

## CAROTID MASTERCLASS

# Identifying the Carotid ‘High Risk’ Plaque: Is it Still a Riddle Wrapped up in an Enigma?

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*The selection of patients for many vascular interventions has largely been based on the severity of luminal narrowing. However, histological data from the coronary and carotid circulations suggest that other plaque features such as inflammation and fibrous cap thickness may be more important in predicting future thrombo-embolic events.*

*This paper reviews the available evidence for identifying carotid atheroma at high risk of being associated with clinical events. Despite a large number of imaging and biomarker studies, ‘presenting symptoms’ remains the most clearly identified risk predictor for ischaemic stroke in patients with carotid stenosis. At present, no imaging modality or plasma biomarker has clearly identified a high risk sub-group of asymptomatic carotid stenoses for which the benefit of carotid intervention is comparable to that of symptomatic atherosclerosis.*

*Emerging developments in MRI, transcranial Doppler and scintigraphic imaging hold some promise for the future. However, the multiple mechanisms and sites determining ischaemic stroke occurrence in association with atherosclerosis suggests that systemic therapies are likely to be the most powerful modality in the management of asymptomatic disease.*

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### Introduction

Atherosclerosis is a common systemic problem with multiple arterial sites affected. Some degree of carotid artery narrowing has been reported in up to 75% of men and 62% of women aged  $\geq 65$  years.<sup>1</sup> At present the selection of patients for carotid revascularisation is, to a large extent, determined by the severity of arterial narrowing.

Approximately 5% of subjects aged  $\geq 65$  years have a 50–99% stenosis.<sup>1–3</sup> Despite evidence from a number of randomised controlled trials demonstrating the benefit of carotid endarterectomy (CEA) over medical therapy, there remains controversy over patient selection.<sup>2–8</sup> This controversy primarily relates to the management of asymptomatic carotid artery disease. While two randomised trials have demonstrated that CEA reduces the long-term stroke risk, the absolute risk reduction is only approximately 1% per

year.<sup>2,3</sup> This combined with the high proportion of subjects who have an asymptomatic carotid stenosis and the loss of benefit resulting from a small increase in perioperative complications commonly reported in other prospective studies, continues to engender concern about patient selection.<sup>9</sup>

Randomised trials of coronary stenting suggest that revascularisation of stable stenoses or occlusions confers no additional benefit over intensive medical therapy alone.<sup>10</sup> Hence the identification of atherosclerotic plaques which confer excess risk of events is fundamental to the selection of patients for vascular intervention. In this review we discuss the concept of the high risk plaque and its identification as relevant to the management of carotid atherosclerosis. The definition of ‘high risk’ in the context of this review is its association with ischaemic stroke and not surgery.

### Pathobiology

There are many mechanisms that determine when/how an ischaemic stroke will occur and all are

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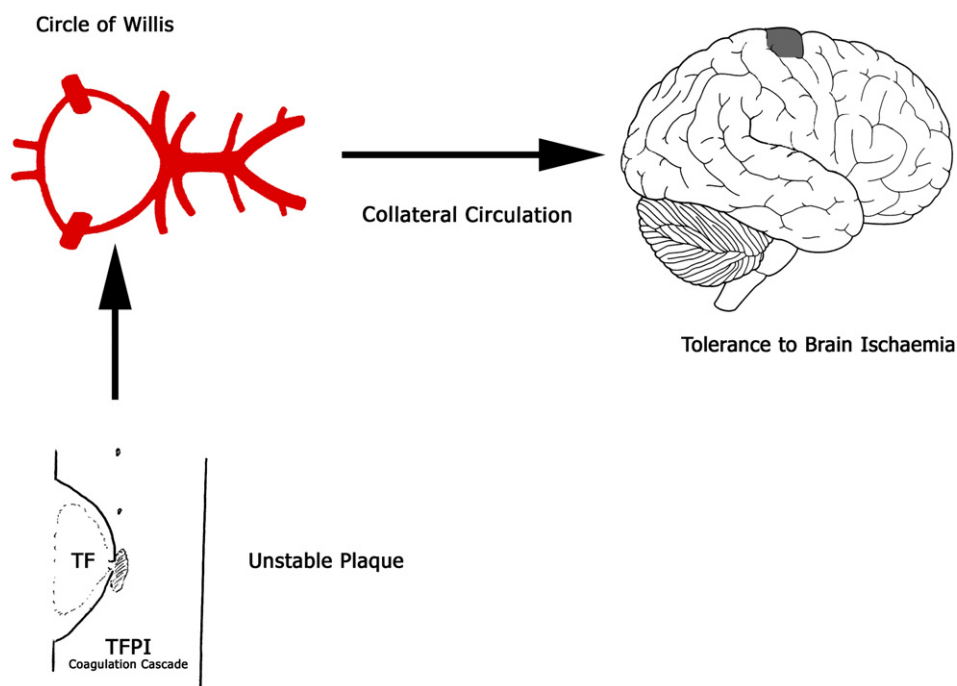
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difficult to predict precisely (Fig. 1). Ischaemic strokes result from thrombo-embolism arising from an array of sites including the heart, aortic arch, carotid, vertebro-basilar system and intra-cranial arteries. Intra-cardiac thrombus is promoted by impaired chamber contractility and stasis and randomised trials have demonstrated a reduced stroke incidence in patients with persistent atrial fibrillation treated with warfarin.<sup>11</sup> Studies of the coronary circulation and, to a lesser degree the carotid system, have suggested that arterial thrombo-embolic events result from rupture or erosion of the fibrous cap overlying an advanced atherosclerotic plaque.<sup>12-14</sup> A number of morphologic features have been associated with plaque rupture, including a thin fibrous cap, accumulation of macrophages within the cap, reduced numbers of matrix producing vascular smooth muscle cells and a large lipid filled core.<sup>12</sup> A summation analysis of small studies comparing carotid atheroma removed from severe symptomatic and asymptomatic stenoses observed that neurological events were significantly associated with plaque rupture, fibrous cap thinning and infiltration by macrophages and T cells.<sup>13</sup> However, highlighting the unpredictable nature of this problem, plaque rupture was only present in 48% of plaques removed from recently symptomatic patients.<sup>13</sup> In the largest histology study of carotid atheroma, Redgrave *et al.* reported a good correlation between plaque

inflammation/cap rupture with time since stroke onset.<sup>14</sup> However, there was little evidence of a relationship between inflammation/cap rupture and time since TIA onset.<sup>14</sup> Quite why there should be such a difference between TIA and stroke patients has not been clarified.

Most published studies (to-date) are limited by the fact that many of the operations were performed months after the index clinical event and therefore pathological findings may not always reflect those that were present at the time of onset of symptoms.<sup>13,14</sup> Accordingly, it is possible that inflammation could follow rather than precede plaque rupture. When and where plaque rupture will occur is also influenced by the wall stress generated. This is dependent upon plaque site, blood pressure and physical features of the arterial wall such as rigidity.<sup>12</sup>

The lipid core contains high concentrations of tissue factor and can promote thrombus development following exposure to flowing blood following cap rupture.<sup>12</sup> The degree of thrombus formation is influenced by the pro-coagulant and anti-coagulant balance within the exposed lipid core and the systemic circulation. This balance, combined with the blood flow characteristics at the arterial site in question, will influence whether an occlusive or non-occlusive thrombosis occurs. These factors will also influence the potential for distal embolisation



**Fig. 1.** Mechanisms important in the development of ischaemic stroke. TF = Tissue factor; TFPI = Tissue factor pathway inhibitor.

and embolisation load. The response of the brain to proximal occlusion or distal embolisation will then depend on many factors including the site of distal embolisation, the anatomy of the circle of Willis, the status of the contralateral carotid artery and vertebro-basilar system and the ability of the brain area to withstand ischaemia (Fig. 1).

### Clinical Presentation and Severity of Carotid Stenosis in Identifying High Risk Carotid Atheroma

At the present time, the most powerful predictor of 'high risk' carotid atherosclerosis is the presence of recent focal neurological symptoms ipsilateral to a carotid stenosis.<sup>2-6,15</sup> Table 1 illustrates the incidence of ipsilateral stroke in patients allocated 'best medical treatment' in four randomised trials of CEA for symptomatic or asymptomatic disease.<sup>2-6</sup> These data (Table 1) illustrate a number of important features regarding carotid atherosclerosis. Following a stroke, transient ischaemic attack (TIA) or retinal infarction (in association with a 80–99% carotid stenosis), one quarter of patients treated medically will experience a stroke over the next three years. In a cohort of patients with similarly severe carotid stenoses (but no symptoms), the incidence of stroke is only about 6% at three years. The risk of stroke associated with an asymptomatic carotid stenosis remains about 2% per year, in contrast to the acute risk which accompanies a symptomatic stenosis.<sup>3,4</sup>

The 'very early' risk associated with a symptomatic stenosis is probably even greater than that detailed in Table 1. This is because the European and North American trials enrolled patients who had experienced a neurological event within 6 months.<sup>4,5</sup> Recent population studies suggest that up to 32% of patients with  $\geq 50\%$  carotid stenoses will suffer a stroke within 12 weeks of the index event and prior to carotid intervention.<sup>16</sup> The vital importance of symptoms in the identification of high risk carotid atherosclerosis is

illustrated by a pooled analysis of the endarterectomy trials.<sup>17</sup> If surgery is carried out within 2 weeks of symptoms, only 5 patients with  $\geq 50\%$  stenoses require surgery in order to prevent one stroke. However, if surgery is delayed for  $>12$  weeks, 125 patients with  $\geq 50\%$  stenoses have to undergo surgery to prevent one stroke. There are other important clinical parameters that determine stroke risk,<sup>17,18</sup> including older age, hypertension, discriminating clinical symptomatology (unilateral weakness as opposed to ocular events, duration  $\geq 60$  minutes) and diabetes.<sup>18</sup> Accordingly, there is already excellent data to select patients with high risk symptomatic stenoses most likely to benefit from carotid intervention.

Conversely, the situation with regard to asymptomatic stenoses is in marked contrast, with relatively little evidence of a clear 'high risk' sub-group. The benefit conferred by CEA for asymptomatic disease does appear to be more apparent in men,<sup>2,8</sup> although this gender disparity could partly reflect worse procedural outcomes in women, rather than a greater risk of events during medical therapy.<sup>2,8</sup> The MRC Asymptomatic Carotid Surgery Trial (ACST) also identified a lack of efficacy of carotid surgery in patients aged  $\geq 75$  years. This was not due to an increased procedural risk but, rather, a limited life expectancy.<sup>3</sup>

### Issues in Identifying 'High Risk' Sub-group of Patients with Asymptomatic Carotid Stenosis

As outlined above, the identification of a 'high risk for stroke' sub-group in patients with recent symptoms is feasible utilising nothing more than clinical presentation and stenosis severity. The same situation does not apply in the management of the great majority of patients who have asymptomatic carotid disease. This has led to marked global disparities in managing these patients.

There are several reasons why it has not been possible to predict the behaviour of carotid atheroma in asymptomatic patients.

**Table 1. Relationship between symptoms, stenosis severity and stroke risk on medical therapy**

NASCET				ECST				ACAS				ACST			
Stenosis	n	time	stroke	stenosis	n	time	stroke	stenosis	n	time	stroke	stenosis	n	time	stroke
<50%	690	60	19%												
50–69%	428	60	22%	60–69%	137	36	11%	60–69%	131	36	6%	60–79%	642	60	10%
70–79%	43	24	21%	70–79%	170	36	9%	70–79%	94	36	5%	80–99%	918	60	10%
80–89%	33	24	27%	80–89%	159	36	21%								
90–99%	24	24	35%	90–99%	60	36	32%								
80–99%	57	24	31%	80–99%	219	36	24%	80–99%	88	36	3%				

NASCET = North American symptomatic carotid endarterectomy trial,<sup>4</sup> ECST = European carotid surgery trial,<sup>5</sup> ACAS = Asymptomatic carotid atherosclerosis study,<sup>2</sup> ACST = Asymptomatic carotid surgery trial.<sup>3</sup> Stenosis severity was measured differently in each trial. ACAS and ACST used ultrasound based criteria.<sup>2,3</sup> NASCET and ECST used angiographic criteria but different measurements methods.<sup>4,5</sup>

- 1) Plaque rupture may remain an 'asymptomatic' phenomenon, either because systemic anti-coagulant factors minimise thrombus development or because thrombus embolises to a 'clinically silent' area of the brain.
- 2) Multiple atheromatous plaques are commonly present in combination. Studies in the coronary circulation have demonstrated that patients often have more than one ruptured plaque in the artery supplying an infarcted myocardium, although usually only one site has evidence of thrombosis.<sup>19</sup> Accordingly, any technique for identifying rupture prone plaque may have difficulty in identifying the most clinically important anatomical lesion.
- 3) There is evidence of a *systemic* inflammatory response in association with unstable plaques at multiple sites.<sup>20,21</sup>
- 4) Any plaque features that are to be useful in identifying patients at high risk of stroke must be present for a sufficient time period before the neurological event occurs in order to permit detection and treatment. Some of the pathological features linked with symptomatic plaques (macrophage accumulation and proteolytic enzyme release) are likely to be present only shortly before fibrous cap rupture.

### Imaging Methods for Identifying High Risk Plaques

An array of imaging modalities (Table 2) have been evaluated in the hope that they might reliably (and accurately) identify features associated with plaque rupture.<sup>19,22</sup>

Ultrasound has been subject to more scrutiny than any other technique, primarily because of its portability, availability and non-invasiveness, together with the fact that it is the commonest means of determining stenosis severity in contemporary clinical practice. A number of subjective carotid plaque criteria (eg grades of echolucency/echogenicity, surface irregularity) were initially assessed as being potential

predictors of increased risk of suffering a stroke. More recently, computer assisted measurement of the gray scale median (GSM) and integrated backscatter has enabled more reproducible assessment of carotid plaques.<sup>20,21,23,24</sup> A number of studies have related carotid plaque echolucency to symptoms in cross sectional comparisons with asymptomatic patients.<sup>23</sup> Prospective studies examining the association of plaque echogenicity and stroke have been limited.<sup>24</sup> Gronholt and colleagues monitored 246 patients (135 symptomatic and 111 asymptomatic) with  $\geq 50\%$  carotid stenosis over a mean of 4.4 years and demonstrated an association between plaques with echolucency above median and subsequent ipsilateral stroke in previously symptomatic patients.<sup>24</sup> The investigators demonstrated no association between plaque echolucency and outcome in asymptomatic patients. Similarly, an evaluation of plaque echolucency did not identify a sub-group in which carotid endarterectomy was more beneficial in the ACST, although details regarding the assessment technique were not provided.<sup>3</sup>

The Asymptomatic Carotid Stenosis and Risk of Stroke (ACSRS) study has recently reported on the incidence of ipsilateral stroke in 1115 patients with an asymptomatic carotid stenosis during a mean follow-up of 37 months.<sup>25</sup> The investigators used image normalisation in order to improve the reproducibility of grading of plaque echogenicity. In this study, patients with a 70–99% stenosis and an echogenic plaque, had a 7-year cumulative risk of stroke of 1%. This compares with a 14% risk of stroke at 7 years in patients with echolucent plaques.<sup>25</sup> Further data incorporating automated gray scale median assessment is expected from ACSRS in due course and corroborative data from similar studies is required prior to concluding that plaque echogenicity can be definitively used to select patients for carotid intervention.<sup>25</sup>

Interestingly a number of studies have also associated carotid plaque echolucency to an increased risk of atherothrombotic events in *unrelated* vascular arteries such as myocardial infarction or contralateral

**Table 2. Imaging methods of potential value in identifying high risk plaques**

Technique	High risk features	Advantages	Disadvantages
Angiography	Stenosis severity; ulcer	Available in most centres	Only lumen visualised, invasive, stroke risk, contrast related complications, radiation required
CT MRI (high resolution)	Calcification <sup>32</sup> Thin cap, cap rupture, intra-plaque haemorrhage, necrotic core <sup>28–31</sup>	Rapid, readily available Non-invasive, able to visualise wall and lumen, less operator dependant than ultrasound	Contrast related complications, radiation required Time required for acquisition, reconstruction and analysis, exclusion of metal implants, availability
Ultrasound	Echolucency, ulcer <sup>20–24</sup>	Non-invasive, able to visualise wall and lumen.	Operator dependent
TCD	Micro embolic signals <sup>33–37</sup>	Non-invasive, theoretically a very direct measure of plaque instability	Very labour intensive, complex analysis, prolonged assessment required

stroke.<sup>26,27</sup> The latter findings support the concept of a systemic destabilizing process which promotes multiple at risk plaques to become unstable.<sup>20,21</sup>

MRI and CT are increasingly being used to evaluate carotid plaque instability.<sup>28–32</sup> MRI features of plaque instability, including a thin fibrous cap, a large necrotic core and marked intraplaque haemorrhage, have been associated with an increased risk of late, ipsilateral ischemic events in asymptomatic patients, although the majority of observed events were TIAs.<sup>29</sup> A more recent study found that intraplaque haemorrhage (detected by MRI) was highly predictive of recurrent ipsilateral ischaemic events. Interestingly, this association was only observed in patients who had previously been symptomatic. MR evidence of intraplaque haemorrhage in the contralateral asymptomatic carotid artery was not associated with an increased long term risk of ischaemic stroke.<sup>30</sup>

Recent advances in MRI to reduce acquisition times, while allowing for movement suggest that this modality may provide the most detailed information about carotid plaque composition.<sup>31</sup> Further studies are required to assess whether this information will be useful in identifying high risk asymptomatic stenoses most likely to benefit from treatment with surgery or angioplasty.

Transcranial Doppler (TCD) is the only method capable of diagnosing micro-embolic signals (MES) in the intracranial arteries<sup>33</sup> and the frequency of MES detected in the ipsilateral middle cerebral artery has been associated with an increased risk of cerebral events in patients with symptomatic carotid stenoses.<sup>34</sup> The detection of MES is also a potentially useful measure of the efficacy of interventions such as medication or surgery.<sup>35</sup>

In a small study of 42 asymptomatic patients, the detection of MES was associated with an increased risk of stroke and TIA (in combination) during follow-up.<sup>36</sup> More recently, Abbott and colleagues studied 202 subjects with asymptomatic 60–99% carotid stenoses every 6 months with TCD for 60 minutes.<sup>37</sup> By Kaplan-Meier analysis, the cumulative risk of ipsilateral stroke/TIA was 17% at 5 years in patients who were positive for MES, as compared with 9% at 5 years in asymptomatic patients who were MES negative ( $p = 0.6$ ).<sup>37</sup>

The latter study, however, illustrated a number of important logistical issues regarding the potential role of TCD for identifying high risk carotid stenoses in asymptomatic patients. Firstly, 11% of patients had an inadequate temporal acoustic window and 2% of completed studies were technically inadequate. A further 5% of studies were not carried out for the planned 60 minutes, presumably due to technical difficulties or problems with the procedure

for the patient or technician. The rate of MES is low in asymptomatic patients (0.16 MES per hour of study) implying that extended examinations are required.<sup>37</sup>

Work continues on developing an ambulatory probe with automated MES monitoring which would significantly enhance the utility of this technique.<sup>38,39</sup>

Other imaging techniques such as angiography, thermography and intra-vascular ultrasound have been demonstrated to identify some features of unstable plaques, but since these methods are generally invasive, they are not likely to be useful in assessing asymptomatic carotid atherosclerosis in the majority of patients.

### Biological Markers of High Risk Carotid Atherosclerosis

Inflammation, proteolysis, lipid accumulation, infection and thrombosis are all processes that have been associated with atherosclerotic plaque instability and secondary clinical events.<sup>12,13,19</sup> With growing awareness that stenosis severity (alone) is a poor guide as to which sites of atherosclerosis should be treated, there has been increasing interest in utilising biological markers of plaque instability to more effectively target interventions, especially in the asymptomatic patient.

Methods of targeting a variety of biological relevant molecules using scintigraphic imaging have been developed.<sup>40</sup> Targeted molecules include oxidized LDL, annexin A5, deoxyglucose and metalloproteinases (MMP).<sup>40</sup> *Ex vivo* and limited *in vivo* studies have demonstrated the potential of single photon emission computed tomography (SPECT) and positron emission tomography (PET) in identifying some of these molecules, however, the value of this type of imaging in selecting high risk plaques for intervention remains unclear.<sup>40,41</sup>

Elevated circulating concentrations of a range of proteins have been related to atherosclerosis.<sup>42</sup> A number of studies have correlated plasma biomarkers in patients presenting with symptomatic and asymptomatic carotid stenoses. Circulating biomarkers that have been found to have elevated concentrations in symptomatic patients in at least one study include; MMP-2, MMP-9, sICAM-1, osteoprotegerin, fibrinogen, homocysteine and anti chlamydia pneumoniae antibodies.<sup>43–48</sup>

However, cross-sectional studies are difficult to interpret, because the elevated levels of biomarkers could be a response to the stroke or TIA rather than being associated with primary plaque instability. Moreover, it is very difficult to reliably match patient subgroups for factors such as age and other cardiovascular risk factors.

Most prospective studies examining the relationship between baseline circulating biomarker

concentrations and subsequent stroke have been performed in patients without documenting or stratifying for the degree of carotid artery stenosis.<sup>49–51</sup> In the Framingham study of healthy elderly subjects, baseline CRP in the top quartile predicted a 2 and 3-fold increased risk of stroke in men and women respectively during a 12 year follow-up of 1462 subjects.<sup>49</sup> In a similar study of healthy 70–79 years old subjects, increased baseline circulating IL-6 concentrations (one standard deviation) predicted a 1.3-fold increase in stroke during a 4 year follow-up after adjusting for other risk factors.<sup>50</sup> In a similar prospective study, lipoprotein-associated phospholipase A2 (Lp-PLA2) was also associated with a higher risk of late stroke.<sup>51</sup> More recently, Eldrup and colleagues examined the relationship between plasma MMP-9 concentrations and the cumulative risk of stroke or cardiovascular death.<sup>52</sup> Patients studied included both symptomatic (56%) and asymptomatic (44%) subjects with  $\geq 50\%$  carotid stenosis in which carotid intervention was not planned. Over a mean follow-up of 4.4 years, plasma MMP-9 predicted combined stroke and cardiovascular death, particularly when combined with plaque echolucency.<sup>52</sup>

For biomarkers to be useful in selecting high risk plaques in a population with an overall level of risk that is low for stroke, a number of important criteria are required. Firstly, the biomarker needs to be highly specific and sensitive for predicting stroke as determined by receiver operator curves. Secondly, these findings need to be tested prospectively in carefully defined patient subgroups eg asymptomatic patients. Thirdly, it has to be demonstrated that any 'high risk' subgroup with high levels of circulating biomarkers should benefit from interventions such as carotid surgery or stenting. It should be borne in mind that elevated biomarkers may simply be predicting the risk of ischaemic stroke as a result of its release from multiple sites of atherosclerosis.

### Conclusions

Overall, the best predictor of 'high risk for stroke' carotid atherosclerosis remains whether or not the patient presented with symptoms (stroke/TIA). The main challenge (for the future) remains the identification of a subgroup of asymptomatic patients who will benefit from angioplasty or surgery. A number of techniques hold promise (high resolution MR, GSM, scintigraphic imaging, TCD and plasma biomarkers) but none have proved reliable enough to be introduced into practice. Since the mechanisms underlying ischaemic stroke are multi-factorial and atherosclerosis is

a systemic disease, it remains a distinct possibility that carotid intervention in such a high risk group may only afford the same absolute risk reduction as that observed within the low risk group.

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