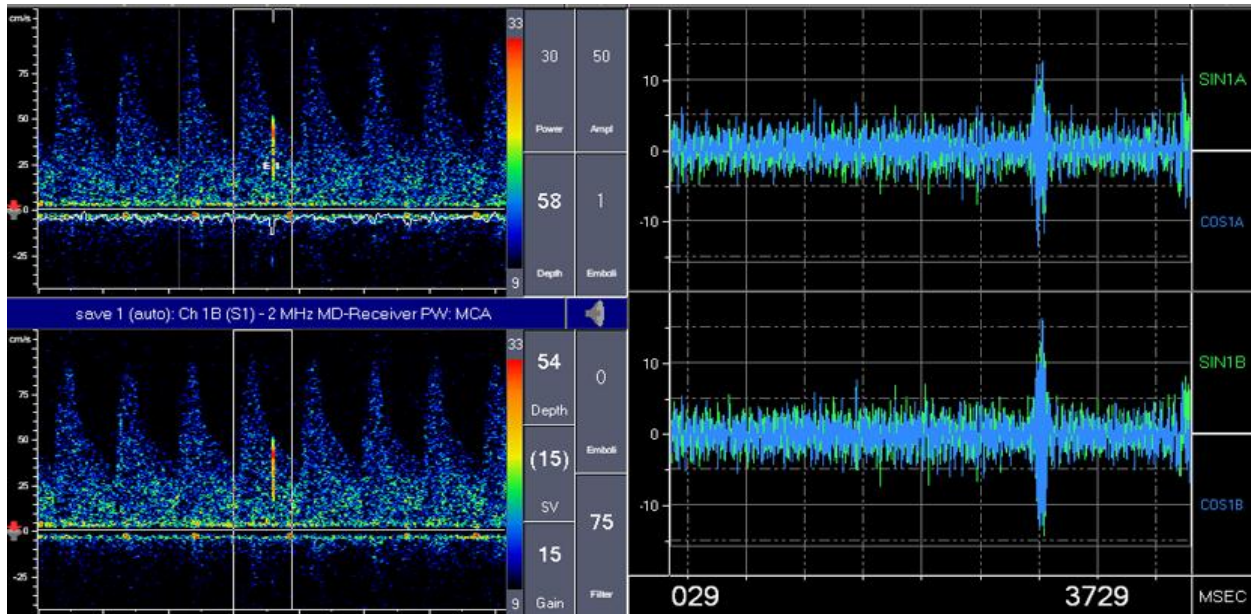
Figure 23: 2 MHz multi-gated transcranial Doppler recording from a 65 year old asymptomatic patient with 70% carotid stenosis through a trans-temporal window. Two gates, 4mm apart are triggered in succession by a discrete embolic particle. Image on the left is the spectral Doppler trace at 58mm (top) and 54mm (bottom) depth from the pterion. Image on the right is an amplitude measurement in dB, demonstrating an audible >10dB chirp coinciding with a discrete transient signal on both spectra. Source: Ankur Thapar (with permission from patient).

Plaque echolucency

Echolucency is the intensity of ultrasound echo from a plaque, coded as the brightness function on B-mode (brightness mode) ultrasound. Several large cohort studies have examined the relationship between echolucency and risk of future stroke (171, 172). In these, plaques were categorised as echolucent (representing lipoidic, haemorrhagic or necrotic constituents [Figure 24]) or echogenic (fibrous or calcified). Alternatively, echolucency has been categorised into plaque types (173, 174): type I (echolucent), type II (echolucent and heterogeneous), type III (echogenic and heterogeneous), type IV (echogenic) and type V (calcified with acoustic shadow). Finally, others have quantified echolucency using the pixel grey scale median (GSM), a continuous linear scale with 0 representing a pixel intensity similar to blood (black) and 190 representing a pixel intensity similar to vessel adventitia (white) (175).

The Cardiovascular Health Study reported in 1985 and followed 4,886 individuals (out of a total of 5888 recruited) aged over 65 for an average of 3.3 years (176). After controlling for cardiovascular risk factors, the adjusted Cox hazard ratio for ipsilateral non-fatal stroke was 2.78 (95% CI 1.36-5.69) for echolucent plaque, in comparison to those without carotid stenosis (note the comparator group was not other plaque types). The hazard ratios for isoechoic and echogenic plaques were not significant. In the echolucent group 30/856 (4%) of patients experienced a carotid territory stroke, versus 53/2382 (2%) of other plaque types.

The Trömso study reported in 2001 and as part of a larger cohort study followed 237 patients with >50% carotid stenosis for 3 years (171). The adjusted hazard ratio (adjusted for stenosis and cardiovascular risk factors) for ipsilateral cerebrovascular events in those with echolucent plaques was 4.56 (95% CI 1.10–18.93) and in those with predominantly echolucent plaques was 3.56 (95% CI 1.02-12.52), in comparison to an age-sex matched control group without carotid stenosis. Hazard ratios for the echogenic or predominantly echogenic groups were again non-significant. In the echolucent or predominantly echolucent groups, 18/130 (14%) of patients experienced an ipsilateral cerebrovascular event, versus 4/93 (4%) of patients in the echogenic and predominantly echogenic group. Inter-sonographer agreement regarding plaque type was moderate (κ=0.56, 95% CI 0.38-0.74). The Trömso investigators also found that total plaque area in the common and internal carotid artery was predictive of ischaemic stroke, however these variables did not add to a prognostic model based on clinical risk factors alone (c-statistic 0.75) (42).

After this point three groups directly compared echolucent and echogenic plaques. Hashimoto et al. reported in 2009 and followed 250 patients for a mean of 22 months (177). Normalized grey scale median values were measured in carotid plaques by a single observer at baseline and plaques grouped into thirds based on grey scale median and percentage of echolucent pixels. Kaplan-Meier analysis showed no significant difference in stroke free survival between grey scale median tertiles. However percentage of echolucent pixels was found to be a better discriminator (HR 3.4, 95% CI 1.2-10.0, p=0.026 after adjustment for stenosis). The plaques with the largest echolucent area had 7/8 strokes, however no details on group sizes were reported.

The Asymptomatic Carotid Stenosis and Risk of Stroke investigators reported in 2010 and followed 1,121 individuals with 12-99% NASCET stenosis for a mean of 48 months (164). Image normalisation allowed compensation for differences in B-mode gain, unlike earlier studies. Before normalisation, in echolucent plaques 24/419 (6%) of patients experienced an ipsilateral stroke versus 27/673 (4%) of other plaque types (178). After image normalisation, 28/409 (7%) of those with type I and II plaques experienced an ipsilateral stroke versus 23/683 (3%) with other plaque types, i.e. approximately the same.

Using grey scale median, plaque area, discrete white areas within an echolucent plaque (reflecting heterogeneity) and history of a contralateral cerebrovascular event, their predictive model gave an area under the curve (concordance or c-statistic) of 0.80 (95% CI 0.74-0.87). Inter-reader reproducibility was moderate (κ=0.61) for plaque type and for GSM the inter-reader intra-class correlation coefficient was 0.93. However no details on inter-sonographer reproducibility were given, the most important reproducibility parameter for real world practice. The ACSRS investigators proposed that plaque texture analysis could form part of a non-invasive risk stratification model, taking into account patient and plaque characteristics, requiring external validation in other cohorts or medical arms of randomised trials. This is the most recent prospectively derived model for asymptomatic disease and is being examined in the medical arm of the ECST-2 trial.

The ACES investigators reported in 2011 and followed 435 individuals with 70-99% stenosis without recent cerebrovascular symptoms for a mean of 1.8 years (179). Plaque images were graded visually by consensus and therefore no reproducibility statistic was available. Plaque types I and II demonstrated a Cox hazard ratio of 6.43 (95% CI 1.36–30.44) for future ipsilateral stroke. This finding was independent of cardiovascular risk factors and co-existent microembolic signals. The combination of microembolic signals and echolucent plaque was a strong predictor of future ipsilateral stroke, even after adjustment for cardiovascular risk factors (HR 10.61, 95% CI 2.98–37.82). In those with type I and II plaque, 8/162 (5%) experienced an ipsilateral stroke versus 2/266 (1%). This improved to 4/27 (15%) with ipsilateral stroke versus 6/401 (1%) when echolucency and microembolic signals were present together. The investigators suggested that these two variables could be used in combination in future studies.

Some studies have not found the same association between echolucent plaques and future stroke. Grönholdt et al. found echolucency (defined as GSM <75, the average value in their cohort) to predict future stroke in 135 symptomatic patients followed for 4.4 years but not in their smaller 111 asymptomatic patient cohort (Cox hazard ratio 1.0, 95% CI 0.4-2.8) (172). This may have been a real finding, or alternatively their subgroup may have been underpowered, the GSM threshold chosen may have been too high, or plaque heterogeneity may have been an issue. Similarly the ACST investigators followed 1,560 patients for a median of 9 years in the medical arm of a randomised trial (5). Plaque echolucency was assessed visually in a proportion of patients (defined as ≥25% of plaque area echolucent, rather than the 50% used in ACSRS and ACES). Echogenic plaques had identical stroke rates to those with echolucent plaques (1.8% per annum). However many patients were excluded from plaque analysis, which was additionally performed and interpreted at each centre, rather than in a core laboratory.

In summary, four large cohort studies have found an increased risk of future ipsilateral stroke with echolucent or predominantly echolucent plaques, with two disagreeing and one showing uncertainty. Two recent independent longitudinal studies agree that type I and II plaques present a higher risk for cerebrovascular events. Importantly, echolucency can be combined with clinical and/or other radiological markers to develop useful prognostic models. Because estimation of plaque echolucency is quick, safe and low cost, it may prove to be a useful gatekeeper examination for more advanced techniques and form part of a risk stratification algorithm.

Figure 24: Echolucent plaque in the internal carotid artery of a 72 year old asymptomatic man. Image analysis is performed using images normalised to blood and adventitia to provide a relative quantitative grey scale value. This helps overcome the subjectivity of different gain settings. This is a type I plaque (uniformly echogenic) with a grey scale median value of 6. Source: Ankur Thapar (with permission from patient).

Progression of stenosis on duplex

Whilst baseline stenosis appears only weakly related to future stroke, one study examined whether progression of stenosis was an independent risk factor for stroke using data from the medical arm of the ACST (180). Data from 1,469 patients with a mean follow up of 5 years was included. Carotid stenosis was graded locally (with varying criteria) into the following categories: 0-49%, 50-69%, 70-89%, 90-99% and occlusion. Fifty patients (3.4%) progressed through two categories and ten (0.7%) progressed through three categories. The adjusted hazard ratios for progression through two categories (seen in 50 patients, 3.4%) were 4.03 (95% CI 1.82 – 8.93) and through 3-4 categories 7.56 (95% CI 1.81–31.56). The confidence intervals were wide due to the small number of events. In patients who progressed through 2 or more groups 11/60 (18%) experienced an ipsilateral stroke, versus 197/2201 (9%) with progression through 1 category, no change, or regression of stenosis.

There are some important limitations to this study. Firstly, stenosis was not graded in a uniform manner in the ACST, e.g. what may be considered progression according to

ECST equivalent velocity measurements may be considered the same stenosis according to NASCET (161). Secondly, progression of stenosis through 2 categories identified a very small proportion (5%) of ipsilateral ischaemic strokes. Finally, this method of risk stratification necessitates repeat attendance, something that is less desirable than a single examination on resource and practicality grounds. For this reason progression of stenosis is unlikely to translate into clinical practice.

Magnetic resonance imaging

Takaya et al. followed 154 patients with 50-79% carotid stenosis without recent symptoms for a mean of 38 months (181). A combination of T1, T2 and proton density weighted 1.5T magnetic resonance images (MRI) was used to image structural vulnerability features of carotid plaques, with a slice thickness of 2mm. The unadjusted hazard ratios were 17.0 (95% CI 2.2-132.0) for a thin or disrupted fibrous cap and 5.2 (95% CI 1.6-17.3) for intraplaque haemorrhage in predicting any ipsilateral ischaemic cerebrovascular event. Adjusted hazard ratios were not calculated in this study, which was a limitation due to the small number of endpoints. The small number of artery-years meant that the confidence intervals were wide. No reproducibility statistics were quoted in this study.

The Sunnybrook group identified 90 men with 50-99% stenosis without recent symptoms retrospectively who had been followed for a mean of 24 months (182). A T1 fat suppression sequence, with a 1.5T magnet and 2mm slice thickness was used to subjectively characterise intraplaque haemorrhage, with an inter-reader κ=0.75. The Cox unadjusted hazard ratio for future ipsilateral stroke or TIA was 3.6 (95% CI 2.5-4.7).

MRI appears to allow in-vivo identification of gross plaque rupture and intraplaque haemorrhage, with promising results. However, along with ultrasound, current clinical scanners do not yet have the resolution to image the thin cap that Virmani et al. propose is the key finding in vulnerable plaques (64). For example, recent publications using a 1.5T magnet report a resolution of 0.39 − 0.49mm, with a slice thickness of 2-3 mm (183). In addition it remains unknown, because of the small number of events in these studies, whether plaque rupture and intraplaque haemorrhage are independent prognostic

variables. Finally, in many healthcare systems MRI will simply not be affordable or available on the large scale required to image the asymptomatic population.

Silent cerebral infarction on computed tomography

Unenhanced cerebral computed tomography (CT) has been used to identify silent brain infarction in the anterior and middle cerebral artery territory that may represent subclinical thromboembolism (Figure 25). Pattern A infarctions are defined as cortical or subcortical infarctions in or adjacent to the anterior or middle cerebral artery territory or basal ganglia/anterior thalamic infarctions and have been demonstrated more frequently in patients with symptomatic carotid stenosis (184) (185).

Two studies from one group reported the association between silent pattern A infarction and future ipsilateral stroke. The smaller study was a pilot study of 138 patients (186). The larger study included 821 European patients followed for a mean of 44 months (187). This showed an increased risk in individuals with pattern A infarction for developing future ipsilateral stroke, in comparison to those without. The larger study found an adjusted hazard ratio of 2.0 (95% CI 1.1-3.8, p=0.033) for pattern A infarction in predicting future ipsilateral stroke after stenosis and other confounders were adjusted for. There were no studies examining the incidence of silent infarction due to a requirement for repeated irradiation in asymptomatic individuals.

Whether this modest increase in hazard ratio justifies the use of CT imaging for silent infarction is debateable. It is approximately the same increase in risk conferred by a previous contralateral carotid atherosclerotic event (164) but certainly less than that conferred by the presence of microembolic signals (20). To the authors' knowledge there are no prospective studies of silent cerebral infarction in patients with carotid stenosis using MRI.
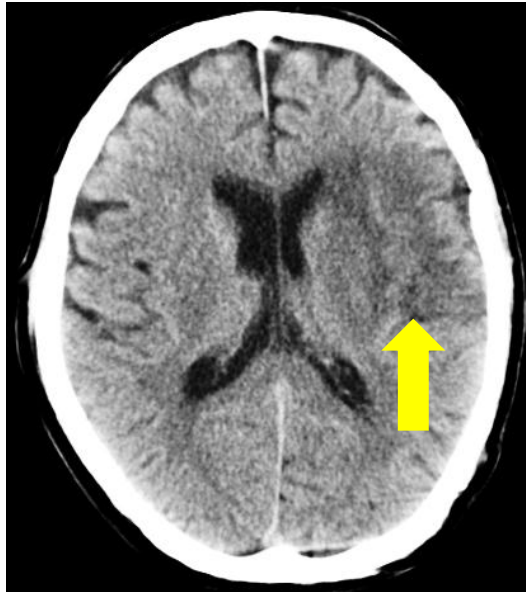
Figure 25: Established hypodense left middle cerebral artery infarction on unenhanced computed tomography. Source: Ankur Thapar (with permission from patient).

Plaque surface irregularity

Irregularities or ulceration thought to correspond to plaque surface rupture associated have been examined prospectively in three large studies. The North Manhattan Study examined 1091 asymptomatic patients with carotid plaque using duplex ultrasound and followed them for a mean of 6.2 years (40). Irregular plaque was an independent but weak predictor of any territory ischaemic stroke with an adjusted HR versus smooth plaque surface of 2.3 (95% CI, 1.0-5.4).

In contrast, the ACSRS investigators examined plaque ulceration using B-mode ultrasound and found no significant relationship between plaque ulceration and ipsilateral stoke (unadjusted univariate Cox HR 0.48, 95% CI, 0.15-1.55). (164)

One of the problems with two dimensional ultrasound is a spatial sampling error due to its uniplanar nature. Madani and Spence used three dimensional ultrasound to summate the total number of ulcers present in both carotid arteries (41). Using this technique the three year risk of stroke was significantly higher in those with 3 or more ulcers (18.2%)

versus those with less than 3 ulcers (1.7, p=0.02). However there were only six strokes in the study and it was a univariate analysis. In addition it is unclear how, using 3D B-mode alone, ulceration within echolucent plaques was assessed, as without luminal contrast ulcers are missed (188). The role of ulceration in predicting future events is therefore currently uncertain, but contrast imaging is likely to be the most reliable way to detect it (Figure 26).
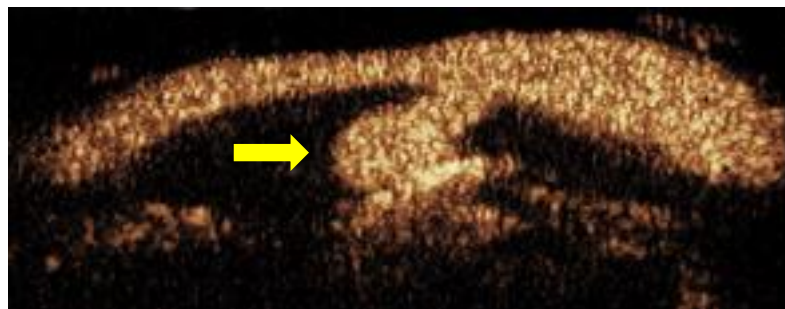
Figure 26: Large ulcer (yellow arrow) in an echolucent carotid plaque on dynamic contrast enhanced ultrasound. This is a longitudinal image taken on a Philips iu22 machine, using an L9-3 transducer a mechanical index of 0.06. Source: Ankur Thapar (with permission from patient).

5.3   New modalities under investigation in cross-sectional studies

Many new technologies under development provide additional functional information regarding neovascularisation, metabolic activity or inflammatory infiltration. Cross-sectional studies are the initial testing ground for these new modalities.   The discriminatory ability of these modalities can be assessed using the area under a receiver operator characteristic curve (c-statistic), or for categorical tests, the sensitivity, specificity, positive and negative predictive value. It must be stressed that cross-sectional studies retrospectively assess the features of plaque rupture. This is not the same as identifying vulnerable plaques, the precursors of future stroke. However cross-sectional studies allow validation of imaging modalities against endarterectomy specimens and are the initial testing ground for feasibility studies of new carotid plaque imaging technologies. However,

it should be recognised that ruptured plaques are a more advanced phenotype than the vulnerable or rupture-prone plaque.

Contrast enhanced carotid ultrasound

Contrast enhanced ultrasound is a hybrid (i.e. structural and functional) imaging technique that is performed using high-end ultrasound platforms, with the additional of an intravascular microbubble tracer. The tracer is injected intravenously during the scan and is commonly a fluorocarbon gas inside a phospholipid membrane. Tiny microbubbles, the size of an erythrocyte improve the Doppler return from the bloodstream. Non-linear pulse sequences used to eliminate static tissues are used to create a map of perfusion, based on change of pulse amplitude or phase inversion. With non-linear pulse sequences, the asymmetrical oscillation of single microbubbles within vessels can be detected at low mechanical indices (those at which microbubbles are not disrupted). Further technical details regarding contrast enhanced ultrasound imaging will be found in the next chapter.

In addition to delineating ulceration and near occlusion (Figure 26), contrast agents provide real-time visualisation of intraplaque microvessels (Figure 27). These are found in increased quantities in cross-sectional studies of culprit plaques (69, 84-86). Current quantification of plaque neovascularisation is hampered by an unknown concentration-time profile of contrast in the carotid lumen and out of plane motion. However qualitative and quantitative increases in plaque neovascularisation have been shown in a pilot study to be present in symptomatic patients (189), however the accuracy, reproducibility and predictive value of the technique have not yet been established. In addition quantification of ultrasound perfusion is as yet in its infancy and requires a skilled operator to acquire a video loop. Microbubbles targeted to cell surface receptors upregulated in atherosclerosis such as P-selectin and VCAM-1 are also currently under study in pre-clinical models (190), however are not currently approved for human use.

Because contrast enhanced ultrasound can image both the plaque surface and intraplaque perfusion and potentially even inflammation at low cost, with relative safety,

speed and in the outpatient setting, this technique is currently being trialled for examination of the carotid.
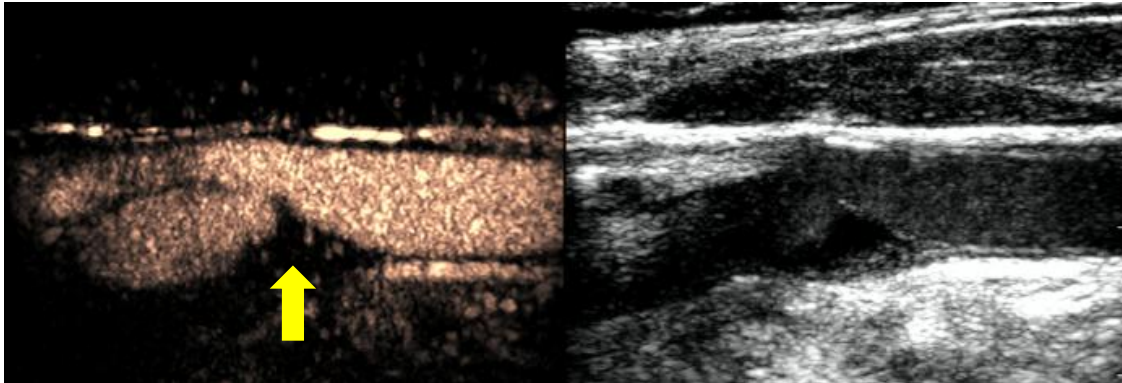
Figure 27: Longitudinal image from a dynamic contrast enhanced ultrasound examination of a symptomatic 75 year old lady demonstrating microbubble movement throughout plaque (yellow arrow). The intravenous contrast agent used is SonoVue™ (Bracco, Milan, Italy) at a dose of 2ml and a non-linear imaging mode is selected with a low mechanical index of 0.06 on a Philips iu22 machine. Source: Ankur Thapar (with permission from patient).

PET – CT of carotid plaque

Positron emission tomography, using radiolabelled ligands has been used to image inflammation within carotid plaques semi-quantitatively. Initially the conventional radioligand 18F-FDG-glucose was used to demonstrate increased glucose metabolism within symptomatic lesions, in comparison with a contralateral asymptomatic artery (191). Furthermore lipid rich plaques displayed more uptake than fibrous or calcified lesions (192). More recently, the radioligand, (11)C-PK11195, targeted to the peripheral benzodiazepine receptor, found in activated macrophages, was successfully used to identify culprit lesions (193). Activity within a region of interest was normalised to that in the adjacent jugular vein. This showed a sensitivity of 78%, specificity of 74%, negative predictive value of 91% and positive predictive value of 50% in a small study of 32 patients. PET-CT has been used in one prospective study on cancer patients with an endpoint of coronary and cerebrovascular events, however there was no carotid or stroke specific analysis (194). Whilst showing good

discriminatory ability in pilot studies, PET-CT has the disadvantages of radiation, cost and need for a cyclotron facility. In asymptomatic patients it is likely to remain a research tool for validation of other, more translational, techniques.

MRI of carotid plaque

Investigators have used a variety of MRI pulse sequences to image carotid plaques in symptomatic and asymptomatic individuals. Intraplaque iron in animals has been demonstrated to accelerate atherosclerosis through oxidation of low-density lipoprotein (195) and has been investigated in a pilot study in humans using T2* MRI, a technique for imaging intraplaque iron (196). Twenty-eight asymptomatic and eleven symptomatic patients with carotid atherosclerosis were imaged and operative specimens examined for iron content. There was a significant shortening of T2* relaxation time to 20.0 ms (SD 1.8) in symptomatic patients versus 34.4 ms (SD 2.7, p=0.0006) and a corresponding decrease in histological Fe^{3+}. The authors concluded that imaging of reactive iron species is feasible and may have discriminatory value in the assessment of atherosclerotic plaque, through identification of areas of intraplaque haemorrhage.

Howarth et al. used the activated macrophage marker Sinerem (an ultra-small paramagnetic particle of iron oxide or USPIO) to assess macrophage infiltration in symptomatic and asymptomatic patients in a 1.5T magnet (197). In a pilot study of 10 symptomatic and 10 asymptomatic patients, mean signal drop (representing USPIO accumulation) was 36% (95% CI 28.2-44.0%, p<0.001) lower in the symptomatic group. Signal drop showed a moderate correlation with fibrous cap thickness (r=0.51). The investigators concluded that USPIO imaging could identify inflamed plaques with a thin cap. USPIO imaging requires two MRI scans performed 36 hours apart and a proportion of scans remain difficult to interpret.

It is clear that MRI represents a powerful functional imaging modality, however due to cost, practicality and expertise has not yet translated into a prognostic study.

Computed tomography of carotid plaque

Computed tomography (CT) in combination with computed tomographic angiography (CTA) is widely used in hyperacute stroke and can be used to grade stenosis and plaque surface and texture characteristics. Several cross-sectional studies have assessed differences in carotid plaque features ipsilateral to a symptomatic cerebral hemisphere using the asymptomatic side as a control. In the largest study to date, 673 patients with acute ischaemic stroke were retrospectively analysed (198). In a multivariate logistic regression model adjusting for age, sex and degree of stenosis, only the presence of luminal thrombus, predicted the side of the symptomatic artery (OR 3.1, p=0.048). Extensive calcification predicted the asymptomatic side (OR=0.69, p=0.047).

The presence of increased calcification in asymptomatic arteries was independently found in another ex-vivo CT study of 48 carotid atheromas which underwent concurrent histological analysis for calcium content and macrophage staining (199). Mean percentage area calcification was 48% (SD19%) in the symptomatic group and 24% (SD20%) in the asymptomatic group (p<0.05).

Serfaty et al. recently quantified attenuation of plaques using Hounsfield units, finding that plaques of higher attenuation were more likely to be found in asymptomatic hemispheres (OR 1.54, 95% CI 1.17 − 2.04, p<0.002) for symptomatic status for each 10 point decrease in Hounsfield units (200).

However not all investigators concur that calcified plaques are stable, with one group demonstrating an exponential increase in odds of symptomatology with increasing calcified plaque volume (201).

Plaque structural components can also be graded on CT. In a retrospective analysis of 40 clinically carotid-territory stroke patients, a computer algorithm was used to extract plaque texture features (202). Multivariate logistic regression demonstrated that fibrous cap thickness (OR 0.11, 95% CI 0.06-0.16, p<0.013, the number of lipid clusters (OR 1.58, 95% CI 1.15-2.18, p<0.005) and carotid wall volume (OR 1.58, 95% CI 1.00-2.49, p<0.017) independently identified the symptomatic artery with a c-statistic of 0.83. The findings of fissured fibrous cap were also reported to increase the odds of symptomatic status by 3.9,

p=0.0032 by another group retrospectively studying CT images of 36 symptomatic and 96 asymptomatic patients. Inter-reader agreement for detecting fibrous cap fissuring was good (κ=0.78).

It appears that CT can characterise plaque texture, with preliminary promise, however again, exposing asymptomatic patients to large disease of ionising radiation around sensitive structures such as the thyroid, is unlikely to be viewed as a safe endeavour. In addition the axial resolution of high resolution CT in current clinical use is at best 0.6mm, rather too large at present to measure the fibrous cap in vulnerable plaques (203).

Juxtaluminal necrotic core

A juxtaluminal necrotic core is a recognised histological feature of culprit carotid lesions (63). In a recent study of 31 plaques, an echolucent area near the plaque luminal surface was shown to be associated with histological evidence of a thin fibrous cap (<80μm) and a juxtaluminal necrotic core (37). This juxtaluminal echolucent area was measured in a cross-sectional study of 139 asymptomatic and 185 symptomatic patients using US with pixels colour mapped to improve visualisation (204). A juxtaluminal echolucent area of 8mm^2 provided a sensitivity of 71% and specificity of 67% for symptomatic status. The c-statistic was 0.73 for juxtaluminal black area >8mm^2 in this study.

In a further recent study, surface echogenicity was compared in 67 symptomatic and 117 asymptomatic carotid plaques of between 50-99% stenosis (205). Using ROC curve analysis, surface echogenicity (c-statistic 0.74, 95% CI 0.67-0.78) appeared marginally more discriminatory of symptomatic status than GSM (c-statistic 0.69, 95% CI 0.62-0.69). However the study was underpowered to determine whether this was a significant difference.

Plaque surface motion analysis

Plaque motion can be tracked with US and is hypothesised to reflect plaque instability. This again is thought to be important within heterogeneous plaques, whose components respond differently to shear stress, leading to fissuring, a precursor to rupture. In a pilot study using 4D (i.e. real-time volumetric) ultrasound, 23 asymptomatic and 22 symptomatic patients were studied with 50-99% carotid stenosis (71). Mean change in surface velocity was calculated through ECG gated, motion tracking of voxels representing the plaque surface. The mean differential surface velocity was significantly higher in symptomatic patients (3.85mm/s [range 2.1-6.3mm/s] versus 0.58mm/s [range 0-1.7mm/s], p<0.001). Similarly in a pilot study using 2D ultrasound to image 10 symptomatic and 9 asymptomatic patients, plaque surface regions of interest were tracked using two dimensional ultrasound video loops of carotid plaques (206). A higher mean relative surface velocity was found in symptomatic patients versus asymptomatic patients: 2.85 (SD 1.69) versus 0.52 (SD 0.53) mm/s, p<0.0003. Plaque motion analysis requires 10 cycles of systole and no movement during the acquisition. In addition analysis can be time consuming and possibly for this reason has not yet been tested in a prospective study.

5.4   Discussion

A carotid-specific imaging biomarker should have the following ideal prerequisites: a good sensitivity and specificity for identification of plaques predating future ipsilateral stroke, safety (*i.e.* non-ionising), practicality, and capacity for large scale testing at an acceptable cost. It is clear that no one imaging modality yet fulfils these criteria (Table 12). The findings of this review are that the most promising modalities to date appear to be microembolic signals and plaque echolucency. These have been used alone or in combination to develop predictive models for ipsilateral ischaemic stroke. Combinations of independent variables derived from these modalities appear to identify patients at highest risk. This is to be expected, as plaque rupture is a multi-stage process and each variable adds a single piece of structural or functional information.

It is thus possible that an algorithm for investigation may be used in the future, analogous to testing ABPI first, before duplex, in patients with peripheral arterial disease. It is likely that carotid ultrasound will be the first line investigation, with either TCD or carotid MRI as a more specific test, based on the criteria above.

There are some criticisms of the use of imaging biomarkers. Firstly, some argue that background stroke rates have fallen so low that testing asymptomatic patients for vulnerable plaque is unlikely to be cost-effective. However, development of risks stratification tools may also benefit recently symptomatic patients with 50-69% stenosis. This population is the perfect testing ground for new imaging biomarkers, as clinicians are uncertain how to select for surgery, the prevalence of future ipsilateral stroke is approximately 3% per annum (161), patients do not require case finding as they present with symptoms and plaques are expected to be at a more advanced stage. Secondly, risk modelling suffers from an accepted paradox. Take the following scenario: a new risk stratification tool identifies 10% of asymptomatic patients with an annual stroke rate of 10%, whilst the remaining 90% have an annual stroke rate of 2%. Therefore after 5 years of follow up, 0.1x10x5 = 5 strokes occur in the high risk group and 0.02x90x5 = 9 strokes occur in the low risk group. Even by operating on all of the high-risk patients successfully, only a minority of strokes will be saved, because the high-risk group is so small. To prevent the majority of strokes, primary prevention measures have a greater potential impact in the low and moderate risk groups. Thirdly, as highlighted by Naylor, selecting higher risk plaques for surgery, means higher risk operations (207). In fact in the European Carotid Surgery Trial, two separate risk models were created, the first for background stroke rates and the second for operative stroke or death (118). Patients and surgeons must be aware that this is an expected consequence of operating on vulnerable plaques and should be taken as a caveat to risk modelling.

Carotid imaging biomarkers are still required in the asymptomatic population and those with moderate symptomatic stenosis. Would we operate on an asymptomatic abdominal aortic aneurysm without asking about vessel diameter? In the same way, we should establish which patients will benefit most from carotid revascularisation in the future.

Imaging Modality	NHS HRG 2010-2011 tariff / £	Benefits	Problems	Future direction
Transcranial Doppler	66	Level 2a evidence for medium term stroke risk prediction	Temporal sampling error, 1 hour examination time	Miniaturization, automation, overnight recording
Carotid ultrasound	51	Evidence of echolucency and increased risk of future stroke from four cohort studies and infrastructure already in place	Standardization of examination required, sonographer training, little functional information gained	Assessment of plaque texture in prospective cohorts and validation with MRI
Contrast enhanced carotid ultrasound	51 + 50 contrast = 101	Low cost, safe, outpatient based, hybrid imaging modality	Non-linear propagation affecting far carotid wall	Improvements in tissue subtraction, pulse sequencing and quantification
Computed tomographic angiography (plaque)	129 scan + 21 = 150	Accurate identification of calcification and ulceration	Limited resolution, radiation exposure	? Stop
Computed tomography (brain)	93 + 21 = 114	Quick acquisition Applicable to most patients	Weak relationship to future stroke, radiation exposure, non-specific	? Stop
Magnetic resonance imaging (plaque)	230 + 23 = 253	Imaging of intraplaque haemorrhage, cap disruption, activated macrophage concentration, non-irradiating	Cost Availability	Validation of ultrasound findings Phase II drug trials
PET-MRI	PET 565 + 57 MRA 230 + 23 = 875	Imaging and localisation of inflammation	Radiation exposure, cost, availability of Cyclotron	Phase II drug trials, targeted ligands

Table 12: NHS reimbursement tariffs 2010-2011 for carotid imaging (3). Costs of CT, MR and PET have additional reporting fees, shown as an additional cost.

6.0   Late phase contrast enhanced ultrasound

6.1   Introduction

Contrast enhanced ultrasound is an attractive modality with which to image carotid atherosclerosis. It has the following advantages: simultaneous structural and functional imaging, low-cost, relative safety, combination with B-mode imaging, minimal requirement for new infrastructure and ability to perform it in the outpatient setting, as part of a one stop service Table (13). It remains relatively unexplored in carotid atherosclerosis and is considered further in the next few chapters.

Ultrasound imaging can be enhanced by the use of contrast agents (microbubbles) in a similar way to x-ray, MRI and CT. This provides the following benefits:

1. Improved echo intensity from the lumen. This can improve detection of structural features such as plaque ulceration and dissection (Figure 28) (208). A further chapter will be devoted to assessment of ulceration with dynamic contrast enhanced ultrasound (DCE-US).

2. The ability to image perfusion semi-quantitatively within plaques (Figure 28) (209). Shah et al. have demonstrated a correlation of 0.6 between ultrasonic neovascularisation (expressed as an ordinal variable from 1-3) and histological microvessel density (210).
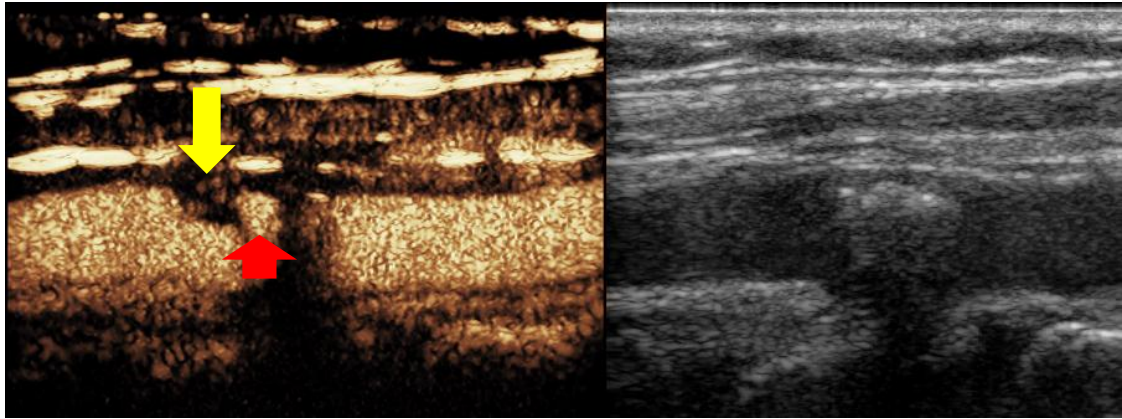
Figure 28: Imaging the microcirculation within carotid atheroma using a 2ml intravenous bolus of SonoVue and a low mechanical index of 0.06 to avoid bubble destruction. The image on the left is a longitudinal, dynamic contrast enhanced ultrasound image of a near wall carotid plaque and that on the right the corresponding B-mode image. Individual resonating microbubbles are seen in the echolucent portion of the plaque (larger yellow arrow) adjacent to an ulcer (smaller red arrowhead). Source: Ankur Thapar (with permission from patient).

3. Microbubble adherence has been demonstrated to activated leucocytes *in-vitro* after 3 minutes and phagocytosis after 15 minutes with the phagocytosed microbubbles remaining detectable with ultrasound (211, 212).

4. The ability of the some lipid coated contrast agents to adhere to activated leucocytes *in-vivo* through complement and integrin mediated binding and thus image sites of inflammation (213).

5. Negatively charged microbubbles have been demonstrated *in-vivo* to adhere to damaged endothelium which has shed its negatively charged glycocalyx (a surface glycoprotein) layer within 30 minutes (214).

Advantages	Disadvantages
Avoidance of radiation	Adds time, cost and training for the sonographers
Quicker examination than TCD / CTA or MRA	Requires skilled operator and reader for video loop acquisition/analysis
Cheaper than cross sectional imaging	Requires intravenous cannula
Real-time information regarding perfusion	Inability to image thin fibrous cap
Ideal if follow up scans required, e.g. treatment monitoring	No targeted microbubble in clinical use, so lack of specific binding
Non-nephrotoxic	Calcification produces a pronounced artefact
Outpatient clinic based	Currently no accepted method of quantification
Portable	Input function unknown, so perfusion measurements are relative not absolute
Pure intravascular agent	Uniplanar

Table 13: Advantages and disadvantages of using contrast enhanced ultrasound for risk stratification.

Contrast agents historically began with agitated saline. This followed from the observation in 1968 that ultrasound signal intensity was augmented by the injection of iodinated contrast (215). This was due to the formation of microscopic gas bubbles (microbubbles) which backscattered ultrasound signal, in a similar fashion to spontaneous microemboli, improving the signal to noise ratio within blood vessels. This method is still used to detect right to left cardiac shunts in clinical practice today (Figure 29):
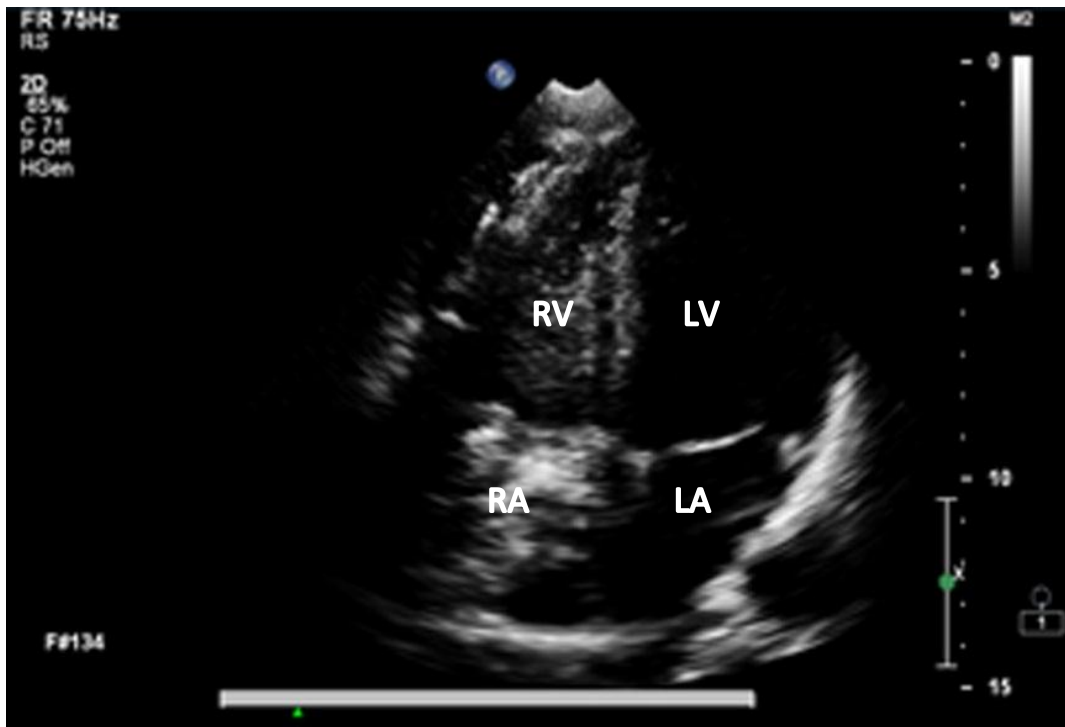
Figure 29: Four chamber echocardiogram demonstrating the effect of lung filtration on saline-air microbubbles. Standard B-mode image using 1ml intravenous bolus of agitated saline. Saline is much less stable across the lungs and therefore the left side of the heart remains black, despite Valsalva to raise intra-thoracic pressure and detect a shunt. Newer third generation contrast agents cross the lungs and opacify the systemic arterial circulation. RA= right atrium, RV=right ventricle, LA=left atrium, LV=left ventricle Source: Ankur Thapar (with permission from patient).

In 1950, Epstein and Plesset demonstrated that air bubbles readily diffuse gas and collapse within seconds to minutes in water (216). This lead to the development of a phospholipid shell and use of a high molecular weight gas to improve microbubble stability by reducing diffusion of gas out of the bubble, e.g. the third generation agent SonoVue contains sulphur hexafluoride. SonoVue is more stable across the pulmonary vasculature and appears in the left ventricle, unlike air bubbles which are larger and less stable (Figure 29) and are filtered out almost completely by pulmonary capillaries, which are in the order of 10µm in diameter (217).

In most patients SonoVue is well tolerated (Table 13):

Frequency >1%	Frequency <1%
Headache	Chest/abdominal/back pain
Nausea	Rash
Injection site pain	Hyperglycaemia
	Transient visual disturbance
	Paraesthesia
	Vasodilation

Table 13: Side-effects of SonoVue (from summary of product characteristics).

Contra-indications to SonoVue include previous adverse reaction to sulphur hexafluoride, recent acute coronary syndrome, recent coronary revascularisation, acute or severe cardiac failure (New York Heart Association grade III-IV), arrhythmia, right to left cardiac shunts, severe pulmonary hypertension, uncontrolled hypertension, adult respiratory distress syndrome, pregnancy and lactation. There have been three reports in cardiac patients of transient shock and arrhythmia (218). In animal studies, bubble disruption does not appear to cause damage to the microcirculation (219). Importantly there are no reports of SonoVue causing stroke.

Technical considerations

Microbubbles oscillate in an asymmetrical fashion in response to an incident ultrasound beam with greater expansion than compression. This is termed a non-linear response. Tissue is relatively incompressible and returns linear echoes. Developments in ultrasound signal processing have allowed subtraction of these linear tissue echoes to produce a non-linear tissue subtraction image depicting perfusion, a similar concept to digital subtraction angiography but at a capillary level. This is achieved by alternating the amplitude or phase (positive and negative deflection) of successive waves and subtracting those that return from those that are sent (220). Waves which return from tissue cancel completely, whereas those that return from microbubbles are phase and amplitude shifted, therefore do not cancel, generating a bright pixel.

SonoVue remains within the blood pool and 75% of a 2ml dose is excreted through the lungs in 11 minutes in healthy persons (221). In a preliminary study the median duration of useful sonographic enhancement in the dynamic phase was 3.9 minutes (208). The frequency at which SonoVue microbubbles resonate is around 5MHz and thus a transducer encompassing this range is required, for the greatest microbubble sensitivity (222).

Mechanical index (MI) is defined as the ratio of the peak negative ultrasound pressure / square root of ultrasound frequency wave (222). At low mechanical indices microbubbles resonate without rupture, e.g. SonoVue microbubbles resonate around 5-7Mhz. At high mechanical indices microbubbles burst leading to a highly non-linear signal, aiding detection of minute concentrations. In the era before low mechanical index imaging, high mechanical index imaging was used to sweep large organs such as the liver, allowing adjacent areas to refill as others were scanned, however today is generally delivered as a short burst, over milliseconds known as "flash" imaging. Imaging can be performed in the dynamic phase (the first few minutes of imaging, when contrast is flowing through the carotid, followed by the jugular) or after luminal contrast levels fall to near zero – the late phase (see later in this chapter).

Dynamic contrast enhanced ultrasound is performed at low mechanical index and when microbubble concentration is high, leading to a greater echo (e.g. the first few minutes in the carotid). It can be performed using bolus injections or a constant infusion, a technique known as disruption-replenishment (223). The advantage of bolus injections are that the technique can be performed by one person, no infusion pump is required, the examination is quicker and visible perfusion is initially entirely arterial. The transducer is held still over the plane of interest and the resulting DICOM (Digital Information and Communications in Medivideo) video loop can be quantified, with parameters such as peak intensity, rise-time and wash-in time derived from the time-intensity curve of a user selected region of interest (Figures 31 and 34). However because bolus injections vary due to mixing within a patient's blood volume and loss through pulmonary transit, a normalisation process is required to overcome variations in the input function (the bolus concentration over time).

Normalisation converts an absolute quantity e.g. intensity, into a relative quantity, e.g. intensity relative to the lumen or adjacent structures. Normalisation is usually done through comparison with an adjacent healthy tissue at a similar depth. This accounts for any effects of depth on the incident ultrasound beam intensity and phase. In the carotid, adventitia returns a strong echo and can have an increase in perfusion itself, making it less suitable for normalisation. Hence the lumen is sometimes used as a reference point. This technique also allows a measure of the input function to be known. However there are problems with using the carotid lumen, in that it is an order of magnitude more intense than the plaque (Figure 30). This means a small variation in carotid intensity can dramatically change the denominator for normalisation. Bolus techniques are more suitable in organs with a dual blood supply (e.g. liver) as isolated arterial perfusion can visualised, instead of mixed arterial and venous inflow.
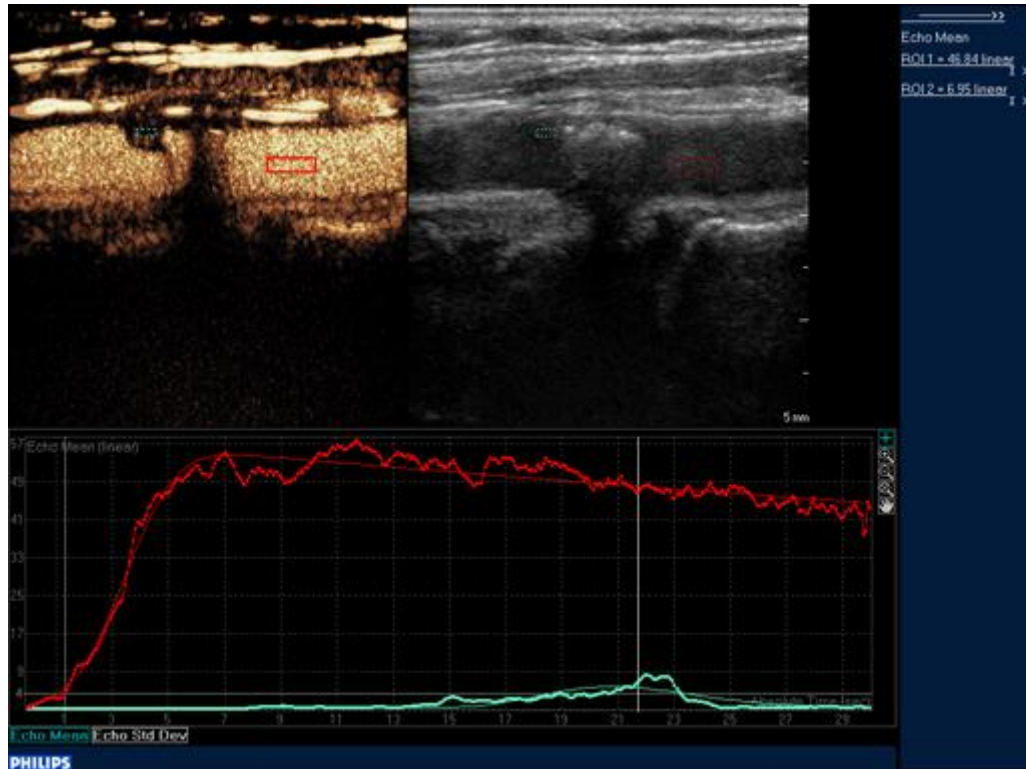
Figure 30: An example of a time-intensity curve of the common carotid lumen (red curve, top) and that from a predominantly near wall atherosclerotic plaque (blue curve, bottom). The raw intensity of each corresponding region of interest (red or blue box on the contrast enhanced image (above left) has been plotted against time in seconds. A thirty second period is depicted (x-axis), to avoid the effects of recirculation, which in effect would cause a second bolus. Perfusion is measured in the near wall of the carotid and is seen to incur a short delay, after the common carotid fills. This short delay is the difference in the arrival time of contrast in the macrocirculation (carotid) and the microcirculation (vasa vasorum) and is the order of a few seconds. Source: Ankur Thapar (with permission from patient).

Conversely with disruption replenishment, an approximate steady state infusion is set up (Figure 31) to which a high mechanical index flash is applied to cause microbubble disruption within the field of view. This allows for refilling (replenishment) of microbubbles with a near constant input function. This allows several planes to be imaged, unlike bolus injection which is strictly uniplanar and is important in heterogeneous structures. The disadvantages of this technique are that it requires an infusion pump which adds time and cost to the examination. In addition, contrast recirculates around the body and back into the carotids, causing in effect a slowly rising input function. For this reason it is easiest to concentrate on the wash in phase of a bolus injection (the upslope and plateau lasting

approximately 30s), before there is an opportunity for recirculation. These two techniques are illustrated in Figures 31 and 32.
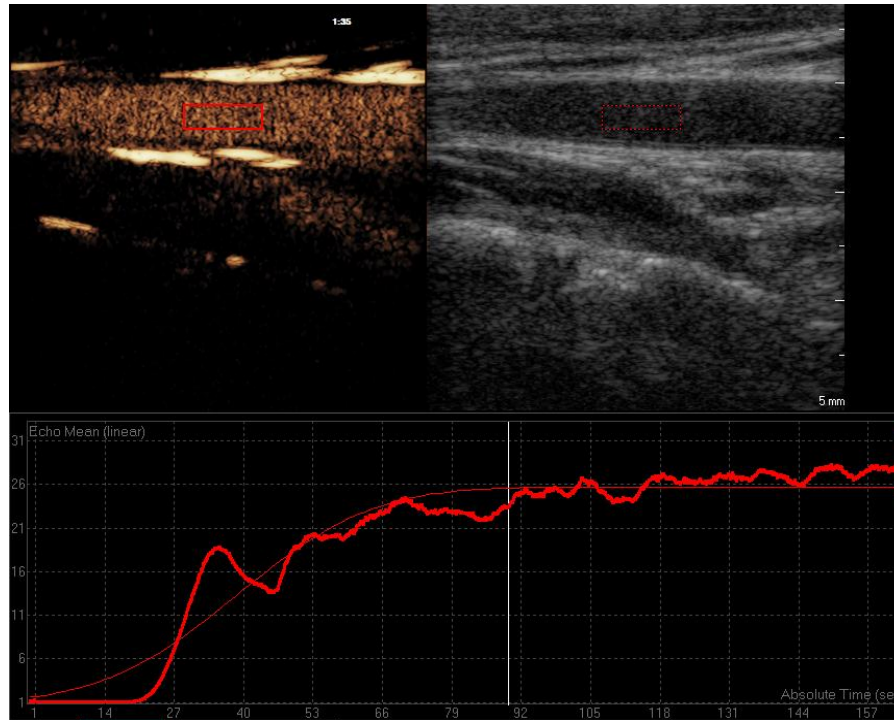
Figure 31: Time-intensity curve of the common carotid using an infusion pump. After 90 seconds steady state was reached in this example as illustrated by the wash in curve. After this point a high mechanical index flash pulse can be given to clear the microcirculation of bubbles. This allows them to refill at a constant velocity from a near steady state input function. Source: Ankur Thapar (with permission from patient).
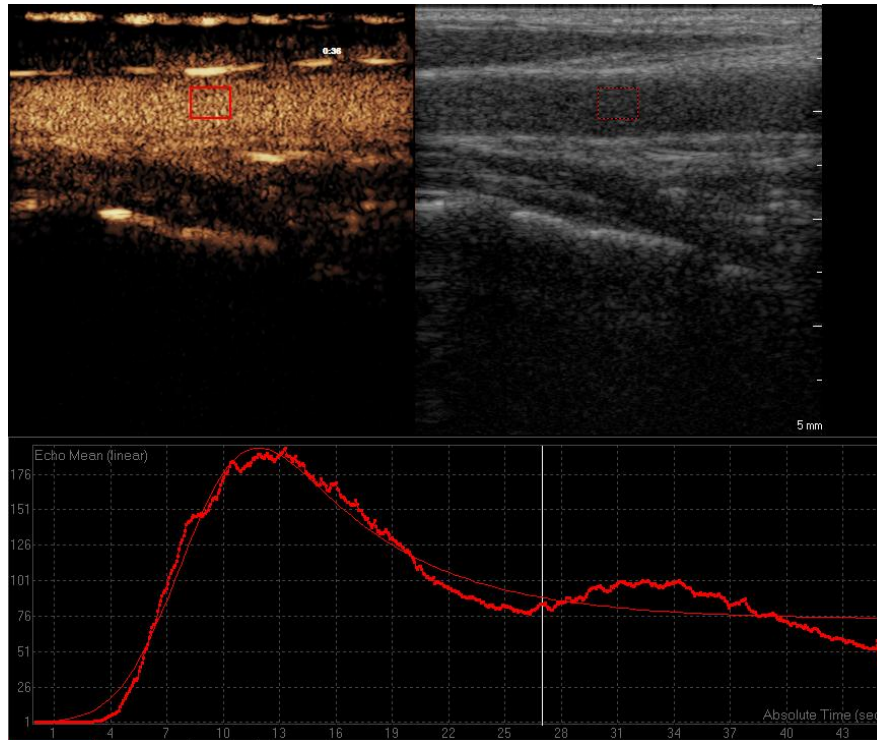
Figure 32: Time-intensity curve demonstrating the effect of a 2ml SonoVue intravenous bolus. Recirculation is seen to occur around 30 seconds after the injection. For this reason analysis of the bolus is performed upto the end of the first peak. Transducer position and lack of movement is critical during this period. Source: Ankur Thapar (with permission from patient).

The analysis of contrast perfusion imaging relies on indicator dilution theory (224). This assumes that the indicator produces a signal proportional to its concentration, there is minimal microbubble destruction and that the microbubbles themselves do not obstruct capillaries. The following time-based or intensity based ratios between the plaque and lumen have been proposed to quantify flow parameters: rise time (reflecting flow velocity), wash in time (flow velocity) and peak intensity (microvessel density) (Figure 33).
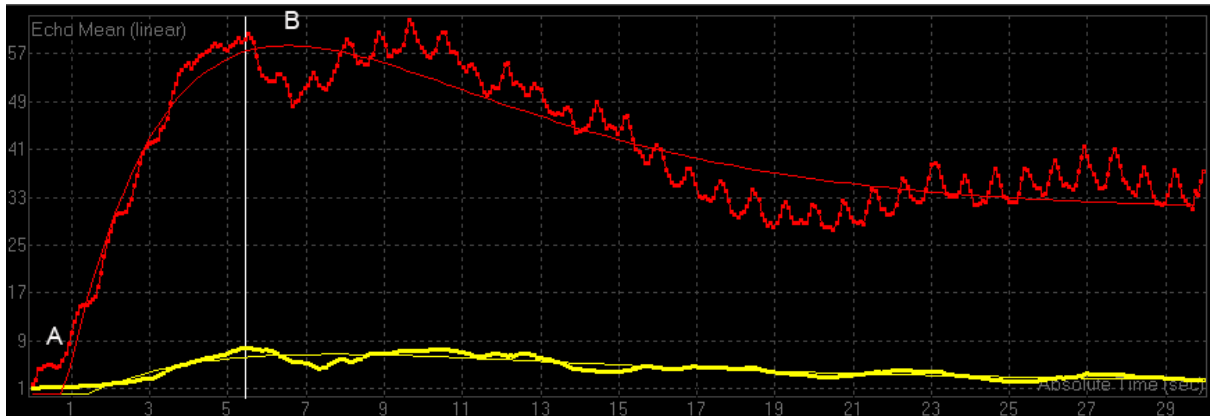
Figure 33: Example time-intensity curve from the common carotid lumen (red top line) and a carotid atherosclerotic plaque (yellow bottom line). A lognormal curve has been added to each data set for analysis of perfusion parameters. The intensity difference at B represents the peak intensity. The time difference between A (5% peak intensity) and B (95% peak intensity) represent rise time. Source: Ankur Thapar (with permission from patient).

Developments in technology have led to the newer third generation contrast agents, of which SonoVue™ (Bracco, Milan, Italy) is one. These agents have the benefits of: remaining biologically inert, having a size similar to an erythrocyte (90% of microbubbles have a diameter less than 6μm, allowing them to cross capillary beds), improved stability (due to a phospholipid shell and the low solubility sulphur hexafluoride gas) and lack of uptake into the extracellular fluid space (225).

One of the key assumptions is that microbubble echo intensity is proportional to concentration (Figure 34). At concentrations relevant to clinical practise this linear relationship has been established, however above this saturation occurs (226).
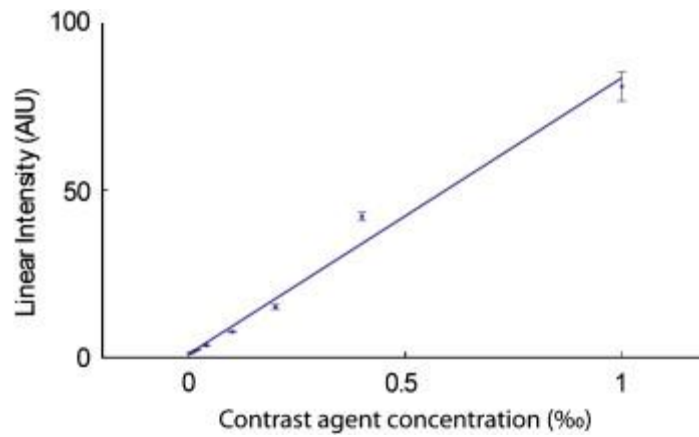
Figure 34: The linear relationship between contrast agent concentration (ml SonoVue / L deionised water) and arbitrary linear intensity units. Source Lampaskis et al. 2010 (226).

Sonographic artefacts

An artefact represents an aberrant ultrasound finding which does not correspond to a real structure. There are several sonographic artefacts associated with the use of contrast agents.

- Blooming – where enhancement is seen outside of the vessel (Figure 35).
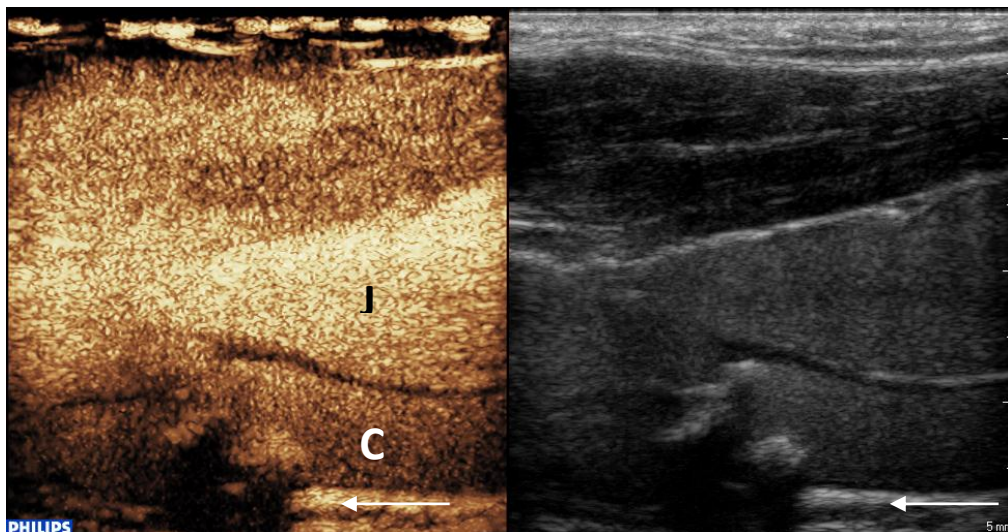
Figure 35: Blooming and saturation of the internal jugular vein (longitudinal section, from dataset). Blooming (spill of echoes outside of the target vessel is seen around the jugular (J). The returned echoes are so strong that the carotid (C) in comparison does not enhance as expected. This image also illustrates that contrast does not visualise calcified plaques well (arrow). Source: Ankur Thapar (with permission from patient).

- Saturation – where the returned echo intensity is above the maximum of the detector causing the image to appear white. Avoiding saturation is important for studies where quantification is used because small increases in returned signal (i.e. from the microcirculation) cannot be detected.

- Near field artefact due to strong echoes from skin (Figure 36).
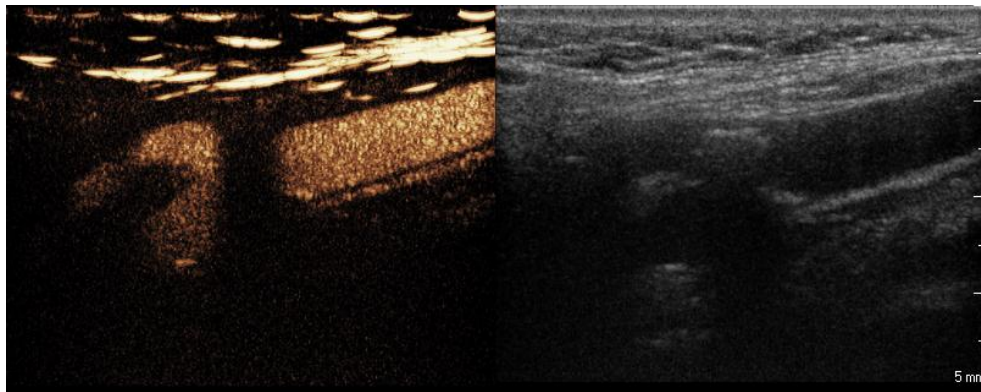
Figure 36: Example of acoustic shadowing obscuring plaque. Near field artefact is also seen at the top of the left hand contrast enhanced image (from dataset). Source: Ankur Thapar (with permission from patient).

- Tissue harmonics at moderate to high mechanical indices caused by incomplete non-linear subtraction, particularly in areas of calcification (Figure 37).
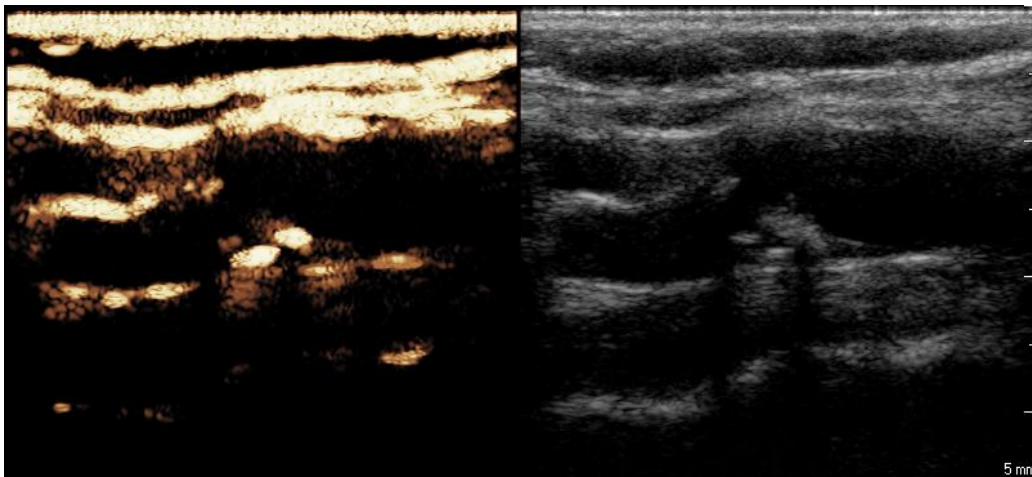
Figure 37: Artefactual tissue echoes in areas of calcification within plaque generated by high mechanical index imaging (longitudinal section). Source: Ankur Thapar (with permission from patient).

Aim of preliminary study

Our group has previously used high mechanical index imaging to image carotid atherosclerosis showing a significant separation in signal from symptomatic and asymptomatic individuals (Figure 38) (227). Late phase is the point at which microbubbles are retained within the capillary bed of interest but not the bloodstream. A high mechanical index is used to disrupt the tiny quantity of retained microbubbles that are found within plaque, returning a plaque signal which is normalised to the lumen.
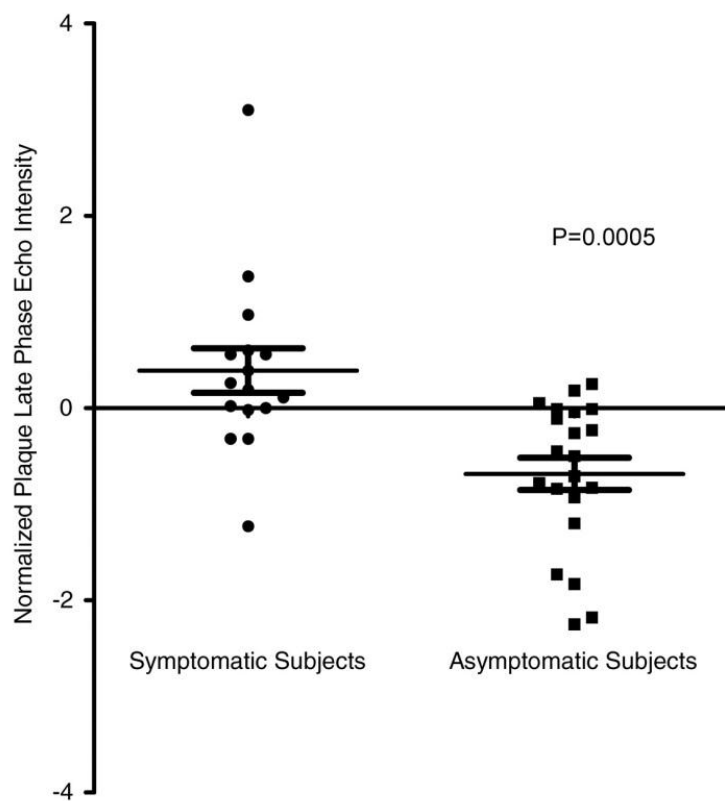
Figure 38: Graph demonstrating separation in normalised plaque signal between symptomatic and asymptomatic individuals (n=37). Reproduced with permission from Owen et al. (227).

The mechanism of contrast retention at 6 minutes is unlikely to be phagocytosis, as this takes around 15 minutes in vitro (211). However, negatively charged albumin coated microbubbles have been shown to adhere to damaged endothelium in-vivo that has shed the negatively charged surface glycoprotein glycocalyx (214). Additionally, in-vivo,

microbubbles have demonstrated integrin and complement mediated binding to TNF-α activated leucocytes adherent to vascular endothelium (213).

The aim of this preliminary study was to check the validity of late-phase contrast enhanced ultrasound for imaging atherosclerotic plaques at the carotid bifurcation The hypothesis was that plaques in symptomatic individuals have an increased late phase signal due to the presence of retained microbubbles at the vascular endothelial surface.

6.2   Methods

Ethical approval was obtained in advance for this prospective case series (ref 09/H0706/89). Written informed consent was obtained from each participant. No formal sample size calculation was performed, however the previous study (n=37) was used as a guide. Forty-nine consecutive patients referred for carotid duplex to a vascular laboratory were screened between March – December 2010 (Figure 40). Inclusion criteria were: a discrete 50-99% carotid stenosis (NASCET equivalent by velocity criteria), for symptomatic patients a recent (<4 weeks) ischaemic stroke, transient ischaemic attack or amaurosis fugax in the ipsilateral carotid territory with no other obvious clinical source after electrocardiogram and troponin I were tested. For asymptomatic patients no prior ipsilateral carotid territory symptoms were permitted. Symptomatic status was assigned by the referring neurologist, in conjunction with an ophthalmologist for patients with amaurosis fugax. Exclusion criteria were an established alternative embolic source e.g. atrial fibrillation, mechanical heart valve, prior endarterectomy, neck radiotherapy, tandem lesions and contrast contraindication. Nine patients (18%) were excluded for the following reasons: SonoVue contraindication (n=4, 8%), severe acoustic shadowing (n=2, 4%), atrial fibrillation (n=1, 2%), prior endarterectomy (n=1, 2%), declined to participate (n=1, 2%). This left a total of 40 patients, of which 15 were symptomatic (2 had an ipsilateral stroke, 6 had an ipsilateral TIA and 7 had ipsilateral amaurosis fugax) and 25 were asymptomatic (control group).
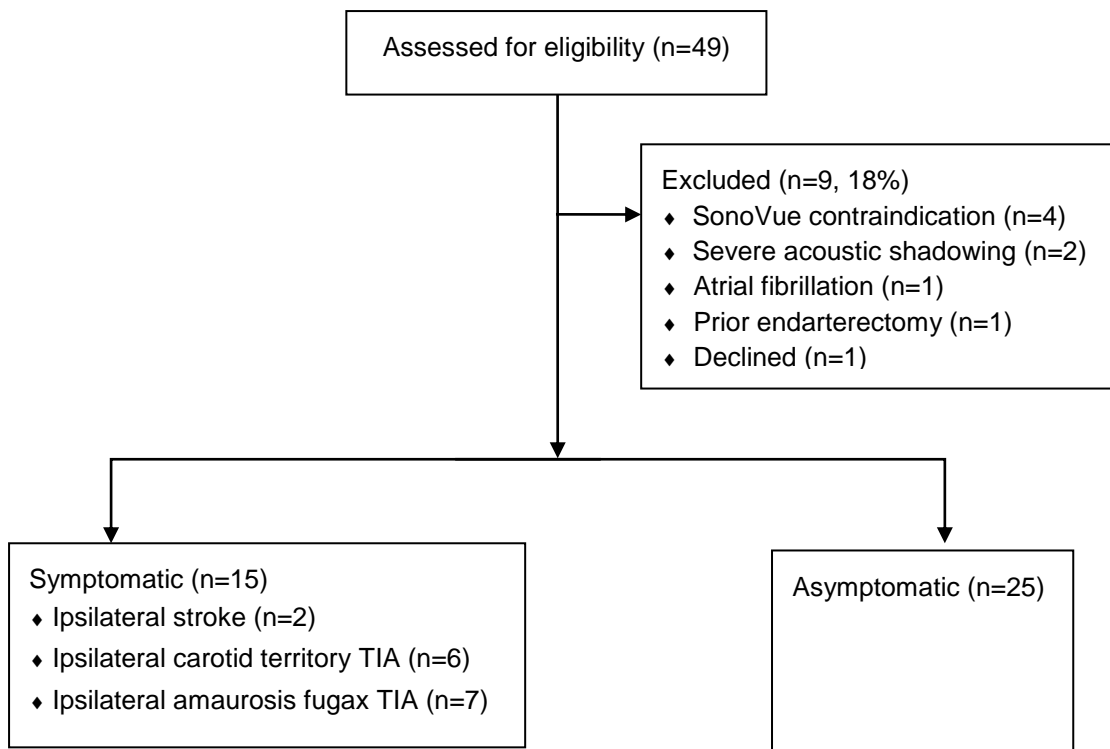
Figure 39: Study flow diagram.

A Philips iu22 ultrasound machine was used for imaging with a linear array L9-3 transducer (Figure 40). This has a close frequency range (3-9MHz) to the resonant frequency of SonoVue (5-7Mhz) (220). B-mode and contrast gain settings were kept constant at 85%, with a 4cm depth field, temporal and spatial averaging disabled (XRES and persistence off) and a vertical, zeroed time-gain curve. These were disabled as they would affect the results of quantification. The internal carotid artery was identified by its higher end diastolic velocity, lack of branches and lack of response to pre-auricular tap.

A dual screen B-mode / non-linear mode (pulse inversion and power modulation) display was used. A B-mode and colour acquisition was performed first for orientation. A baseline, axial, pre-contrast image was taken flashing the plaque at approximately the lower, middle and upper thirds at a mechanical index of 0.33. A bolus of SonoVue was given through a 20 gauge cannula (dead space <0.1ml, flow rate 1.1ml/sec) in the right arm. The lower, middle and upper thirds of the plaque were flash imaged as before. After a 15 minute interval to allow contrast elimination this sequence was repeated. Images were exported as anonymised DICOM for offline analysis.

112

Figure 40: Room setup for contrast enhanced ultrasound with single sonographer. A cannula in the right arm provides easy access for the sonographer to inject and then find the correct plane for imaging.

Image Analysis

Quantification was performed offline by A.T. using QLab v8 software (Philips, Bothel, USA) on raw, linear DICOM data (utilising the linear relationship between pixel intensity and contrast concentration) (226). Quantification was performed blind to clinical data at the end of the study, using images labelled with study ID numbers (Figure 41). Regions of interest were manually drawn around the axial sections of plaque and lumen using the "spline" tool. Care was taken to avoid areas of saturation artefact, i.e. areas where tissue calcification did not cancel out completely. These areas were identified by a characteristic shining white appearance and an intensity which was approximately ten times greater than that of the lumen and an order of magnitude greater than the surrounding plaque. The highest mean pixel intensity from the three sections was used for analysis, as there was no reason for inflammation and stenosis to co-localise. These were entered into Excel 2010 (Microsoft, California, USA) for normalisation. Normalisation was performed through the formula shown in Figure 42 (227).
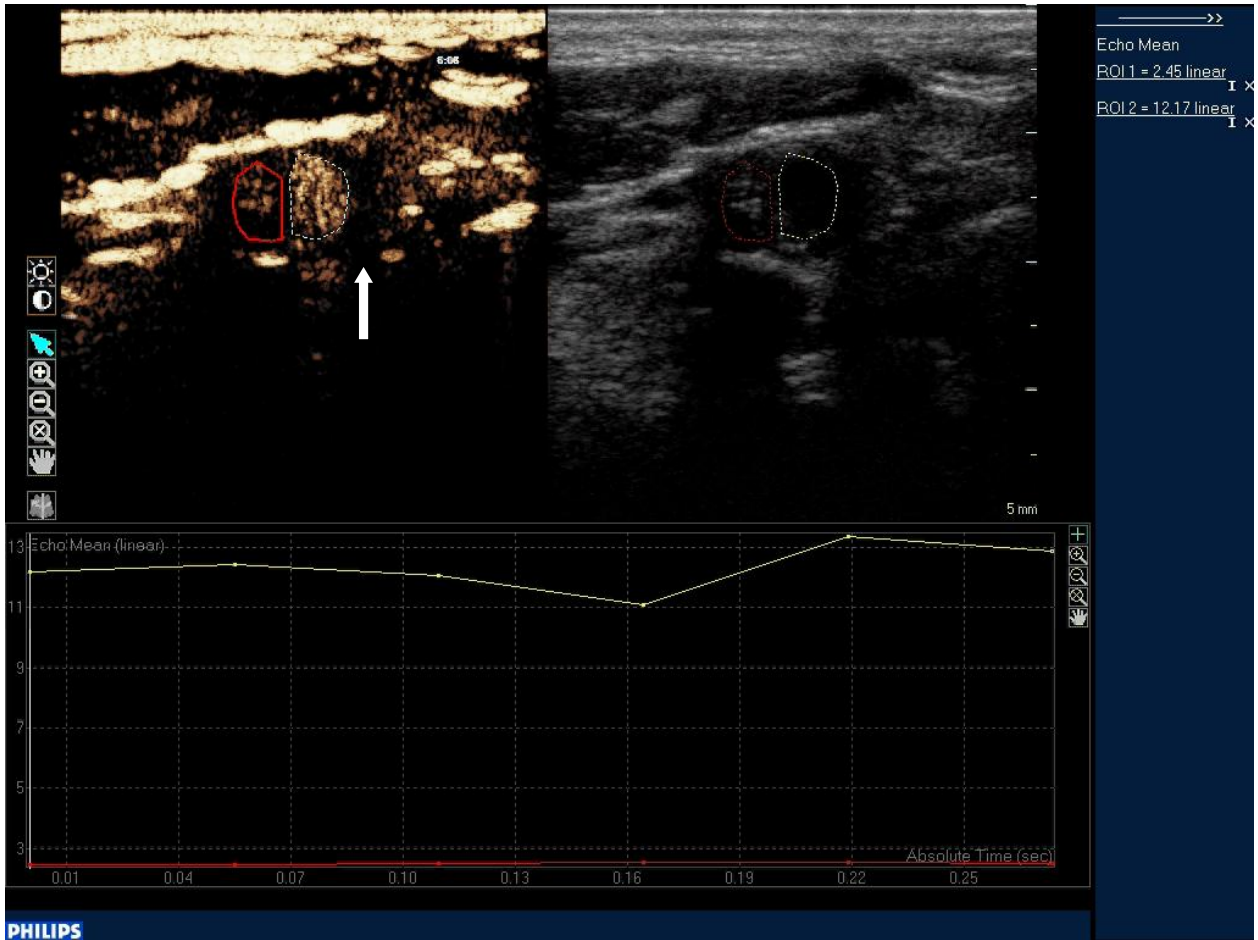
Figure 41: QLab screenshot depicting axial late phase image (above, left). Plaque has been outlined in red and lumen in yellow. A saturation artefact is seen (white arrow). This was identified visually and also because of an intensity typically an order of magnitude greater than the surrounding plaque. In fact the saturated area is brighter than the lumen, which is highly improbable. The time-intensity curve over 0.28ms is depicted for the lumen (yellow top line) and plaque (bottom red line). Source: Ankur Thapar (with permission from patient).

$$NormalisedLP = \log 10 \frac{PI}{LI}$$

Figure 42: Normalisation to the lumen was achieved using the formula above. PI=plaque intensity, LI=lumen intensity. Logarithmic units are used as there is an order of magnitude difference between plaque and lumen intensity.

The aim of late phase normalisation was to use the lumen as a reference for any remaining microbubbles in circulation. This was because each 2ml bolus can vary slightly in the volume of contrast delivered and in elimination kinetics due to individual differences in cardiorespiratory function and blood volume (see Appendix 2) - hence the concentration of contrast reaching the carotid may vary as a result. For this reason relative rather than absolute intensity values were used. This allowed the concentration in the tissues to be gauged against the local input function in the carotid.

Statistical analysis was performed using Prism v5 (GraphPad software, California, USA and MedCalc v11, Mariakerke, Belgium). Data were checked for normality, two-tailed tests used and statistical significance taken as $p < 0.05$. A receiver operator characteristic (ROC) curve was constructed for the variable late phase signal versus symptom status.

Intra and inter-reader reproducibility were calculated for the normalised late phase signal from 20 patient images and analysed using the intra-class correlation coefficient.

6.3   Results

The demographic details of the patients in the study are shown in Table 14. The patients in the symptomatic group had a significantly higher degree of stenosis (88%, IQR 80-95 v 60 %, IQR 53-75, p=0.001).

	Symptomatic (n=15)	Asymptomatic (n=25)	p-value
Age	67 (SD 11)	73 (SD 12)	0.58
Male	67%	80%	0.46
Diabetes	13%	24%	0.69
Hypertension	73%	68%	1.00
Parental stroke	7%	32%	0.12
Current Smoker	40%	28%	0.50
Stenosis (NASCET)	88 (IQR 80 – 95)	60 (IQR 53- 75)	0.001*
Statin > 1 week	73%	80%	0.71

Table 14: Demographic details of patients in the clinical study. * indicates a statistically significant difference between groups. SD=standard deviation, IQR=inter-quartile range.
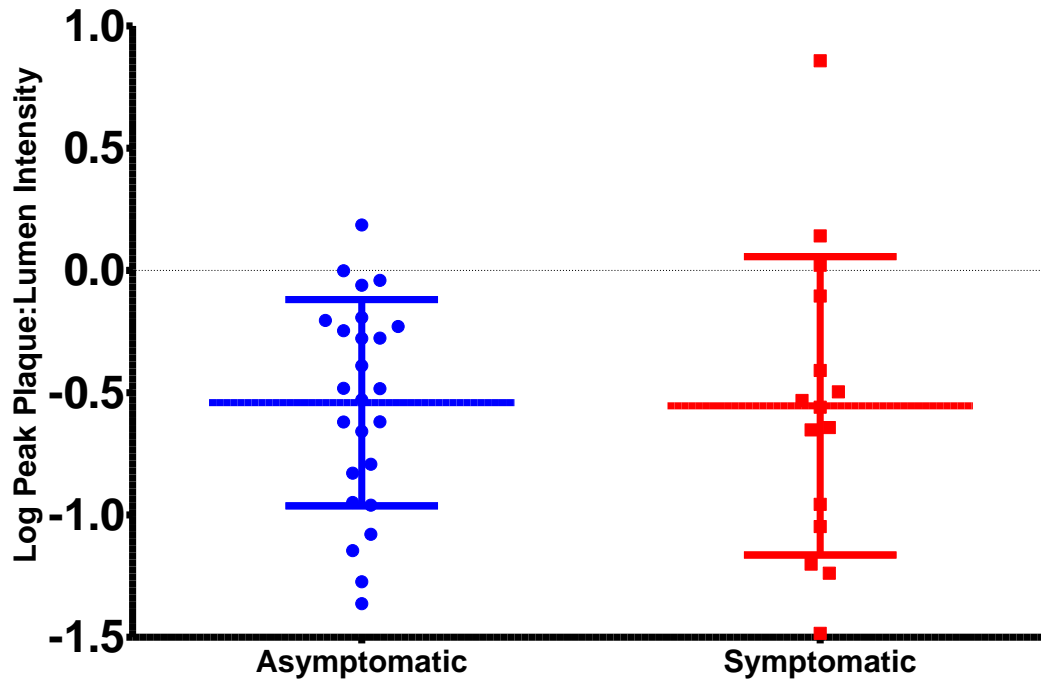
Figure 43: Peak normalised late phase plaque signal ratio (n=25, maximum plaque intensity taken per patient). There was no statistically significant difference between the symptomatic patients and the asymptomatic patients, with respect to the peak normalised plaque intensity (p=0.94).

The mean normalised $\log_{10}$ plaque signal was -0.54 (SD 0.42) in the asymptomatic group and -0.55 (SD 0.61) in the symptomatic group (Figure 43). There was no statistically significant difference between groups using the student's t-test (p=0.94). Receiver operator characteristic curve analysis yielded a non-significant c-statistic of 0.55 (p=0.41).

The intra-reader intra-class correlation coefficient was 0.98 and the inter-reader intra-class correlation coefficient was 0.70 (performed with E.L.).
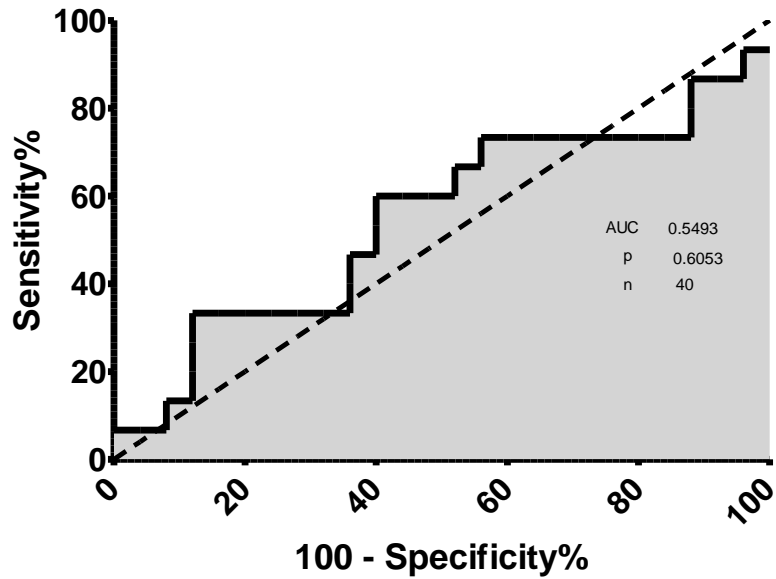
Figure 44: Receiver operator characteristic curve for late phase plaque signal in the identification of patients with ipsilateral symptoms. The area under the curve was no better than chance, (c-statistic or AUC=0.55, p=0.61, n=40).

6.4　Discussion

The results of this study did not reproduce previous findings that late phase high mechanical index imaging is a discriminatory imaging biomarker for culprit plaques. The accuracy of the technique was no better than chance and this was not simply a function of inter-reader reproducibility.

In both experiments the mean pixel intensities of the plaque and lumen were quantified and normalised with a similar protocol. However in the present experiment very few plaques were brighter than the lumen at 6 minutes, a very different finding to the previous study (Figure 44). Secondly there was no significant difference in the plaque signal between symptomatic and asymptomatic patients. It is unlikely the difference in stenosis was responsible for this, as the symptomatic group had a higher average stenosis and was thus likely to consist of more culprit plaques.

An alternative explanation is that the areas of focal high signal intensity may have been found elsewhere in the plaques and were not imaged. However, in the previous experiment, only one single axial section was quantified, again making this unlikely.

This led to the following questions: firstly was six minutes the optimum time point for late phase imaging? Secondly, was an MI of 0.33 the optimum mechanical index for imaging carotid plaque?

6.5   When is the end of the arterial phase?

The six minute cut-off was based on low mechanical index imaging in the liver, where Küpffer cells retain contrast, rather than high mechanical index imaging in the carotid. To establish a reliable time point for the end of the arterial phase, and hence the start of the late phase, the following experiment was performed. Eight patients with 50-99% carotid stenosis were administered a 2ml intravenous bolus of SonoVue. Flash imaging of the lumen was performed at one minute intervals at an MI of 0.33 for 15 minutes. The lumen was imaged in an axial orientation at the carotid bifurcation and a luminal time-intensity curve derived.
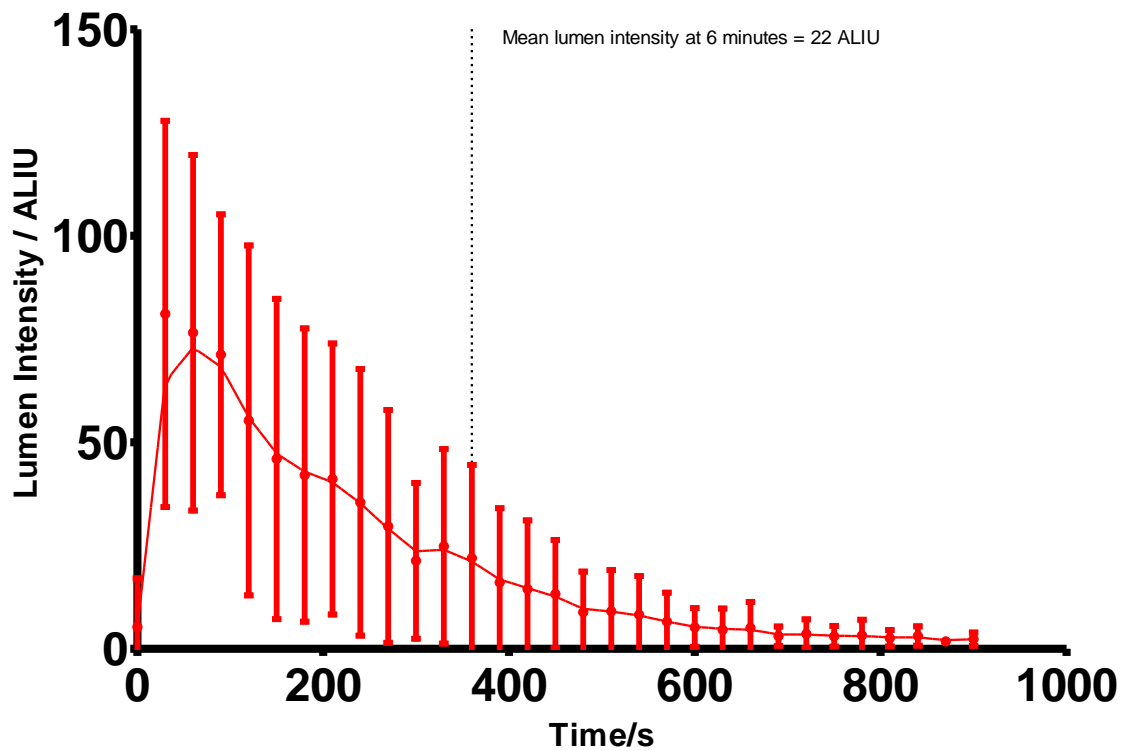
Figure 45: Time-intensity curve for the carotid lumen at a mechanical index of 0.33 (n=8 patients). At 6 minutes the mean lumen intensity was 22.0 (SD 22.6) arbitrary linear intensity units. Error bars=standard deviation. ALIU=arbitrary linear intensity units.

The high MI washout curve in Figure 45 demonstrated that at 6 minutes (360 seconds) at a high mechanical index the raw lumen intensity was a mean of 22.0 (SD 22.6) ALIU. To fall close to zero, around 12 minutes or longer was required. This was surprisingly high and was repeated at a low mechanical index, after a 15 minute interval.
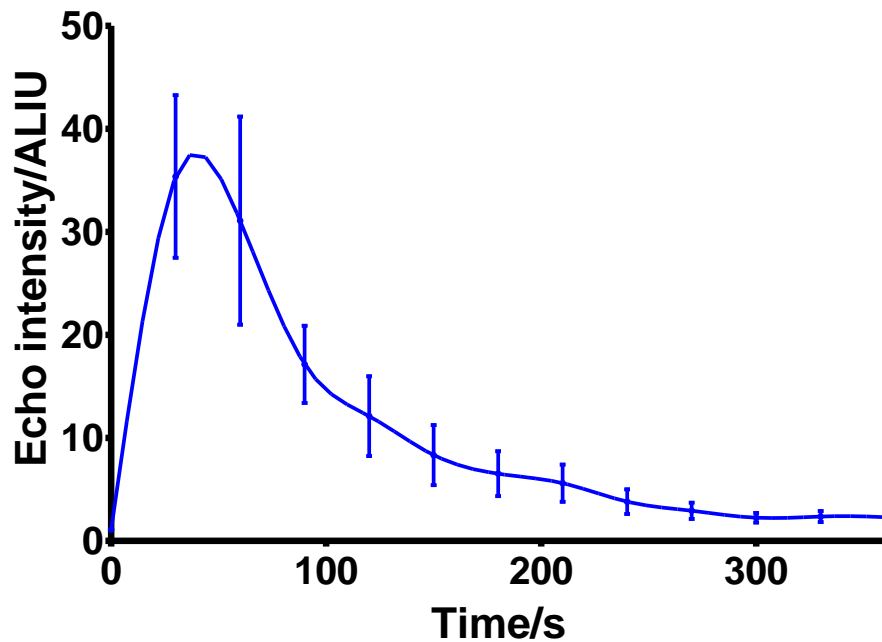
Figure 46: 2ml dynamic time-intensity curve of carotid lumen over 6 minutes using a mechanical index of 0.06 (n=8 patients). At 6 minutes the mean lumen intensity was 2.3 (SD 1.5). Error bars = standard deviation. ALIU = arbitrary linear intensity units.

The low MI washout curve in Figure 46 demonstrated that at a low mechanical index of 0.06, lumen intensity fell to a mean of 2.3 (SD 1.5) linear intensity units at 6 minutes (i.e. near baseline). This experiment demonstrated that far from 6 minutes being considered late phase at a high mechanical index, the lumen was still full of visible contrast. A second question now emerged, which was below which MI should imaging take place?

6.6   Relationship of plaque signal to mechanical index

It was noted qualitatively, with high mechanical index imaging there was an abundance of saturation artefact in the surrounding tissues (Figure 47). To examine whether this affected the plaque signal the following experiment was performed. 10 patients had flash imaging of the plaque at the point of greatest carotid stenosis with a mechanical index varying between 0.01 – 1.0. For the MI range used in clinical practice

results are shown in Figure 48. Further details are available at even higher mechanical indices in Appendix 2.
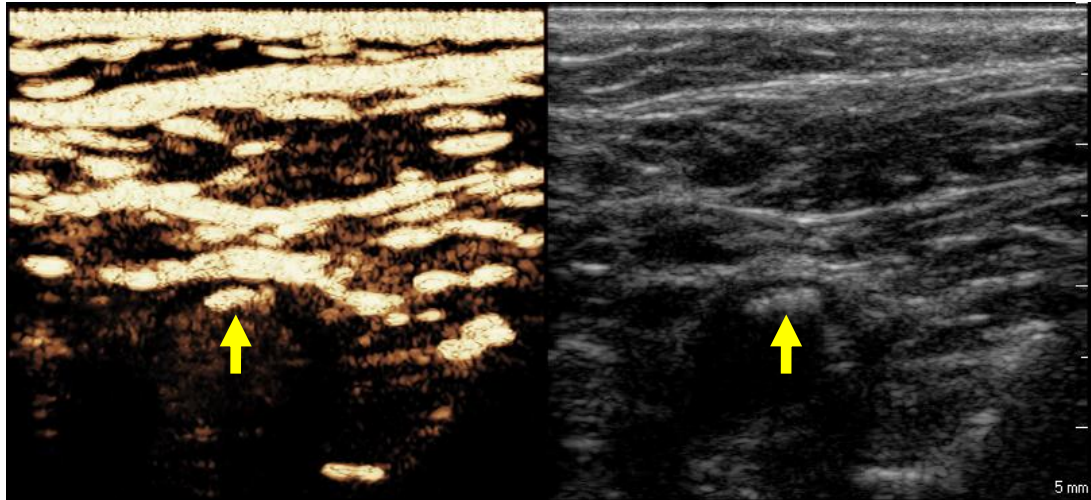
Figure 47: Axial image through a common carotid plaque using a non-linear imaging mode before administration of contrast. Flash imaging at high mechanical index (0.33) created a saturation artefact at tissue interfaces and in a calcified portion of carotid plaque itself (yellow arrow). Note that this is also visible in the soft tissues superficial to the carotid. This saturation artefact appears to be caused by incomplete cancellation of linear echoes at tissue interfaces or in areas of calcification, i.e. in areas with a strong acoustic impedance mismatch. Source: Ankur Thapar (with permission from patient).
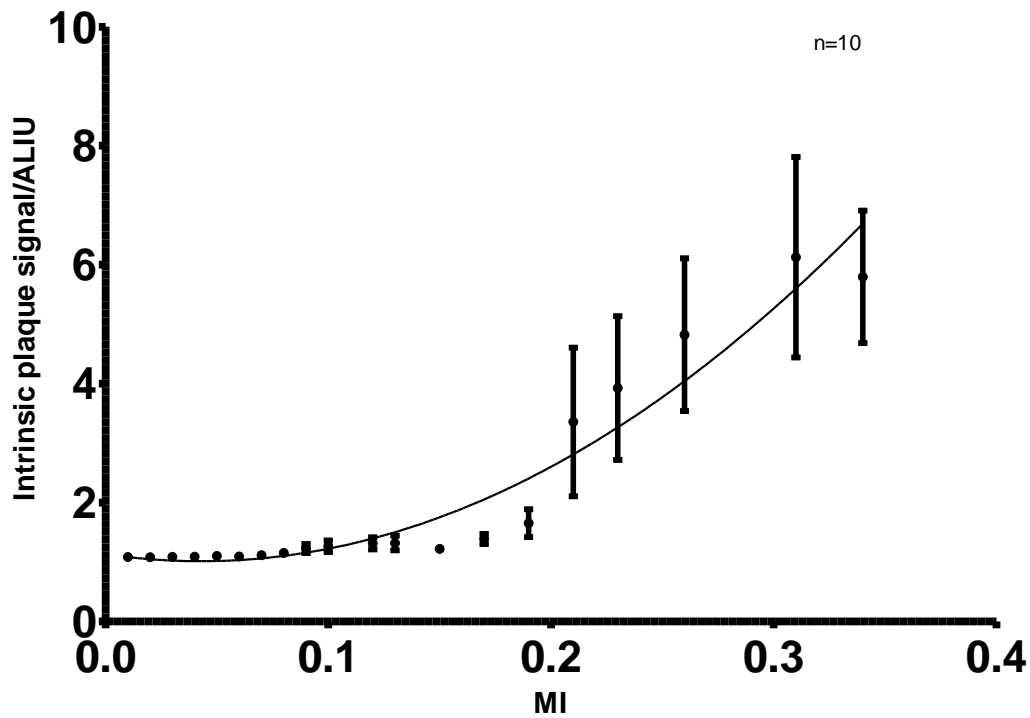
Figure 48: Increasing mechanical index causes an exponential increase in signal within the plaque before contrast administration. ALIU=arbitrary linear intensity units. The artefactual signal became visually apparent above an MI of 0.19.

To identify whether the saturation artefact was in fact the signal observed, an additional experiment was performed. Twenty plaques of between 50-99% stenosis were flash imaged in axial section at the point of greatest stenosis, before and after a 2ml intravenous injection of SonoVue, at an MI of 0.33 (Figure 49).
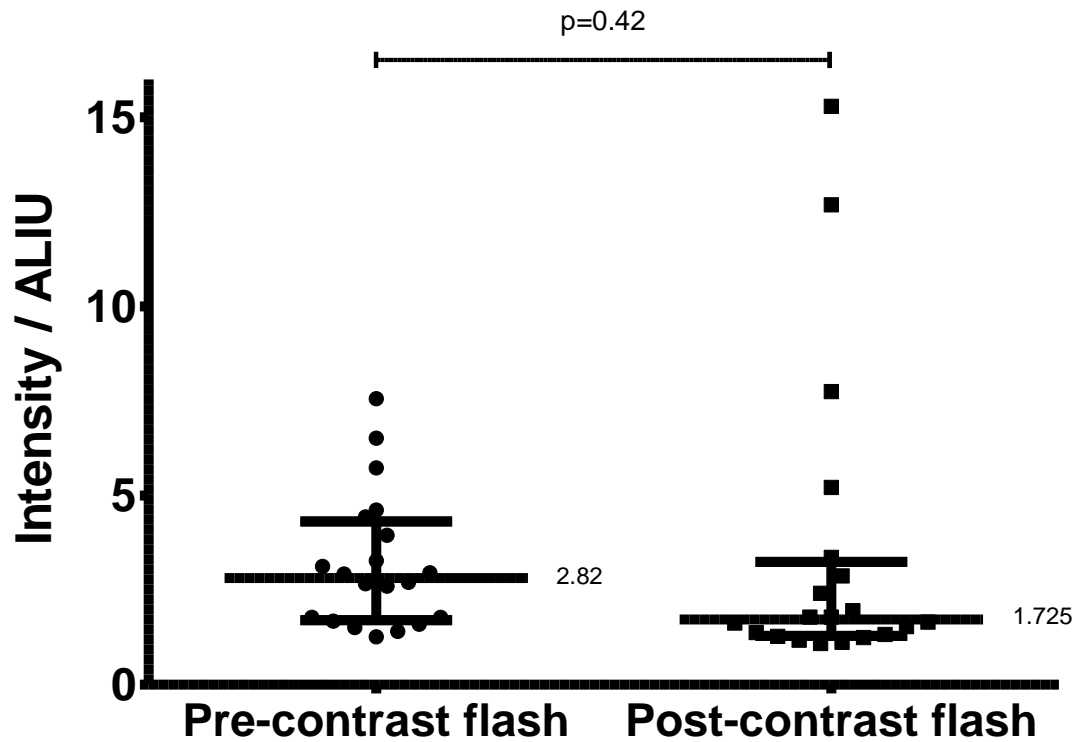
Figure 49: 20 plaques were flash imaged before and after an intravenous injection of 2ml of SonoVue at a mechanical index of 0.33. There was no significant difference in normalised plaque intensity, p=0.42. The three plaques returning the highest intensity signal were all calcified plaques.

There was no significant difference in peak plaque signal before and after administration of contrast: 2.8 (IQR 1.7-4.3) versus 1.7 (IQR 1.3-3.2), p=0.42, Wilcoxon matched pairs test. Three plaques with heavy calcification returned the highest signal both pre and post contrast injection.

6.7   Conclusion

These experiments demonstrated that:

i.   At a high mechanical index an artefactual plaque signal is created by incomplete cancellation of linear tissue echoes. This is most apparent at areas where there is a strong acoustic reflector, e.g. calcification within carotid plaque. However it is present even in non-calcified areas.

ii.   At a high mechanical index, contrast is still detectable in the lumen at 6 minutes, unlike low mechanical index imaging. This implies that any signal return from the plaque may be perfusion rather than adherent contrast.

The results of this experiment suggest that high mechanical index imaging using the current non-linear imaging mode was unreliable for use in the human carotid without major improvements in tissue suppression and bubble detection. From here on, attempts at performing a cohort study were postponed until a better imaging method could be developed. Focus now shifted to low mechanical index dynamic imaging of plaque perfusion.

7.0 The effect of non-linear propagation on the quantification of dynamic contrast enhanced ultrasound

7.1 Introduction

Microbubble movement within the microcirculation is visible with dynamic contrast enhanced ultrasound due to its excellent spatial and temporal resolution (228). Previous investigators have found an association between peak enhancement within carotid plaque and histological microvessel density (210, 229), prior cardiovascular events (230) and prior ipsilateral ischaemic stroke or transient ischaemic attack (231, 232). Proliferation of intraplaque vasa vasorum (neovascularisation) has been linked to recent ipsilateral stroke (85), intraplaque haemorrhage (84), plaque rupture (86) and a higher rate of future cardiovascular events (69). These studies hypothesised that because increased microvessel density and intraplaque haemorrhage cluster, that the former may be responsible for the latter and may represent a destabilising pathway in vulnerable plaques.

When considering ultrasound studies, most focus on the far wall, rather than the near wall of the carotid artery as the impedance mismatch between luminal blood and the fibrous cap acts a good acoustic reflector. However in studies in other organs, pseudo-enhancement is noted to cause a bright artefact at depths of 5-6cm (233). This phenomenon can mimic perfusion, which is important when considering quantification (234). It is possible that pseudoenhancement complicates quantification of carotid plaque perfusion, even at depths of 2-4cm, typical of the carotid.

The aim of this study was to establish if pseudo-enhancement in the carotid artery affected quantification of perfusion in carotid plaques. The primary hypothesis was that pseudoenhancement would affect the far wall of the carotid artery, which is commonly used for quantification of neovascularisation and render it invalid.

7.2   Methods

Clinical study

Ethical approval was obtained prior to commencing the study (ref 09/H0706/89). Patients gave written informed consent to participate in this prospective case series. Sample size calculation determined 28 patients were necessary to demonstrate an intensity difference of 2 linear intensity units, between the near and far wall with a type I error rate of 5% and power of 90%.

Thirty-five patients were screened for the study from vascular and acute stroke clinics at Imperial College Healthcare NHS Trust between March 2010 and February 2011 (Figure 50).   Thirty-one patients (31/35, 89%) with a discrete (NASCET equivalent (31)) 50-99% internal carotid artery stenosis were eligible to take part.   Patients were excluded if they were <18 years of age, had NYHA ≥III heart failure (1 patient), had experience an acute coronary syndrome within 2 weeks (0 patients), had a sulphur allergy (1 patient) or declined to participate (2 patients) .

One artery per patient was insonated to avoid data clustering.   In the case of bilateral disease, the side with the greatest stenosis was imaged.   Patients were defined as symptomatic if they had experienced an ipsilateral carotid territory stroke, transient ischaemic attack, amaurosis fugax or retinal infarction within 4 weeks and had a normal 24 hour electrocardiogram and echocardiogram.   Patients were defined as asymptomatic if there were no prior neurological events in the territory of the carotid artery under examination.

```
┌─────────────────────────────────────────────────┐        ┌──────────────────────────────┐
│ Eligible patients with 50- 99% carotid          │        │ Parallel in-vitro phantom    │
│ atherosclerosis (n=35)                          │        │ study                        │
└─────────────────────────────────────────────────┘        └──────────────────────────────┘

┌─────────────────────────────────────────┐
│ Excluded (n=4, 11%)                      │
│ ♦ SonoVue contraindication (n=2)         │
│ ♦ Declined (n=2)                         │
└─────────────────────────────────────────┘

┌─────────────────────────────────────────────────┐
│ DCE-US and quantification of near and far wall   │
│ intensities.                                     │
│ Symptomatic (n=12), Asymptomatic (n=19)          │
└─────────────────────────────────────────────────┘
```

Figure 50: Study flow diagram. The diagram on the left depicts the clinical study and that on the right the in-vitro validation study. The clinical study used a mechanical index of 0.06 to image carotid atheroma in longitudinal section. The in-vitro study used SonoVue concentrations of 0.02, 0.1, 0.5, 1, and 2 ‰ sequentially, to cover the range of contrast concentrations used in clinical practice.

Phantom study

A tissue phantom was used to validate in-vivo findings (Figure 51). A single flow channel tissue phantom consisting of latex tubing with a diameter of 8 mm, placed at a depth of 12 mm in a tissue mimicking fluid was used. The fluid was composed of equal volumes of water and glycerine and had similar attenuation properties to soft tissue (0.3dB/cm/MHz). The enclosure was made from 10mm thick acrylic and the bottom was lined with an anechoic material (Aptflex F28, Precision Acoustics, Dorchester, UK) which reduced reflected ultrasound waves. SonoVue™ microbubbles at concentrations of 0.02, 0.1, 0.5, 1, and 2 ‰ were injected into a reservoir sequentially. These concentrations were chosen to cover the range of *in vivo* contrast use. A peristaltic pump (Cole-Parmer, Vernon Hills, IL, USA) was used to pump contrast continuously through the flow channel.
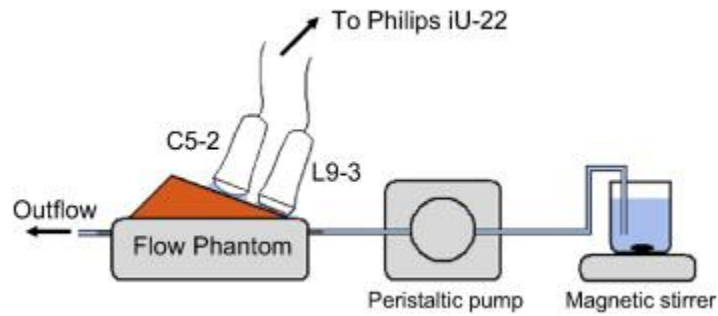
Figure 51: Diagram of flow phantom experiment. A reservoir of agitated contrast agent was pumped through a rubber based flow phantom. In this experiment only the L9-3 transducer was used. Reproduced with permission from Averkiou et al. (235).

Ultrasound imaging

A Philips iU22 ultrasound platform and an L9-3 transducer (linear array, 3-9MHz frequency range) (Philips, Bothel, WA, USA) with a standard power modulation, pulse inversion non-linear sequence was used. Imaging was performed by A.T. under the supervision of E.L. for the clinical study and by M.A. and C.M for the *in vitro* experiment. A low mechanical index of 0.06 was used to prevent microbubble destruction. The time-gain compensation curve was kept vertical and centred with dynamic range set at 36 dB, and 2D gain at 85%. Machine parameters remained unchanged between examinations to avoid the effect of increasing gain on absolute pixel intensity in body tissues. This was essential because pixel intensity in this experiment was a non-normalised measurement, comparing the near and far wall directly.

Each patient received a 2 ml bolus of intravenous SonoVue (Bracco, Milan, Italy) via a 20 gauge cannula in the right antecubital fossa. A one minute uniplanar video loop of the carotid bifurcation was recorded using the contrast / B-mode side-by-side display, in longitudinal section at the point of greatest stenosis. In the final 10 patients, a second bolus of 1 ml of SonoVue was given and the examination repeated. This was performed after 15 minutes, to ensure elimination of the previous dose. Patients were observed for 30 minutes post-dose. The dose range was chosen to replicate those used in clinical studies previously (189, 232, 236).

Tissue phantom imaging was performed by attaching the L9-3 transducer to an articulated arm such that the transducer surface was 3 mm under the air-fluid interface. Two video loops were acquired at each concentration, one at 25 ml/min and one with no flow, to establish whether flow velocity altered the findings.

Quantification

Raw DICOM data was exported to an offline workstation for quantification using QLab software v8 (Phillips, Bothel, USA). Quantification was performed by A.T. and repeated by E.L. for the clinical study and by M.A. for the *in vitro* study.

The time-intensity curve was analysed from the contrast arrival time in the carotid to the time at which jugular enhancement overshadowed the near wall adventitia (video loop cropped to 6 seconds post arrival time). Regions of interest were drawn in the common carotid lumen, in the common carotid near wall adventitia and in the common carotid far wall adventitia, directly above and below that of the lumen, using the 'freeform polygon' tool (Figure 53 [A]). Regions of interest in the adventitia were placed away from plaque in order to avoid confusion with neovascularisation. Pre and post peak contrast intensities were measured from the raw time-intensity data (Figure 52). Similar regions of interest were then placed on the *in vitro* video loops. Linear intensity units were used, in order that pixel intensity was directly proportional to contrast concentration, up to a concentration of about 1‰.
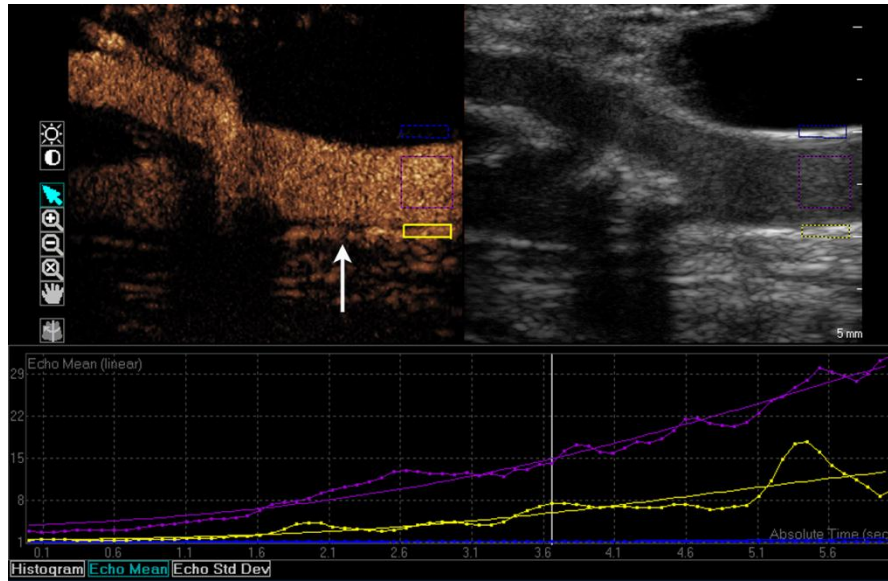
Figure 52: Longitudinal image of the left common carotid bifurcation in an asymptomatic 79 year old male with a 50% internal carotid artery stenosis. Quantification of intensity was performed using the dual-display mode with the contrast enhanced image to the left of the screen and the B-mode reference to the right. Adventitia is seen as an echogenic line on B-mode, aiding identification. Pseudoenhancement mimicking neovascularisation in the region of the far wall carotid adventitia is seen (white arrow). Plaque is seen as a filling defect in the internal carotid artery. Regions of interest have been drawn: blue (near wall adventitia); purple (common carotid lumen); and yellow (far wall adventitia). The time-intensity curves are shown below in linear units in the same colours as the regions of interest. The lumen and far wall adventitia intensity curves rose synchronously however the near wall did not follow this pattern. This was unusual because the near and far wall adventitia are in fact contiguous structures. Source: Ankur Thapar (with permission from patient).
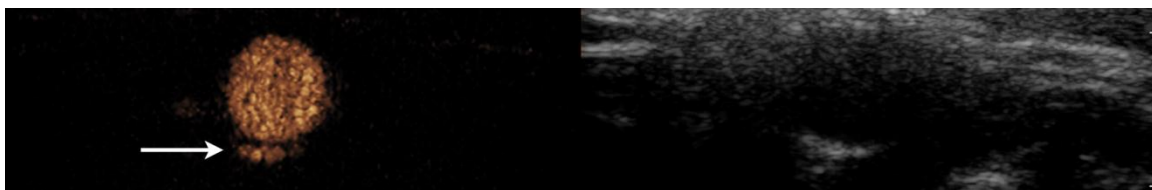
Figure 53: Axial image of the left common carotid artery, demonstrating a far wall artefact in an asymptomatic 33 year old female. Pseudoenhancement is seen as a semicircle under the lumen (white arrow). Regions of interest have been removed to illustrate the visual appearance of pseudoenhancement. It became apparent that pseudoenhancement was strongest where there was an echogenic structure on B-mode. Source: Ankur Thapar (with permission from patient).

Statistical Analysis

The raw peak intensities of the far and near wall were compared using the Wilcoxon signed rank test. For the ten patients given varying doses of the contrast, the difference between the near and far wall was used to determine the multiplication factor by which the two intensities differed. Additionally, a paired t-test was used to determine whether there was a significant difference in intensity at increasing contrast doses. Intra and inter-reader agreement was calculated using the intra-class correlation coefficient for all 31 patients at the dose of 2ml. Data were analysed using Prism v5 (GraphPad Software, LaJolla, USA) and MedCalc v11 (MedCalc software Mariakerke, Belgium). Data were checked for normality, two-tailed tests used and statistical significance was inferred below $p<0.05$.

7.3 Results

Clinical study

The demographic details of the patients are shown in Table 15 and are typical of those with carotid stenosis with a mean age of 71 years (SD 11), a predominantly male demographic and a high prevalence of anti-hypertensive and statin use.

Demographic variable	Number of patients (%) or mean (SD)
Age	71 years (SD 11)
Male gender	24 (77%)
Ipsilateral neurological symptoms	12 (39%)
Statin use	22 (71%)
Anti-hypertensive use	22 (71%)
Diabetes	11 (35%)
Current smoker	9 (29%)
Family history of stroke	7 (23%)

Table 15: Demographic details of the 31 patients enrolled into the clinical study. Of the 12 symptomatic patients, 3 had amaurosis fugax, 4 had a transient ischaemic attack and 5 had a stroke.

An echogenic strip of adventitia was consistently seen on longitudinal and axial views of the common carotid artery in the region of the far wall adventitia (Figures 52 and 53). The time-intensity curve for the far wall adventitia rose synchronously with that of the lumen, however this was not the case for the near wall, which was consistently darker (Figure 53). At a dose of 2 ml of SonoVue, the far wall was significantly more echogenic than the near wall ($p<0.0001$, n=31) (Table 16).

	Near wall intensity (IQR)	Far wall intensity (IQR)	Difference (IQR)	Two - tailed p value
Peak intensity / linear units	1.30 (1.16 to 1.80)	15.16 (5.16 to 29.49)	13.11 (4.03 to 28.01)	<0.0001

Table 16: Median near and far wall carotid adventitia peak intensity measurements using 2 ml of SonoVue. The far wall was significantly more echogenic. All intensity measurements are given in arbitrary linear units.

In the 10 patients given increasing doses of contrast, the magnitude of the difference between the near wall and far wall intensities was a factor of 0.91 (SD 0.14) at 0 ml, 4.44 (SD 2.97) at 1ml and 12.68 (SD 10.41) at 2 ml (Table 17, Figure 54). The intensity difference between near and far walls was significantly higher at 2 ml in comparison to 1 ml (p=0.012, n=10).

	Near wall intensity Mean (SD)	Far wall intensity Mean (SD)	Far wall - near wall Mean (SD)	Multiplication factor Far wall – near wall Mean (SD)
0ml	1.25 (0.25)	1.11 (0.11)	-0.14 (0.22)	0.91 (0.14)
1ml	1.76 (0.58)	7.67 (5.59)	5.91 (5.35)	4.44 (2.97)
2ml	1.49 (0.40)	17.94 (14.91)	16.96 (14.27)	12.68 (10.41)

Table 17: Mean near and far wall carotid adventitia peak intensity measurements with increasing doses of contrast. The difference between the near and far wall rose significantly with dose, up to a multiplication factor of 12 at a dose of 2ml. All intensity measurements are given in arbitrary linear units.
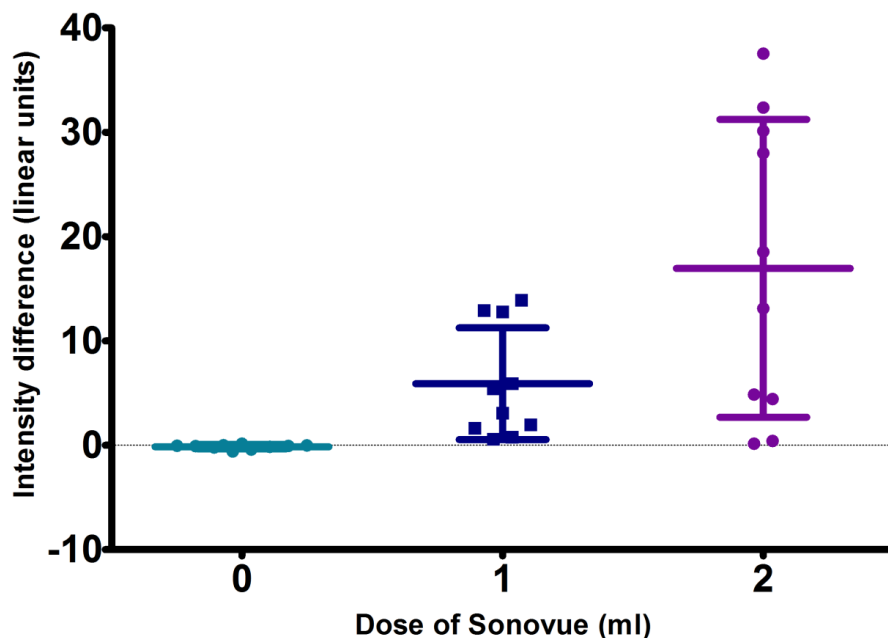
Figure 54: Graphical representation of the absolute mean difference between far and near wall peak intensity with increasing doses of contrast *in-vivo* (error bars represent standard deviation).

For intra-reader agreement (A.T.) the intra-class correlation coefficient was 0.54 for the near wall and 0.92 for the far wall. For inter-observer agreement (A.T. versus E.L.) these values were 0.49 for the near wall and 0.89 for the far wall.

In vitro study

In the flow phantom, a similar echogenic strip was seen at the far wall of the flow phantom, in an area devoid of vasa vasorum (Figure 55). With increasing contrast concentrations, this effect became more pronounced both visually and upon quantification. The magnitude of the difference between the near and far wall intensity rose from a factor of 1.47 at a concentration of 0.02 ‰ to 12.67 at 2 ‰ at a flow rate of 25 ml/min. With static contrast, the magnitude of the difference was a factor of 1.40 at a concentration of 0.02 ‰ and 15.63 at 2 ‰.
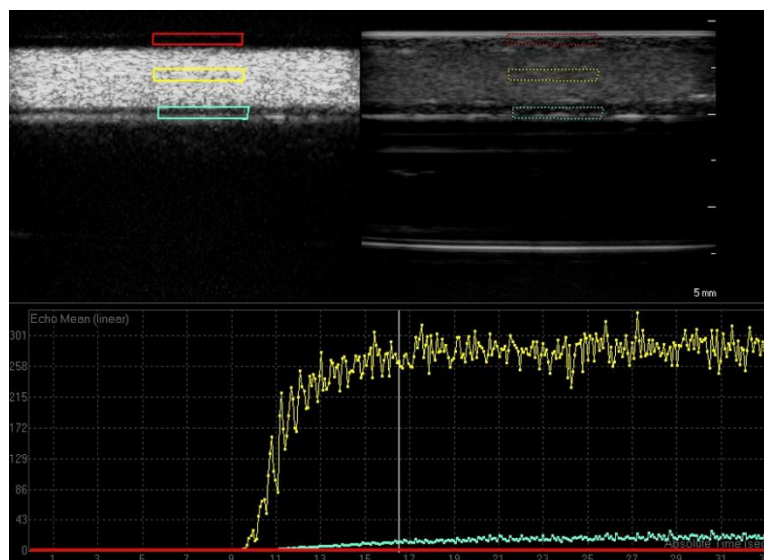
Figure 55: Longitudinal image in flow phantom using a concentration of 0.2 ‰ SonoVue. Regions of interest for quantification have been drawn: red (near wall); yellow (lumen); and blue (far wall). The respective time-intensity curves are shown below in the same colours. The far wall intensity is seen to rise in parallel with the lumen. The near wall does not exhibit this phenomenon. Source: Professor Averkiou.

7.4   Discussion

The results of this study indicate that there is a pronounced intensity difference between the near and far wall adventitia of the carotid artery during DCE-US examination. Furthermore, the far wall adventitia and lumen time-intensity curves rise synchronously, without the time delay one would expect between the macro and microcirculation (237). In addition, the walls of the latex flow phantom do not contain vasa vasorum and yet the same pattern of far wall enhancement was observed. This was despite the use of a non-linear pulsing scheme designed to subtract static linear targets. The magnitude of this phenomenon is biologically implausible as the near and far wall adventitia are a contiguous structure, separated only by the contrast filled lumen. In reality, therefore, this is likely to represent a non-linear propagation artefact.

The artefact we have described is not present before the arrival of contrast and is dose-dependent. It is visible in both longitudinal and axial planes, and *in vitro* and *in vivo* simulating the appearance of neovascularisation. Static and pulsatile flow conditions both demonstrate the phenomenon. It affects quantitative more than qualitative visual assessment because quantification software cannot distinguish moving microbubble clusters from static pseudo-enhancement.

This artefact limits the use of the far wall of the carotid for assessment of neovascularisation, as used in previous studies (189, 209, 230-232, 238). This is important to recognise for those embarking on a quantitative contrast enhanced ultrasound programme. Measurements from the same patient at the near and far will give quite different signal intensities. In clinical practise this will lead to inaccurate quantification and increase the variability of analysis. It should not lead to clinical confusion, particularly when assessing plaque vulnerability, or considering intervention.

Various strategies may be employed to minimise this problem. Firstly, for visual assessment, observers may rely on moving microbubble clusters to separate real from artefactual signal. However in reality, plaque perfusion is rarely completely absent or present, but is a continuum, lending itself to quantitative analysis. Secondly, the near wall alone can be used for quantification; the limitation is the adjacent jugular vein which can

overshadow it. This may be reduced by sitting the patient at 45° and applying gentle pressure with the transducer during the examination. Lower doses of contrast agent may be considered; however this may affect image quality by lowering signal to noise ratio.

This phenomenon should be considered when interpreting the results of previous studies utilizing DCE-US of the far wall for risk stratification. Time-based parameters such as time to peak intensity are expected to encounter a similar problem. This is despite the utility of identifying if a lag time between plaque and lumen time-intensity curves exists (*i.e.* confirming a change from macro- to micro-circulation) (237).

This study is limited in that it was an un-blinded, non-consecutive case series of patients performed with one ultrasound system. However this phenomenon is important and can be visualised on other ultrasound platforms (Figure 56).
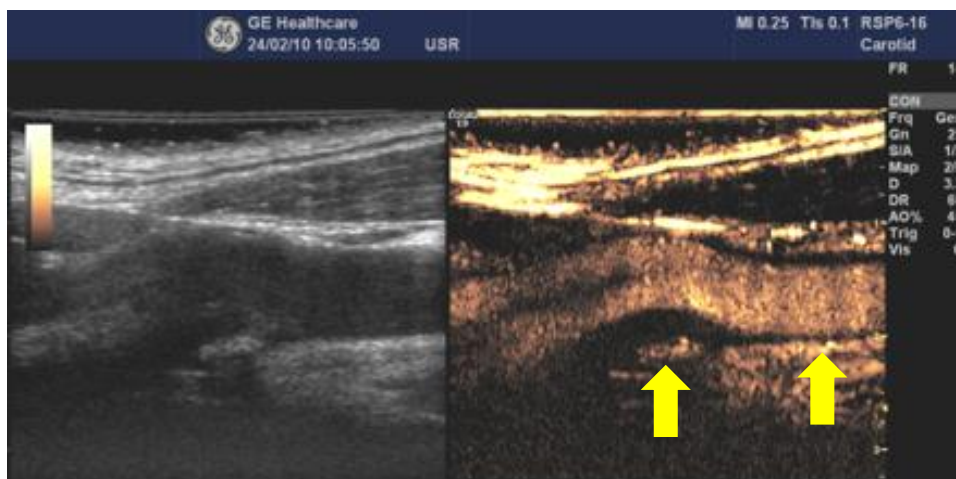
Figure 56: Example of non-linear propagation artefact using the General Electric Logiq E9 ultrasound system (yellow arrows). The intense area of enhancement in the plaque is not perfusion, but non-linear echoes. Source: Ankur Thapar (with permission from patient).

In conclusion, a dose-dependent artefact caused by non-linear propagation of ultrasound in contrast filled media causes pseudo-enhancement of the far wall of the carotid artery. This limits accurate quantitative assessment of neovascularisation at the far wall of any contrast filled blood vessel and should not be mistaken for neovascularisation.

The magnitude of this effect on carotid plaques is currently being investigated, however a similar pattern has been observed in echogenic areas of plaque (Figure 57).
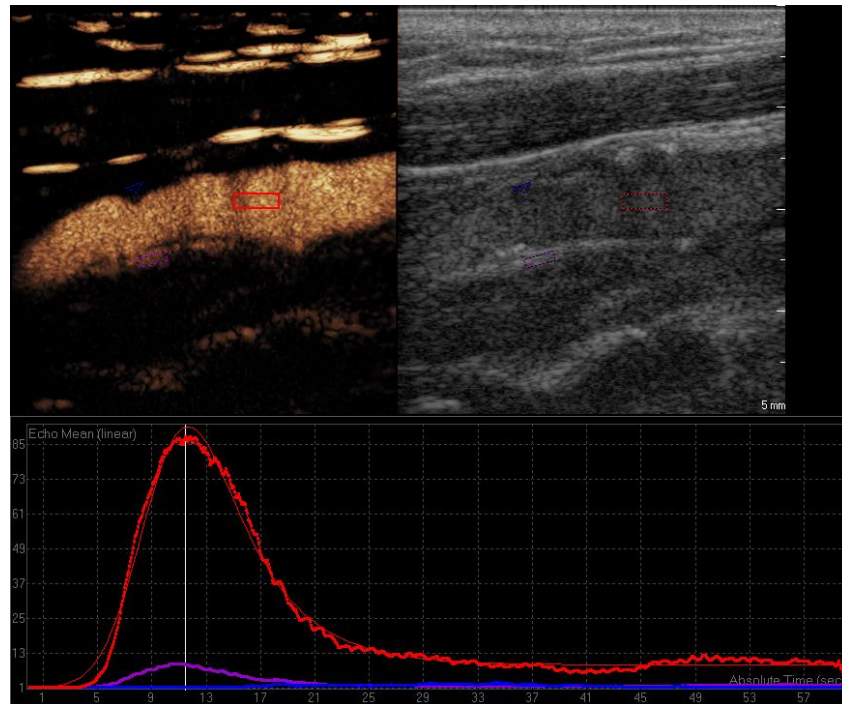
Figure 57: Effect of non-linear propagation within far wall carotid plaque using the Philips iu22 ultrasound system (longitudinal image). The far wall displays a static echogenic strip on both B-mode and contrast enhanced imaging. The time-intensity curve shows the far wall (purple) peaking at the same time as the lumen (red) with a peak intensity of 8 linear units. In contrast the near wall plaque demonstrated moving microbubbles with a peak intensity of only 3 linear units (blue). Source: Ankur Thapar (with permission from patient).

8.0   Assessment of carotid plaque perfusion and ulceration

8.1   Introduction

Dynamic contrast enhanced ultrasound (DCE-US) is a low cost, safe, clinic-based, structural and functional imaging modality to assess carotid atherosclerosis (239). Histological studies of ruptured carotid plaques have demonstrated a number of characteristic features which may be imaged in real-time using DCE-US: firstly a proliferation of abnormal, immature, intraplaque microvessels, termed neovascularisation (84-87, 240) and secondly macroscopic plaque surface defects, termed ulcers found in ruptured lesions (188).

Large ulcers may be visualized with colour Doppler, however DCE-US may have the potential to visualize small ulcers more easily, analogous to the use of luminal contrast for angiography. These features may have future potential as risk markers allowing therapy to be tailored to individual patients.

The aim of this study was to assess the potential benefits of DCE-US in the assessment of plaque perfusion and ulceration. The primary hypothesis of this study was that generalised plaque perfusion would be found in ≥50% of recently symptomatic individuals. The exploratory hypothesis was that ulceration on DCE-US would also represent a risk factor for symptomatic status.
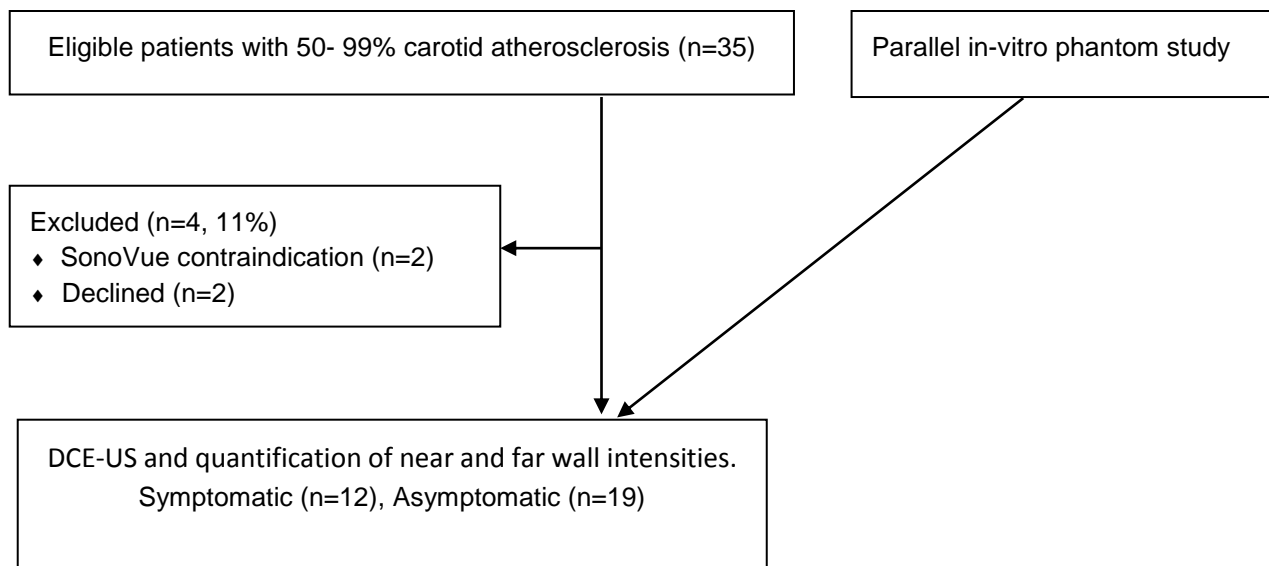
8.2   Methods

Ethical approval was obtained prior to commencing the study (Ref 09/H0706/89). Patients gave written informed consent to participate in this prospectively recruited, cross-sectional study. From a previous study it was anticipated that generalised perfusion plaque would be found in 6% of asymptomatic individuals (209). Sample size calculation

determined 22 patients per group were required to demonstrate a difference of 50% versus 5% in generalised plaque perfusion, between symptomatic and asymptomatic patients with 90% power and a 5% false positive rate.

Seventy-two consecutive patients with a discrete 50-99% internal carotid artery stenosis (NASCET equivalent (31)) were recruited from neurovascular clinics between January 2011 and January 2012 (Figure 58). Inclusion criteria were: male or female patients aged 18 years or over with a discrete 50-99% internal carotid artery stenosis. Exclusion criteria were: atrial fibrillation, mechanical heart valve, cardiomyopathy, elevated troponin, contrast contraindication, NYHA III/IV cardiac failure, myocardial infarction within 3 months and ischaemic symptoms >4 weeks prior to recruitment (to avoid the effects of remodelling post rupture) . Sixteen patients (22%) were excluded for the following reasons: 5 declined, 3 severe cardiorespiratory co-morbidity, 3 severe calcification, 2 alternate embolic source, 1 tandem carotid lesions, 1 other contraindication to SonoVue, 1 recurrent stenosis following endarterectomy, leaving a total of 56 patients (78%) who entered the study. Of the symptomatic patients, 11/26 (42%) had an ipsilateral carotid territory stroke, 10/26 (38%) had an ipsilateral carotid territory transient ischaemic attack and 5/26 (19%) had ipsilateral amaurosis fugax. Of the control patients who had been tested for asymptomatic carotid disease, 18/30 (47%) were referred from a neurologist, 10/30 (33%) were referred from a vascular surgeon because of arterial disease in another territory and 2/30 (7%) were referred because of risk factors. An independent stroke physician assigned the diagnosis of carotid territory ischaemic symptoms after a workup including intracranial computed tomography, 24 hour electrocardiogram, troponin I result and for cases of amaurosis fugax, an ophthalmic opinion. If no history of stroke, transient ischaemic attack or amaurosis fugax was present, an asymptomatic status was recorded.

An initial colour Doppler scan was performed by an independent vascular scientist (minimum 2 years of carotid ultrasound experience) where luminal stenosis and plaque ulceration (>1.5mm discrete surface indentation with pulsating colour fill) were recorded live at the time of examination.

DCE-US was performed using a Philips iU22 system by A.T. (1 year of carotid DCE-US experience). An L9-3 transducer and the following standard settings were used: mechanical

index 0.06, 2D gain 80%, focal depth 4cm, vertical centred time-gain curve, XRES on and persistence at medium (as quantification was not being performed, these last two served to improve the image quality). A 2ml bolus of intravenous SonoVue (Bracco, Milan, Italy) was injected into a 20 gauge cannula in the right antecubital fossa. A dose of 2ml was used, rather than the 1ml suggested for quantification, as it proved insufficient for the detection of moving intraplaque microbubbles in very elderly patients, or those on beta-blockers. A one minute video loop from the point of contrast injection was captured, centred on the plaque in longitudinal section. After 10 minutes this was repeated. Patients were observed for 30 minutes post injection for adverse events.

Assessed for eligibility (n=72)

Excluded (n=16, 22%)
♦ SonoVue contraindication (n=4)
♦ Severe acoustic shadowing (n=3)
♦ Alternate embolic source (n=3)
♦ Prior endarterectomy (n=1)
♦ Declined (n=5)

Symptomatic (n=26)
♦ Ipsilateral stroke (n=11)
♦ Ipsilateral carotid territory TIA (n=10)
♦ Ipsilateral amaurosis fugax TIA (n=5)
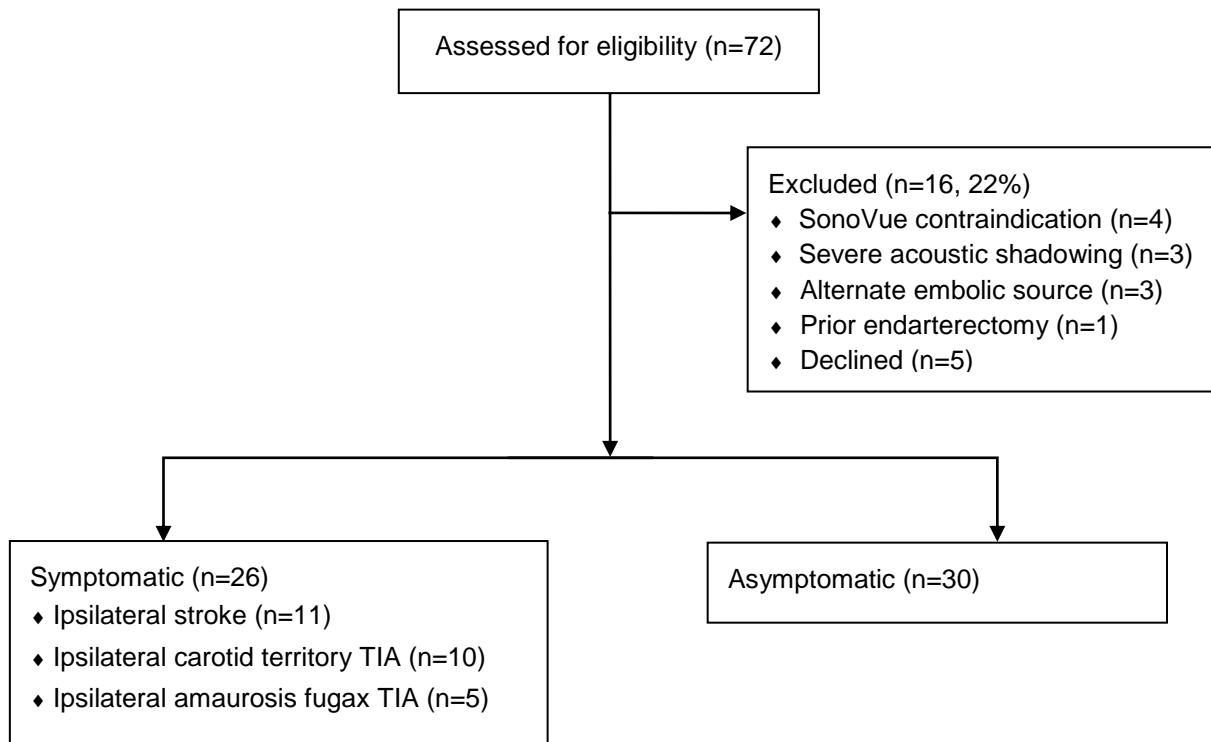
Asymptomatic (n=30)

Figure 58: Study flow diagram. Seventy-eight percent of those screened were scanned as part of the clinical study, with approximately a 1:1 symptomatic to asymptomatic breakdown.

Image analysis

DICOM video loops were exported for blinded, offline analysis by an independent vascular radiologist (Y.Z. 10 years of vascular carotid ultrasound experience). This was repeated by the sonographer (A.T.) for inter-reader comparison. Images were graded acceptable or unacceptable according to whether there was a clear view of the plaque and adequate enhancement, judged by the presence of moving microspheres in the soft tissue superficial to the carotid. Randomly moving microspheres were considered as real perfusion, whereas static enhancement was considered artefact. A training phase where ten pre-recorded image loops were graded in consensus was performed before the main study.

The previous chapter identified problems with quantification of signal intensity in the far wall of the carotid artery. Therefore in this study a simple binary grading system for perfusion was used: grade 0 representing 0-50% of plaque area containing moving microspheres and grade 1 representing >50% area of plaque area containing moving microspheres (Figures 59 and 60). Ulceration was recorded if a ≥1.5mm focal surface indentation communicating with the vessel lumen was visible on DCE-US (Figure 59). This depth was chosen because the axial resolution of the system was 1mm and both investigators were confidently able to agree on the presence of ulceration of 1.5mm. Below this depth it was difficult to distinguish ulceration from irregularity (analogous to the situation with plaque and intima-media thickness). It is noted that other investigators have used a range of 1-2mm to define ulceration with colour Doppler (102, 241).
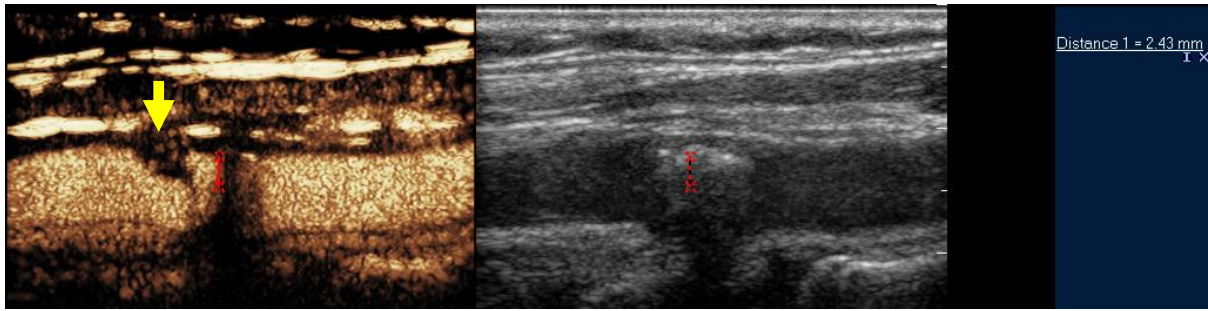
Figure 59: Dynamic contrast enhanced ultrasound image of patient with ipsilateral stroke demonstrating a 2.4mm deep ulcer (red line) and generalised plaque perfusion (yellow arrow). Source: Ankur Thapar (with permission from patient).
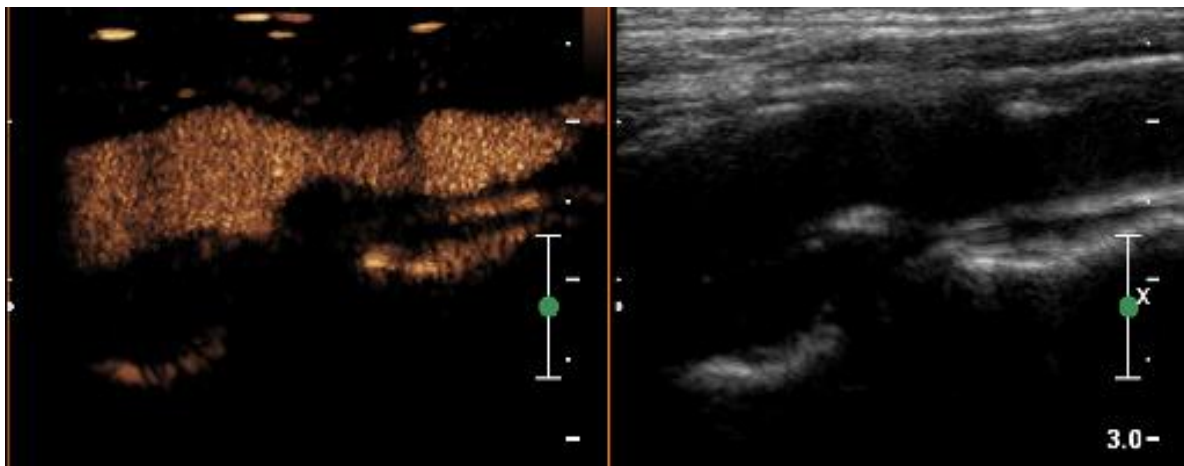
Figure 60: Dynamic contrast enhanced ultrasound image of an asymptomatic patient with smooth plaque and minimal plaque perfusion. Source: Ankur Thapar (with permission from patient).

Statistical analysis

Sample size calculation was performed using PASSv11 (NCSS, Utah, USA) using a one degree of freedom chi-squared test, inferring statistical significance below $p < 0.05$. Statistical analysis was performed using MedCalc v11 (MedCalc software, Mariakerke, Belgium). Continuous variables were checked for normality and differences between the groups assessed using the student's t-test or the Mann-Whitney test. Differences in

proportions were assessed using Fisher's exact test. Intra and inter-observer reproducibility were assessed using Cohen's kappa.

8.3   Results

Baseline demographics and univariate analysis

Demographic details of the 56 patients are shown in Table 18. Symptomatic patients had a higher degree of stenosis (77% SD 16 versus 67% SD 15, p=0.03) and were less likely to be on antiplatelet therapy (17/26 [65%] versus 28/30 [93%], p=0.02).

Variable	Asymptomatic	Symptomatic	p value
Number of patients	30	26	-
Age (years)	68 (SD 9)	73 (SD 11)	0.06
Male gender	21 (70%)	15 (58%)	0.41
Ipsilateral NASCET stenosis (%)	67 (SD 15)	77 (SD 16)	0.03*
Prior ipsilateral symptoms	2 (7%)	4 (15%)	0.40
Prior contralateral symptoms	8 (27%)	2 (8%)	0.09
Diabetes	6 (20%)	6 (23%)	1.00
Antihypertensive therapy	22 (73%)	20 (77%)	1.00
Statin therapy	24 (80%)	18 (69%)	0.38
Antiplatelet therapy	28 (93%)	17 (65%)	0.02*
History of smoking	20 (67%)	12 (46%)	0.18
Parental CVA	8 (27%)	4 (15%)	0.35
PAD	11 (37%)	5 (19%)	0.24
MI	5 (17%)	1 (4%)	0.20

Table 18: Univariate analysis of baseline risk factors. Of the 26 symptomatic patients, 11 (42%) had an ipsilateral carotid territory stroke, 10 (38%) had an ipsilateral carotid territory transient ischaemic attack and 5 (19%) had ipsilateral amaurosis fugax. *indicates a statistically significant difference between the groups using a t-test or Fisher's exact test. Prior symptoms refer to carotid territory events such as stroke, transient ischaemic attack, amaurosis fugax and retinal infarction.

Generalised plaque perfusion was seen in 9/26 (35%) of symptomatic and 12/30 (40%) of asymptomatic patients (Figure 61). There was no significant difference in the

proportion of patients with generalised plaque perfusion between the groups (OR 0.79, 95% CI 0.27 – 2.36, p=0.78).

Ulceration was seen in 4/56 (7%) of patients with colour Doppler and 9/56 (16%) with DCE-US (p=0.24) (Figure 61). The concordance between the two modalities was 47/56 (84%). The sensitivity, specificity, positive predictive value and negative predictive value of ulceration on colour Doppler for identifying patients with ipsilateral symptoms were 25%, 52%, 4% and 90% respectively. Using colour Doppler there was no significant difference in ulceration between symptomatic or asymptomatic patients (1/26 v 3/30, p=0.62). With DCE-US there was a trend towards more ulceration in symptomatic patients (7/26 v 2/30, OR 5.2, 95% CI 0.96 – 27.6, p=0.07) (Figure 62). The sensitivity, specificity, positive predictive value and negative predictive value of ulceration alone with DCE-US for identifying patients with ipsilateral symptoms were 27%, 93%, 78% and 60% respectively.
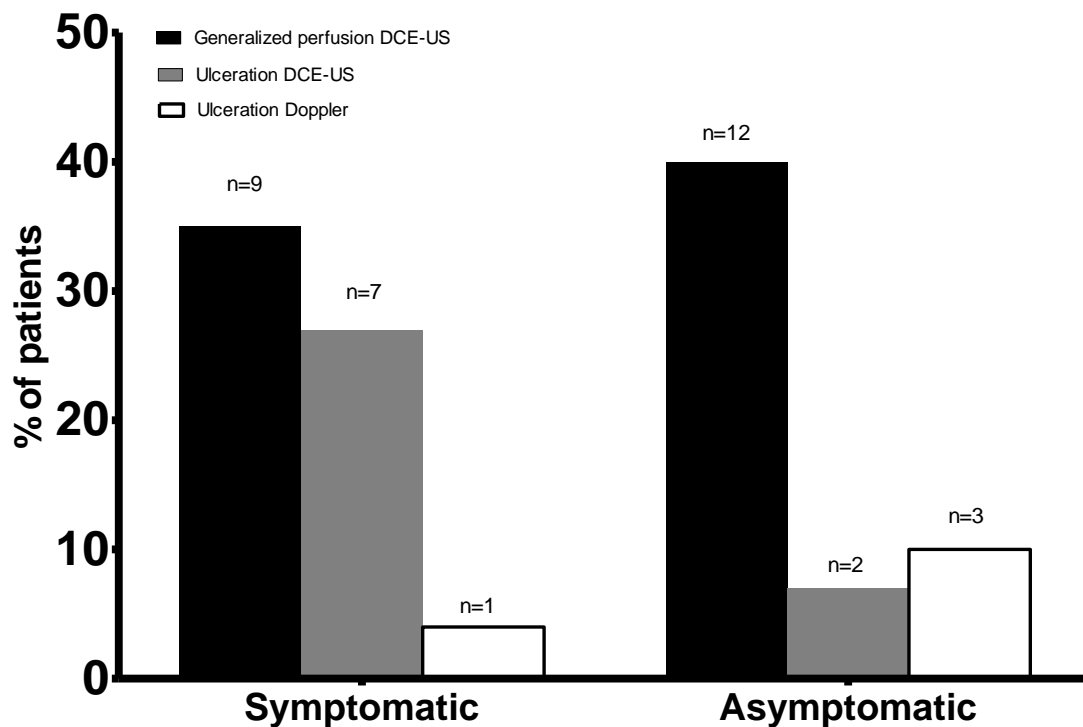
Figure 61: Proportion (n=absolute number) of patients with generalised perfusion and ulceration on dynamic contrast enhanced ultrasound and Doppler. There was a trend towards more ulcers in symptomatic patients, but only with DCE-US (p=0.07).

146

Ulceration on DCE-US and generalised perfusion co-localised in 6 patients, of whom 4 were symptomatic and 3 were asymptomatic.

Reproducibility

For plaque perfusion intra-reader reproducibility was moderate (κ=0.43) and inter-reader reproducibility was moderate (κ=0.49). For ulceration on DCE-US, intra-reader reproducibility was good (κ=0.87) and inter-reader reproducibility was good (κ=0.64).

Multivariate analysis

Variables with a p-value <0.1 were checked for collinearity in a correlation matrix (Table 19). Of particular interest was the relationship between ulceration and other risk factors such as stenosis and generalised perfusion. Three multivariate models were constructed to identify if ulceration was an independent predictor of symptomatic status (Tables 20 and 21). After adjustment for significant confounders (stenosis and lack of antiplatelet therapy), ulceration showed only a trend towards an increased odds of predicting symptomatic status (OR 2.77, 95% CI 0.41-18.94).

		Age	Antiplatelet	Contralateral event	Generalised perfusion	Stenosis	Ulceration
Age	Correlation Coefficient	-	0.013	-0.020	-0.016	0.090	0.016
	Significance Level p		0.9219	0.8856	0.9083	0.5081	0.9057
	n		56	56	56	56	56
Antiplatelet	Correlation Coefficient	0.013	-	0.113	0.104	0.117	-0.151
	Significance Level p	0.9219		0.4063	0.4436	0.3891	0.2673
	n	56		56	56	56	56
Contralateral event	Correlation Coefficient	-0.020	0.113	-	0.024	0.103	-0.077
	Significance Level p	0.8856	0.4063		0.8602	0.4515	0.5723
	n	56	56		56	56	56
Generalised perfusion	Correlation Coefficient	-0.016	0.104	0.024	-	-0.128	0.264
	Significance Level p	0.9083	0.4436	0.8602		0.3486	0.0496
	n	56	56	56		56	56
Stenosis	Correlation Coefficient	0.090	0.117	0.103	-0.128	-	0.300
	Significance Level p	0.5081	0.3891	0.4515	0.3486		0.0248
	n	56	56	56	56		56
Ulceration	Correlation Coefficient	0.016	-0.151	-0.077	0.264	0.300	-
	Significance Level p	0.9057	0.2673	0.5723	0.0496	0.0248	
	n	56	56	56	56	56	

Table 19: Correlation matrix demonstrating the weak positive correlation between ulceration and stenosis and between ulceration and generalised perfusion (shaded). All variables with a p<0.1 have been included. Pearson correlation coefficient for parametric variables.

Model	1	2	3
Ulceration on DCE-US	5.16 (0.97 – 27.56)	3.69 (0.65-21.06)	2.77 (0.41-18.94)
NASCET Stenosis (per %)	-	1.03 (0.99-1.07)	1.05 (1.01-1.10)
No prior antiplatelet therapy	-	-	11.96 (1.85-77.52)
c-statistic	0.60 (0.46-0.73)	0.68 (0.55-0.80)	0.80 (0.67-0.89)

Table 20: Results of logistic regression analysis for the outcome of symptomatic status. Numbers in the table refer to odds ratios and 95% confidence intervals. Model 1 = univariate analysis using ulceration on DCE-US. Model 2 = ulceration adjusted for stenosis. Model 3 = ulceration adjusted for stenosis and antiplatelet therapy. C-statistic = concordance statistic, representing model fit. Ulceration was not an independent predictor of symptomatic status. Antiplatelet therapy was the most powerful predictor of symptomatic status.

Further details of the coefficients for model 3 are shown in Table 21:

Variable	Coefficient	Std. Error	p
Ulceration	1.02	0.98	0.30
Stenosis per %	0.05	0.02	0.03
Lack of prior antiplatelet therapy	2.48	0.95	0.01

Table 21: Regression coefficients with standard error and statistical significance level of independent variables in model 3, consisting of ulceration, stenosis and lack of prior antiplatelet therapy. Note that after adjustment for confounders, the regression coefficient for ulceration had a large standard error and was non-significant.

8.4   Discussion

The results of this study demonstrate that visual grading of plaque perfusion appeared subjective and did not confer any benefits in the identification of culprit carotid atheroma.   DCE-US reported more ulcers than colour Doppler, with improved reproducibility. This could be expected as the addition of luminal contrast is the basis for detection of ulcers on angiography. Plaque ulceration on DCE-US was more specific to patients with ipsilateral symptoms. This requires histological validation. However plaque ulceration on DCE-US showed only a trend towards increased prevalence in symptomatic individuals on univariate analysis. Ulceration was not a significant predictor of symptomatic status on multivariate analysis and was weakly collinear with stenosis and lack of antiplatelet therapy. Antiplatelet therapy was the major clinical risk factor in this study for symptoms.

The finding that 7% of asymptomatic persons and 27% of symptomatic patients have ulcerated plaques is interesting as they roughly represent the number of patients needed to treat with endarterectomy to prevent one stroke. This may be a coincidence or may reflect a high risk subset within these two groups, who go on to suffer stroke. In a population with a high prevalence of ulceration, for example, symptomatic patients with moderate stenosis, it may be interesting to assess baseline ulceration and ipsilateral neurovascular events in a prospective fashion. In NASCET, angiographic ulceration was independently been shown to be prognostic for future events in symptomatic patients and therefore this is a potential hypothesis for further study (242), perhaps particularly in those with moderate symptomatic stenosis.

Ulceration on pre-operative angiography has been demonstrated in a large histopathological study of endarterectomy specimens of patients with cerebrovascular symptoms, to be strongly associated with the presence of microscopic plaque rupture and intraplaque haemorrhage (188). This supports the argument that ulceration on luminal contrast imaging is a risk marker for a culprit plaque. However it added only borderline discriminatory information regarding symptom status in the present study, perhaps because ulceration is less prevalent than microscopic fibrous cap rupture.

Other investigators have found rates of generalised plaque enhancement in 42-80% of symptomatic patients in comparison with 5-30% of asymptomatic controls (209, 232). There may be several reasons why we did not find a difference between these two groups. Firstly, ultrasound is uniplanar and plaque perfusion may be heterogeneous. Secondly, other investigators excluded calcified plaques without acoustic shadow, a few of which were included in this study. Thirdly, there were more smokers in the asymptomatic group and this may have increased the levels of neovascularisation. Finally the technique itself was somewhat subjective (intra-reader κ=0.43).

The strengths of this study are that it was reader blinded, using DCE-US to examine risk markers which previously been determined histologically. The limitations of this study are firstly, that plaque perfusion was graded visually and this system lacked reproducibility. Other groups have used either a two or three point grading system (209, 230), however it is difficult in practice to distinguish between absent (grade 0) and low grade (grade 1) perfusion. Indeed this group now also have switched to a two point system (230). Quantification was not attempted in this study due to the problem of non-linear propagation (243). It was noted that 1ml of contrast, although more suitable for quantification, was insufficient with current settings for visual plaque assessment of perfusion in those with a lower cardiac output (elderly or beta-blocked). To ensure there was adequate enhancement 2ml was used, however this much contrast was not required in most patients. Secondly, DCE-US was unsuccessful in assessing heavily calcified lesions, 3 of which were excluded in this study. For these lesions, other risk stratification techniques such as transcranial Doppler may be more appropriate. Thirdly, this study lacked a histological reference standard for ulceration. When considering whether this should be macroscopic assessment of ulceration or microscopic assessment of plaque rupture with luminal thrombus, it should be noted that agreement between visual macroscopic assessment and angiography for ulceration was poor in NASCET and the same is likely to be true for contrast enhanced ultrasound (244). If ulcerated plaques do not demonstrate microscopic plaque rupture on serial sectioning, then there is little biological basis for their use as a vulnerability marker. Fourthly, the lower stenosis range in asymptomatic patients may reflect a selection bias, as patients with high grade asymptomatic stenosis are considered for endarterectomy at our institution. Finally, the reference standard was

clinical and it may be that some patients were incorrectly classified. For symptomatic patients this was minimised by excluding those with abnormal clinical, electrocardiogram or troponin results. With respect to asymptomatic patients, only a small proportion of patients with asymptomatic stenosis in the UK (≈1% per annum) (165), progress to symptomatic status and therefore the number of vulnerable plaques in this group was likely to be low .

The findings of this study are that visual assessment of intraplaque perfusion did not add significant benefit in identifying patients with ipsilateral hemispheric symptoms. Ulceration demonstrated a trend towards an increased prevalence in symptomatic individuals and was reproducible. Future research could focus on the validity and predictive value of ulceration on DCE-US for future stroke in symptomatic patients with moderate (50-69%) stenosis.

9.0   Carotid plaque texture analysis

9.1   Introduction

Practical tests for vulnerable carotid plaque are sought to reduce the number needed to treat with endarterectomy for asymptomatic carotid stenosis. As the first line investigation for carotid stenosis in the United Kingdom, carotid ultrasound(30) , is ideally suited to characterising plaque structural features which are associated with ipsilateral symptoms. In 2009, the European Society for Vascular Surgery recommended "plaque morphology should be assessed in all cases before invasive treatment" (44). This is particularly important in populations where stenosis is not a clinically useful measure of risk, e.g. symptomatic patients with 50-69% stenosis and asymptomatic patients with 50-99% stenosis.

Plaque morphology analysis began with a binary separation of plaques on B (brightness) mode images into echolucent, isoechoic and echodense (176), followed by categorisation into plaque types by Gray-Weale (173) and subsequently Geroulakos (174), then into a continuous measure grey scale median (175) and recently by measuring the juxtaluminal echolucent component (204). Investigators are split as to which classification is the most discriminatory and all are in current use in prognostic trials (164, 179, 245, 246).

The aim of this study was to examine whether echolucency was a stronger discriminator than stenosis was in the previous chapter.

The primary hypothesis was that grey scale median identifies symptomatic patients more accurately than stenosis. The exploratory hypothesis was that a spatial measure of echolucency, percentage juxtaluminal echolucent area would show a better discriminatory ability than grey scale median for identifying images from symptomatic patients. Total juxtaluminal echolucent area was chosen as it accounts for 2 component plaques (near and far wall) and takes into account the overall area of the plaque, unlike largest juxtaluminal echolucent area used by other authors (204).

9.2   Methods

Ethical approval was gained in advance to perform this prospectively recruited cross-sectional study (ref 09/H0706/89). Written informed consent to participate was obtained from participants.   A sample size calculation was performed (MedCalc v11, MedCalc software, Mariakerke, Belgium). This determined that 46 patients per group were required to demonstrate a difference between c-statistic (the area under a receiver operator characteristic curve) of 0.8 (for grey scale median) and 0.6 (for stenosis, from previous chapter) with 80% power and a 10% false positive rate. The power and false positive rates were set in light of the number of patients which were likely to be recruited over one year from a single centre.

Inclusion criteria were 50-99% haemodynamic (NASCET equivalent) internal carotid artery stenosis on ultrasound. For symptomatic patients an ipsilateral carotid territory stroke, transient ischaemic attack, episode of amaurosis fugax or retinal infarction within 28 days of recruitment confirmed by an independent stroke neurologist was required, along with no evidence an alternative thromboembolic source on 24 hour electrocardiogram, full blood count, coagulation profile and troponin I. The time point of four weeks was chosen as a cut-off to avoid substantial plaque remodelling (decrease in echolucent constituents such as intraplaque haemorrhage) in symptomatic patients (34). Echocardiography and 24 hour electrocardiography were performed on those who had a clinical indication: bilateral cerebral infarcts, palpitations, cardiomegaly, left ventricular hypertrophy or raised troponin I. For asymptomatic patients no symptoms, signs or history of prior clinical events were permissible in the ipsilateral carotid territory. A flow diagram is shown in Figure 62.

Exclusion criteria were free floating thrombus, tandem carotid lesions, established alternative embolic source, recurrent stenosis, prior irradiation, masking neurological condition and acoustic shadowing concealing >50% of the plaque area due to calcification.

Patients were imaged using a Philips iu22 ultrasound platform and an L9-3 linear array broadband transducer (A.T.). Anterolateral, lateral and posterolateral scanning angles were tried to give the best image. Gain settings were maintained between at 30-40%, time-

gain curve gently sloping to the right at depth, except for the lumen section which was kept vertical, focus encompassing the plaque which was kept horizontal in the centre of the image. The section of the plaque chosen for capture was the largest area of plaque where the plaque and intima media thickness was sharply in focus and a colour Doppler signal was visible through the lumen (Figure 63). Images were anonymised using a study identifier. One artery per patient was imaged to avoid data clustering.
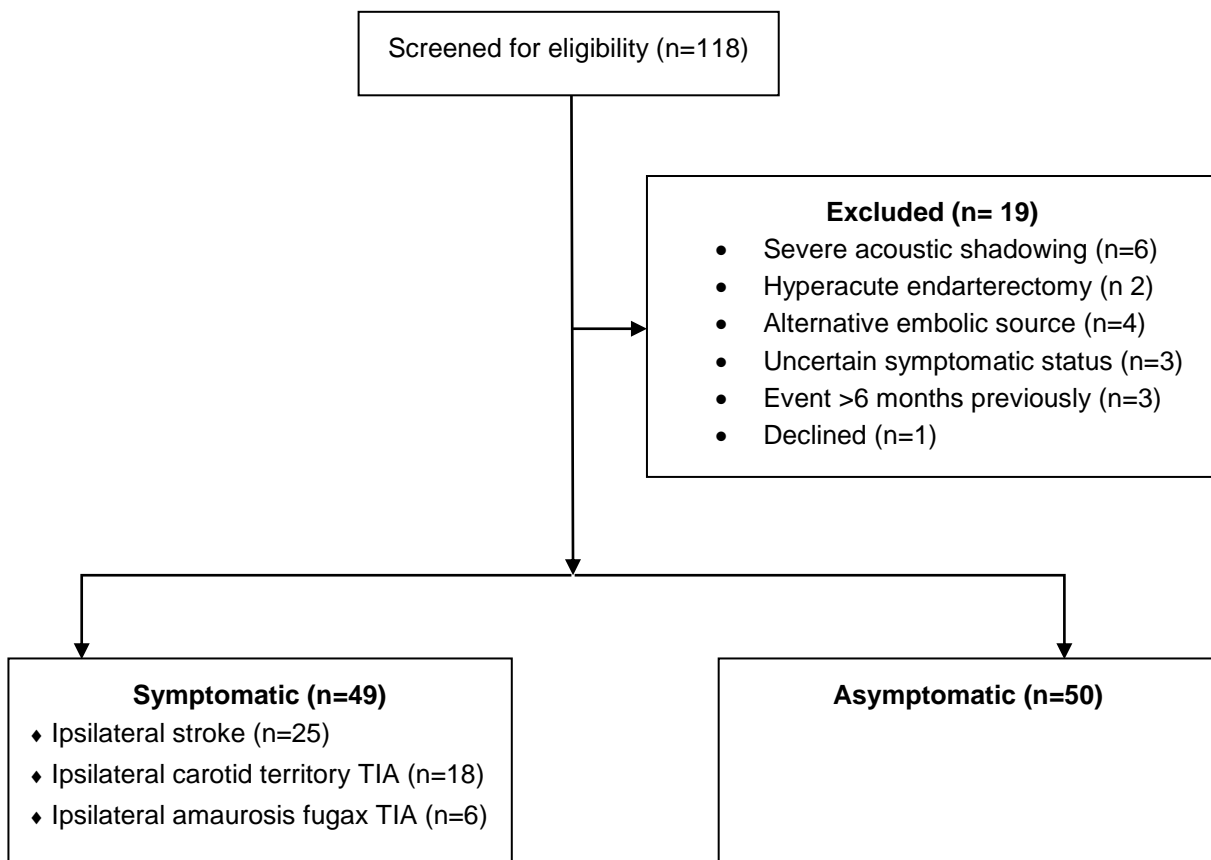
Figure 62: Flow diagram of recruitment for the study. Out of 118 patients screened, 99 (84%) underwent ultrasound imaging and entered the study whilst 19 (16%) were excluded for reasons shown above. Each patient underwent ultrasound imaging of one carotid plaque, which was analysed by an independent radiologist.

Image analysis

Images were analysed offline by an independent vascular radiologist (Y.Z. – ten years' carotid ultrasound experience), who had undergone training in plaque texture analysis before the study commenced. Plaque was identified as a focal structure encroaching into the lumen of >50% of the surrounding intima-media complex (25). Where acoustic shadowing was severe, plaques were excluded. For those where only a small portion of the plaque was obscured, texture analysis was performed on the calcified cap, and an estimate of plaque area was made (Figure 3). Images were adjusted for gain settings by setting the pixel intensity of the lumen as a reference point of 0 arbitrary units and the brightest adventitial segment as 190 arbitrary units. Images were adjusted for differing depth settings by manually selecting a 10mm distance on the image and re-pixelating to 20 pixels/mm, enabling standardised area measurements to be calculated.
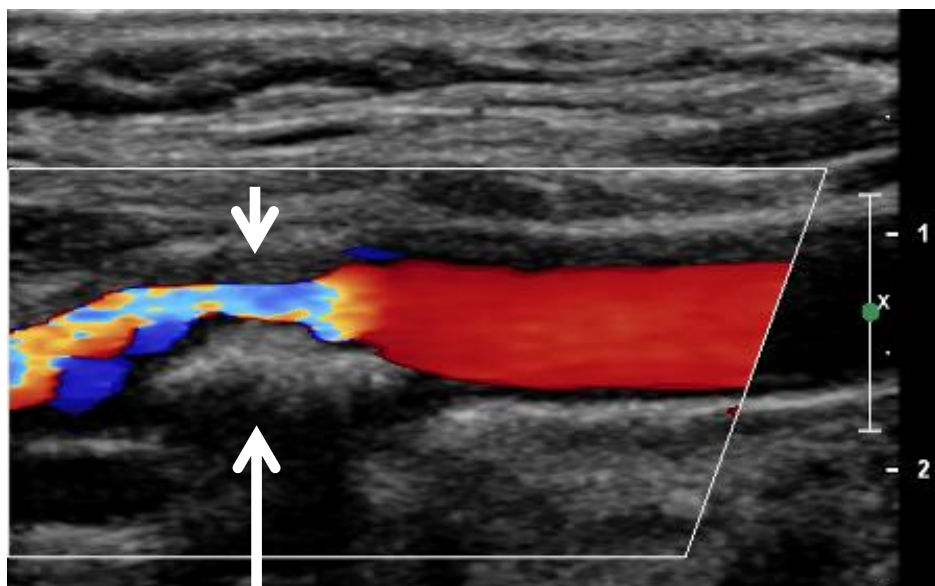
Figure 63: Colour Doppler image capture of a type V carotid plaque. The artery was captured running horizontally through the field of view. The adventitia is sharply defined as an echogenic stripe, a focal thickening (plaque) with a calcified cap and acoustic shadow is visible (type V plaque). A small near wall component (small arrow) and a larger far wall component (large arrow) are visible. For calculation of are, both segments of the plaque was outlined manually. However for calculation of grey scale median only the echogenic area was used, to avoid inclusion of the acoustic shadow. Source: Ankur Thapar (with permission from patient).

Grey scale median measurements were extracted after manual outlining of the plaque, as the normalised median pixel intensity of the plaque. Juxtaluminal echolucent areas were manually outlined as a black area (normalised pixel intensity of 0-25) on parametric imaging (Figure 64). In plaques with distinct near and far wall components, the total juxtaluminal black area and the largest single near or far wall juxtaluminal black area were also measured. Plaque type was automatically classified using acoustic shadow-free areas of the plaque (Figure 64): type I plaques had <15% of plaque area comprising pixel grayscale values >25, type II plaques had 15-50% of plaque area comprising pixel grayscale values >25, type III plaques had pixel 51-85% of plaque area comprising pixel grayscale values >25, type IV plaques had 86-100% of plaque area comprising pixel grayscale values >25. Type V plaques resembled type IV plaques however acoustic shadowing obscured the base of the plaque. Type 1 and 2 plaque were categorised as echolucent and types III, IV and V as echogenic. Plaque area was manually outlined as the sum of any plaques at the carotid bifurcation using power Doppler still images and video loops as a guide. Stenosis was calculated as per consensus UK guidelines by the vascular scientist primarily scanning the patient, using peak systolic velocities, but using velocity ratios or after computed tomographic angiography if unclear (31). Figure 65 depicts a sample analysis.
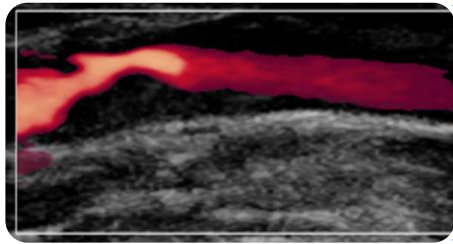
Echolucent plaque, type I

Ultrasound cannot differentiate lipid from necrosis or intraplaque haemmorhage, or measure cap thickness.
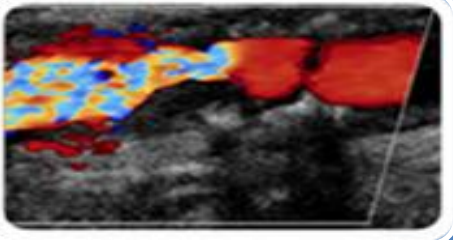
Heterogeneous plaque, type II
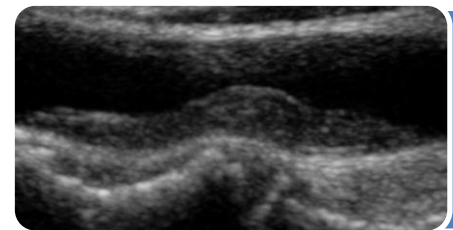
Fibrous plaque, type III

Ultrasound cannot reliably differentiate fibrous plaque from fibroatheroma
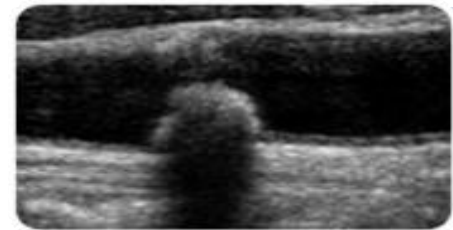
Calcified plaque type IV

Ultrasound cannot easily image the core of the plaque due to reflection of sound waves from echogenic cap
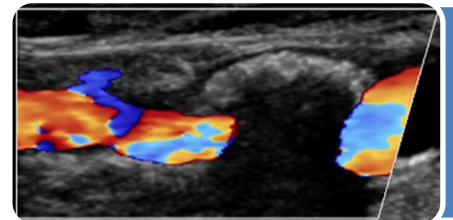
Plaque with acoustic shadow type V

Far wall plaque component inaccessible to ultrasound due to acoustic shadow

Figure 64: Pictorial representation of the 5 conventional plaque types using ultrasound (174). Where plaques are difficult to visualise, colour Doppler images have been used. Note that when actually determining plaque type only normalised B-mode images are used. This is because the use of colour Doppler artificially lowers the B-mode gain and decreases B-mode resolution. Source: Ankur Thapar (with permission from patients).
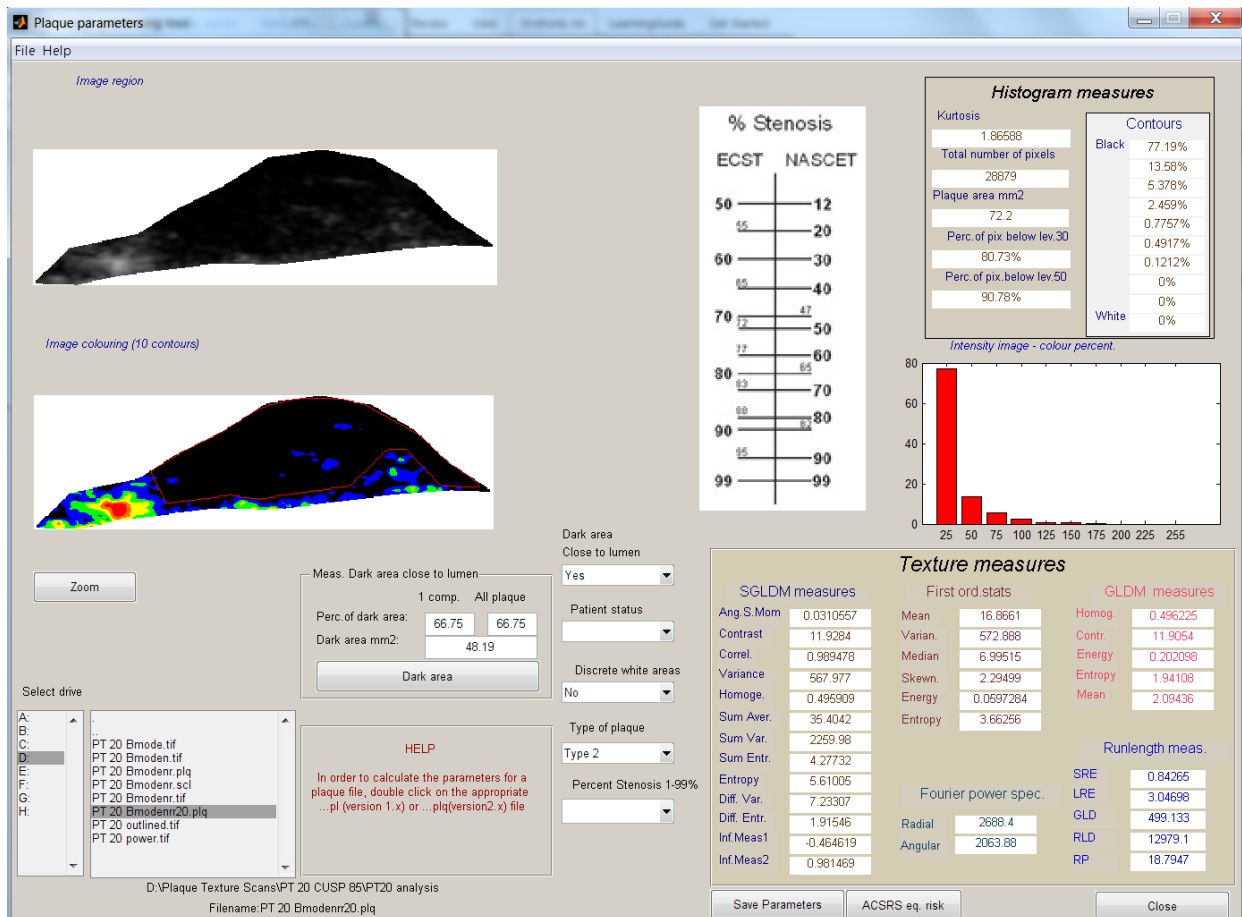
Figure 65: Example of plaque texture analysis screen from Plaque Texture Analysis software version 4 (Iconsoft, UK). An echolucent, type 2 plaque with a single discrete white area is seen (top). The plaque type, area and grayscale pixel median intensity were automatically calculated after the plaque border was manually outlined using a colour or power video clip and still image. Below a parametric image (a colour mapped representation of B-mode pixel intensity, with lighter colours representing bright pixels) has been used to aid manual outlining of the juxtaluminal black area (48mm^2). Source: Ankur Thapar.

Statistical Analysis

Data were checked for normality and appropriate parametric (t-test) or non-parametric (Man-Whitney) tests used. Statistical significance for univariate analysis was inferred below $p<0.05$. The sensitivity, specificity, diagnostic odds ratio and area under the receiver operator characteristic (ROC) curve were calculated for stenosis, grey scale median, echolucency and type II plaque. Inter-sonographer reproducibility was calculated using the intra-class correlation coefficient and Bland-Altman methodology.

Candidate imaging variables were checked for collinearity using a correlation matrix. Independent variables were entered into a logistic regression model manually, starting with those that were statistically significant on univariate analysis. The output of the models was the area under a receiver operator characteristic curve (concordance or c-statistic), estimating the predictive ability of the model for symptomatic status.

9.3   Results

118 consecutive patients were screened for eligibility for this study between May 2011 and May 2012 (Figure 62). After screening 19 (16%) patients were excluded (6 had severe acoustic shadowing, 2 underwent hyperacute endarterectomy before image capture could be performed, 2 had concurrent atrial fibrillation and were not anticoagulated, 2 had tandem carotid lesions, 3 had uncertain symptomatic status, 3 had an ipsilateral stoke >6 months previously and 1 patient declined).

In total, 99 arteries from 99 patients were analysed. The mean age of patients was 71 years (SD 11) and 62/99 (63%) were male. These patients had carotid duplex performed due to the presence of peripheral arterial or aortic aneurysmal disease (n=30, 30%) or new neurological symptoms (n=68, 69%) or through private screening (n=1, 1%). Fifty were asymptomatic and 49 were symptomatic. Of the symptomatic group, 6 (12%) had amaurosis fugax, 18 (37%) had a transient ischaemic attack and 25 (51%) had ischaemic stroke. The median time from last symptom was 4 days (IQR 1-18 days).

Patient demographics

Baseline demographics are shown in Table 22:

Variable	Asymptomatic group n=50	Symptomatic group n=49	p value
Age	70 (SD 12)	72 (SD 10)	0.28
Male gender	34 (69%)	28 (57%)	0.30
Regular antihypertensive use	42 (84%)	39 (80%)	0.61
Regular statin use	40 (80%)	24 (49%)	0.003*
Regular antiplatelet use	38 (76%)	25 (51%)	0.01*
Diabetes	16 (33%)	8 (16%)	0.10
Parental stroke	11 (22%)	10 (20%)	1.00
Current smoker	8 (16%)	16 (33%)	0.06
Prior ipsilateral stroke/TIA/amaurosis	1 (2%)	6 (12%)	0.06
Prior myocardial infarction	6 (12%)	4 (8%)	0.74
Peripheral arterial disease	8 (16%)	6 (12%)	0.77
Aortic aneurysm	2 (4%)	1 (2%)	1.00

Table 22: Results of univariate analysis of baseline demographics. Statistically significant differences are shown in grey with an asterisk.

Plaque texture characteristics

Plaque texture characteristics are shown in Table 23:

Variable	Asymptomatic group n=50	Symptomatic group n=49	p value
NASCET stenosis (%)	60 (IQR 50-80)	70 (58-90)	0.09
Echolucent plaque type (1 or 2)	18 (36%)	29 (59%)	0.03*
Type II plaque	14 (28%)	26 (53%)	0.01*
Plaque area at bifurcation (mm^2)	47 (SD 19)	52 (SD 22)	0.21
Grey scale median (0-256)	33 (IQR 22-49)	22 (11-52)	0.21
Juxtaluminal echolucent proportion (%)	17 (6-38)	30 (0-47)	0.55
Largest juxtaluminal echolucent area (mm^2)	7 (2-14)	10 (1-22)	0.17

Table 23: Results of univariate analysis of plaque texture features. Statistically significant differences are highlighted with an asterisk.

Accuracy of grey scale median versus stenosis for identification of symptomatic status

To estimate the accuracy of grey scale median and stenosis for identification of plaques from symptomatic individuals, a receiver operator characteristic curve was plotted. The c-statistic for stenosis was 0.60 (95% CI 0.49-0.71) and for grey scale median 0.57 (95% CI 0.46-0.69). Neither was significantly better than chance for identification of symptomatic status (p=0.09 for stenosis and p=0.21 for grey scale median respectively). Results are depicted in Figures 66-68.

ROC curve for stenosis and symptomatic status

Area	0.5984
Std. Error	0.05724
95% confidence interval	0.4862 to 0.7106
P value	0.09173
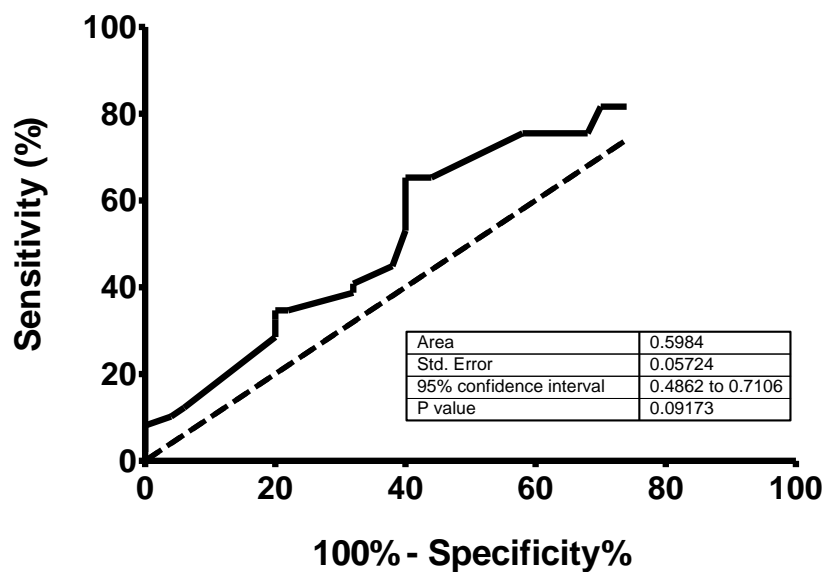
Figure 66: Receiver operator characteristic (ROC) curve for luminal stenosis in identifying plaque images from symptomatic patients. The area under the curve (c-statistic) was 0.60 and was not significantly better than chance (p=0.09).

ROC curve for GSM and symptomatic status

Area	0.5737
Std. Error	0.05879
95% confidence interval	0.4584 to 0.6889
P value	0.2066
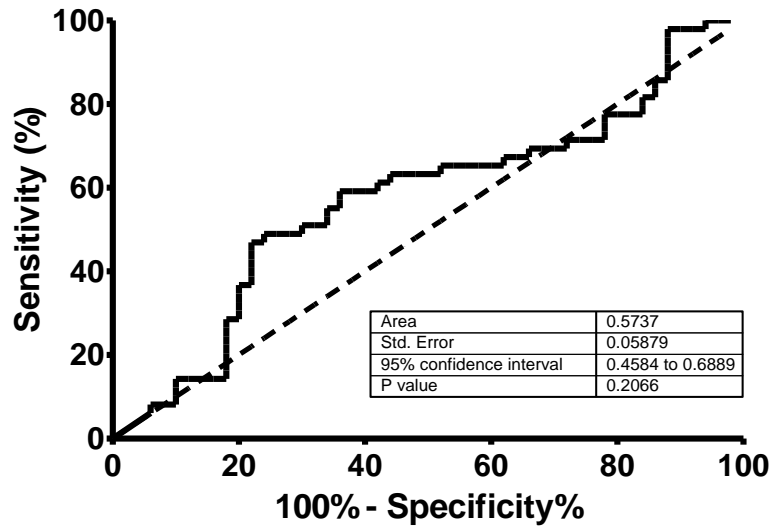
Figure 67: Receiver operator characteristic (ROC) curve for grey scale median (GSM) in identifying plaque images from symptomatic patients. The area under the curve (c-statistic) was 0.57 and was not significantly better than chance (p=0.21).

ROC curve for % juxtaluminal echolucent area and symptomatic status

Area	0.5347
Std. Error	0.05925
95% confidence interval	0.4185 to 0.6509
P value	0.5520
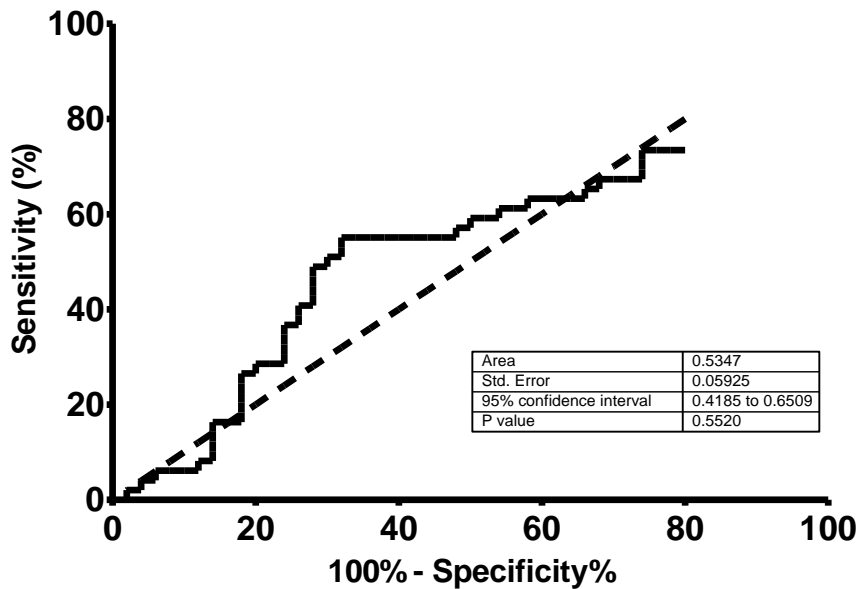
Figure 68: Receiver operator characteristic (ROC) curve for percentage total (upper and lower plaque components) juxtaluminal black area in identifying symptomatic patients. The area under the curve (c-statistic was 0.53 and this was not significantly better than chance p=0.55).

Breakdown of plaque types by symptomatic status
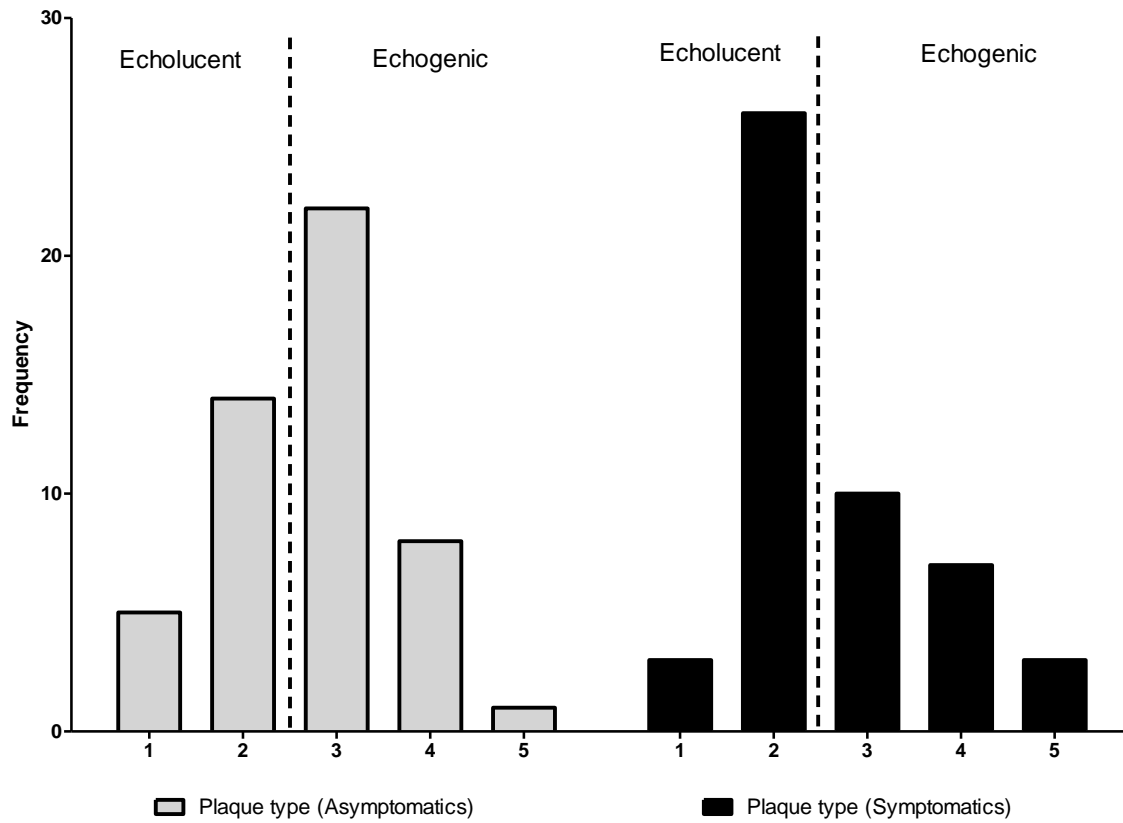
Figure 69: Breakdown of plaque types by symptomatic or asymptomatic status. There were more type II and less type III plaques in the symptomatic group. Plaque type refers to the Geroulakos classification (174).

For the categorical variables echolucency and proportion of type II plaques, diagnostic accuracy statistics were calculated as shown in Table 24:

	Echolucency	Type II plaque
Sensitivity	59%	65%
Specificity	64%	61%
Positive predictive value	62%	53%
Negative predictive value	62%	72%
Diagnostic odds ratio (95% CI)	2.58 (1.15-5.80)	2.91 (1.23-6.70)

Table 24: Test statistics for echolucency and type II plaque for identifying symptomatic patients.

Reproducibility

To ascertain the inter-separately sonographer reproducibility of plaque texture analysis, 20 random patients were scanned separately by A.T. and the on-duty vascular scientist. The results were read offline by the same vascular radiologist (Y.Z.).

To ascertain the inter-reader reproducibility of plaque texture analysis, the full 99 images taken by A.T. were analysed separately by Y.Z. and A.T. Results are shown in Tables 25 and 26. Bland-Altman plots for continuous variables are available in Appendix 3.

Variable	Reproducibility Measure	Statistic
Plaque type	Cohen's kappa with linear weighting	0.65 (95% CI 0.47-0.83)
Echolucent plaque	Cohen's kappa	0.60 (95% CI 0.26-0.94)
Grey scale median	Intra-class correlation coefficient	0.84 (95% CI 0.65-0.93)
Plaque area	Intra-class correlation coefficient	0.80 (95% CI 0.57-0.92)
Largest juxtaluminal echolucent area	Intra-class correlation coefficient	0.59 (95% CI 0.22-0.81)
Percentage juxtaluminal echolucent area	Intra-class correlation coefficient	0.67 (95% CI 0.33 – 0.85)

Table 25: Inter-sonographer reproducibility statistics. Kappa statistics of 0.41-0.60 correspond to moderate agreement, 0.61-0.80 to good agreement and 0.81-1.00 to near perfect agreement.

Variable	Reproducibility Measure	Statistic
Plaque type	Cohen's kappa with linear weighting	0.80 (95% CI 0.72 – 0.88)
Echolucent plaque	Cohen's kappa	0.84 (95% CI 0.73 - 0.95)
Grey scale median	Intra-class correlation coefficient	0.91 (95% CI 0.87 – 0.94)
Plaque area	Intra-class correlation coefficient	0.75 (95% CI 0.65 – 0.83)
Largest juxtaluminal echolucent area	Intra-class correlation coefficient	0.77 (95% CI 0.68 – 0.84)
Percentage juxtaluminal echolucent area	Intra-class correlation coefficient	0.72 (95% CI 0.61 – 0.80)

Table 26: Inter-reader reproducibility statistics. Kappa statistics of 0.41-0.60 correspond to moderate agreement, 0.61-0.80 to good agreement and 0.81-1.00 to near perfect agreement.

Multivariate analysis

Multivariate analysis was undertaken to identify if plaque type was independently associated with symptomatic status.

		Antiplatelet	Area (mm²)	Largest echolucent area (mm²)	Echolucent plaque	Stenosis (%)	GSM (ALIU)	Total % JLEA	Prior ipsilateral event	Smoker	Statin	Type II plaque
Antiplatelet	Correlation Coefficient Significance Level p		-0.029 0.7727 99	-0.151 0.1358 99	-0.107 0.2921 99	-0.074 0.4682 99	0.071 0.4878 99	-0.104 0.3044 99	0.045 0.6606 99	-0.237 0.0180 99	**0.559 <0.0001 99**	-0.019 0.8485 99
Area (mm²)	Correlation Coefficient Significance Level p n	-0.029 0.7727 99		0.343 0.0005 99	0.076 0.4566 99	0.214 0.0333 99	-0.135 0.1816 99	0.047 0.6446 99	0.085 0.4001 99	0.000 1.0000 99	-0.106 0.2943 99	0.126 0.2138 99
Largest echolucent area (mm²)	Correlation Coefficient Significance Level p n	-0.151 0.1358 99	0.343 0.0005 99		**0.731 <0.0001 99**	0.258 0.0101 99	**-0.840 <0.0001 99**	**0.862 <0.0001 99**	-0.134 0.1860 99	0.120 0.2366 99	-0.290 0.0036 99	**0.530 <0.0001 99**
Echolucent plaque	Correlation Coefficient Significance Level p n	-0.107 0.2921 99	0.076 0.4566 99	**0.731 <0.0001 99**		0.152 0.1341 99	**-0.866 <0.0001 99**	**0.749 <0.0001 99**	-0.110 0.2788 99	0.087 0.3897 99	-0.192 0.0567 99	**0.849 <0.0001 99**
Stenosis (%)	Correlation Coefficient Significance Level p n	-0.074 0.4682 99	0.214 0.0333 99	0.258 0.0101 99	0.152 0.1341 99		-0.205 0.0419 99	0.203 0.0443 99	0.055 0.5891 99	0.048 0.6370 99	-0.101 0.3181 99	0.170 0.0925 99
GSM (ALIU)	Correlation Coefficient Significance Level p n	0.071 0.4878 99	-0.135 0.1816 99	**-0.840 <0.0001 99**	**-0.866 <0.0001 99**	-0.205 0.0419 99		**-0.828 <0.0001 99**	0.102 0.3148 99	-0.109 0.2827 99	0.116 0.2539 99	**-0.671 <0.0001 99**
Total % JLEA	Correlation Coefficient Significance Level p n	-0.104 0.3044 99	0.047 0.6446 99	**0.862 <0.0001 99**	**0.749 <0.0001 99**	0.203 0.0443 99	**-0.828 <0.0001 99**		-0.142 0.1623 99	0.123 0.2259 99	-0.178 0.0775 99	**0.508 <0.0001 99**
Prior Ipsilateral event	Correlation Coefficient Significance Level p n	0.045 0.6606 99	0.085 0.4001 99	-0.134 0.1860 99	-0.110 0.2788 99	0.055 0.5891 99	0.102 0.3148 99	-0.142 0.1623 99		0.021 0.8360 99	0.034 0.7418 99	-0.147 0.1470 99
Smoker	Correlation Coefficient Significance Level p n	-0.237 0.0180 99	0.000 1.0000 99	0.120 0.2366 99	0.087 0.3897 99	0.048 0.6370 99	-0.109 0.2827 99	0.123 0.2259 99	0.021 0.8360 99		-0.216 0.0317 99	0.043 0.6755 99
Statin	Correlation Coefficient Significance Level p n	**0.559 <0.0001 99**	-0.106 0.2943 99	-0.290 0.0036 99	-0.192 0.0567 99	-0.101 0.3181 99	0.116 0.2539 99	-0.178 0.0775 99	0.034 0.7418 99	-0.216 0.0317 99		-0.141 0.1626 99
Type II plaque	Correlation Coefficient Significance Level p n	-0.019 0.8485 99	0.126 0.2138 99	**0.530 <0.0001 99**	**0.849 <0.0001 99**	0.170 0.0925 99	**-0.671 <0.0001 99**	**0.508 <0.0001 99**	-0.147 0.1470 99	0.043 0.6755 99	-0.141 0.1626 99	

Table 27: Correlation matrix using the non-parametric Spearman rank correlation to assess the putative independent variables for collinearity. **Bold and shaded**, cells indicate moderate to strong, statistically significant collinearity, whereas shaded cells alone represent weak statistically significant collinearity. Where this existed, the variable with the lowest p-value on univariate analysis was chosen. For example in the case of type II plaque and echolucent plaque, type II plaque was chosen for multivariate analysis.

Model	1	2	3	4	5
	Stenosis alone	Type II plaque	Stenosis & type II plaque	Clinical variables	Best fit
NASCET Stenosis (per % increase)	1.02 (1.00-1.05)	-	1.02 (0.99-1.04)	-	-
Type II plaque	-	2.91 (1.26-6.70)	2.71 (1.16-6.30)	-	3.22 (1.30-7.94)
Lack of statin therapy	-	-	-	3.72 (1.47-9.41)	3.86 (1.50-9.90)
Current smoker	-	-	-	-	-
Prior ipsilateral event	-	-	-	8.52 (0.94-76.90)	11.92 (1.28-111.34)
c-statistic (95% CI)	0.60 (0.50-0.70)	0.63 (0.52-0.72)	0.66 (0.56-0.75)	0.68 (0.58-0.77)	0.75 (0.65-0.83)

Table 28: Odds ratios for 5 logistic regression models with symptomatic status as the outcome variable. Model 3 illustrates that when type II plaque is used as a covariate, stenosis ceases to have an effect on outcome. Model 4 illustrates the best model that could be achieved without plaque imaging, whilst model 5 (best fit) combines clinical and imaging features to give an area under the curve of 0.75.

Variable	Coefficient	Std. Error	P
Type II plaque	1.17	0.46	0.01
Lack of statin therapy	1.35	0.48	0.005
Prior ipsilateral CORI	2.48	1.14	0.03

Table 29: Regression coefficients for the main independent variables used in model 5.

9.4   Discussion

The principal results of this study were that neither stenosis nor indeed grey scale median were clinically useful discriminators in plaque images from symptomatic patients. The use of total juxtaluminal echolucent percentage area did not add useful discriminatory information. However the presence of echolucent and especially type II plaque in particular, significantly increased the odds of symptomatic status, independently of stenosis. Assessment of plaque type also demonstrated good inter-sonographer reproducibility. When clinical and imaging variables were combined in a multivariate model, lack of prior statin therapy, a prior ipsilateral event and type II plaque were independent predictors of symptomatic status with reasonable accuracy.   The best fit model is exploratory and requires validation in another population.

As expected stenosis was not a powerful discriminator for symptomatic status. Grey scale median showed no statistically significant difference between groups because type II plaques are not the most echolucent plaque type (type I). The finding that echolucent plaques increased the risk of future symptoms has been reported by others in prospective studies (164, 171, 179). However in this study, an increase in the numbers of type II (echolucent and heterogeneous) plaque was the main difference between symptomatic and asymptomatic individuals suggesting that focal areas of heterogeneity may be important. These may represent areas of old intraplaque haemorrhage, an isoechoic plaque with an accumulation of echolucent surface thrombus, or a boundary between soft and hard

components where shear stress changes abruptly, leading to areas where rupture may occur. However as juxtaluminal echolucent area was not discriminatory in the present study, this argues against surface thrombus adhering to an underlying isoechoic plaque. Or this may simply represent a chance finding in the data. Investigators are split as to whether plaque heterogeneity increases or decreases risk (38, 164, 177). However, histologically, little is known about these focal areas of echogenicity.

The strengths of this study are that it was a practical approach to risk stratification, using a low-technology approach performed on an adequate number of individuals, with blinded semi-automated analysis. The imaging marker with most promise, plaque type, was reproducible enough to facilitate clinical translation.

There are several limitations to this study. Overall this study was designed to identify variables with a strong association with symptomatic status for prospective study. Therefore it cannot be concluded that type II plaques are responsible for symptoms, as they might be the effect of plaque rupture. Secondly an 80% power was chosen to allow a recruitment to finish within a year. There is therefore a 20% chance of a false negative result for grey scale median identifying symptomatic patients more accurately than stenosis. However there was no trend seen towards this in the results. Thirdly, ultrasound is a uniplanar technique and therefore it was seen that some quantitative variables such as grey scale median and juxtaluminal hypoechoic area were prone to change with the plane of image capture. Features such as plaque type are less prone to this problem. The situation will only change with the advent of volumetric acquisition, which at present is under study. Fourthly, ultrasound cannot image the intracranial internal carotid artery which may also have atheroma present. As ultrasound is now the primary imaging technique for internal carotid stenosis at our institution, only a handful of patients received alternative imaging.

The implications of this study are that echolucency or plaque type should be included in future prospective studies of plaque vulnerability. They represent reproducible and simple measures that other groups also suggest have prognostic benefit. Plaque morphology would be complemented by a simple marker of previous rupture or activity, perhaps ulceration on DCE-US could be considered here. Researchers could consider plaque type or echolucency a screening tool for further investigation such as transcranial Doppler or MRI.

For clinicians, it is recommended those with a type II plaque or a previous ipsilateral event, receive adequate statin therapy to lower LDL to the high risk target of 1.8mmol/L as per ESVS guidelines (95).

Conclusion

The results of this study suggest that grey scale median does not add significant extra discriminatory information for symptomatic status, over and above luminal stenosis. In this study, lack of statin use, prior event and type II plaque were independent risk factors for symptomatic status. Further histological study of type II plaques is required to identify if histologically they correspond to a thin cap fibroatheroma and to understand the nature of any echodense segments. Clinicians should ensure that patients with type II plaques receive adequate dose statin therapy.

10.0   Final discussion

10.1 Significance of findings

The premise of this thesis was that there exists a subset of asymptomatic patients with a vulnerable carotid plaque in whom early endarterectomy is beneficial and that ultrasound may have a role in their identification.

The findings of this thesis were that if the results of the Asymptomatic Carotid Surgery Trial were extrapolated to the United Kingdom, carotid endarterectomy was likely to be cost-effective, according to current thresholds but that this was conditional on background stroke rates (which are currently falling). Modelling the benefits of testing and treating claudicants, an easily accessible, high prevalence population, would result in a very small number of strokes saved, with a high cost per stroke saved due to a high number needed to treat, relative to symptomatic individuals. This introduced the idea of selection through imaging plaque vulnerability markers, of which detection of microembolic signals, imaging of plaque echolucency with ultrasound and imaging intraplaque haemorrhage with magnetic resonance imaging currently appear to hold promise. However, a low cost screening modality may be useful as a gatekeeper for more expensive, more accurate imaging tests.

The use of high mechanical index contrast-enhanced ultrasound was found to have a problem with tissue suppression and therefore was not used further for plaque assessment. At low mechanical indices, perfusion in the far carotid wall, demonstrated a non-linear propagation artefact which affect quantification of at least half of the visible atheroma, which was a problem. Imaging of perfusion, the proposed precursor to intraplaque haemorrhage, did not appear to show a large difference between symptomatic and asymptomatic patients and the technique was subjective. Imaging of ulceration with dynamic contrast enhanced ultrasound was reproducible and was very specific for symptomatic individuals. However, it was not an independent predictor of risk. Finally a specific type of echolucent plaque (type II) was found to cluster in symptomatic individuals

and was an independent risk marker. However it should be noted that simple clinical risk factors such as prior antiplatelet and statin therapy were probably more important in predicting symptomatic status than the sonographic appearance of the atheroma.

This thesis added new information regarding the cost-effectiveness of endarterectomy for asymptomatic carotid atherosclerosis from a UK perspective. It also added information for those planning vascular services on the cost and impact of testing for asymptomatic carotid disease. The contrast enhanced ultrasound chapters identified important issues for ultrasound engineers designing the next generation of non-linear pulse sequences. An important finding was that the simpler the risk stratification variable, the more likely it was to be reproducible and also discriminatory.

There were many limitations to this thesis, which are described in the individual chapters. The main limitations were that the cost-effectiveness analysis relied on inferred costs and trial data, rather than micro-costing data and real world results, e.g. from the National Vascular Database. There are limitations with the concept of risk stratification through carotid specific imaging biomarkers, which should be noted. Firstly, many existing modalities measure the end product of rupture, e.g. microemboli and ulceration. These are more likely to be useful in risk prediction in symptomatic patients with moderate carotid stenosis, who are more likely to have these findings. Imaging the true precursors to rupture, e.g. inflammation, plaque surface shear stress, intraplaque haemorrhage and microvessel density are in their infancy and will take time to develop. There is a possibility that by the time these modalities have been developed and implemented, there may be no rationale for their use in the vast majority of asymptomatic patients, in whom risk factors have been intensively treated. However they may add information to patients with moderate symptomatic stenosis and may be important as surrogate endpoints for testing novel therapies for atherosclerosis in phase II trials. An example of this type of is the dal-PLAQUE trial of cholesteryl ester transfer protein, which raises high density lipoprotein cholesterol, whose vessel specific effects were monitored using PET-MRI in a randomised phase II trial (247), leading on to a larger phase III study post-acute coronary syndrome (248). Thirdly, the small population selected for risk stratification will be dwarfed by the much larger population who are not selected for testing and who, simply by virtue of their

increased size will incur more strokes. This population can only be reached through improved active monitoring of primary prevention, an important consideration for the preventative vascular specialist. In this respect it is important for the Vascular Society to work with the Joint British Societies preventative task force, as the European and US Society for Vascular Surgery already do. Fourthly, once we identify high risk plaque phenotypes, it is worth noting that they may be associated with a higher risk during endarterectomy. The perioperative stroke rate in any high risk group requires national audit, to ensure that it remains low enough to ensure benefit remains.

Moving on and considering the differing results of late phase imaging between successive students, it is apparent that either the acquisition technique is extremely subjective, or that without measuring baseline plaque intensity, a false positive was detected, that was misinterpreted as retained contrast within plaque. This will no doubt be clarified in future work by other investigators. There were limitations to all of the plaque ultrasound studies, most importantly that they could have been prospective. This was originally planned with late phase contrast enhanced ultrasound, however was discontinued after the preliminary results. The last two chapters could have been combined into one large study, however there two obstacles to this: firstly not all of the patients suitable for plaque texture analysis were suitable for contrast agents and secondly, the plaque texture software was only available in the last year of work. A larger sample may have clarified the role of ulceration on dynamic contrast enhanced ultrasound as a putative risk marker. Finally, it is apparent that all quantitative or semi-quantitative ultrasound studies suffer from a sampling error as they are uniplanar. This makes it difficult to reproduce certain features such as greyscale median and juxtaluminal echolucent area. These static structural features will only be made truly objective with the use of three-dimensional ultrasound, for which a linear, electronic array (matrix) transducer suitable for vascular imaging, is being developed by Philips.

Contrast enhanced ultrasound requires several technological developments before it can be successfully used for quantification. Firstly, improved tissue suppression and single microbubble detection at high mechanical indices is required to enable imaging of retained contrast, following adherence and ingestion by phagocytes. It certainly is possible to detect

single microbubbles in the carotid and therefore this avenue remains open to exploration (Figure 74).
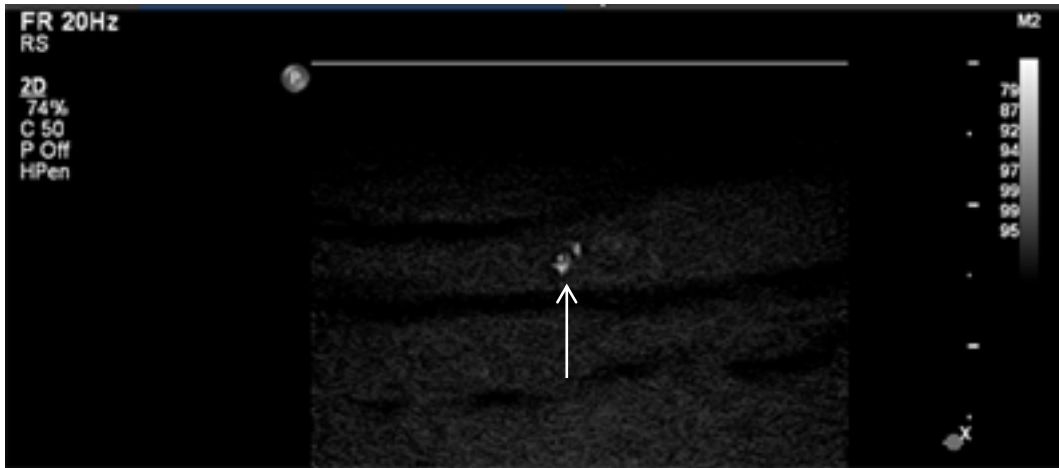
Figure 70: High mechanical index (MI 1.3) bubble specific longitudinal image with complete tissue subtraction (black background) in the human carotid during the late phase showing two resonating microbubbles (arrow). Taken by Ankur Thapar and Professor Averkiou using a custom pulse sequence on a Philips iu22 machine. Note that the microbubbles are not within a plaque (this has not yet been tested).

Secondly, considering low mechanical index techniques, a method of eliminating the troublesome non-linear propagation artefact that affects perfusion quantification is required. This is under investigation at the ERASMUS centre in Rotterdam, however there are no published records of a solution as yet. Finally, a three dimensional assessment of carotid plaque morphology has been performed by our group (Figure 75), which may help identify ulceration (249).
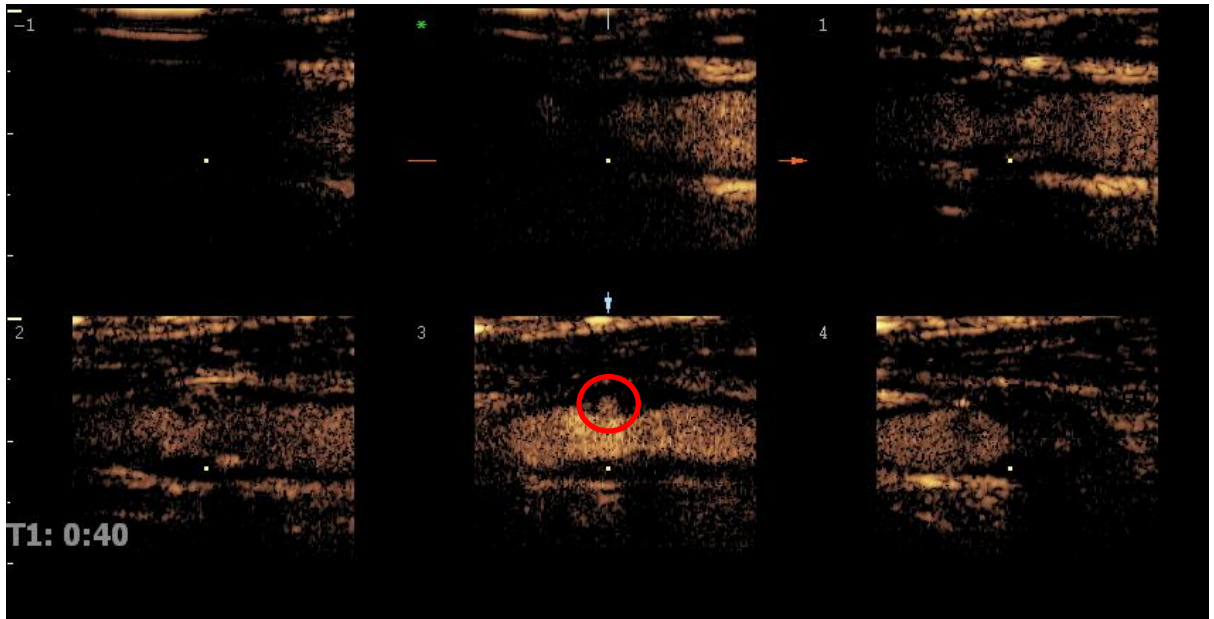
Figure 71: Static three dimensional multi-slice longitudinal contrast enhanced image of ulcerated carotid atheroma. Technology such as this could be used to improve the detection of ulceration (seen on one slice only - red circle). Taken at a mechanical index of 0.27 at 40 seconds post contrast injection in the carotid on a GE Logiq E9 ultrasound platform using the L6-16 linear transducer. Source: Ankur Thapar (with permission from patient) (249).

Overall, although stroke secondary to carotid atherosclerosis will remain a problem, into the next century, the role of risk stratification and surgery for asymptomatic individuals is waning. Surgeons of today should become the vascular physicians of tomorrow, treating their patients with aggressive and novel medical therapy. Quantitative imaging biomarkers still have a role in phase II studies of these drugs. However there is still a need for risk stratification for symptomatic patients with 50-69% stenosis, in whom the prognostic value of these quantitative imaging biomarkers can be studied.

10.2 Future work

There are a number of interesting possible avenues to explore both with asymptomatic carotid cohorts and with contrast enhanced ultrasound.   The first question

is how low is the stroke rate of an unselected European cohort of patients on modern medical therapy? If it is less than 1% per annum, it is likely that we should stop testing for the disease and focus on standard clinical risk factors. As alluded to earlier, a more relevant population in the future for prognostic study is symptomatic patients with 50-69% carotid stenosis. A cohort study using plaque texture, assessment of ulceration, along with microembolic signals or intraplaque haemorrhage on MRI may be a worthwhile international endeavour. Unfortunately because microembolic signals have been demonstrated to be an independent prognostic risk factor to echolucency (179), there may a problem in using them as a surrogate endpoint. It is ten years since landmark trials examined the stroke rates in this group, who have a higher risk than asymptomatic individuals (161), have readily identifiable vulnerability features (245) and who present to stroke units in reasonable numbers without the need for extra case finding costs.

Considering the use of contrast enhanced ultrasound in vascular diagnostics, there are two main avenues to pursue. Firstly a quantitative approach necessitates development and testing of new high MI tissue suppression imaging modes (Figure 1) and low MI pseudoenhancement suppression modes in carotid atheroma. These are issues for ultrasound engineers to work on with industry in collaboration with clinicians. However imaging the vasa vasorum may have equal benefit in another area: vasculitis. In this group of conditions, a safe, repeatable vessel specific marker of disease activity is lacking and could help target an appropriate dose of drug therapy. Secondly there still be a use for qualitative contrast enhanced imaging in areas where patients are currently exposed to repeated doses of ionising radiation and nephrotoxic contrast agents, e.g. detection of occult endoleaks after endovascular aneurysm repair.

Considering echolucency, further work should focus on histological examination of echolucent plaques in asymptomatic individuals, particularly the type II plaque, to establish whether it has characteristics similar to the thin cap fibroatheroma. Considering the main discriminator between stable and unstable plaques is fibrous cap rupture, it would be interesting to see if either high frequency ultrasound could non-invasively quantify fibrous cap thickness.

10.3 Conclusion

Asymptomatic carotid atherosclerosis is becoming a relatively safer condition, however the absolute numbers of strokes secondary to plaque rupture will increase as the population ages and the number of years at risk rise. Risk stratification will still be necessary but may be better targeted, in the future, to patients with moderate symptomatic carotid stenosis. These new imaging biomarkers are likely to be incorporated into the phase II testing of new medical therapies in atherosclerosis. In asymptomatic disease there is likely to be a role for vascular physicians to diagnose, medicate and monitor patients and to run clinical trials of new medical therapies.

References

1.   Fowkes FG, Housley E, Cawood EH, Macintyre CC, Ruckley CV, Prescott RJ. Edinburgh Artery Study: prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. Int J Epidemiol. 1991;20(2):384-92. Epub 1991/06/01.

2.   Offce NS. Population projections for the office of national statistics 2010 http://www.statistics.gov.uk/hub/population/population-change/population-projections accessed 1/8/2012.

3.   DoH. Healthcare Resource Group Payment by Results Tariffs 2011-2012 http://www.dh.gov.uk/health/category/policy-areas/nhs/resources-for-managers/payment-by-results/ accessed 1/3/2012.

4.   Ahmed B, Al-Khaffaf H. Prevalence of significant asymptomatic carotid artery disease in patients with peripheral vascular disease: a meta-analysis. Eur J Vasc Endovasc Surg. 2009;37(3):262-71. Epub 2008/12/09.

5.   Halliday A, Harrison M, Hayter E, Kong X, Mansfield A, Marro J, et al. 10-year stroke prevention after successful carotid endarterectomy for asymptomatic stenosis (ACST-1): a multicentre randomised trial. Lancet. 2010;376(9746):1074-84. Epub 2010/09/28.

6.   Goessens BM, Visseren FL, Algra A, Banga JD, van der Graaf Y. Screening for asymptomatic cardiovascular disease with noninvasive imaging in patients at high-risk and low-risk according to the European Guidelines on Cardiovascular Disease Prevention: the SMART study. J Vasc Surg. 2006;43(3):525-32. Epub 2006/03/08.

7.   Cerebrovascular disorders: a clinical and research classification. Geneva: World Health Organisation; 1978.

8.   Tyrrell P, Swain S, Rudd A. Diagnosis and initial management of transient ischaemic attack. Clin Med. 2010;10(2):164-7. Epub 2010/05/05.

9.   Organisation WH. The global burden of disease: 2004 update http://www.who.int/evidence/bod accessed on 2/1/2012.

10.   Accounts CoP. Progress in Improving Stroke Care, 26th Report of Session 2009-2010. In: Health Do, editor. London: The Stationery Office; 2010.

11.   Adamson J, Beswick A, Ebrahim S. Is stroke the most common cause of disability? J Stroke Cerebrovasc Dis. 2004;13(4):171-7. Epub 2007/10/02.

12.   Office NA. Reducing brain damage: faster access to better stroke care. In: Health Do, editor. London: The Stationery Office; 2005.

13.   Saka O, McGuire A, Wolfe C. Cost of stroke in the United Kingdom. Age Ageing. 2009;38(1):27-32. Epub 2009/01/15.

14.   Health Do. Department of Health Annual Resource Accounts. London: The Stationery Office; 2009-2010.

15.   Wolfe CD, Rudd AG, Howard R, Coshall C, Stewart J, Lawrence E, et al. Incidence and case fatality rates of stroke subtypes in a multiethnic population: the South London Stroke Register. J Neurol Neurosurg Psychiatry. 2002;72(2):211-6. Epub 2002/01/18.

16.   Hajat C, Heuschmann PU, Coshall C, Padayachee S, Chambers J, Rudd AG, et al. Incidence of aetiological subtypes of stroke in a multi-ethnic population based study: the South London Stroke Register. J Neurol Neurosurg Psychiatry. 2010. Epub 2010/10/27.

17.   Jahromi AS, Cina CS, Liu Y, Clase CM. Sensitivity and specificity of color duplex ultrasound measurement in the estimation of internal carotid artery stenosis: a systematic review and meta-analysis. J Vasc Surg. 2005;41(6):962-72. Epub 2005/06/10.

18.   Nederkoorn PJ, van der Graaf Y, Hunink MG. Duplex ultrasound and magnetic resonance angiography compared with digital subtraction angiography in carotid artery stenosis: a systematic review. Stroke. 2003;34(5):1324-32. Epub 2003/04/12.

19.   Kolominsky-Rabas PL, Weber M, Gefeller O, Neundoerfer B, Heuschmann PU. Epidemiology of ischemic stroke subtypes according to TOAST criteria: incidence, recurrence, and long-term survival in ischemic stroke subtypes: a population-based study. Stroke. 2001;32(12):2735-40. Epub 2001/12/12.

20.   Markus HS, King A, Shipley M, Topakian R, Cullinane M, Reihill S, et al. Asymptomatic embolisation for prediction of stroke in the Asymptomatic Carotid Emboli Study (ACES): a prospective observational study. Lancet Neurol. 2010;9(7):663-71. Epub 2010/06/18.

21.   de Weerd M, Greving JP, Hedblad B, Lorenz MW, Mathiesen EB, O'Leary DH, et al. Prevalence of asymptomatic carotid artery stenosis in the general population: an individual participant data meta-analysis. Stroke. 2010;41(6):1294-7. Epub 2010/05/01.

22.   Randomised trial of endarterectomy for recently symptomatic carotid stenosis: final results of the MRC European Carotid Surgery Trial (ECST). Lancet. 1998;351(9113):1379-87. Epub 1998/05/21.

23.   Barnett HJ, Taylor DW, Eliasziw M, Fox AJ, Ferguson GG, Haynes RB, et al. Benefit of carotid endarterectomy in patients with symptomatic moderate or severe stenosis. North American Symptomatic Carotid Endarterectomy Trial Collaborators. N Engl J Med. 1998;339(20):1415-25. Epub 1998/11/13.

24.   Lopez A.D. MCD, Ezzati M., Jamison D.T., Murray C.J.L.(ed). Global burden of disease and risk factors: Oxford University Press; 2006.

25.   Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. Cerebrovasc Dis. 2007;23(1):75-80. Epub 2006/11/17.

26.   Gerraty RP, Bowser DN, Infeld B, Mitchell PJ, Davis SM. Microemboli during carotid angiography. Association with stroke risk factors or subsequent magnetic resonance imaging changes? Stroke. 1996;27(9):1543-7. Epub 1996/09/01.

27.   Endarterectomy for asymptomatic carotid artery stenosis. Executive Committee for the Asymptomatic Carotid Atherosclerosis Study. JAMA. 1995;273(18):1421-8. Epub 1995/05/10.

28.   Blackshear WM, Phillips DJ, Chikos PM, Harley JD, Thiele BL, Strandness DE, Jr. Carotid artery velocity patterns in normal and stenotic vessels. Stroke. 1980;11(1):67-71. Epub 1980/01/01.

29.   Nicolaides AN, Shifrin EG, Bradbury A, Dhanjil S, Griffin M, Belcaro G, et al. Angiographic and duplex grading of internal carotid stenosis: can we overcome the confusion? J Endovasc Surg. 1996;3(2):158-65. Epub 1996/05/01.

30.   Wardlaw J, Chappell FM, Stevenson M, De Nigris E, Thomas S, Gillard J, et al. Accurate, practical and cost-effective assessment of carotid stenosis in the Uk. Health Technol Assess. 2006;10(30):1-200.

31.   Oates CP, Naylor AR, Hartshorne T, Charles SM, Fail T, Humphries K, et al. Joint recommendations for reporting carotid ultrasound investigations in the United Kingdom. Eur J Vasc Endovasc Surg. 2009;37(3):251-61. Epub 2008/12/03.

32.   Saba L, Potters F, van der Lugt A, Mallarini G. Imaging of the fibrous cap in atherosclerotic carotid plaque. Cardiovasc Intervent Radiol. 2010;33(4):681-9. Epub 2010/03/20.

33.   Makris GC, Lavida A, Griffin M, Geroulakos G, Nicolaides AN. Three-dimensional ultrasound imaging for the evaluation of carotid atherosclerosis. Atherosclerosis. 2011;219(2):377-83. Epub 2011/06/15.

34.   Carotid artery plaque composition--relationship to clinical presentation and ultrasound B-mode imaging. European Carotid Plaque Study Group. Eur J Vasc Endovasc Surg. 1995;10(1):23-30. Epub 1995/07/01.

35.   Gray-Weale AC, Graham JC, Burnett JR, Byrne K, Lusby RJ. Carotid artery atheroma: comparison of preoperative B-mode ultrasound appearance with carotid endarterectomy specimen pathology. J Cardiovasc Surg (Torino). 1988;29(6):676-81. Epub 1988/11/01.

36.   El-Barghouty NM, Levine T, Ladva S, Flanagan A, Nicolaides A. Histological verification of computerised carotid plaque characterisation. Eur J Vasc Endovasc Surg. 1996;11(4):414-6. Epub 1996/05/01.

37.   Sztajzel R, Momjian S, Momjian-Mayor I, Murith N, Djebaili K, Boissard G, et al. Stratified gray-scale median analysis and color mapping of the carotid plaque: correlation with endarterectomy specimen histology of 28 patients. Stroke. 2005;36(4):741-5. Epub 2005/02/12.

38.   Tegos TJ, Stavropoulos P, Sabetai MM, Khodabakhsh P, Sassano A, Nicolaides AN. Determinants of carotid plaque instability: echoicity versus heterogeneity. Eur J Vasc Endovasc Surg. 2001;22(1):22-30. Epub 2001/07/20.

39.   Pedro LM, Pedro MM, Goncalves I, Carneiro TF, Balsinha C, Fernandes e Fernandes R, et al. Computer-assisted carotid plaque analysis: characteristics of plaques associated with cerebrovascular symptoms and cerebral infarction. Eur J Vasc Endovasc Surg. 2000;19(2):118-23. Epub 2000/03/23.

40.   Prabhakaran S, Rundek T, Ramas R, Elkind MS, Paik MC, Boden-Albala B, et al. Carotid plaque surface irregularity predicts ischemic stroke: the northern Manhattan study. Stroke. 2006;37(11):2696-701. Epub 2006/09/30.

41.   Madani A, Beletsky V, Tamayo A, Munoz C, Spence JD. High-risk asymptomatic carotid stenosis: ulceration on 3D ultrasound vs TCD microemboli. Neurology. 2011;77(8):744-50. Epub 2011/08/19.

42.   Mathiesen EB, Johnsen SH, Wilsgaard T, Bonaa KH, Lochen ML, Njolstad I. Carotid plaque area and intima-media thickness in prediction of first-ever ischemic stroke: a 10-year follow-up of 6584 men and women: the Tromso Study. Stroke. 2011;42(4):972-8. Epub 2011/02/12.

43.   Spence JD, Hackam DG. Treating Arteries Instead of Risk Factors A Paradigm Change in Management of Atherosclerosis. Stroke. 2010;41(6):1193-9.

44.   Liapis CD, Bell PR, Mikhailidis D, Sivenius J, Nicolaides A, Fernandes e Fernandes J, et al. ESVS guidelines. Invasive treatment for carotid stenosis: indications, techniques. Eur J Vasc Endovasc Surg. 2009;37(4 Suppl):1-19. Epub 2009/04/16.

45.   Naylor AR, Gaines PA, Rothwell PM. Who benefits most from intervention for asymptomatic carotid stenosis: patients or professionals? Eur J Vasc Endovasc Surg. 2009;37(6):625-32. Epub 2009/04/07.

46.   Bouri S, Thapar A, Shalhoub J, Jayasooriya G, Fernando A, Franklin IJ, et al. Hypertension and the Post-carotid Endarterectomy Cerebral Hyperperfusion Syndrome. Eur J Vasc Endovasc Surg. 2011;41(2):229-37. Epub 2010/12/07.

47.   Alpers BJ, Berry RG, Paddison RM. Anatomical studies of the circle of Willis in normal brain. AMA archives of neurology and psychiatry. 1959;81(4):409-18. Epub 1959/04/01.

48.   Fawcett E, Blachford JV. The Circle of Willis: an Examination of 700 Specimens. Journal of anatomy and physiology. 1905;40(Pt 1):63 2-70. Epub 1905/10/01.

49.   Brott TG, Hobson RW, 2nd, Howard G, Roubin GS, Clark WM, Brooks W, et al. Stenting versus endarterectomy for treatment of carotid-artery stenosis. N Engl J Med. 2010;363(1):11-23. Epub 2010/05/28.

50.   Reiff T, Stingele R, Eckstein HH, Fraedrich G, Jansen O, Mudra H, et al. Stent-protected angioplasty in asymptomatic carotid artery stenosis vs. endarterectomy: SPACE2 -

a three-arm randomised-controlled clinical trial. Int J Stroke. 2009;4(4):294-9. Epub 2009/08/20.

51.   Snell RJ. ACT -1 website http://act1trial.com/ accessed on 27/9/2012.

52.   Rudarakanchana N, Dialynas M, Halliday A. Asymptomatic Carotid Surgery Trial-2 (ACST-2): rationale for a randomised clinical trial comparing carotid endarterectomy with carotid artery stenting in patients with asymptomatic carotid artery stenosis. Eur J Vasc Endovasc Surg. 2009;38(2):239-42. Epub 2009/06/23.

53.   Tindall GT, Goree JA, Lee JF, Odom GL. Effect of common carotid ligation on size of internal carotid aneurysms and distal intracarotid and retinal artery pressures. J Neurosurg. 1966;25(5):503-11. Epub 1966/11/01.

54.   Rautenberg W, Mess W, Hennerici M. Prognosis of asymptomatic carotid occlusion. J Neurol Sci. 1990;98(2-3):213-20. Epub 1990/09/01.

55.   Jayasooriya GS, Shalhoub J, Thapar A, Davies AH. Patient preference survey in the management of asymptomatic carotid stenosis. J Vasc Surg. 2011;53(6):1466-72. Epub 2011/04/12.

56.   Eastcott HH, Pickering GW, Rob CG. Reconstruction of internal carotid artery in a patient with intermittent attacks of hemiplegia. Lancet. 1954;267(6846):994-6. Epub 1954/11/13.

57.   Castaigne P, Lhermitte F, Gautier JC, Escourolle R, Derouesne C. Internal carotid artery occlusion. A study of 61 instances in 50 patients with post-mortem data. Brain. 1970;93(2):231-58. Epub 1970/01/01.

58.   Pessin MS, Hinton RC, Davis KR, Duncan GW, Roberson GH, Ackerman RH, et al. Mechanisms of acute carotid stroke. Ann Neurol. 1979;6(3):245-52. Epub 1979/09/01.

59.   Siebler M, Sitzer M, Steinmetz H. Detection of intracranial emboli in patients with symptomatic extracranial carotid artery disease. Stroke. 1992;23(11):1652-4. Epub 1992/11/01.

60.   Doux JD, Yun AJ. The link between carotid artery disease and ischemic stroke may be partially attributable to autonomic dysfunction and failure of cerebrovascular autoregulation triggered by Darwinian maladaptation of the carotid baroreceptors and chemoreceptors. Med Hypotheses. 2006;66(1):176-81. Epub 2005/11/09.

61.   Inzitari D, Eliasziw M, Gates P, Sharpe BL, Chan RK, Meldrum HE, et al. The causes and risk of stroke in patients with asymptomatic internal-carotid-artery stenosis. North American Symptomatic Carotid Endarterectomy Trial Collaborators. N Engl J Med. 2000;342(23):1693-700. Epub 2000/06/08.

62.   Bassiouny HS, Sakaguchi Y, Mikucki SA, McKinsey JF, Piano G, Gewertz BL, et al. Juxtalumenal location of plaque necrosis and neoformation in symptomatic carotid stenosis. J Vasc Surg. 1997;26(4):585-94. Epub 1997/11/14.

63.   Golledge J, Greenhalgh RM, Davies AH. The symptomatic carotid plaque. Stroke. 2000;31(3):774-81. Epub 2000/03/04.

64.   Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the vulnerable plaque. J Am Coll Cardiol. 2006;47(8 Suppl):C13-8. Epub 2006/04/25.

65.   Carr S, Farb A, Pearce WH, Virmani R, Yao JS. Atherosclerotic plaque rupture in symptomatic carotid artery stenosis. J Vasc Surg. 1996;23(5):755-65; discussion 65-6. Epub 1996/05/01.

66.   Fisher M, Paganini-Hill A, Martin A, Cosgrove M, Toole JF, Barnett HJ, et al. Carotid plaque pathology: thrombosis, ulceration, and stroke pathogenesis. Stroke. 2005;36(2):253-7. Epub 2005/01/18.

67.   Virmani R, Ladich ER, Burke AP, Kolodgie FD. Histopathology of carotid atherosclerotic disease. Neurosurgery. 2006;59(5 Suppl 3):S219-27; discussion S3-13. Epub 2006/10/21.

68.   Naghavi M, Falk E, Hecht HS, Jamieson MJ, Kaul S, Berman D, et al. From vulnerable plaque to vulnerable patient--Part III: Executive summary of the Screening for Heart Attack Prevention and Education (SHAPE) Task Force report. Am J Cardiol. 2006;98(2A):2H-15H. Epub 2006/07/18.

69.   Hellings WE, Peeters W, Moll FL, Piers SR, van Setten J, Van der Spek PJ, et al. Composition of carotid atherosclerotic plaque is associated with cardiovascular outcome: a prognostic study. Circulation. 2010;121(17):1941-50. Epub 2010/04/21.

70.   Vengrenyuk Y, Carlier S, Xanthos S, Cardoso L, Ganatos P, Virmani R, et al. A hypothesis for vulnerable plaque rupture due to stress-induced debonding around cellular microcalcifications in thin fibrous caps. Proc Natl Acad Sci U S A. 2006;103(40):14678-83. Epub 2006/09/28.

71.   Meairs S, Hennerici M. Four-dimensional ultrasonographic characterization of plaque surface motion in patients with symptomatic and asymptomatic carotid artery stenosis. Stroke. 1999;30(9):1807-13. Epub 1999/09/02.

72.   Doyle B, Caplice N. Plaque neovascularization and antiangiogenic therapy for atherosclerosis. J Am Coll Cardiol. 2007;49(21):2073-80. Epub 2007/05/29.

73.   Michel JB, Virmani R, Arbustini E, Pasterkamp G. Intraplaque haemorrhages as the trigger of plaque vulnerability. Eur Heart J. 2011. Epub 2011/03/15.

74.   Loftus IM, Naylor AR, Goodall S, Crowther M, Jones L, Bell PR, et al. Increased matrix metalloproteinase-9 activity in unstable carotid plaques. A potential role in acute plaque disruption. Stroke. 2000;31(1):40-7. Epub 2000/01/08.

75.   Molloy KJ, Thompson MM, Jones JL, Schwalbe EC, Bell PR, Naylor AR, et al. Unstable carotid plaques exhibit raised matrix metalloproteinase-8 activity. Circulation. 2004;110(3):337-43. Epub 2004/07/01.

76.   Morgan AR, Rerkasem K, Gallagher PJ, Zhang B, Morris GE, Calder PC, et al. Differences in matrix metalloproteinase-1 and matrix metalloproteinase-12 transcript levels among carotid atherosclerotic plaques with different histopathological characteristics. Stroke. 2004;35(6):1310-5. Epub 2004/04/10.

77.   Stevenson A, Waite M. Oxford English Dictionary 12th Edition: Oxford University Press; 2011.

78.   Stary HC. Natural history and histological classification of atherosclerotic lesions: an update. Arterioscler Thromb Vasc Biol. 2000;20(5):1177-8. Epub 2000/05/16.

79.   Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, Jr., et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation. 1995;92(5):1355-74. Epub 1995/09/01.

80.   Underhill HR, Yuan C, Yarnykh VL, Chu B, Oikawa M, Polissar NL, et al. Arterial remodeling in [corrected] subclinical carotid artery disease. JACC Cardiovasc Imaging. 2009;2(12):1381-9. Epub 2010/01/20.

81.   Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. Circulation. 2003;108(14):1664-72. Epub 2003/10/08.

82.   Dodge JT, Jr., Brown BG, Bolson EL, Dodge HT. Lumen diameter of normal human coronary arteries. Influence of age, sex, anatomic variation, and left ventricular hypertrophy or dilation. Circulation. 1992;86(1):232-46. Epub 1992/07/11.

83.   Krejza J, Arkuszewski M, Kasner SE, Weigele J, Ustymowicz A, Hurst RW, et al. Carotid artery diameter in men and women and the relation to body and neck size. Stroke. 2006;37(4):1103-5. Epub 2006/02/25.

84.   Mofidi R, Crotty TB, McCarthy P, Sheehan SJ, Mehigan D, Keaveny TV. Association between plaque instability, angiogenesis and symptomatic carotid occlusive disease. Br J Surg. 2001;88(7):945-50. Epub 2001/07/10.

85.   Dunmore BJ, McCarthy MJ, Naylor AR, Brindle NP. Carotid plaque instability and ischemic symptoms are linked to immaturity of microvessels within plaques. J Vasc Surg. 2007;45(1):155-9. Epub 2007/01/11.

86.   McCarthy MJ, Loftus IM, Thompson MM, Jones L, London NJ, Bell PR, et al. Angiogenesis and the atherosclerotic carotid plaque: an association between symptomatology and plaque morphology. J Vasc Surg. 1999;30(2):261-8. Epub 1999/08/07.

87.   Mofidi R, Powell TI, Crotty T, Mehigan D, Macerlaine D, Keaveny TV. Angiogenesis in carotid atherosclerotic lesions is associated with timing of ischemic neurological events and presence of computed tomographic cerebral infarction in the ipsilateral cerebral hemisphere. Ann Vasc Surg. 2008;22(2):266-72. Epub 2008/03/19.

88.   Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010;376(9753):1670-81. Epub 2010/11/12.

89.   Sipahi I, Swaminathan A, Natesan V, Debanne SM, Simon DI, Fang JC. Effect of Antihypertensive Therapy on Incident Stroke in Cohorts With Prehypertensive Blood Pressure Levels: A Meta-Analysis of Randomized Controlled Trials. Stroke. 2011. Epub 2011/12/14.

90.   Hobson RW, 2nd, Weiss DG, Fields WS, Goldstone J, Moore WS, Towne JB, et al. Efficacy of carotid endarterectomy for asymptomatic carotid stenosis. The Veterans Affairs Cooperative Study Group. N Engl J Med. 1993;328(4):221-7. Epub 1993/01/28.

91.   Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, Peto R, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet. 2009;373(9678):1849-60. Epub 2009/06/02.

92.   Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet. 2010. Epub 2010/12/15.

93.   Engelter S, Lyrer P. Antiplatelet therapy for preventing stroke and other vascular events after carotid endarterectomy. Stroke. 2004;35(5):1227-8. Epub 2004/04/03.

94.   Paraskevas KI, Liapis CD, Hamilton G, Mikhailidis DP. Can statins reduce perioperative morbidity and mortality in patients undergoing non-cardiac vascular surgery? Eur J Vasc Endovasc Surg. 2006;32(3):286-93. Epub 2006/05/13.

95.   Liapis CD, Bell PF, Mikhailidis DP, Sivenius J, Nicolaides A, Fernandes e Fernandes J, et al. ESVS Guidelines: Section A--prevention in patients with carotid stenosis. Curr Vasc Pharmacol. 2010;8(5):673-81. Epub 2010/06/25.

96.   Ricotta JJ, Aburahma A, Ascher E, Eskandari M, Faries P, Lal BK. Updated Society for Vascular Surgery guidelines for management of extracranial carotid disease. J Vasc Surg. 2011;54(3):e1-31. Epub 2011/09/06.

97.   Davies KJ, Thapar A, Kasivisvanathan V, Shalhoub J, Davies AH. Review of Trans-Atlantic Cardiovascular Best Medical Therapy Guidelines Recommendations for Asymptomatic Carotid Atherosclerosis. Curr Vasc Pharmacol. 2012. Epub 2012/06/26.

98.   Marquardt L, Geraghty OC, Mehta Z, Rothwell PM. Low risk of ipsilateral stroke in patients with asymptomatic carotid stenosis on best medical treatment: a prospective, population-based study. Stroke. 2010;41(1):e11-7. Epub 2009/11/21.

99.   Goessens BM, Visseren FL, Kappelle LJ, Algra A, van der Graaf Y. Asymptomatic carotid artery stenosis and the risk of new vascular events in patients with manifest arterial disease: the SMART study. Stroke. 2007;38(5):1470-5. Epub 2007/03/17.

100.   Abbott AL. Medical (nonsurgical) intervention alone is now best for prevention of stroke associated with asymptomatic severe carotid stenosis: results of a systematic review and analysis. Stroke. 2009;40(10):e573-83. Epub 2009/08/22.

101.   Chambers BR, Norris JW. Outcome in patients with asymptomatic neck bruits. N Engl J Med. 1986;315(14):860-5. Epub 1986/10/02.

102.   Johnson JM, Kennelly MM, Decesare D, Morgan S, Sparrow A. Natural history of asymptomatic carotid plaque. Arch Surg. 1985;120(9):1010-2. Epub 1985/09/01.

103.   Longstreth WT, Jr., Shemanski L, Lefkowitz D, O'Leary DH, Polak JF, Wolfson SK, Jr. Asymptomatic internal carotid artery stenosis defined by ultrasound and the risk of subsequent stroke in the elderly. The Cardiovascular Health Study. Stroke. 1998;29(11):2371-6. Epub 1998/11/06.

104.   Abbott AL, Chambers BR, Stork JL, Levi CR, Bladin CF, Donnan GA. Embolic signals and prediction of ipsilateral stroke or transient ischemic attack in asymptomatic carotid stenosis: a multicenter prospective cohort study. Stroke. 2005;36(6):1128-33. Epub 2005/05/10.

105.   Mackey AE, Abrahamowicz M, Langlois Y, Battista R, Simard D, Bourque F, et al. Outcome of asymptomatic patients with carotid disease. Asymptomatic Cervical Bruit Study Group. Neurology. 1997;48(4):896-903. Epub 1997/04/01.

106.   Nicolaides AN, Kakkos SK, Kyriacou E, Griffin M, Sabetai M, Thomas DJ, et al. Asymptomatic internal carotid artery stenosis and cerebrovascular risk stratification. J Vasc Surg. 2010;52(6):1486-96 e1-5. Epub 2010/12/15.

107.   Rerkasem K, Rothwell PM. Patch angioplasty versus primary closure for carotid endarterectomy. Cochrane Database Syst Rev. 2009(4):CD000160. Epub 2009/10/13.

108.   Kasivisvanathan V, Thapar A, Davies KJ, Dharmarajah B, Shalhoub J, Davies AH. Periprocedural outcomes after surgical revascularization and stenting for postradiotherapy carotid stenosis. J Vasc Surg. 2012. Epub 2012/07/24.

109.   Taylor DW, Barnett HJ, Haynes RB, Ferguson GG, Sackett DL, Thorpe KE, et al. Low-dose and high-dose acetylsalicylic acid for patients undergoing carotid endarterectomy: a randomised controlled trial. ASA and Carotid Endarterectomy (ACE) Trial Collaborators. Lancet. 1999;353(9171):2179-84. Epub 1999/07/07.

110.   Hopkins LN, Roubin GS, Chakhtoura EY, Gray WA, Ferguson RD, Katzen BT, et al. The Carotid Revascularization Endarterectomy versus Stenting Trial: credentialing of interventionalists and final results of lead-in phase. J Stroke Cerebrovasc Dis. 2010;19(2):153-62. Epub 2010/03/02.

111.   Physicians RCo. UK Audit of vascular surgical services and carotid endarterectomy. 2010 July 2010. Report No.

112.   Ederle J, Dobson J, Featherstone RL, Bonati LH, van der Worp HB, de Borst GJ, et al. Carotid artery stenting compared with endarterectomy in patients with symptomatic carotid stenosis (International Carotid Stenting Study): an interim analysis of a randomised controlled trial. Lancet. 2010;375(9719):985-97. Epub 2010/03/02.

113.   Murad MH, Shahrour A, Shah ND, Montori VM, Ricotta JJ. A systematic review and meta-analysis of randomized trials of carotid endarterectomy vs stenting. J Vasc Surg. 2011;53(3):792-7. Epub 2011/01/11.

114.   Klein A, Solomon CG, Hamel MB. Clinical decisions. Management of carotid stenosis--polling results. N Engl J Med. 2008;358(20):e23. Epub 2008/05/16.

115.   Yadav JS, Wholey MH, Kuntz RE, Fayad P, Katzen BT, Mishkel GJ, et al. Protected carotid-artery stenting versus endarterectomy in high-risk patients. N Engl J Med. 2004;351(15):1493-501. Epub 2004/10/08.

116.   Walker J, Isherwood J, Eveson D, Naylor AR. Triaging TIA/minor stroke patients using the ABCD2 score does not predict those with significant carotid disease. Eur J Vasc Endovasc Surg. 2012;43(5):495-8. Epub 2012/03/02.

117.   Giles MF, Albers GW, Amarenco P, Arsava MM, Asimos A, Ay H, et al. Addition of brain infarction to the ABCD2 Score (ABCD2I): a collaborative analysis of unpublished data on 4574 patients. Stroke. 2010;41(9):1907-13. Epub 2010/07/17.

118.   Rothwell PM, Warlow CP. Prediction of benefit from carotid endarterectomy in individual patients: a risk-modelling study. European Carotid Surgery Trialists' Collaborative Group. Lancet. 1999;353(9170):2105-10. Epub 1999/06/26.

119.   NICE. Guide to the method of technology appraisa 2008l http://www.nice.org.uk/media/B52/A7/TAMethodsGuideUpdatedJune2008.pdf.

120.   DoH. Reforming NHS Financial Flows: Introducing payment by results 2002
http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/di
gitalasset/dh_4018704.pdf.

121.   NICE. Measuring effectiveness and cost effectiveness: the QALY
http://www.nice.org.uk/newsroom/features/measuringeffectivenessandcosteffectivenessth
eqaly.jsp accessed 29/9/2012. 2010.

122.   EuroQol--a new facility for the measurement of health-related quality of life. The
EuroQol Group. Health Policy. 1990;16(3):199-208. Epub 1990/11/05.

123.   Devlin N, Parkin D. Does NICE have a cost-effectiveness threshold and what other
factors influence its decisions? A binary choice analysis. Health Econ. 2004;13(5):437-52.
Epub 2004/05/06.

124.   Thapar A, Shalhoub J, Dharmarajah B, Davies AH. Should we stop testing for
asymptomatic carotid atherosclerosis? Journal of neurointerventional surgery. 2012. Epub
2012/09/07.

125.   Henriksson M, Lundgren F, Carlsson P. Cost-effectiveness of endarterectomy in
patients with asymptomatic carotid artery stenosis. Br J Surg. 2008;95(6):714-20. Epub
2008/04/17.

126.   Haacke C, Althaus A, Spottke A, Siebert U, Back T, Dodel R. Long-term outcome after
stroke: evaluating health-related quality of life using utility measurements. Stroke.
2006;37(1):193-8. Epub 2005/12/13.

127.   Duncan PW, Lai SM, Keighley J. Defining post-stroke recovery: implications for design
and interpretation of drug trials. Neuropharmacology. 2000;39(5):835-41. Epub 2000/03/04.

128.   DoH. NHS Costing Manual 2012
http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuid
ance/DH_132395.

129.   Wardlaw JM, Keir SL, Seymour J, Lewis S, Sandercock PA, Dennis MS, et al. What is
the best imaging strategy for acute stroke? Health Technol Assess. 2004;8(1):iii, ix-x, 1-180.
Epub 2004/01/21.

130.   Chambers M, Hutton J, Gladman J. Cost-effectiveness analysis of antiplatelet therapy
in the prevention of recurrent stroke in the UK. Aspirin, dipyridamole and aspirin-
dipyridamole. PharmacoEconomics. 1999;16(5 Pt 2):577-93. Epub 2000/02/08.

131.   Patel A, Knapp M, Evans A, Perez I, Kalra L. Training care givers of stroke patients: economic evaluation. BMJ. 2004;328(7448):1102. Epub 2004/05/08.

132.   Curtis L. Unit costs of health and social care. University of Kent, 2010.

133.   DoH. NHS Reference Costs 2010-2011 http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_131140 accessed 1/6/2011.

134.   Sandercock P, Berge E, Dennis M, Forbes J, Hand P, Kwan J, et al. A systematic review of the effectiveness, cost-effectiveness and barriers to implementation of thrombolytic and neuroprotective therapy for acute ischaemic stroke in the NHS. Health Technol Assess. 2002;6(26):1-112. Epub 2002/11/16.

135.   Statistics ON. UK Interim Life Tables 2007-2009 http://www.ons.gov.uk/ons/taxonomy/index.html?nscl=Life+Tables accessed 1/6/2011. 2010.

136.   Office NS. Mortality Statistics: Deaths registered in England and Wales 2009 http://www.ons.gov.uk/ons/rel/vsob1/mortality-statistics--deaths-registered-in-england-and-wales--series-dr-/2009/index.html accessed 1/6/2011.

137.   Dennis MS, Burn JP, Sandercock PA, Bamford JM, Wade DT, Warlow CP. Long-term survival after first-ever stroke: the Oxfordshire Community Stroke Project. Stroke. 1993;24(6):796-800. Epub 1993/06/01.

138.   Hardie K, Hankey GJ, Jamrozik K, Broadhurst RJ, Anderson C. Ten-year survival after first-ever stroke in the perth community stroke study. Stroke. 2003;34(8):1842-6. Epub 2003/07/05.

139.   Gough MJ, Bodenham A, Horrocks M, Colam B, Lewis SC, Rothwell PM, et al. GALA: an international multicentre randomised trial comparing general anaesthesia versus local anaesthesia for carotid surgery. Trials. 2008;9:28. Epub 2008/05/23.

140.   Leichtle SW, Mouawad NJ, Welch K, Lampman R, Whitehouse WM, Jr., Heidenreich M. Outcomes of carotid endarterectomy under general and regional anesthesia from the American College of Surgeons' National Surgical Quality Improvement Program. J Vasc Surg. 2012;56(1):81-8 e3. Epub 2012/04/07.

141.   Silver FL, Mackey A, Clark WM, Brooks W, Timaran CH, Chiu D, et al. Safety of stenting and endarterectomy by symptomatic status in the Carotid Revascularization Endarterectomy Versus Stenting Trial (CREST). Stroke. 2011;42(3):675-80. Epub 2011/02/11.

142.   Rudarakanchana N, Halliday AW, Kamugasha D, Grant R, Waton S, Horrocks M, et al. Current practice of carotid endarterectomy in the UK. Br J Surg. 2012;99(2):209-16. Epub 2011/12/23.

143.   Halliday A, den Hartog AG, S.J. lC, C.M. W, A.C. N, editors. Is carotid intervention in trials becoming safer? Some early results from the ACST-2 trial. ESVS; 2012; Bologna.

144.   Robless P, Baxter A, Byrd S, Emson M, Halliday A. The prevalence of cerebral infarcts in the Asymptomatic Carotid Surgery Trial (ACST) in relation to prior contralateral symptoms. Int Angiol. 1998;17(3):187-93. Epub 1998/11/20.

145.   Jones R. Is the health resource group (HRG) tariff fit for purpose? Br J Health Man. 2012;18(1):52-3.

146.   Cronenwett JL, Birkmeyer JD, Nackman GB, Fillinger MF, Bech FR, Zwolak RM, et al. Cost-effectiveness of carotid endarterectomy in asymptomatic patients. J Vasc Surg. 1997;25(2):298-309; discussion 10-1. Epub 1997/02/01.

147.   Benade MM, Warlow CP. Costs and benefits of carotid endarterectomy and associated preoperative arterial imaging: a systematic review of health economic literature. Stroke. 2002;33(2):629-38. Epub 2002/02/02.

148.   Ashton HA, Buxton MJ, Day NE, Kim LG, Marteau TM, Scott RA, et al. The Multicentre Aneurysm Screening Study (MASS) into the effect of abdominal aortic aneurysm screening on mortality in men: a randomised controlled trial. Lancet. 2002;360(9345):1531-9. Epub 2002/11/22.

149.   Qureshi AI, Alexandrov AV, Tegeler CH, Hobson RW, 2nd, Dennis Baker J, Hopkins LN. Guidelines for screening of extracranial carotid artery disease: a statement for healthcare professionals from the multidisciplinary practice guidelines committee of the American Society of Neuroimaging; cosponsored by the Society of Vascular and Interventional Neurology. J Neuroimaging. 2007;17(1):19-47. Epub 2007/01/24.

150.   Risk of stroke in the distribution of an asymptomatic carotid artery. The European Carotid Surgery Trialists Collaborative Group. Lancet. 1995;345(8944):209-12. Epub 1995/01/28.

151.   Physicians RCo. Draft 4th National Clinical Guideline for Stroke. 2012.

152.   Stansby G, Macdonald S, Allison R, de Belder M, Brown MM, Dark J, et al. Asymptomatic carotid disease and cardiac surgery consensus. Angiology. 2011;62(6):457-60. Epub 2011/03/23.

153.   Naylor AR, Cuffe RL, Rothwell PM, Bell PR. A systematic review of outcomes following staged and synchronous carotid endarterectomy and coronary artery bypass. Eur J Vasc Endovasc Surg. 2003;25(5):380-9. Epub 2003/04/26.

154.   Naylor AR, Bown MJ. Stroke after cardiac surgery and its association with asymptomatic carotid disease: an updated systematic review and meta-analysis. Eur J Vasc Endovasc Surg. 2011;41(5):607-24. Epub 2011/03/15.

155.   Endarterectomy for moderate symptomatic carotid stenosis: interim results from the MRC European Carotid Surgery Trial. Lancet. 1996;347(9015):1591-3. Epub 1996/06/08.

156.   Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). Eur J Vasc Endovasc Surg. 2007;33 Suppl 1:S1-75. Epub 2006/12/05.

157.   Brott TG, Halperin JL, Abbara S, Bacharach JM, Barr JD, Bush RL, et al. 2011 ASA/ACCF/AHA/AANN/AANS/ACR/ASNR/CNS/SAIP/SCAI/SIR/SNIS/SVM/SVS Guideline on the Management of Patients With Extracranial Carotid and Vertebral Artery Disease: Executive Summary: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, and the American Stroke Association, American Association of Neuroscience Nurses, American Association of Neurological Surgeons, American College of Radiology, American Society of Neuroradiology, Congress of Neurological Surgeons, Society of Atherosclerosis Imaging and Prevention, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of NeuroInterventional Surgery, Society for Vascular Medicine, and Society for Vascular Surgery. Circulation. 2011. Epub 2011/02/02.

158.   Ebrahim S, Harwood R. Stroke: Epidemiology, Evidence and Clinical Practice 2nd Ed.: Oxford University Press; 1999.

159.   Spence JD. Is there a role for revascularisation in asymptomatic carotid stenosis? No. BMJ. 2010;341:c4900. Epub 2010/09/17.

160.   Roffi M. Is there a role for revascularisation in asymptomatic carotid stenosis? Yes. BMJ. 2010;341:c4898. Epub 2010/09/17.

161.   Rothwell PM, Eliasziw M, Gutnikov SA, Fox AJ, Taylor DW, Mayberg MR, et al. Analysis of pooled data from the randomised controlled trials of endarterectomy for symptomatic carotid stenosis. Lancet. 2003;361(9352):107-16. Epub 2003/01/18.

162.   Chambers BR, Donnan GA. Carotid endarterectomy for asymptomatic carotid stenosis. Cochrane Database Syst Rev. 2005(4):CD001923. Epub 2005/10/20.

163.   Rothwell PM, Mehta Z, Howard SC, Gutnikov SA, Warlow CP. Treating individuals 3: from subgroups to individuals: general principles and the example of carotid endarterectomy. Lancet. 2005;365(9455):256-65. Epub 2005/01/18.

164.   Nicolaides AN, Kakkos SK, Kyriacou E, Griffin M, Sabetai M, Thomas DJ, et al. Asymptomatic internal carotid artery stenosis and cerebrovascular risk stratification. J Vasc Surg. 2010;52(6):1486-96 e5. Epub 2010/12/15.

165.   Marquardt L, Fairhead JF, Rothwell PM. Lower rates of intervention for symptomatic carotid stenosis in women than in men reflect differences in disease incidence: a population-based study. Stroke. 2010;41(1):16-20. Epub 2009/11/17.

166.   Orlandi G, Parenti G, Landucci Pellegrini L, Sartucci F, Paoli C, Puglioli M, et al. Plaque surface and microembolic signals in moderate carotid stenosis. Italian journal of neurological sciences. 1999;20(3):179-82. Epub 1999/12/14.

167.   Spence JD, Coates V, Li H, Tamayo A, Munoz C, Hackam DG, et al. Effects of intensive medical therapy on microemboli and cardiovascular risk in asymptomatic carotid stenosis. Arch Neurol.67(2):180-6. Epub 2009/12/17.

168.   Jayasooriya G, Thapar A, Shalhoub J, Davies AH. Silent cerebral events in asymptomatic carotid stenosis. J Vasc Surg. 2011;54(1):227-36. Epub 2011/07/05.

169.   Molloy J, Khan N, Markus HS. Temporal variability of asymptomatic embolization in carotid artery stenosis and optimal recording protocols. Stroke. 1998;29(6):1129-32. Epub 1998/06/17.

170.   Wong KS, Chen C, Fu J, Chang HM, Suwanwela NC, Huang YN, et al. Clopidogrel plus aspirin versus aspirin alone for reducing embolisation in patients with acute symptomatic cerebral or carotid artery stenosis (CLAIR study): a randomised, open-label, blinded-endpoint trial. Lancet Neurol.9(5):489-97. Epub 2010/03/26.

171.   Mathiesen EB, Bonaa KH, Joakimsen O. Echolucent plaques are associated with high risk of ischemic cerebrovascular events in carotid stenosis: the tromso study. Circulation. 2001;103(17):2171-5. Epub 2001/05/23.

172.   Gronholdt ML, Nordestgaard BG, Schroeder TV, Vorstrup S, Sillesen H. Ultrasonic echolucent carotid plaques predict future strokes. Circulation. 2001;104(1):68-73. Epub 2001/07/04.

173.   Steffen CM, Gray-Weale AC, Byrne KE, Lusby RJ. Carotid artery atheroma: ultrasound appearance in symptomatic and asymptomatic vessels. Aust N Z J Surg. 1989;59(7):529-34. Epub 1989/07/01.

174.   Geroulakos G, Ramaswami G, Nicolaides A, James K, Labropoulos N, Belcaro G, et al. Characterization of symptomatic and asymptomatic carotid plaques using high-resolution real-time ultrasonography. Br J Surg. 1993;80(10):1274-7. Epub 1993/10/01.

175.   Sabetai MM, Tegos TJ, Nicolaides AN, Dhanjil S, Pare GJ, Stevens JM. Reproducibility of computer-quantified carotid plaque echogenicity: can we overcome the subjectivity? Stroke. 2000;31(9):2189-96. Epub 2000/09/08.

176.   Polak JF, Shemanski L, O'Leary DH, Lefkowitz D, Price TR, Savage PJ, et al. Hypoechoic plaque at US of the carotid artery: an independent risk factor for incident stroke in adults aged 65 years or older. Cardiovascular Health Study. Radiology. 1998;208(3):649-54. Epub 1998/09/02.

177.   Hashimoto H, Tagaya M, Niki H, Etani H. Computer-assisted analysis of heterogeneity on B-mode imaging predicts instability of asymptomatic carotid plaque. Cerebrovasc Dis. 2009;28(4):357-64. Epub 2009/07/25.

178.   Nicolaides AN, Kakkos SK, Griffin M, Sabetai M, Dhanjil S, Thomas DJ, et al. Effect of image normalization on carotid plaque classification and the risk of ipsilateral hemispheric ischemic events: results from the asymptomatic carotid stenosis and risk of stroke study. Vascular. 2005;13(4):211-21. Epub 2005/10/19.

179.   Topakian R, King A, Kwon SU, Schaafsma A, Shipley M, Markus HS. Ultrasonic plaque echolucency and emboli signals predict stroke in asymptomatic carotid stenosis. Neurology. 2011;77(8):751-8. Epub 2011/08/19.

180.   Hirt LS. Progression Rate and Ipsilateral Neurological Events in Asymptomatic Carotid Stenosis. Stroke. 2011. Epub 2011/07/30.

181.   Takaya N, Yuan C, Chu B, Saam T, Underhill H, Cai J, et al. Association between carotid plaque characteristics and subsequent ischemic cerebrovascular events: a prospective assessment with MRI--initial results. Stroke. 2006;37(3):818-23. Epub 2006/02/14.

182.   Singh N, Moody AR, Gladstone DJ, Leung G, Ravikumar R, Zhan J, et al. Moderate carotid artery stenosis: MR imaging-depicted intraplaque hemorrhage predicts risk of cerebrovascular ischemic events in asymptomatic men. Radiology. 2009;252(2):502-8. Epub 2009/06/11.

183.   Kwee RM, Truijman MT, van Oostenbrugge RJ, Mess WH, Prins MH, Franke CL, et al. Longitudinal MRI study on the natural history of carotid artery plaques in symptomatic patients. PLoS One. 2012;7(7):e42472. Epub 2012/08/04.

184.   Stevens JM, Barber CJ, Kerslake R, Broz M, Barter S. Extended use of cranial CT in the evaluation of patients with stroke and transient ischaemic attacks. Neuroradiology. 1991;33(3):200-6. Epub 1991/01/01.

185.   Sabetai MM, Tegos TJ, Clifford C, Dhanjil S, Belcaro G, Kakkos S, et al. Carotid plaque echogenicity and types of silent CT-brain infarcts. Is there an association in patients with asymptomatic carotid stenosis? Int Angiol. 2001;20(1):51-7. Epub 2001/05/09.

186.   Tegos TJ, Kalodiki E, Nicolaides AN, Sabetai MM, Stevens JM, Thomas DJ. Brain CT infarction in patients with carotid atheroma. Does it predict a future event? Int Angiol. 2001;20(2):110-7. Epub 2001/09/05.

187.   Kakkos SK, Sabetai M, Tegos T, Stevens J, Thomas D, Griffin M, et al. Silent embolic infarcts on computed tomography brain scans and risk of ipsilateral hemispheric events in patients with asymptomatic internal carotid artery stenosis. J Vasc Surg. 2009;49(4):902-9. Epub 2009/02/19.

188.   Lovett JK, Gallagher PJ, Hands LJ, Walton J, Rothwell PM. Histological correlates of carotid plaque surface morphology on lumen contrast imaging. Circulation. 2004;110(15):2190-7. Epub 2004/10/07.

189.   Huang PT, Chen CC, Aronow WS, Wang XT, Nair CK, Xue NY, et al. Assessment of neovascularization within carotid plaques in patients with ischemic stroke. World J Cardiol. 2010;2(4):89-97. Epub 2010/12/17.

190.   Kaufmann BA, Carr CL, Belcik JT, Xie A, Yue Q, Chadderdon S, et al. Molecular imaging of the initial inflammatory response in atherosclerosis: implications for early detection of disease. Arterioscler Thromb Vasc Biol. 2010;30(1):54-9. Epub 2009/10/17.

191.   Rudd JH, Warburton EA, Fryer TD, Jones HA, Clark JC, Antoun N, et al. Imaging atherosclerotic plaque inflammation with [18F]-fluorodeoxyglucose positron emission tomography. Circulation. 2002;105(23):2708-11. Epub 2002/06/12.

192.   Silvera SS, Aidi HE, Rudd JH, Mani V, Yang L, Farkouh M, et al. Multimodality imaging of atherosclerotic plaque activity and composition using FDG-PET/CT and MRI in carotid and femoral arteries. Atherosclerosis. 2009;207(1):139-43. Epub 2009/05/27.

193.   Gaemperli O, Shalhoub J, Owen DR, Lamare F, Johansson S, Fouladi N, et al. Imaging intraplaque inflammation in carotid atherosclerosis with 11C-PK11195 positron emission tomography/computed tomography. Eur Heart J. 2012;33(15):1902-10. Epub 2011/09/22.

194.   Rominger A, Saam T, Wolpers S, Cyran CC, Schmidt M, Foerster S, et al. 18F-FDG PET/CT identifies patients at risk for future vascular events in an otherwise asymptomatic cohort with neoplastic disease. J Nucl Med. 2009;50(10):1611-20. Epub 2009/09/18.

195.   Araujo JA, Romano EL, Brito BE, Parthe V, Romano M, Bracho M, et al. Iron overload augments the development of atherosclerotic lesions in rabbits. Arterioscler Thromb Vasc Biol. 1995;15(8):1172-80. Epub 1995/08/01.

196.   Raman SV, Winner MW, 3rd, Tran T, Velayutham M, Simonetti OP, Baker PB, et al. In vivo atherosclerotic plaque characterization using magnetic susceptibility distinguishes symptom-producing plaques. JACC Cardiovasc Imaging. 2008;1(1):49-57. Epub 2008/01/01.

197.   Howarth SP, Tang TY, Trivedi R, Weerakkody R, J UK-I, Gaunt ME, et al. Utility of USPIO-enhanced MR imaging to identify inflammation and the fibrous cap: a comparison of symptomatic and asymptomatic individuals. Eur J Radiol. 2009;70(3):555-60. Epub 2008/03/22.

198.   Eesa M, Hill MD, Al-Khathaami A, Al-Zawahmah M, Sharma P, Menon BK, et al. Role of CT angiographic plaque morphologic characteristics in addition to stenosis in predicting the symptomatic side in carotid artery disease. AJNR Am J Neuroradiol. 2010;31(7):1254-60. Epub 2010/04/03.

199.   Shaalan WE, Cheng H, Gewertz B, McKinsey JF, Schwartz LB, Katz D, et al. Degree of carotid plaque calcification in relation to symptomatic outcome and plaque inflammation. J Vasc Surg. 2004;40(2):262-9. Epub 2004/08/07.

200.   Serfaty JM, Nonent M, Nighoghossian N, Rouhart F, Derex L, Rotaru C, et al. Plaque density on CT, a potential marker of ischemic stroke. Neurology. 2006;66(1):118-20. Epub 2006/01/13.

201.   Nandalur KR, Baskurt E, Hagspiel KD, Finch M, Phillips CD, Bollampally SR, et al. Carotid artery calcification on CT may independently predict stroke risk. AJR Am J Roentgenol. 2006;186(2):547-52. Epub 2006/01/21.

202.   Wintermark M, Arora S, Tong E, Vittinghoff E, Lau BC, Chien JD, et al. Carotid plaque computed tomography imaging in stroke and nonstroke patients. Ann Neurol. 2008;64(2):149-57. Epub 2008/08/30.

203.   Mollet NR, Cademartiri F, van Mieghem CA, Runza G, McFadden EP, Baks T, et al. High-resolution spiral computed tomography coronary angiography in patients referred for diagnostic conventional coronary angiography. Circulation. 2005;112(15):2318-23. Epub 2005/10/06.

204.   Griffin MB, Kyriacou E, Pattichis C, Bond D, Kakkos SK, Sabetai M, et al. Juxtaluminal hypoechoic area in ultrasonic images of carotid plaques and hemispheric symptoms. J Vasc Surg. 2010;52(1):69-76. Epub 2010/06/12.

205.   Momjian-Mayor I, Kuzmanovic I, Momjian S, Bonvin C, Albanese S, Bichsel D, et al. Accuracy of a novel risk index combining degree of stenosis of the carotid artery and plaque surface echogenicity. Stroke. 2012;43(5):1260-5. Epub 2012/03/10.

206.   Stoitsis J, Golemati S, Nikita KS, Nicolaides AN. Characterization of carotid atherosclerosis based on motion and texture features and clustering using fuzzy c-means. Conf Proc IEEE Eng Med Biol Soc. 2004;2:1407-10. Epub 2007/02/03.

207.   Naylor AR. Time is brain! Surgeon. 2007;5(1):23-30.

208.   Sidhu PS, Allan PL, Cattin F, Cosgrove DO, Davies AH, Do DD, et al. Diagnostic efficacy of SonoVue, a second generation contrast agent, in the assessment of extracranial carotid or peripheral arteries using colour and spectral Doppler ultrasound: a multicentre study. Br J Radiol. 2006;79(937):44-51. Epub 2006/01/20.

209.   Staub D, Partovi S, Schinkel AF, Coll B, Uthoff H, Aschwanden M, et al. Correlation of Carotid Artery Atherosclerotic Lesion Echogenicity and Severity at Standard US with Intraplaque Neovascularization Detected at Contrast-enhanced US. Radiology. 2010. Epub 2010/10/26.

210.   Shah F, Balan P, Weinberg M, Reddy V, Neems R, Feinstein M, et al. Contrast-enhanced ultrasound imaging of atherosclerotic carotid plaque neovascularization: a new surrogate marker of atherosclerosis? Vasc Med. 2007;12(4):291-7. Epub 2007/12/01.

211.   Lindner JR, Dayton PA, Coggins MP, Ley K, Song J, Ferrara K, et al. Noninvasive imaging of inflammation by ultrasound detection of phagocytosed microbubbles. Circulation. 2000;102(5):531-8. Epub 2000/08/02.

212.   Yanagisawa K, Moriyasu F, Miyahara T, Yuki M, Iijima H. Phagocytosis of ultrasound contrast agent microbubbles by Kupffer cells. Ultrasound Med Biol. 2007;33(2):318-25. Epub 2007/01/09.

213.   Lindner JR, Coggins MP, Kaul S, Klibanov AL, Brandenburger GH, Ley K. Microbubble persistence in the microcirculation during ischemia/reperfusion and inflammation is caused by integrin- and complement-mediated adherence to activated leukocytes. Circulation. 2000;101(6):668-75. Epub 2000/02/15.

214.   Lindner JR, Ismail S, Spotnitz WD, Skyba DM, Jayaweera AR, Kaul S. Albumin microbubble persistence during myocardial contrast echocardiography is associated with microvascular endothelial glycocalyx damage. Circulation. 1998;98(20):2187-94. Epub 1998/11/17.

215.   Gramiak R, Shah PM. Echocardiography of the aortic root. Invest Radiol. 1968;3(5):356-66. Epub 1968/09/01.

216.   Epstein PS, Plesset MS. On the stability of gas bubbles in liquid-gas solutions. J Chem Phys. 1950;18(11):1505-9.

217.   Singhal SS, Cumming G, Horsfield K, Harking LK. Morphometric study of pulmonary arterial tree and its haemodynamics. J Assoc Physicians India. 1973;21(9):719-22. Epub 1973/09/01.

218.   Geleijnse ML, Nemes A, Vletter WB, Michels M, Soliman OI, Caliskan K, et al. Adverse reactions after the use of sulphur hexafluoride (SonoVue) echo contrast agent. J Cardiovasc Med (Hagerstown). 2009;10(1):75-7. Epub 2009/01/16.

219.   Haggag KJ, Russell D, Walday P, Skiphamn A, Torvik A. Air-filled ultrasound contrast agents do not damage the cerebral microvasculature or brain tissue in rats. Invest Radiol. 1998;33(3):129-35. Epub 1998/04/03.

220.   Lencioni R. Enhancing the role of ultrasound with contrast agents. Milan: Springer; 2006. 252 p.

221.   Morel DR, Schwieger I, Hohn L, Terrettaz J, Llull JB, Cornioley YA, et al. Human pharmacokinetics and safety evaluation of SonoVue, a new contrast agent for ultrasound imaging. Invest Radiol. 2000;35(1):80-5. Epub 2000/01/19.

222.   Yip GW, Chandrasekaran K, Miller TD, Hagen ME, Langins AP, Khandheria BK. Feasibility of continuous venous infusion of SonoVue for qualitative assessment of reversible coronary perfusion defects in stress myocardial contrast echocardiography. Int J Cardiovasc Imaging. 2003;19(6):473-81. Epub 2003/12/24.

223.   Williams R, Hudson JM, Lloyd BA, Sureshkumar AR, Lueck G, Milot L, et al. Dynamic microbubble contrast-enhanced US to measure tumor response to targeted therapy: a proposed clinical protocol with results from renal cell carcinoma patients receiving antiangiogenic therapy. Radiology. 2011;260(2):581-90. Epub 2011/05/11.

224.   Meier P, Zierler KL. On the theory of the indicator-dilution method for measurement of blood flow and volume. J Appl Physiol. 1954;6(12):731-44. Epub 1954/06/01.

225.   Feinstein SB. The powerful microbubble: from bench to bedside, from intravascular indicator to therapeutic delivery system, and beyond. Am J Physiol Heart Circ Physiol. 2004;287(2):H450-7. Epub 2004/07/28.

226.   Lampaskis M, Averkiou M. Investigation of the relationship of nonlinear backscattered ultrasound intensity with microbubble concentration at low MI. Ultrasound Med Biol. 2010;36(2):306-12. Epub 2010/01/05.

227.   Owen DR, Shalhoub J, Miller S, Gauthier T, Doryforou O, Davies AH, et al. Inflammation within carotid atherosclerotic plaque: assessment with late-phase contrast-enhanced US. Radiology. 2010;255(2):638-44. Epub 2010/04/24.

228.   Cosgrove D, Lassau N. Imaging of perfusion using ultrasound. Eur J Nucl Med Mol Imaging. 2010;37(Suppl 1):S65-85. Epub 2010/07/20.

229.   Faggioli GL, Pini R, Mauro R, Pasquinelli G, Fittipaldi S, Freyrie A, et al. Identification of carotid 'vulnerable plaque' by contrast-enhanced ultrasonography: correlation with

plaque histology, symptoms and cerebral computed tomography. Eur J Vasc Endovasc Surg. 2011;41(2):238-48. Epub 2010/12/15.

230.   Staub D, Patel MB, Tibrewala A, Ludden D, Johnson M, Espinosa P, et al. Vasa vasorum and plaque neovascularization on contrast-enhanced carotid ultrasound imaging correlates with cardiovascular disease and past cardiovascular events. Stroke. 2010;41(1):41-7. Epub 2009/11/17.

231.   Coli S, Magnoni M, Sangiorgi G, Marrocco-Trischitta MM, Melisurgo G, Mauriello A, et al. Contrast-enhanced ultrasound imaging of intraplaque neovascularization in carotid arteries: correlation with histology and plaque echogenicity. J Am Coll Cardiol. 2008;52(3):223-30. Epub 2008/07/12.

232.   Xiong L, Deng YB, Zhu Y, Liu YN, Bi XJ. Correlation of carotid plaque neovascularization detected by using contrast-enhanced US with clinical symptoms. Radiology. 2009;251(2):583-9. Epub 2009/03/24.

233.   Yu H, Jang HJ, Kim TK, Khalili K, Williams R, Lueck G, et al. Pseudoenhancement within the local ablation zone of hepatic tumors due to a nonlinear artifact on contrast-enhanced ultrasound. AJR Am J Roentgenol. 2010;194(3):653-9. Epub 2010/02/23.

234.   Tang MX, Eckersley RJ. Nonlinear propagation of ultrasound through microbubble contrast agents and implications for imaging. IEEE Trans Ultrason Ferroelectr Freq Control. 2006;53(12):2406-15. Epub 2006/12/26.

235.   Averkiou M, Lampaskis M, Kyriakopoulou K, Skarlos D, Klouvas G, Strouthos C, et al. Quantification of tumor microvascularity with respiratory gated contrast enhanced ultrasound for monitoring therapy. Ultrasound Med Biol. 2010;36(1):68-77. Epub 2009/11/11.

236.   Giannoni MF, Vicenzini E, Citone M, Ricciardi MC, Irace L, Laurito A, et al. Contrast carotid ultrasound for the detection of unstable plaques with neoangiogenesis: a pilot study. Eur J Vasc Endovasc Surg. 2009;37(6):722-7. Epub 2009/03/31.

237.   Averkiou M, Mannaris C, Nicolaides A. Vascular ultrasound imaging with contrast agents: carotid plaque neovascularization and the hyperplastic vasa vasorum network. In: Nicolaides A, Beach K, Pattichis C, Kyriacou E, editors. Ultrasound and Carotid Bifurcation Disease: Springer; 2012.

238.   Magnoni M, Coli S, Marrocco-Trischitta MM, Melisurgo G, De Dominicis D, Cianflone D, et al. Contrast-enhanced ultrasound imaging of periadventitial vasa vasorum in human carotid arteries. Eur J Echocardiogr. 2009;10(2):260-4. Epub 2008/09/02.

239.   Shalhoub J, Owen DR, Gauthier T, Monaco C, Leen EL, Davies AH. The use of contrast enhanced ultrasound in carotid arterial disease. Eur J Vasc Endovasc Surg. 2010;39(4):381-7. Epub 2010/01/12.

240.   Russell DA, Abbott CR, Gough MJ. Vascular endothelial growth factor is associated with histological instability of carotid plaques. Br J Surg. 2008;95(5):576-81. Epub 2008/03/18.

241.   de Bray JM, Baud JM, M. D. Consensus Concerning the Morphology and the Risk of Carotid Plaques. Cerebrovasc Dis. 1997;7(5):289-96.

242.   Eliasziw M, Streifler JY, Fox AJ, Hachinski VC, Ferguson GG, Barnett HJ. Significance of plaque ulceration in symptomatic patients with high-grade carotid stenosis. North American Symptomatic Carotid Endarterectomy Trial. Stroke. 1994;25(2):304-8. Epub 1994/02/01.

243.   Thapar A, Shalhoub J, Averkiou M, Mannaris C, Davies AH, Leen EL. Dose-dependent artifact in the far wall of the carotid artery at dynamic contrast-enhanced US. Radiology. 2012;262(2):672-9. Epub 2011/12/14.

244.   Streifler JY, Eliasziw M, Fox AJ, Benavente OR, Hachinski VC, Ferguson GG, et al. Angiographic detection of carotid plaque ulceration. Comparison with surgical observations in a multicenter study. North American Symptomatic Carotid Endarterectomy Trial. Stroke. 1994;25(6):1130-2. Epub 1994/06/01.

245.   Salem MK, Sayers RD, Bown MJ, West K, Moore D, Nicolaides A, et al. Patients with recurrent ischaemic events from carotid artery disease have a large lipid core and low GSM. Eur J Vasc Endovasc Surg. 2012;43(2):147-53. Epub 2011/12/14.

246.   Reiter M, Effenberger I, Sabeti S, Mlekusch W, Schlager O, Dick P, et al. Increasing carotid plaque echolucency is predictive of cardiovascular events in high-risk patients. Radiology. 2008;248(3):1050-5. Epub 2008/08/20.

247.   Fayad ZA, Mani V, Woodward M, Kallend D, Abt M, Burgess T, et al. Safety and efficacy of dalcetrapib on atherosclerotic disease using novel non-invasive multimodality imaging (dal-PLAQUE): a randomised clinical trial. Lancet. 2011;378(9802):1547-59. Epub 2011/09/13.

248.   Schwartz GG, Olsson AG, Ballantyne CM, Barter PJ, Holme IM, Kallend D, et al. Rationale and design of the dal-OUTCOMES trial: efficacy and safety of dalcetrapib in patients with recent acute coronary syndrome. Am Heart J. 2009;158(6):896-901 e3. Epub 2009/12/05.

249.   Thapar A, J. S, Owen D, Davies AH, Leen E. 3D contrast enhanced ultrasound of carotid atherosclerosis. Int J Surg. 2011;9(7):558.

Appendix 1: Supplementary information for cost-effectiveness analysis

Analysis excluding crossovers for patient or physician preference

This supplementary information describes the method used to estimate the rate of non-perioperative stroke if crossovers for patient or physician preference were excluded.

The ACST study provides rates of non-perioperative stroke from the intention to treat analysis. The aim of this appendix is to estimate the rates of stroke following surgery (r_cea_t, measured as events per person-year) and medical management (r_mm_t) at time points $t=1$ (at 5 years) and $t=2$ (at 10 years), in patients who comply with allocated treatment.

The ACST found that the treatment effect diminished over time. One reason may be the gradually increasing number of crossovers, which would be expected to bias the treatment effect towards the null. At time period t, one can express the observed rate of events in the intervention arm of the trial (R_1_t) as:

$$R\_1\_t = p(cea\_1\_t)\ r\_cea\_t + (1-p(cea\_1\_t))\ r\_mm\_t$$

Where p(cea_1_t) is the proportion of patients who have received surgery in the intervention arm by time t. Similarly, the observed rate of events in the control arm of the trial at time t (R_2_t) can be expressed as:

$$R\_2\_t = p(cea\_2\_t)\ r\_cea\_t + (1-p(cea\_2\_t))\ r\_mm\_t$$

Where p(cea_2_t) is the proportion of patients who have received surgery in the control arm by time t . This assumes that the rate of events for non-medical crossovers in the control arm is the same as the rate for those who complied with the allocation of surgery in the intervention arm.

At each time point t, there are in effect two simultaneous equations with two unknown parameters (for a given probability of crossover). The unknown parameters (and their standard errors) were estimated using WinBUGS 1.4 software. The number of stroke events in each arm of the trial by each time point was assumed to be generated by a Poisson process. Log-normal distributions were given to the unknown rate parameters.

The clinical trial provides the following data shown in Table 30.

Parameter	Abbreviation	Data
Rate of non-perioperative stroke in the intervention arm (with crossovers) per patient-year over the first 5 years	R_1_1	56/6540 (0.021)
Rate of non-perioperative stroke in the control arm (with crossovers) per patient-year over the first 5 years	R_2_1	140 / 6553 (0.009)
Rate of non-perioperative stroke in the intervention arm (with crossovers) per patient-year at 10 years	R_1_2	43/3042 (0.014)
Rate of non-perioperative stroke in the control arm (with crossovers) per patient-year at 10 years	R_2_2	48 / 3003 (0.016)
Proportion of elective surgery in the intervention arm at 5 years (assumed to be the same as at 1 year)	$P(cea\_1\_1)$	89.8%
Proportion of elective surgery in the intervention arm at 10 years (assumed to be the same as at 1 year)	$P(cea\_1\_2)$	89.8%
Proportion of elective surgery in the control arm by 5 years for non-medical reasons	$P(cea\_2\_1)$	15.8%
Proportion of elective surgery in the control arm by 10 years for non-medical reasons	$P(cea\_2\_2)$	22.4%

Table 30: Data for per the protocol analysis from the ACST (5).

Appendix 2: Supplementary information for late phase contrast enhanced ultrasound

Effect of mechanical index on background peak intensity of carotid atheroma

It was noted qualitatively, that at high mechanical index imaging there was an abundance of artefact in the tissues surrounding the carotid. To examine the magnitude of this effect on carotid plaque itself, axial flash images of 1 patient with a heterogeneous carotid atheroma were captured at increasing mechanical indices, whilst remaining on approximately the same axial section of plaque.
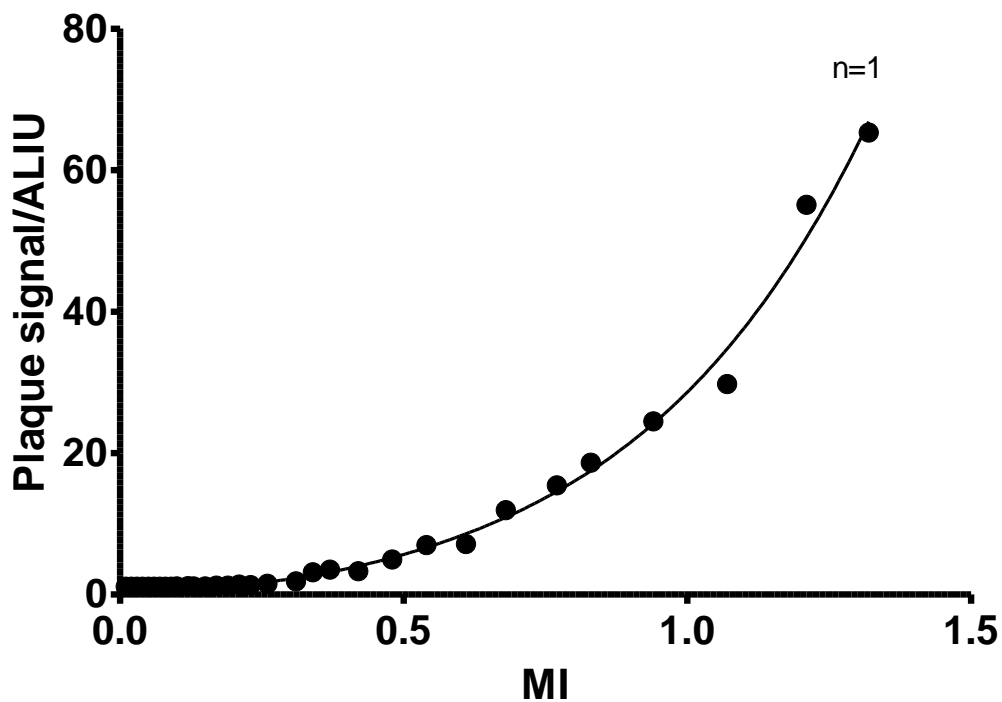
Figure 72: Graph demonstrating background plaque signal before administration of contrast in a heterogeneous carotid atheroma (n=1). This illustrates that at high mechanical index, there is incomplete cancellation of static reflectors within plaque, leading to artefactual signal. MI=mechanical index, ALIU=arbitrary linear intensity units. Source: Ankur Thapar.

Variability in the input function of an intravenous 2ml bolus of SonoVue

The input function of a bolus of intravenous contrast describes its time-intensity profile, which can be described using a lognormal curve fit function. However the variability of the input function in the carotid may be important when measuring the time-intensity profile of a carotid plaque.

To establish the magnitude of the difference between 2 identical boluses in the same person at the same sitting the following experiment was performed. 10 patients with carotid atherosclerosis were administered a 2ml bolus of intravenous SonoVue. Imaging of the carotid bifurcation was performed using standard low mechanical index settings described in Chapter 7. The common carotid lumen intensity was measured using the rectangle tool. Offline quantification was performed using linear data, fitted to a lognormal curve. One further 2ml bolus was administered after 10 minutes to allow lumen intensity at low MI to return to baseline.

The range of the intensity differences between bolus 1 and bolus 2 was -17.26 to 17.38 ALIU (arbitrary linear intensity units). These are the same order of magnitude as that of carotid plaque. The range of the time to peak intensity between bolus 1 and bolus 2 was -2.60 to 3.08 seconds. This again is the variation between the lumen and plaque.

The results of this experiment showed that there are clinically relevant differences in the input function between two identical 2ml boluses. These are important when quantifying the perfusion characteristics of carotid plaque, as the input function will determine the contrast density within the carotid during each examination.

This must be taken into account through normalisation of the plaque value to for example, the corresponding lumen value, i.e. use of a ratio not an absolute number.

Subject	Lumen peak intensity bolus 1 (ALIU)	Lumen WIT bolus 1 (s)	Lumen peak intensity bolus 2 (ALIU)	Lumen WIT bolus 2 (s)	Δ Intensity (ALIU)	Δ WIT (s)
1	61.56	7.11	59.74	9.71	1.82	-2.60
2	48.26	21.78	51.08	18.7	-2.82	3.08
3	26.60	12.16	39.97	14.31	-13.37	-2.15
4	45.11	5.58	62.37	4.97	-17.26	0.61
5	37.04	6.26	19.66	7.71	17.38	-1.45
6	45.82	11.04	36.07	13.07	9.75	-2.03
7	34.99	10.46	37.05	11.17	-2.06	-0.71
8	29.59	5.51	36.83	5.43	-7.24	0.08
9	80.21	4.79	65.89	5.96	14.32	-1.17
10	72.40	8.80	73.31	8.80	-0.91	0.00

Table 31: Characteristics of a 2ml intravenous bolus of SonoVue in ten patients with carotid atherosclerosis. ALIU = arbitrary linear intensity units, WIT=wash in time (representing time from a linear intensity of 2, representing the start of the upslope, to the peak intensity). The difference in the two bolus measurements is shown in the last 2 columns. These are important because they represent the local input function in the carotid, from which the microcirculation feed. Because there are important clinical differences with the same bolus, parameters from the microcirculation need to be normalised to either the lumen or the adjacent sternocleidomastoid (unfortunately not in view in all images).

Appendix 3: Supplementary information for plaque texture analysis

Inter-sonographer error of continuous plaque texture variables
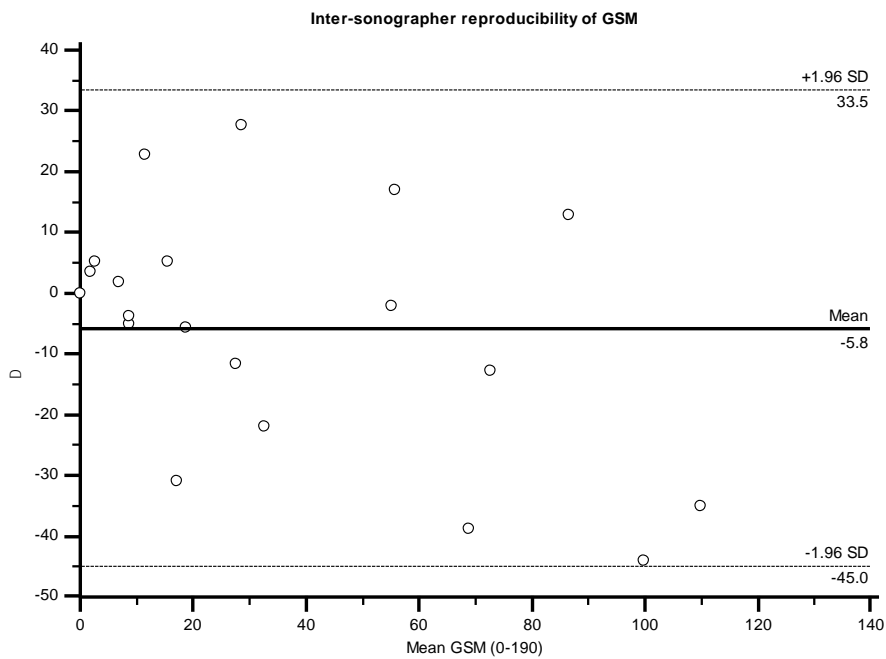
Figure 73: Bland-Altman plot of inter-sonographer error for carotid plaque grey scale median (GSM) from 20 individuals. The 95% limits of agreement were -45 to +34 pixel intensity units.
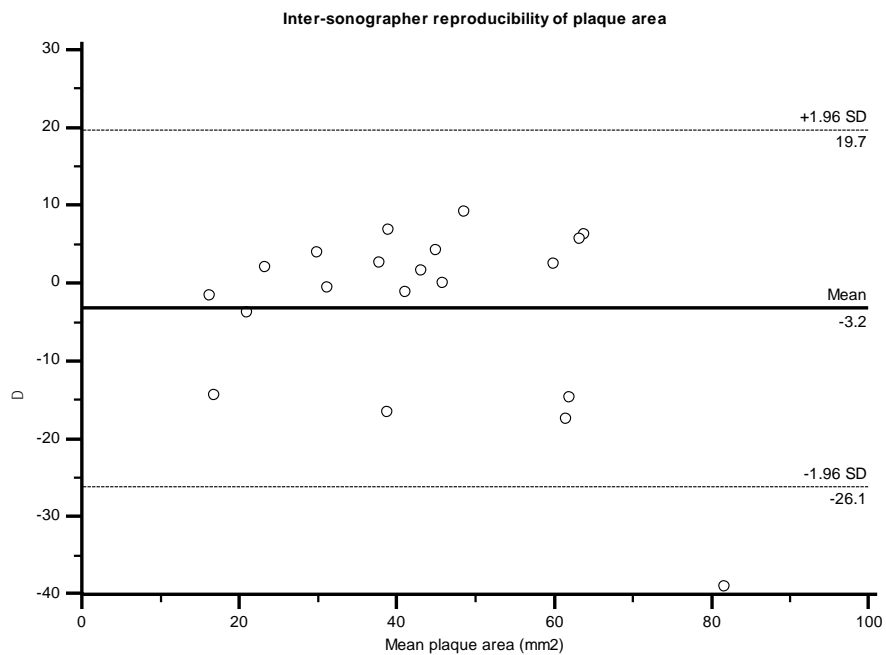
Figure 74: Bland-Altman plot for inter-sonographer error of carotid plaque area from 20 individuals. The 95% limits of agreement were -26 to +20 mm^2.

Inter-sonographer reproducibility of juxtaluminal echolucent area

Figure 75: Bland-Altman plot for inter-sonographer error of carotid plaque juxtaluminal echolucent area from 20 individuals. The 95% limits of agreement were -22 to +17 mm^2.

Inter-sonographer reproducibility for %juxtaluminal echolucent area

Figure 76: Bland-Altman plot for Inter-sonographer error of carotid plaque percentage juxtaluminal echolucent area from 20 individuals, including both near and far wall components. The 95% limits of agreement were -47 to 43%.
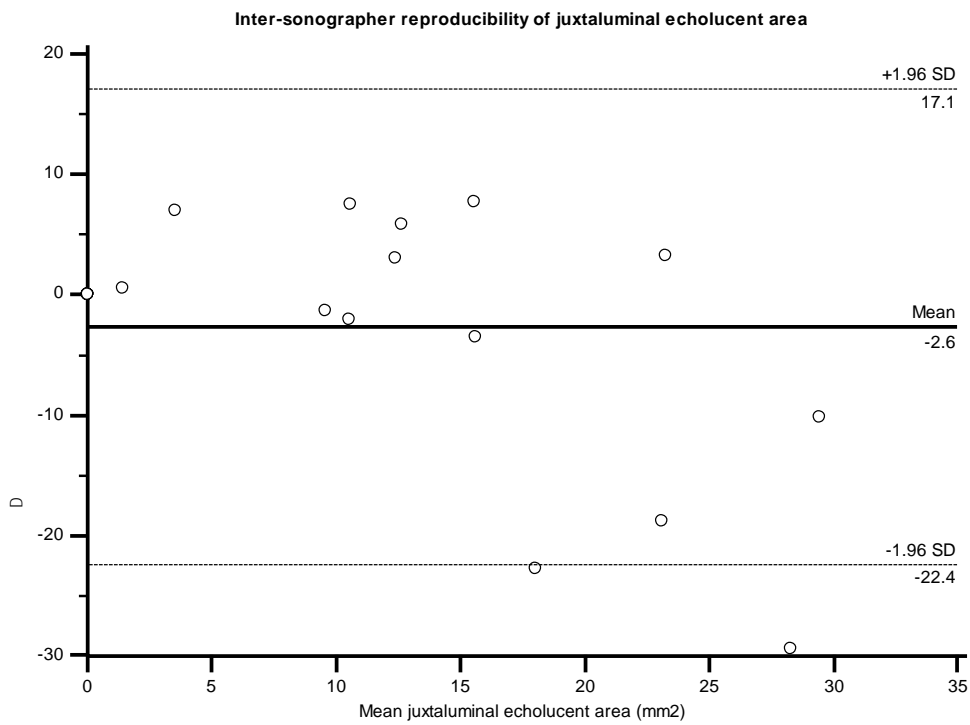
Inter-reader error of continuous plaque texture variables

Figure 77: Bland-Altman plot for inter-reader error of carotid plaque area from 99 individuals. The 95% limits of agreement were -31 to 30 mm^2.
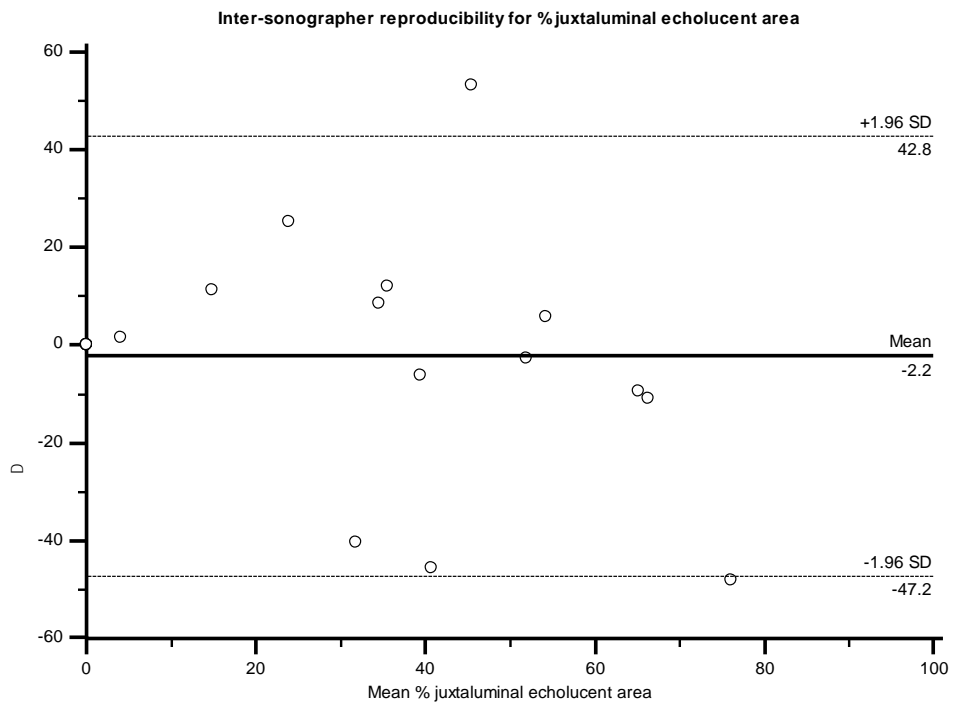

Figure 78: Bland-Altman plot for inter-reader error of carotid plaque grey scale median (GSM) from 99 individuals. The 95% limits of agreement were -20 to +24 pixel intensity units.
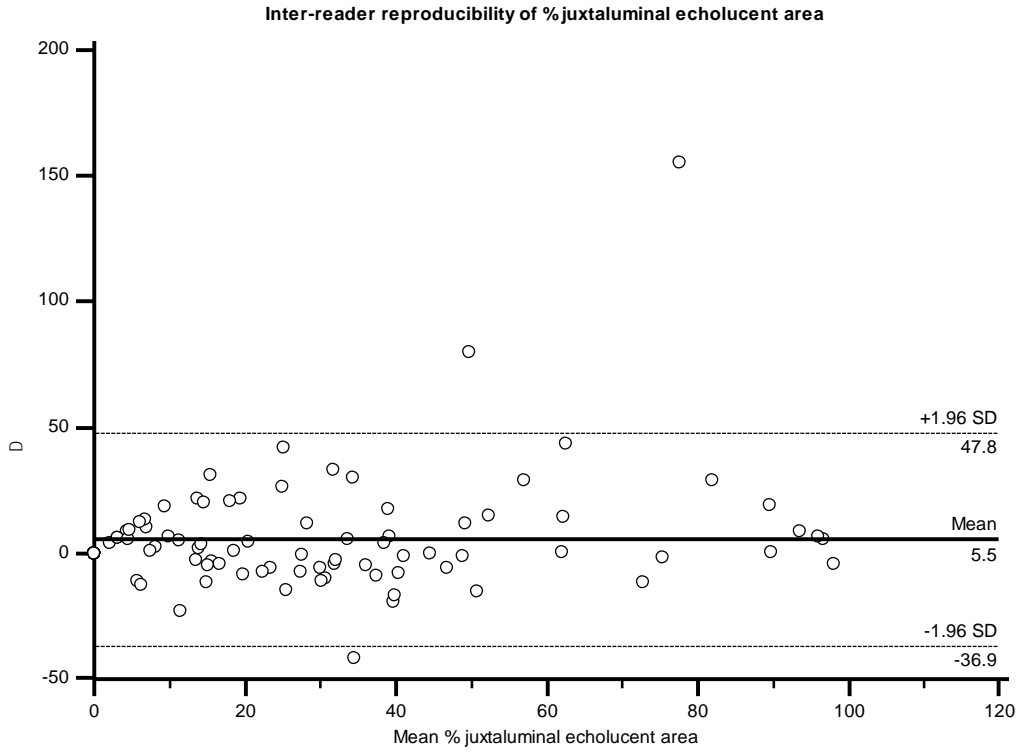
Figure 79: Bland-Altman plot for inter-reader error of carotid plaque juxtaluminal echolucent area from 99 individuals. The 95% limits of agreement were -37 to 48 %.
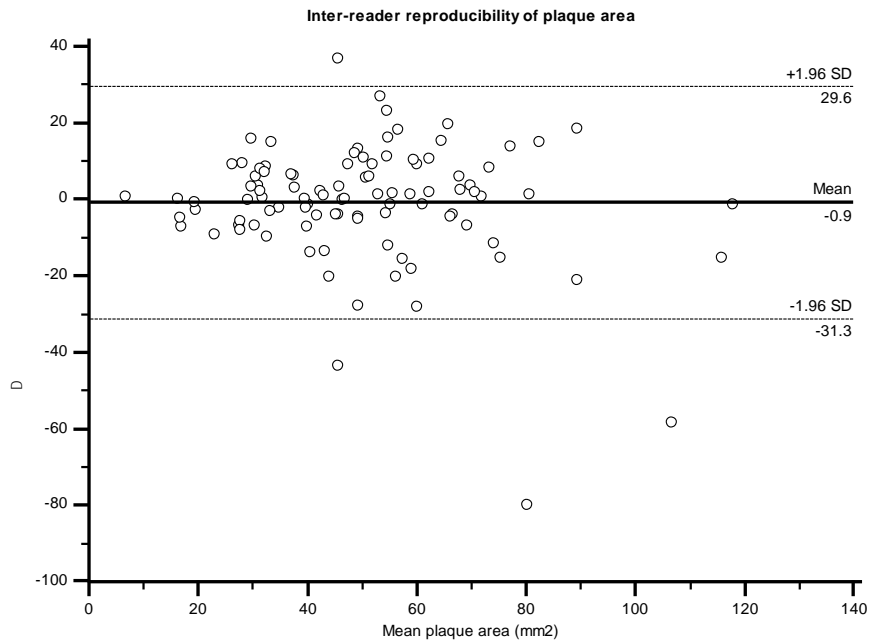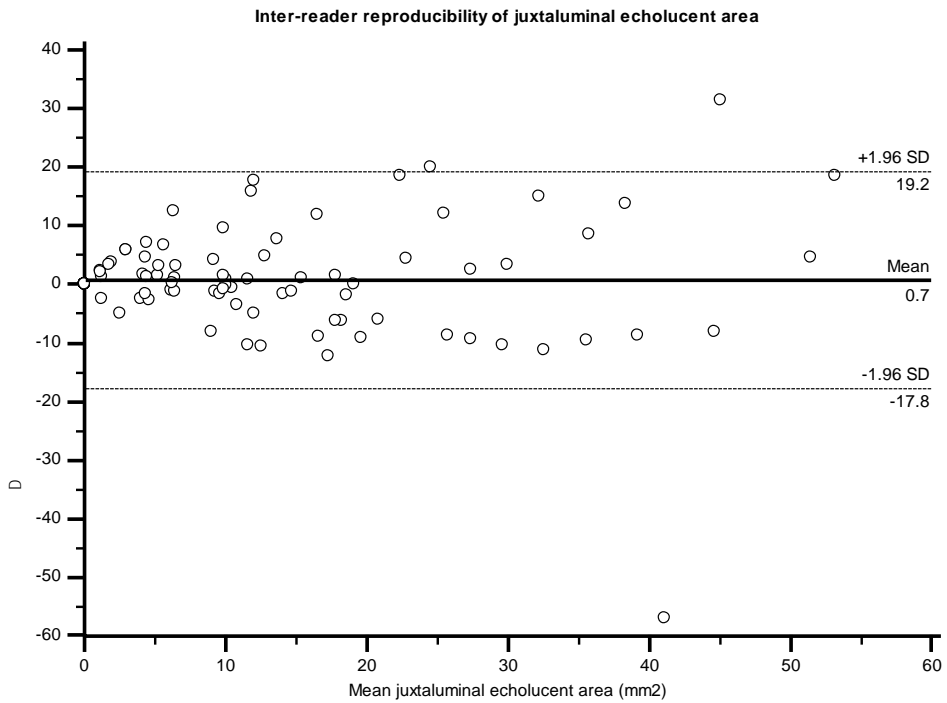

Figure 80: Bland-Altman plot for inter-reader error of carotid plaque juxtaluminal echolucent area from 99 individuals. The 95% limits of agreement were -18 to +19 mm^2.
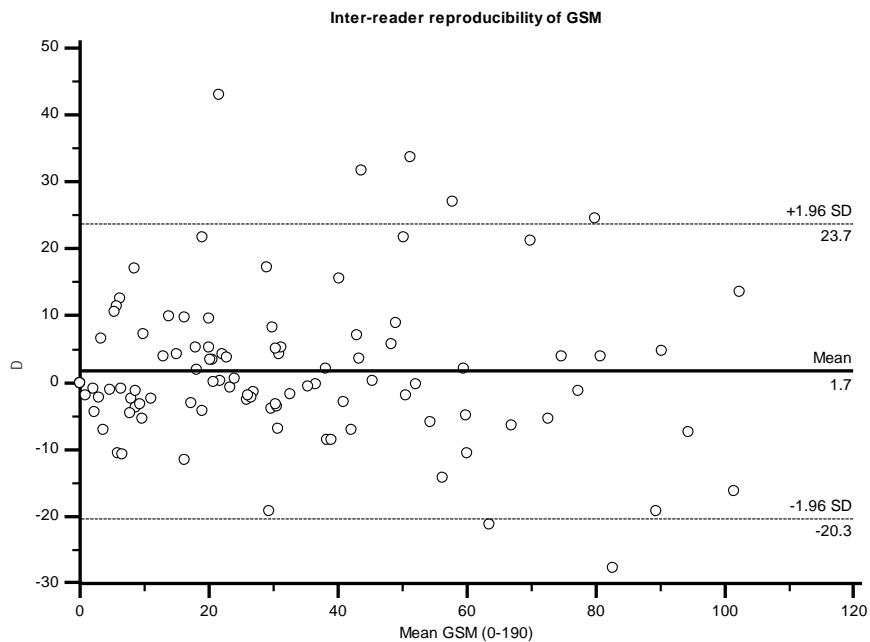
Appendix 4: Prizes

European Federation of Societies for Ultrasound in Medicine and Biology Young Investigator Award 2012

Graham-Dixon Prize for Cardiovascular Surgery 2012

Venous Forum Travelling Fellowship 2011

Royal College of Surgeons England Mary Dunhill Fellowship 2010-2012

The Circulation Foundation Mary Davies Award 2011

London Surgical Symposium 1st Prize 2011

Association of Surgeons Great Britain and Ireland E-poster 1st prize 2011

Rouleaux Club ASIT Prize 2010

Appendix 5: Publications and Presentations

Scientific publications related to thesis

Thapar A, Garcia-Mochon L, Epstein D, Shalhoub J, Davies A H.

Cost-effectiveness of asymptomatic carotid endarterectomy

Accepted British Journal of Surgery 2012 (in press)

Thapar A, Shalhoub J, Dharmarajah B, Davies A H.

Should we stop testing for asymptomatic carotid atherosclerosis?

Journal of Neurointerventional Surgery Sep 5 2012. [Epub ahead of print]

Thapar A, Shalhoub J, Averkiou M, Mannaris C, Davies A H, Leen E.

Dose dependent non-linear artefact in the far wall of the carotid artery with dynamic contrast enhanced ultrasound

Radiology 2012 Jan;262(2):672-9

Davies K, **Thapar A**, Shalhoub J, Davies A H.

Best medical therapy for asymptomatic carotid stenosis

Curr Vasc Pharmacol. 2012 Jun 22. [Epub ahead of print]

Jayasooriya G & **Thapar A,** Shalhoub J, Davies A H.

Silent cerebral events in asymptomatic carotid stenosis

Journal of Vascular Surgery 2011 Jul;54(1):227-36

Jayasooriya G, Shalhoub J, **Thapar A**, Davies A H.

Patient preference in the management of asymptomatic carotid stenosis

Journal of Vascular Surgery 2011 Jun;53(6):1466-72

Thapar A, Shalhoub J, Owen D, Davies A H, Leen E L.

3D contrast enhanced ultrasound of carotid atherosclerosis (abstract only)

International Journal of Surgery 2011:9(7):558

Book chapters

Shalhoub J, **Thapar A**, Davies A H.

Plaque type – does it affect therapy?

Charing Cross Symposium 2012

Thapar A, Shalhoub J, Dharmarajah B, Davies A H.

Testing for asymptomatic carotid disease in patients with symptomatic disease elsewhere

Charing Cross Symposium 2013

Other carotid publications unrelated to thesis

Khan U, **Thapar A**, Shalhoub J, Davies A H.

Risk of rupture of intracranial aneurysms during carotid revascularisation

Accepted Journal of Vascular Surgery 2012

Kasvisvanathan V, **Thapar A**, Shalhoub J, Dharmarajah B, Davies K, Davies A H.

Outcomes following intervention for post-radiotherapy carotid stenosis

J Vasc Surg. 2012 Jul 21. [Epub ahead of print]

Abbott A L, et al. (co-signatory)

Why the US Centre for Medicare and Medicaid should not extend the reimbursement of carotid artery angioplasty/stenting

European Journal of Vascular Surgery 2012 43:247-251

Bouri S, **Thapar A**, Shalhoub J, Jayasooriya G, Fernando A, Franklin I J, Davies A H.

Hypertension and the post-carotid endarterectomy cerebral hyperperfusion syndrome.

European Journal of Vascular Surgery 2011:4(2):229-237

Maruthappu M, Shalhoub J, **Thapar** A, Jayasooriya G, Franklin I J, Davies A H.

The patients' perspective of carotid endarterectomy.

Vasc Endovascular Surg. 2010 Oct;44(7):529-34

Scientific presentations

Thapar A, Y Zheng, Dharmarajah B, Averkiou M, Shalhoub J, Davies A H, Leen E.

Dynamic contrast-enhanced ultrasound for the assessment of perfusion and ulceration in carotid plaque

Radiological Society of North America 2012

Thapar A

Dose dependent non-linear artefact in the far wall of the carotid artery with dynamic contrast enhanced ultrasound

EUROSON 2012

Thapar A

Contrast carotid

Society for Vascular Technologists 2011

Thapar A, Garcia-Mochon L, Epstein D, Shalhoub J, Davies A H.

Cost-effectiveness of asymptomatic carotid endarterectomy

Asymptomatic Carotid Surgery Trial 2 Collaborators Meeting 2011

& London Surgical Symposium 2011

Thapar A, Shalhoub J, Averkiou M, Mannaris C, Davies A H, Leen E.

Dose dependent non-linear artefact in the far wall of the carotid artery with dynamic contrast enhanced ultrasound

British Medical Ultrasound Society 2011

Dose-Dependent Artifact in the Far Wall of the Carotid Artery at Dynamic Contrast-enhanced US[1]

Radiology

Ankur Thapar, BSc, MBBS, MRCS
Joseph Shalhoub, BSc, MBBS, MRCS
Michalakis Averkiou, BS, MS, PhD
Christophoros Mannaris, BS, MS
Alun H. Davies, MA, DM, FRCS, FHEA
Edward L. S. Leen, MB BCh, BAO, MD, FRCR

Purpose: To quantify a pseudoenhancement phenomenon observed during dynamic contrast material–enhanced ultrasonography (US) of the carotid artery, both in vitro and in vivo.

Materials and Methods: Ethical approval was obtained prior to commencing this prospective case series, and each patient gave written informed consent. Thirty-one patients with 50%–99% internal carotid artery stenosis underwent dynamic contrast-enhanced US of the carotid bifurcation with use of 2 mL of microbubbles. In the final 10 patients, an additional 1 mL bolus was administered after 15 minutes. Raw linear digital imaging and communications in medicine data were analyzed offline. Regions of interest were drawn within the common carotid artery lumen and immediately adjacent to the lumen in the near and far wall adventitia. Peak intensity was measured. An in vitro experiment with a single–channel flow phantom was also performed. This apparatus consisted of an 8-mm-diameter latex tube placed in a tissue-mimicking fluid. Microbubble concentrations of 0.02%, 0.1%, 0.5%, 1%, and 2% were pumped into the tube. Regions of interest were drawn in a similar fashion to the in vivo experiments, and peak intensity was measured. The Wilcoxon signed rank and paired t tests were used to compare the difference between the near and far wall signal intensities at each dose; a multiplication factor comparing near and far wall signal intensity was derived.

Results: The far wall of the common carotid artery was significantly more echogenic than the near wall at 2 mL contrast agent doses ($P < .0001$, $n = 31$), and the far wall signal intensity increased synchronously with that of the lumen. The difference in signal intensity between near and far wall regions was significantly greater at 2 mL than at 1 mL ($P = .012$, $n = 10$). In vitro, the phantom tubing demonstrated a similar pattern and magnitude of enhancement to that seen in vivo.

Conclusion: A dose-dependent, nonlinear propagation artifact known as pseudoenhancement occurs in the far wall adventitia of the carotid artery and should not be mistaken as a marker of plaque vulnerability.

©RSNA, 2011

220

Dynamic contrast material–enhanced carotid ultrasonography (US) is an emerging risk-stratification modality for quantifying neovascularization in carotid atherosclerosis (1,2). Proliferation of intraplaque and adventitial vasa vasorum (neovascularization) has been linked to atheroma rupture (3,4), intraplaque hemorrhage (5), and plaque progression (6). Enhancement within the microcirculation is visible with dynamic contrast-enhanced US due to its excellent spatial and temporal resolution (7). Previous studies have linked peak enhancement within carotid plaque and adventitia to histologic microvessel density (8,9), presence or absence of carotid atherosclerosis (10), prior cardiovascular events (11) and, more specifically, ipsilateral ischemic stroke or transient ischemic attack (12,13).

Most B-mode US studies focus on the far wall rather than the near wall of the artery because plaque and adventitia are readily identified due to luminal blood acting as a sonographic window. With dynamic contrast-enhanced US, the far wall is also more convenient to image than the near wall because of the position of the jugular vein, which can cause shadowing. However, in previous in vitro and in vivo studies, artifactual pseudoenhancement has been noted to occur at depths of 5–6 cm (14,15). This artifact results from echoes generated through nonlinear propagation of ultrasound waves through a region of high contrast material concentration. The pulses used for nonlinear imaging are highly distorted during their passage through the lumen, resulting in incomplete cancellation of the linear targets, which are then misinterpreted as perfusion (16).

The aim of this study was to quantify a pseudoenhancement phenomenon observed during dynamic contrast-enhanced US of the carotid artery, both in vitro and in vivo.

Materials and Methods

Clinical Study

Ethical approval was obtained prior to commencing the study. Patients gave written informed consent to participate in this prospective case series. Sample size calculation determined that 28 patients were necessary to demonstrate an intensity difference of 2 linear units between the near and far wall, with a type I error rate of 5% and power of 90%.

Thirty-one patients with a discrete 50%–99% internal carotid artery stenosis (equivalent to North American Symptomatic Carotid Endarterectomy Trial criteria) (17) were recruited from neurovascular clinics between March 2010 and February 2011. Patients were excluded if they were younger than 18 years of age, had New York Heart Association, or NYHA, grade III or higher heart failure class (one patient), and/or had acute coronary syndrome within 2 weeks or sulfur allergy (one patient) (18). The overall mean age was 71 years (range, 46–92 years), with a mean age of 69 years for men (range, 46–84 years) and of 78 years for women (range, 69–92 years). The series was nonconsecutive with respect to those who declined to participate (two patients as above) and those who had contrast material contraindication (two patients).

One artery per patient was insonated to avoid data clustering. In the case of bilateral disease, the side with the greatest stenosis was imaged. Patients were defined as symptomatic if they had experienced an ipsilateral carotid territory stroke, transient ischemic attack, or amaurosis fugax within 4 weeks of dynamic contrast-enhanced US and had a normal 24-hour electrocardiogram and echocardiogram. Patients were defined as asymptomatic if there were no prior neurologic events in the territory of the carotid artery under examination.

In Vitro Study

A single–channel flow tissue phantom was made from latex tubing with a diameter of 8 mm and was placed at a depth of 12 mm in a tissue-mimicking fluid. The fluid was composed of equal volumes of water and glycerine and had similar attenuation properties as tissue (0.3 dB/cm/MHz). The enclosure was made of 10-mm-thick acrylic and the bottom was lined with an anechoic tank-lining material (Aptflex F28; Precision Acoustics, Dorchester, UK) that reduced reflection. Microbubbles (SonoVue; Bracco, Milan, Italy) at concentrations of 0.02%, 0.1%, 0.5%, 1%, and 2% were injected sequentially into a reservoir. These concentrations were chosen to correspond to the range of contrast material used in vivo. In addition, up to a concentration of 1%, there is an established linear relationship between signal intensity and contrast material

Advances in Knowledge

- A dose-dependent artifact known as pseudoenhancement results from an examination of the far wall of the carotid artery with dynamic contrast-enhanced US.

- Current nonlinear pulse sequences do not allow distinction between neovascularization and pseudoenhancement caused by nonlinear propagation of ultrasound waves through a region of high contrast material concentration.

Implication for Patient Care

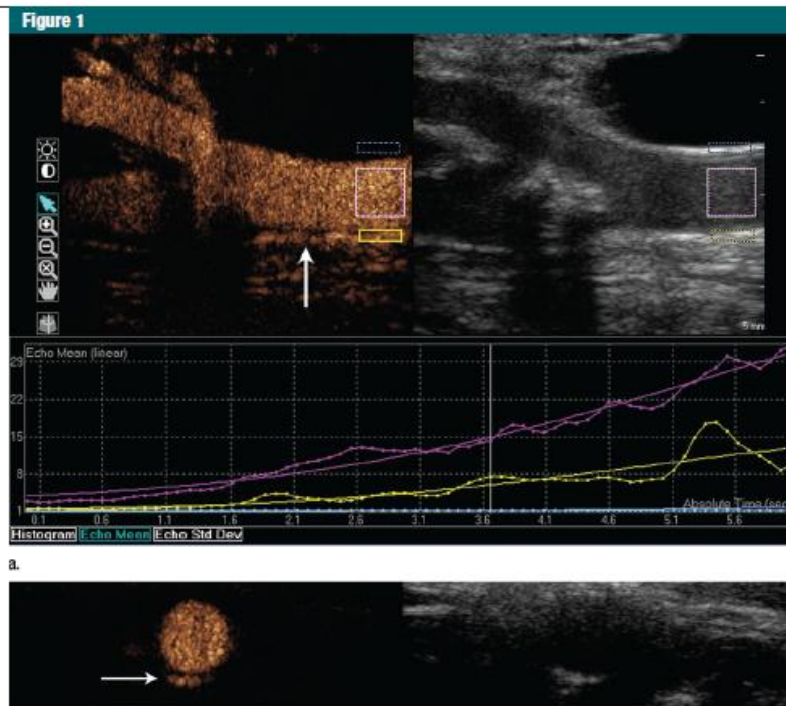- The apparent appearance of neovascularization isolated to the far wall adventitia of the carotid artery should not be interpreted as a marker of plaque vulnerability, as the finding may represent a pseudoenhancement artifact.

221

Radiology

a.

b.

Figure 1: (a) Longitudinal image of left common carotid bifurcation in asymptomatic 79-year-old man with 50% internal carotid artery stenosis. Quantification of signal intensity was performed by using dual-display mode: contrast-enhanced image (left) and B-mode reference (right). Adventitia is seen as an echogenic line on B-mode image, aiding identification. Pseudoenhancement mimicking neovascularization is seen in the region of far wall carotid adventitia (arrow). Plaque is seen as a filling defect in the internal carotid artery. Regions of interest are near wall adventitia (blue), common carotid lumen (purple), and far wall adventitia (yellow). The time-intensity curves are shown below in linear units in the same colors as the regions of interest. The lumen and far wall adventitia time–intensity curves increase synchronously, however the near wall does not. (b) Axial image of left common carotid artery far wall artifact in asymptomatic 33-year-old woman. Pseudoenhancement is seen as a semicircle under the lumen (arrow). Regions of interest have been removed to illustrate the visual appearance of pseudoenhancement.

concentration (19). A peristaltic pump (Cole-Parmer, Vernon Hills, Ill) was used to pump contrast material through the flow channel.

US Imaging

US examination with an 1.9–3 probe (Philips iU22; Philips, Bothel, Wash), a standard power modulation, and nonlinear pulse sequence was performed. Imaging was performed by A.T. under the supervision of E.L. (19 years of dynamic contrast-enhanced US experience) for the clinical study and by

M.A. (15 years of dynamic contrast-enhanced US experience) and C.M. for the in vitro experiment. A low mechanical index of 0.06 was used to prevent microbubble destruction. The time-gain compensation curve was kept vertical and centered, with dynamic range set at 36 dB and two-dimensional gain set at 85%. Machine parameters remained unchanged between the examinations and were checked before the imaging of each patient.

Each patient received intravenously a 2 mL bolus of microbubbles via a

20-gauge cannula in the right antecubital fossa. A 1-minute uniplanar cine loop of the carotid bifurcation was recorded by using the side-by-side display of contrast-enhanced and B-mode images in longitudinal section at the point of greatest stenosis. In the final 10 patients, a second bolus of 1 mL of microbubbles was administered and the examination was repeated. This was performed after 15 minutes to ensure elimination of the previous dose (19). Patients were observed for 30 minutes after the administration of the dose. The dose range was chosen

222

Radiology
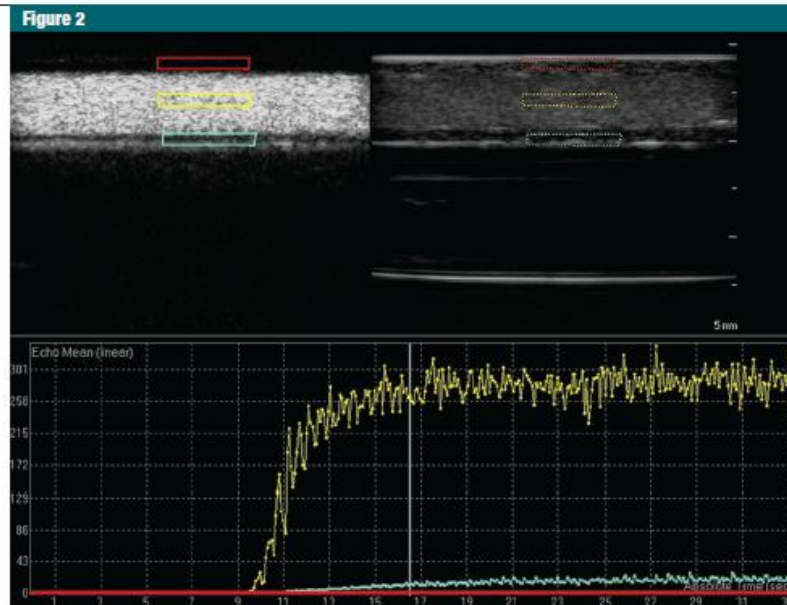
Figure 2: Longitudinal image in flow phantom with a 0.2% concentration of microbubbles. Regions of interest for quantification have been drawn: near wall (red), lumen (yellow), and far wall (green). The respective time-intensity curves are shown below in the same colors.

to replicate the range used previously in clinical studies (20).

Tissue phantom imaging was performed by attaching the probe to an articulated arm such that the transducer surface was 3 mm under the air-fluid interface. Two cine loops were acquired for each concentration, one at 25 mL/min and one without flow, to establish whether flow velocity altered the findings.

Quantification

Raw digital imaging and communications in medicine data were exported to an offline workstation for quantification by using software (QLab; Philips). Quantification was performed by A.T. and was repeated by E.L. for the clinical study and by M.A. for the in vitro study.

The time-intensity curve was analyzed from the contrast material arrival time to the time at which jugular enhancement overshadowed the near wall adventitia (cine loop cropped to

6 seconds after arrival time). Regions of interest were drawn in the common carotid lumen, the common carotid near wall adventitia, and the common carotid far wall adventitia, directly above and below that of the lumen, by using the freeform polygon tool (Fig 1a). Regions of interest in the adventitia were placed away from plaque to avoid confusion with neovascularization. Pre- and post-peak contrast material intensities were measured from the raw time-intensity data (Fig 1a). Similar regions of interest were then placed on the in vitro cine loops (Fig 2). Linear intensity units were used (ie, logarithmic compression was reversed).

Statistical Analysis

In the clinical study, for assessing the 2 mL dose, the intensities of the far and near wall were compared by using the Wilcoxon signed rank test. For the 10 patients administered varying doses of the contrast material, the difference

between the near and far wall was used to determine the multiplication factor by which the two intensities differed. Additionally, a paired t test was used to determine whether there was a significant difference in the paired differences between doses. Intra- and interreader agreement was calculated by using the intraclass correlation coefficient for 30 patients at a dose of 2 mL. Data were analyzed by using Prism v5 (GraphPad, La Jolla, Calif) and MedCalc v11 (Med-Calc, Mariakerke, Belgium) software. Data were checked for normality, and statistical significance was obtained at $P < .05$.

Results

Clinical Study

The demographic details of the patients are shown in Table 1.

An echogenic strip of adventitia was consistently seen on longitudinal and

223

Radiology

Table 1

Demographic Details of the 31 Patients

Demographic	Patient Datum
Age (y)*	71 ± 11
Male sex	24 (77)
Ipsilateral neurologic symptoms	12 (39)
Statin use	22 (71)
Hypertension	22 (71)
Diabetes	11 (35)
Current smoker	9 (29)
Family history of stroke	7 (23)

Note.—Unless otherwise indicated, data are the number of patients and data in parentheses are percentages. Of 12 symptomatic patients, three (25%) had amaurosis fugax, four (33%) had transient ischemic attack, and five (42%) had a stroke.

* Data are the mean age ± standard deviation.

Table 2

Near and Far Wall Carotid Adventitia Peak Intensity Measurements with Increasing Doses of Contrast Material

Dose (mL)	Near Wall Intensity*	Far Wall Intensity*	Difference between Far and Near Wall Intensity*	Multiplication Factor between Near and Far Wall†
0	1.25 ± 0.25	1.11 ± 0.11	−0.14 ± 0.22	0.91 ± 0.14
1	1.76 ± 0.58	7.67 ± 5.59	5.91 ± 5.35	4.44 ± 2.97
2	1.49 ± 0.40	17.94 ± 14.91	16.96 ± 14.27	12.68 ± 10.41

Note.—The difference between the near and far wall signal intensity increased significantly with dose.

* Values are mean linear units ± standard deviation.

† Values are mean ± standard deviation.

axial views of the common carotid artery in the region of the far wall adventitia (Fig 1). The time-intensity curve for the far wall adventitia increased synchronously with that of the lumen; however, this was not apparent for the near wall (Fig 1a). At a dose of 2 mL of microbubbles, the far wall was significantly more echogenic (median peak intensity, 15.16 [interquartile range, or IQR, 5.16–29.49]) than the near wall (median peak intensity, 1.30 [IQR, 1.16–1.80]) (two-tailed $P < .0001$, $n = 31$); the median difference between the far and near wall was 13.11 (IQR, 4.03–28.01).

In the 10 patients administered varying doses of contrast material, the difference between the near wall and far wall signal intensities was a multiplication factor of 0.91 ± 0.14 at 0 mL, 4.44 ± 2.97 at 1 mL, and 12.68 ± 10.41 at 2 mL (Table 2, Fig 3). The signal intensity difference between near and far walls was significantly higher at 2 mL compared with 1 mL ($P = .012$, $n = 10$).

Thirty scans were analyzed for reproducibility at the 2 mL dose. For intrareader reproducibility (A.T.), the intraclass correlation coefficient was 0.54 for the near wall and 0.92 for the far wall. For interreader reproducibility (A.T. and E.L.), the values were 0.49 for the near wall and 0.89 for the far wall.
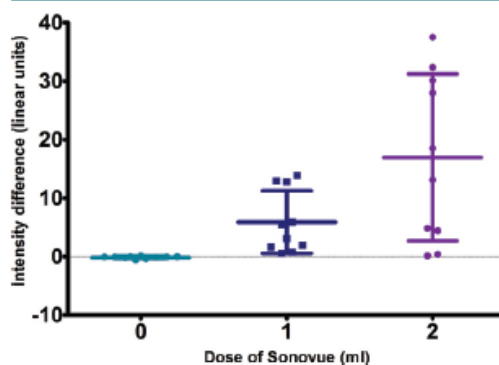
Figure 3

Figure 3: Graph of the absolute mean difference between far and near wall peak signal intensity with increasing doses of contrast material *(Sonovue)*. Error bars = standard deviation.

In Vitro Study

In the flow phantom, a similar echogenic strip to that seen at in vivo imaging was seen at the far wall of the flow phantom (Fig 2). With increasing contrast material concentrations, this effect became more pronounced both visually and on quantification (Figs 4, 5). The magnitude of the difference between the near and far wall signal intensity increased from a factor of 1.47 at a concentration of 0.02% to a factor of 12.67 at a concentration of 2% and a flow rate of 25 mL/min (Fig 5a). With static contrast medium, the magnitude of the difference was a factor of 1.40 at a concentration of 0.02% and of 15.63 at a concentration of 2% (Fig 5b). Figure 5c demonstrates the corresponding lumen signal intensities for reference purposes.

Discussion

The results of this study indicate that there is a pronounced signal intensity difference between the near and far wall adventitia of the carotid artery during dynamic contrast-enhanced US. Furthermore, the far wall adventitia and lumen time–intensity curves increase synchronously in time, without the time delay one would expect between the macro- and microcirculation (16). In addition, the walls of the latex flow phantom, which do not contain vasa vasorum, demonstrated the same pattern of far wall enhancement as that seen clinically.

224
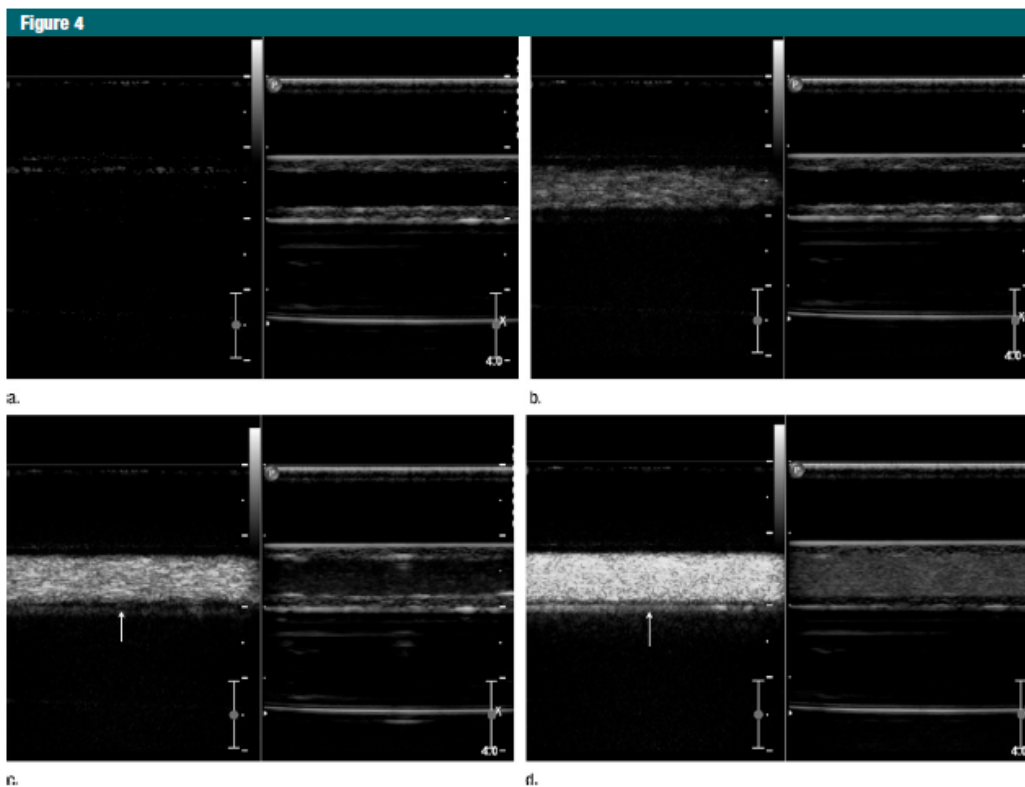
Figure 4

Figure 4: Images with microbubble concentration of (a) 0%, (b) 0.02%, (c) 0.1%, and (d) 2% show increasing visual far wall artifact (arrow) with increasing concentration of microbubbles within the flow phantom. Contrast-enhanced image is on the left and B-mode reference is on the right. The flow rate was maintained at 25 mL/min.

This latter observation was present despite the use of a nonlinear pulsing scheme designed to subtract static linear targets. The magnitude of this phenomenon is biologically implausible as the near and far wall adventitia are a contiguous structure, separated only by contrast material–filled lumen. In reality, therefore, this artifact, termed *pseudoenhancement*, is likely to represent nonlinear propagation unrelated to the presence or absence of vascularized plaque. Because the artifact is coincident with the arrival of intraarterial contrast material, we propose that it is related to nonlinear propagation of ultrasound waves through a region of high contrast material concentration.

The pseudoenhancement artifact described here may affect the use of the far wall of the carotid artery for assessment of neovascularization, as used in previous studies (10–12,20,21). This is important to recognize for those embarking on a quantitative contrast-enhanced US program. Measurements from the same patient at the near and far will result in different signal intensities. In clinical practice this will lead to inaccurate quantification and increase variability of analysis. It should not lead to clinical confusion, particularly when plaque vulnerability is being assessed or intervention is being considered.

Even though we did not explicitly test strategies to diminish the pseudoen-

hancement artifact in the current study, we propose that various strategies may be used to minimize it. First, for visual assessment, observers may rely on moving microbubble clusters to separate real from artifactual signal intensity (8,21). However, plaque perfusion is rarely completely absent or present, but is a continuum, lending itself to quantitative analysis. Second, the near wall alone can be used for quantification, except in cases where the adjacent jugular vein causes substantial shadowing. This jugular shadowing may be reduced by sitting the patient upright and applying gentle pressure with the transducer during the examination. Third, low doses of contrast material may be considered, but

225

Radiology
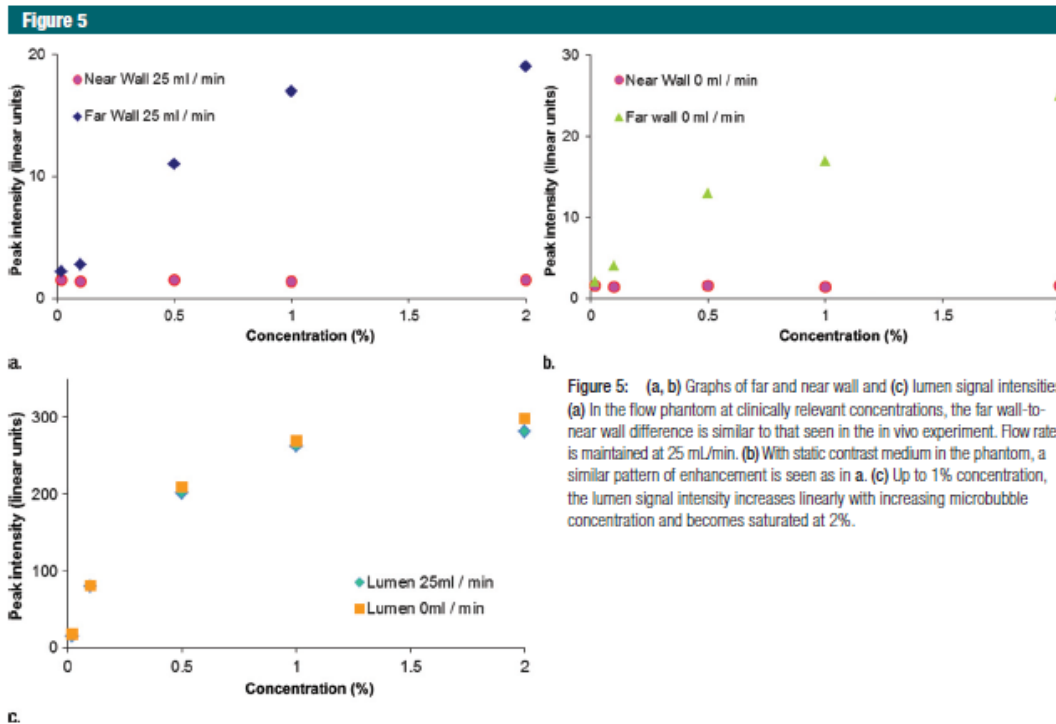
Figure 5

Figure 5: (a, b) Graphs of far and near wall and (c) lumen signal intensities. (a) In the flow phantom at clinically relevant concentrations, the far wall-to-near wall difference is similar to that seen in the in vivo experiment. Flow rate is maintained at 25 mL/min. (b) With static contrast medium in the phantom, a similar pattern of enhancement is seen as in a. (c) Up to 1% concentration, the lumen signal intensity increases linearly with increasing microbubble concentration and becomes saturated at 2%.

this lowering of dose may affect image quality by diminishing signal-to-noise ratios, as compared with higher contrast material doses. Finally, imaging during the late phase, after the lumen has emptied of contrast material (22), offers an alternative to a perfusion-based approach.

This study was limited in that it was an unblinded, nonconsecutive case series of patients who were imaged with one US system. Further, the exact clinical relevance of our proposed artifact remains unclear, and our proposed strategies to reduce the artifact remain unproven.

In conclusion, a dose-dependent artifact caused by nonlinear propagation of ultrasound waves through a region of high contrast material concentration causes pseudoenhancement of the far wall of the carotid artery. This limits accurate quantitative assessment of

neovascularization at the far wall of any contrast material—filled blood vessel and should not be mistaken for neovascularization.

References

1. Shalhoub J, Owen DR, Gauthier T, Monaco C, Leen EL, Davies AH. The use of contrast enhanced ultrasound in carotid arterial disease. Eur J Vasc Endovasc Surg 2010; 39(4):381–387.

2. Staub D, Schinkel AF, Coll B, et al. Contrast-enhanced ultrasound imaging of the vasa vasorum: from early atherosclerosis to the identification of unstable plaques. JACC Cardiovasc Imaging 2010;3(7):761–771.

226

Radiology

3. Moreno PR, Purushothaman KR, Fuster V, et al. Plaque neovascularization is increased in ruptured atherosclerotic lesions of human aorta: implications for plaque vulnerability. Circulation 2004;110(14): 2032–2038.

4. Dunmore BJ, McCarthy MJ, Naylor AR, Brindle NP. Carotid plaque instability and ischemic symptoms are linked to immaturity of microvessels within plaques. J Vasc Surg 2007;45(1):155–159.

5. Michel JB, Virmani R, Arbustini E, Pasterkamp G. Intraplaque haemorrhages as the trigger of plaque vulnerability. Eur Heart J 2011;32(16):1977–1985, 1985a, 1985b, 1985c.

6. Doyle B, Caplice N. Plaque neovascularization and antiangiogenic therapy for atherosclerosis. J Am Coll Cardiol 2007;49(21): 2073–2080.

7. Cosgrove D, Lassau N. Imaging of perfusion using ultrasound. Eur J Nucl Med Mol Imaging 2010;37(Suppl 1):S65–S85.

8. Shah F, Balan P, Weinberg M, et al. Contrast-enhanced ultrasound imaging of atherosclerotic carotid plaque neovascularization: a new surrogate marker of atherosclerosis? Vasc Med 2007;12(4):291–297.

9. Faggioli GL, Pini R, Mauro R, et al. Identification of carotid 'vulnerable plaque' by contrast-enhanced ultrasonography: correlation with plaque histology, symptoms and cerebral computed tomography. Eur J Vasc Endovasc Surg 2011;41(2):238–248.

10. Magnoni M, Coli S, Marrocco-Trischitta MM, et al. Contrast-enhanced ultrasound imaging of periadventitial vasa vasorum in human carotid arteries. Eur J Echocardiogr 2009; 10(2):260–264.

11. Staub D, Partovi S, Schinkel AF, et al. Correlation of carotid artery atherosclerotic lesion echogenicity and severity at standard US with intraplaque neovascularization detected at contrast-enhanced US. Radiology 2011;258(2):618–626.

12. Coli S, Magnoni M, Sangiorgi G, et al. Contrast-enhanced ultrasound imaging of intraplaque neovascularization in carotid arteries: correlation with histology and plaque echogenicity. J Am Coll Cardiol 2008;52(3):223–230.

13. Xiong L, Deng YB, Zhu Y, Liu YN, Bi XJ. Correlation of carotid plaque neovascularization detected by using contrast-enhanced US with clinical symptoms. Radiology 2009; 251(2):583–589.

14. Tang MX, Eckersley RJ. Nonlinear propagation of ultrasound through microbubble contrast agents and implications for imaging. IEEE Trans Ultrason Ferroelectr Freq Control 2006;53(12):2406–2415.

15. Yu H, Jang HJ, Kim TK, et al. Pseudoenhancement within the local ablation zone of hepatic tumors due to a nonlinear artifact on contrast-enhanced ultrasound. AJR Am J Roentgenol 2010;194(3):653–659.

16. Averkiou M, Mannaris C, Nicolaides A. Vascular ultrasound imaging with contrast agents: carotid plaque neovascularization

and the hyperplastic vasa vasorum network. In: Nicolaides A, Beach KW, Kyriacou E, Pattichis CS, eds. Ultrasound and carotid bifurcation atherosclerosis. London, England: Springer (in press).

17. Oates CP, Naylor AR, Hartshorne T, et al. Joint recommendations for reporting carotid ultrasound investigations in the United Kingdom. Eur J Vasc Endovasc Surg 2009; 37(3):251–261.

18. Geleijnse ML, Nemes A, Vletter WB, et al. Adverse reactions after the use of sulphur hexafluoride (SonoVue) echo contrast agent. J Cardiovasc Med (Hagerstown) 2009;10(1): 75–77.

19. Morel DR, Schwieger I, Hohn L, et al. Human pharmacokinetics and safety evaluation of SonoVue, a new contrast agent for ultrasound imaging. Invest Radiol 2000;35(1):80–85.

20. Huang PT, Chen CC, Aronow WS, et al. Assessment of neovascularization within carotid plaques in patients with ischemic stroke. World J Cardiol 2010;2(4):89–97.

21. Staub D, Patel MB, Tibrewala A, et al. Vasa vasorum and plaque neovascularization on contrast-enhanced carotid ultrasound imaging correlates with cardiovascular disease and past cardiovascular events. Stroke 2010; 41(1):41–47.

22. Owen DR, Shalhoub J, Miller S, et al. Inflammation within carotid atherosclerotic plaque: assessment with late-phase contrast-enhanced US. Radiology 2010;255(2): 638–644.

227

Should we stop testing for asymptomatic carotid atherosclerosis?

Ankur Thapar, Joseph Shalhoub,
Brahman Dharmarajah, Alun Huw Davies

WHO IS THE TARGET POPULATION?

In European men aged 60–79 years, moderate to severe carotid atherosclerosis (50–99% using North American Symptomatic Carotid Endarterectomy Trial (NASCET) measurements) is found in 2.3–6.0%,[1] a similar prevalence to aortic aneurysmal disease (4.9%)[2] for which a UK National Screening Programme has recently been introduced for men aged 65.

In high-risk populations seen in a vascular clinic, the prevalence of asymptomatic 50–99% carotid atherosclerosis is much higher: 15–25% in peripheral arterial disease,[3] 12% in patients with an abdominal aortic aneurysm[4] and 6% in those with contralateral carotid territory cerebral or retinal ischemic events.[5] Opinion is divided as to whether these individuals should be tested for asymptomatic carotid atherosclerosis. The American Society for Neuroimaging[6] and the US Society for Vascular Surgery[7] strongly recommend testing in high prevalence populations such as those with symptomatic peripheral arterial disease and persons aged 65 or over with multiple cardiovascular risk factors (table 1). Conversely, the UK Royal College of Physicians draft 4th National Clinical Guideline for Stroke[8] does not recommend testing at all. For cardiac patients in the UK, further observational data are awaited regarding prevalence and outcomes of synchronous carotid and coronary revascularization before evidence-based recommendations can be made.[9]

Patients with claudication are already under the care of a vascular surgeon, have a low rate of disease progression,[10] have the highest prevalence of carotid atherosclerosis[3] and a 1.9% annual risk of non-fatal ischemic stroke,[11] and are therefore an ideal population in which to consider testing. Ultrasound represents a low-cost readily-available imaging modality for these patients with a sensitivity of 86–90% and specificity of 87–94% in comparison with angiography for the detection of 70–99% carotid stenosis.[12][13] For those with ≥60–70% asymptomatic carotid stenosis, evidence from three randomized controlled trials has to date supported carotid endarterectomy for stroke prevention[14] in persons aged <75 years. However, the number needed to treat is high at 1 in 20 to prevent one stroke at 10 years.[15]

COST OF PREVENTING ONE IPSILATERAL STROKE IN SUBJECTS WITH CLAUDICATION

For the purposes of this estimate we have assumed the recommended European surgical intervention threshold of 70% NASCET stenosis,[16] a prevalence of 70–99% asymptomatic carotid stenosis of 15% in those with symptomatic peripheral arterial disease,[3] a number needed to treat of 1 in 20 to prevent one stroke at 10 years[15] and costings derived from NHS reimbursement tariffs 2011–2012, reflecting average national resource use.[17] The results of this analysis are shown in box 1 and approximate £76 000 per stroke saved. One can compare this with the approximate cost per stroke saved using warfarin for atrial fibrillation of $28 000–68 000 (£18 000–44 000, conversion rate as of 22 July, 2012).[18] The figure for carotid stenosis is higher primarily because of the 20 operations required to prevent one stroke.

COST OF TESTING PATIENTS WITH CLAUDICATION AGED 60 YEARS

To minimize upfront costs, a narrow age window for testing is desirable. From the European Carotid Surgery Trial, the mean age for presentation with carotid territory symptoms was established as 62 years.[19] Hence, to save at least half of potential strokes, testing should occur beforehand at around the age of 60.

If this strategy was adopted, the number of patients with claudication who would be eligible together with approximate costs and sonographer time required is shown in box 2. The approximate costs would be £17.5 m to prevent 231 strokes by performing 4613 carotid endarterectomies. This cost would be spread over a number of years to minimize the impact

Table 1 Recommendations from the American Society of Neuroimaging,[6] the US Society for Vascular Surgery[7] and the US Joint Societies[25] on testing for asymptomatic carotid atherosclerosis

American Society of Neuroimaging 2007	Society of Vascular Surgery 2011	US Joint Societies 2011
Grade A recommendation	Grade 1 recommendation	Grade 1 recommendation
Symptomatic peripheral arterial disease	Symptomatic peripheral arterial disease	–
Persons ≥65 years with ≥3 cardiovascular risk factors	Persons ≥65 years old with ≥1 cardiovascular risk factors	–
–	Asymptomatic carotid territory cerebral infarction	–
Grade B recommendation	Grade 2 recommendation	Grade 2a recommendation
Patients undergoing coronary artery bypass aged ≥65 years with left main stem disease, peripheral arterial disease, carotid bruit, previous carotid surgery or multiple cardiovascular risk factors	Patients undergoing coronary artery bypass aged ≥65 years with left main stem disease, peripheral arterial disease	Carotid bruit
Head and neck irradiation >10 years previously	–	Grade 2b recommendation
–	–	Symptomatic peripheral, coronary or aneurysmal arterial disease
–	–	≥2 cardiovascular risk factors

Grade A or 1 is the strongest recommendation and Grade B or 2 represents a moderate recommendation. The recommendations for asymptomatic disease are shown.

Academic Section of Vascular Surgery, Imperial College London, Charing Cross Hospital, London, UK

Correspondence to Ankur Thapar, Academic Section of Vascular Surgery, Imperial College London, 4 North, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK; a.thapar09@imperial.ac.uk

Editorial

Box 1   Estimated costs of preventing one stroke through testing patients with symptomatic peripheral arterial disease (figures are rounded to the nearest whole number in this box, however exact values have been used for calculation)

► Prevalence of ≥70% NASCET stenosis in persons with symptomatic peripheral arterial disease = 15%[5]

► Number of patients with ≥70% stenosis that require surgery to prevent one stroke = 1 in 20, allowing for perioperative stroke or death[15]

► Number of individuals screened to prevent one stroke = 6.7 × 20 = 133 persons

► Cost of preventing one stroke = (UK reimbursement tariff for carotid ultrasound code RA23Z[17] × 133 patients scanned) + (UK reimbursement tariff for carotid endarterectomy code QZ04Z[17] × 20 patients undergoing operation) = (£49 × 133) + (£3473 × 20) = £6533 + £69 460 = £75 993 ($116 908)

Box 2   Estimated costs of testing and operating on patients with claudication aged 60 for 70–99% asymptomatic carotid atherosclerosis in England and Wales (figures are rounded to the nearest whole number in this box, however exact values have been used for calculation)

► Prevalence of intermittent claudication in the UK = 4.6%[27]

► General population of England and Wales aged 60–64 years in 2010 = 3 343 000 persons[28]

► Estimate of number of persons in England and Wales aged 60 years = 3 343 000/5 = 668 600 persons

► Estimated number of patients with claudication in England and Wales aged 60 years = 0.046 × 668 600 = 30 756 persons

► Estimated cost of carotid ultrasound = 30 756 × £49[17] = £1 507 024

► Estimated time requirement for carotid ultrasound (assuming 15 min per scan) = (30 756 × 0.25 h)/24 = 320 days

► Claudication patients with 70–99% stenosis identified = 4613 endarterectomy candidates

► Estimated cost of 4613 endarterectomies performed = 4613 × £3473 = £16 020 949

► Estimated number of strokes prevented at 10 years = 231 strokes

► Estimated total cost = £17 527 973 ($26 956 826)

on the healthcare budget. As a comparator, in the UK 774 carotid endarterectomies were performed in asymptomatic patients in 2011.[20] The total number of strokes saved would only be approximately 0.2% of the 110 000 in 2010.[21]

DO THE ENDS JUSTIFY THE MEANS?
Should we spend such a sum of money on performing such a large number of unnecessary endarterectomies and preventing so few strokes? There are several points to note. First, the main cost driver is not investigation but resultant surgery. A better surgical selection tool would make the cost per stroke saved more acceptable. Second, there is an inherent prevention paradox in that more strokes from carotid atherosclerosis will occur outside the population selected for testing. These individuals can only be reached through active risk factor modification.

Some argue that we should restrict asymptomatic surgery to the 6% of persons found to have an incidental contralateral stenosis when they present with a cerebrovascular event.[5] However, recent UK data from the OXVASC study demonstrated that the annual stroke rate associated with a 50–99% NASCET stenosis in the territory of the contralateral carotid artery to the presenting symptom was only 0.3%.[22] This is lower than the 1.9% contralateral stroke rate found in the European Carotid Surgery Trial.[5] Other international cohorts on intensive medical regimes have also demonstrated ipsilateral stroke rates of <1% per annum and suggest there is less rationale for surgery than ever before.[23 24]

In summary, preventing one stroke requires scanning 133 claudicants, operating on 20 of them, 19 of whom will undergo an unnecessary operation at a cost of approximately £76 000 per stroke saved. The hazards of overdiagnosis have recently been highlighted,[25] and perhaps it is time to realize why it has been recommended that we stop testing for asymptomatic carotid atherosclerosis in the UK.

REFERENCES
1. **de Weerd M**, Greving JP Hedblad B, et al. Prevalence of asymptomatic carotid artery stenosis in the general population: an individual participant data meta-analysis. Stroke 2010;**41**:1294–7.
2. **Ashton HA**, Buxton MJ, Day NE, et al. The Multicentre Aneurysm Screening Study (MASS) into the effect of abdominal aortic aneurysm screening on mortality in men: a randomised controlled trial. Lancet 2002;**360**:1531–9.
3. **Ahmed B**, Al-Khaffaf H. Prevalence of significant asymptomatic carotid artery disease in patients with peripheral vascular disease: a meta-analysis. Eur J Vasc Endovasc Surg 2009;**37**:262–71.
4. **Goessens BM**, Visseren R, Algra A, et al. Screening for asymptomatic cardiovascular disease with noninvasive imaging in patients at high-risk and low-risk according to the European Guidelines on Cardiovascular Disease Prevention: the SMART study. J Vasc Surg 2006;**43**:525–32.
5. **The European Carotid Surgery Trialists Collaborative Group.** Risk of stroke in the distribution of an asymptomatic carotid artery. Lancet 1995;**345**:209–12.
6. **Qureshi AI**, Alexandrov AV, Tegeler CH, et al. Guidelines for screening of extracranial carotid artery disease: a statement for healthcare professionals from the multidisciplinary practice guidelines committee of the American Society of Neuroimaging; cosponsored by the Society of Vascular and Interventional Neurology. J Neuroimaging 2007;**17**:19–47.
7. **Ricotta JJ**, Aburahma A, Ascher E, et al. Updated society for vascular surgery guidelines for management of extracranial carotid disease. J Vasc Surg 2011;**54**:e1–31.
8. **Royal College of Physicians.** National clinical guideline for stroke (draft). London: Royal College of Physicians, 2012.
9. **Stansby G**, Macdonald S, Allison R, et al. Asymptomatic carotid disease and cardiac surgery consensus. Angiology 2011;**62**:457–60.
10. **McDaniel MD**, Cronenwett JL. Basic data related to the natural history of intermittent claudication. Ann Vasc Surg 1989;**3**:273–7.
11. **Steg PG**, Bhatt DL, Wilson PW, et al. One-year cardiovascular event rates in outpatients with atherothrombosis. JAMA 2007;**297**:1197–206.
12. **Jahromi AS**, Cina CS, Liu Y, et al. Sensitivity and specificity of color duplex ultrasound measurement in the estimation of internal carotid artery stenosis: a systematic review and meta-analysis. J Vasc Surg 2005;**41**:962–72.
13. **Nederkoorn PJ**, van der Graaf Y, Hunink MG. Duplex ultrasound and magnetic resonance angiography compared with digital subtraction angiography in carotid artery stenosis: a systematic review. Stroke 2003;**34**:1324–32.
14. **Chambers BR**, Donnan GA. Carotid endarterectomy for asymptomatic carotid stenosis. Cochrane Database Syst Rev 2005;(4):CD001923.

15. **Halliday A**, Harrison M, Hayter E, *et al*. 10-year stroke prevention after successful carotid endarterectomy for asymptomatic stenosis (ACST-1): a multicentre randomised trial. *Lancet* 2010;**376**:1074–84.
16. **Liapis CD**, Bell PR, Mikhailidis D, *et al*. ESVS guidelines. Invasive treatment for carotid stenosis: indications, techniques. *Eur J Vasc Endovasc Surg* 2009;**37**(4 Suppl):1–19.
17. **NHS.** Healthcare Resource Group Payment by Results Tariffs. London: Department of Health, 2011–2012.
18. **Ebrahim S**, Harwood R. *Stroke: epidemiology, evidence and clinical practice.* 2nd edn. Oxford: Oxford University Press, 1999.
19. **European Carotid Surgery Trialists' Collaborative Group** . *Randomised trial of endarterectomy for recently symptomatic carotid stenosis: final results of the MRC European Carotid Surgery Trial (ECST).* Lancet 1998;**351**:1379–87.
20. **Royal College of Physicians of London.** UK carotid endarterectomy audit round 3 public report, June 2011.
21. **Committee of Public Accounts, House of Commons.** *Progress in improving stroke care.* 26th Report of Session 2009–10. 30 March 2010.
22. **Marquardt L**, Geraghty OC, Mehta Z, *et al*. Low risk of ipsilateral stroke in patients with asymptomatic carotid stenosis on best medical treatment: a prospective, population-based study. *Stroke* 2010;**41**: e11–17.
23. **Madani A**, Beletsky V, Tamayo A, *et al*. High-risk asymptomatic carotid stenosis: ulceration on 3D ultrasound vs TCD microemboli. *Neurology* 2011;**77**:744–50.
24. **Goessens BM**, Visseren FL, Kappelle LJ, *et al*. Asymptomatic carotid artery stenosis and the risk of new vascular events in patients with manifest arterial disease: the SMART study. *Stroke* 2007;**38**:1470–5.
25. **Moynihan R**, Doust J, Henry D. Preventing overdiagnosis: how to stop harming the healthy. *BMJ* 2012;**344**:e3502.
26. **Brott TG**, Halperin JL, Abbara S, *et al*. Guideline on the management of patients with extracranial carotid and vertebral artery disease: executive summary. A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines and the American Stroke Association, American Association of Neuroscience Nurses, American Association of Neurological Surgeons, American College of Radiology, American Society of Neuroradiology, Congress of Neurological Surgeons, Society of Atherosclerosis Imaging and Prevention, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of NeuroInterventional Surgery, Society for Vascular Medicine, and Society for Vascular Surgery Developed in Collaboration with the American Academy of Neurology and Society of Cardiovascular Computed Tomography. *J Am Coll Cardiol* 2011;**57**:1002–44.
27. **Fowkes FG**, Housley E, Cawood EH, *et al*. Edinburgh Artery Study: prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. *Int J Epidemiol* 1991;**20**:384–92.
28. **Office for National Statistics.** Population projections for the Office for National Statistics–England and Wales,2010.