Chapter 4

Carotid Plaque Morphology: Plaque Instability and Correlation with Development of Ischaemic Neurological Events

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Additional information is available at the end of the chapter

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1. Introduction

Stroke is one of the major health care problems in the world today. It is the third leading cause of mortality in the western countries and the most common cause of mortality of any neurological disorder. Incidence of stroke is 160 per 100,000 population per year; 40 percent of victims require some type of special services and 10 percent require total care. [1, 2] Consequently, stroke rehabilitation places a large drain on national health care resources.

A significant proportion of strokes are ischemic in nature, one of leading causes for which is internal carotid artery (ICA) atherosclerosis. It is estimated that 20-25 percent of all strokes can be attributed directly to carotid bifurcation atherosclerosis. [1, 2]

Both internal carotid artery endarterectomy and carotid stenting in patients with preoperative ocular or cerebral embolic events are well established as procedures that reduce the risk of future ischaemic events. [3-7] In addition to the management of hypertension and commencement of antiplatelet and statin therapy, these interventions form the corner stone of stroke prevention policy in patients with significant ICA stenosis. As it is recognised that a significant proportion of patients have a disabling embolic stroke attributable to severe ICA stenosis without any prior symptoms, [8, 9] it would be advantageous if patients who are at highest risk of stroke from ICA stenosis could be identified and treated in advance of any ischaemic neurological events.

2. Atherosclerotic plaque morphology

Atherosclerotic plaques are not static lesions; they undergo dynamic changes in their size and morphological characteristics. These changes manifest themselves as changes in plaque volume and consistency, otherwise known as *plaque progression* and *regression*. These, together with adaptive responses of the arterial wall, determine the degree of stenosis in the diseased artery. [10-12] This degree of stenosis is the measurable clinical finding which, together with timing and nature of symptoms and co-morbidities, correlates with the risk of developing further neurological events. [13]

Over the last 20 years a lot has been learned about the morphological characteristics of an atherosclerotic plaque responsible for plaque progression and instability. [10-12] Morphological characteristics of atherosclerotic plaques can be discussed in the context of plaque surface characteristics and the composition of the atherosclerotic lesion.

3. Plaque surface characteristics

Julian *et al* in 1963 were the first to discuss the issue of carotid plaque ulceration. They reported 17 cases of macroscopic plaque ulceration with thrombosis in the ulcer crater and suggested this as a source for embolisation. [12] Ulceration has been described as an observable disruption of intima exposing the adjacent atheromatous plaque or media [13] (figure 1).

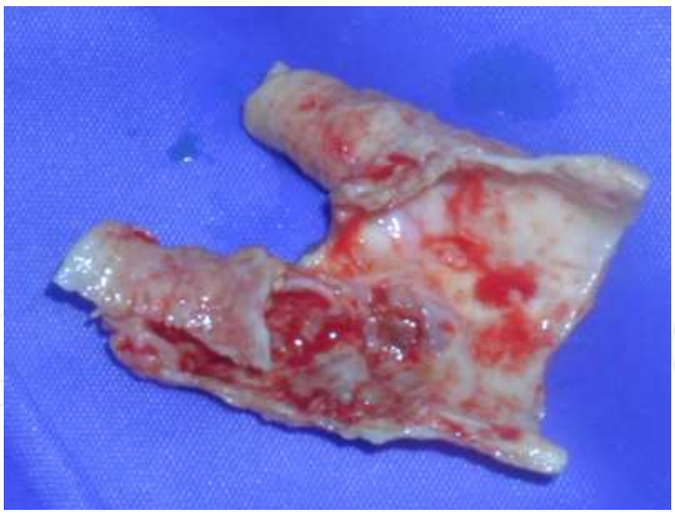

Figure 1. A carotid endarterectomy specimen containing a large ulcer which is associated with intra-plaque haemorrhage.

There has been conflicting evidence regarding the significance of plaque ulceration in the evolution of symptomatic disease. In a large study, Imparato *et al* did not find any significant difference in the incidence of ulceration between symptomatic and asymptomatic groups. [14-16]

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Other researchers have found a definite increase in the incidence of plaque ulceration in patients with symptomatic carotid artery disease. Most notably, following review of 593 angiograms in the North American Symptomatic Carotid Endarterectomy Trial (NASCET), 34 percent of medically treated patients were found to have ulcerated plaques with reasonable certainty; this figure was 36 percent for those assigned to surgery. At 2 years, 30 percent of the medically treated group with ulceration had suffered a non-fatal stroke or vascular death compared to only 17 percent of those without plaque ulceration [17].

One reason for this difference may be the time interval between the duration of symptoms and surgery in some of the studies. Lusby *et al* reported re-endothelialisation of several plaques, and suggested that this is particularly likely when the time span between the onset of symptoms and endarterectomy exceeds 3 weeks. [18]

The strength of the evidence from the NASCET trial has meant that most clinicians recognise plaque cap ulceration as a risk factor for development of further carotid territory embolic neurological events. Preoperative evidence of plaque cap ulceration has been included in stroke risk calculators such as the Oxford Stroke Risk calculator. [19]

4. Composition of an atherosclerotic plaque

The raised lesion or fibro-lipid plaque is the archetypal lesion of atherosclerosis and complications of this lesion (fissure and ulceration) form the basis of the vast majority of cases of occlusive arterial disease. All atherosclerotic plaques share two basic morphological components:

Fibrous cap: a thick layer of fibrous connective tissue, which is significantly thicker and much less cellular than the normal intima and contains lipid-filled macrophages, collagen and smooth muscle cells;

Atheroma: A necrotic mass of lipid that forms the core of the lesion. Loss of continuity of the endothelium is the main step in the progression of a plaque and increases the permeability of the intima to lipoproteins, permits platelet-vessel wall interaction and release of growth factors leading to formation of thrombus on the vessel wall.

Leahy demonstrated that various elements of the plaque are available as potential emboli. [20] This includes the fibrous cap overlying complex plaques, the contents that include cholesterol crystals, the breakdown products of intra-plaque haemorrhage, and fibrous or cartilaginous material as well as calcified tissue. Hollenhorst showed the presence of cholesterol emboli in the retinal artery of patients suffering from amaurosis fugax, in the form of bright plaques that were seen in the extra-cranial carotid vessels. [21] Bock *et al* reported that soft plaques (lipid laden and haemorrhagic plaque) behave in an unstable way and tend to ulcerate, whilst fibrous or calcified plaques behave differently. [22]

Based on the natural history and pathological changes within the plaque the American Heart Association (AHA) has classified atherosclerotic lesions [23] (table 1). This classification that

has been modified by Virmani and Naghavi *et al* divides the atheromatous lesions into non-atherosclerotic intimal lesions and progressive atherosclerotic plaques. [24, 25] The classification, although not particularly directed at the carotid atherosclerotic lesions, is however applicable when classifying carotid plaques. Progressive atherosclerotic plaques (AHA plaque types V and VI) are relevant in the setting of clinically significant carotid disease (figure-2).

Type I	Adaptive Lesion: Intimal Smooth Muscle Cells (SMC)
Type II	Fatty Streak, Foamy Macrophages and underlying SMCs
Type III	Pre Atheroma, Intimal Macrophages, deeper pools of extracellular lipid
Type IV	Atheroma (Fibrous Plaque), dense large extra cellular lipid core deep to intima, and close to media.
Type V	Fibroatheroma, multiple layers of lipid core encased in a fibrous cap
Type VI	Complicated plaque: VI a Disruption of the intimal surface VI b Intra Plaque Haemorrhage VI c Thrombosis related to the atherosclerotic plaque

Table 1. American Heart Association has classification of atherosclerotic lesions [23] (*Circulation* 1995;92: 1355-74)

Figure 2. American Heart Association Type VI (Complicated atherosclerotic lesion) obtained from a carotid endarterectomy specimen. (*Br J Surg.* 2001;88:945–950.)

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5. The concept of unstable atherosclerotic plaque

The concept that a sub-group of atherosclerotic plaques are prone to embolisation or thrombosis is not new. As early as 1926, Benson postulated that coronary thrombosis results from disruption of intima that exposes lipids to flowing blood. [26] Constantinides was the first to establish conclusively that plaque rupture was the immediate cause of coronary thrombosis. [27] In a series of subsequent studies Davies *et al* established the importance of plaque fissuring, ulceration and subsequent thrombosis in the development of acute coronary syndromes. [28-30] Further clinical and angiographic work has led to progression of this concept and introduction of thrombolytic therapy in the treatment of coronary artery atherosclerosis. [31-34]

Atherosclerotic plaques that are prone to rupture are known to have certain cellular, molecular and structural features. Notably these include an intense inflammatory process within the plaque, angiogenesis, and intra-plaque haemorrhage with gradual thinning of the fibrous cap, subsequent loss of plaque cap integrity and ulceration. [35] Burke *et al* defined a vulnerable plaque in the coronary arteries as a lesion with a cap thickness of less than 65 μM [36]. Gertz *et al* noted that the lipid cores were much larger in areas of atherosclerotic plaque disruption than in lesions with intact surfaces. [37-38] Inflammatory activity within the plaque is associated with plaque ulceration and has a role in pathogenesis of intimal damage. [39]

The evolution of atheroma is modulated by innate and adaptive immune responses which are recognized histologically as presence of an inflammatory infiltrate within the lesion [40]. These processes are responsible for replication and phenotypic change within the smooth muscle cell from contractile to secretory which results in formation of plaque cap and lesion growth. Intimal endothelial cell activation results in recruitment of macrophages and lymphocytes (predominantly CD4 positive T-cells) into evolving lesion. [40] Activation of Th-1 T-cells is known to initiate a potent inflammatory cascade which in turn leads to plaque instability [41]. Inflammatory cell infiltrate is a marker for plaque vulnerability. [42-47] Several factors such as oxidized lipoproteins, infectious agents or auto-antigens *(heat shock protein)* have been considered as the putative cause of the chronic inflammatory reaction in an atherosclerotic plaque. [40] This in turn results in weakening of the connective tissue framework of the plaque. [48, 49] Smooth muscles may help to counteract some of these effects by producing matrix protein, collagen and inhibitors of matrix degrading enzymes known as metalloproteinases. [50, 51] The net result of these two processes is thought to define whether or not the plaque ruptures or remains contained by the fibrous cap.

6. Angiogenesis in carotid atherosclerotic lesions

Normal human intima is devoid of blood vessels, [52] however newly formed blood vessels are often seen within atherosclerotic plaques [53-56] (figure-3). The presence and density of these new blood vessels in carotid atherosclerotic lesions has been associated with the histological features of plaque instability and intra-plaque haemorrhage as well as the

Structural:
Large Lipid rich core
Thin Fibrous Cap
Reduces Collagen content
Cellular:
Local Chronic inflammation
Increased macrophage density and activity
T Lymphocyte accumulation near site of rupture
Increased Neovascularity
Reduced Smooth muscle cell density
Increased number and activity of mast cells
Expression of markers of inflammatory activation
Molecular
Matrix Metalloproteinase Secretion
Increased Tissue Factor Expression

Table 2. Features of Rupture prone (Unstable) Plaques [40]

development and timing of ipsilateral ischaemic or ocular events and presence of ipsilateral cerebral infarction on computer tomography (CT) scanning. [57-62] Microarray gene chip analysis revealed that the presence of newly formed vessels is associated with increased angiogenic gene expression. [63, 64] These new blood vessels are weak and could be responsible for intraplaque haemorrhage. Moreover, the endothelial lining of these microvessels express high levels of E-Selectin, ICAM-1, and VCAM-1, which indicates that these endothelial cells are in an activated state. Activated endothelial cells act as local site of inflammatory cell recruitment into the atherosclerotic plaque, perpetuating the inflammatory process within the lesion and contribute to plaque destabilization. [65-69]

7. Plaque haemorrhage

Haemorrhage is a common feature of unstable carotid atherosclerotic lesions. [68-72] Intraplaque haemorrhage has been associated with the development and growth of the necrotic plaque core, rapid changes in plaque volume, development of plaque instability, and ischaemic neurological events. [73-76]

The origin of plaque haemorrhage is uncertain. It has been suggested that it may occur from fissures within the plaque cap. [76] Alternatively the new blood vessels within the atherosclerotic plaque may represent the first site of morphologic change that leads to intra-plaque haemorrhage; features such as microvessel density and perivascular inflammatory infiltrate have been associated with the presence and quantity of intra-plaque haemorrhage. [55, 77, 78]

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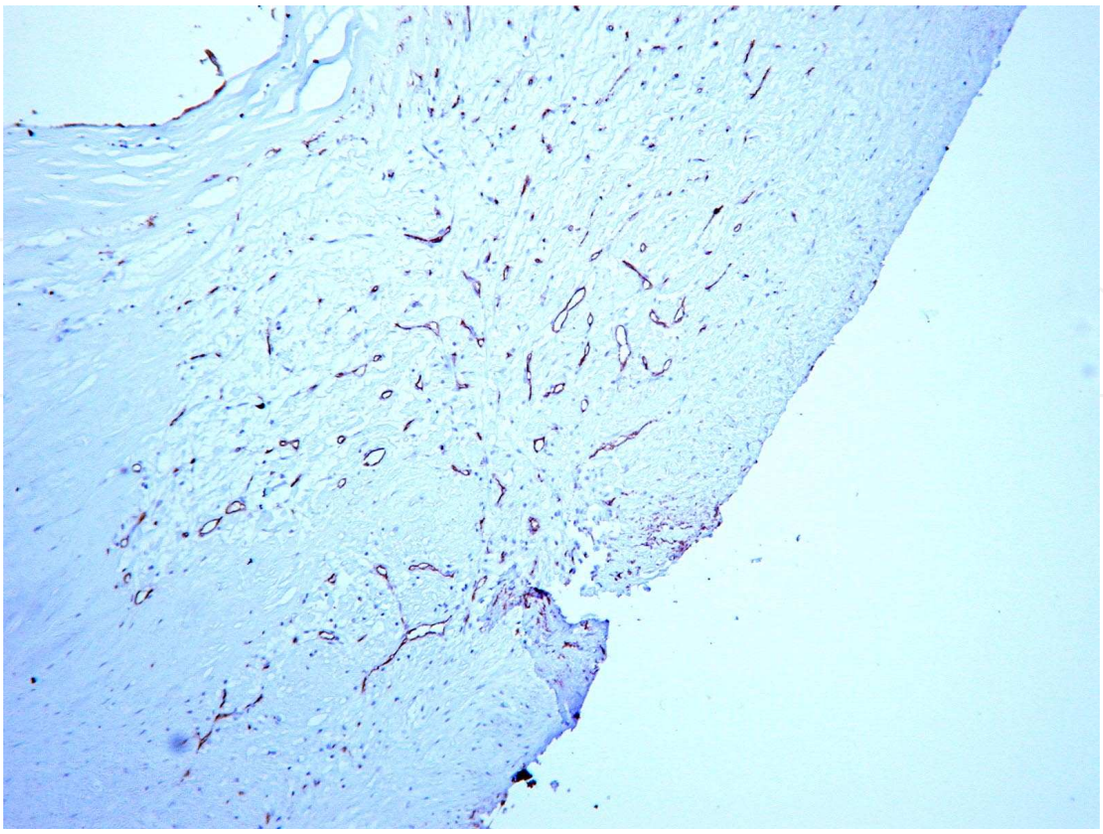

Figure 3. New blood vessels in a carotid atherosclerotic plaque. (*Ann Vasc Surg* 2008; 22(2): 266–272.)

There is ample clinical and histological evidence that carotid atherosclerotic plaques with large necrotic lipid core, thin plaque cap or ulceration, dense inflammatory infiltrate, intra-plaque haemorrhage and angiogenesis are vulnerable to rupture and development of ischaemic neurological and ocular events. In vivo identification of these changes within carotid atherosclerotic plaques gives these findings clinical significance in the context of patients with significant carotid atherosclerosis. For over two decades, non-invasive imaging modalities such as duplex ultrasound and magnetic resonance imaging have been in clinical use. They have been used for the measurement of internal carotid artery stenosis. [79-81] These imaging modalities can also be used to study morphological changes associated with plaque instability and development of ischaemic neurological events. [82-85]

8. Duplex ultrasound assessment of carotid plaque morphology

Duplex ultrasound is arguably the most important imaging modality for preoperative assessment of patients with carotid atherosclerotic disease. It is non-invasive, relatively inexpensive and very accurate at identification of significant ICA stenosis. [87-92] In measuring the degree of stenosis, the flow and velocity characteristics assessed by colour flow Doppler are utilized. Duplex devices also generate high resolution B-mode ultrasound images of the atherosclerotic lesion. These images do not contribute significantly to the assessment of carotid

artery stenosis. However the B-mode ultrasound image can be used to assess morphologic characteristics of an atherosclerotic lesion. It has been known for some time that plaques that have low echogenicity (appear dark on Duplex ultrasound) or a high degree of heterogeneity are associated with histologic characteristics of plaque instability, ipsilateral neurological or ocular events, [93] CT evidence of carotid territory cerebral infarction or evidence of embolisation on trans-cranial ultrasound. [94] These ultrasound characteristics can be assessed subjectively and classified by a trained observer.

B-mode ultrasound assessment of atherosclerotic plaque morphology started some 30 years ago. Reilly *et al* recognised two distinct types of carotid atherosclerotic lesion. The first was termed homogenous and was defined as lesion with uniformly high or medium level echoes. Histologically, homogenous plaques are fibrous lesions. [95] The second type was termed heterogeneous and was defined as plaque with high, medium and low level echoes. [95]

Histologically heterogeneous plaques contain variable amounts of intra-plaque haemorrhage, lipids, cholesterol crystal and a loose stroma. A further refinement of subjective assessment of plaque morphology was the Gray-Wheal classification method (table-3). [96] In the Cardiovascular Health Study, which enrolled 5,201 individuals aged 65 years and over without prior cerebrovascular symptoms, and followed them for an average of 3.3 years demonstrated a significantly increased incidence of stroke in individuals who had echo-lucent plaques. [97]

Plaque Type	Ultrasound characteristics
Type-1	Predominantly echolucent with a thin echogenic cap
Type-2	Intermediate echolucent lesions with small areas of echogenicity
Type-3	Intermediate echogenic lesions with small areas of echolucency (<25%)
Type-4	Uniformly echogenic lesions (equivalent to homogenous).

Table 3. Gray-Wheal Classification of atherosclerotic plaques

Subjective observer dependent assessment of plaque morphology, whilst useful, is limited by high inter- and intra-observer variability, significantly limiting its clinical application. [98-99] Echogenicity and heterogeneity of an atherosclerotic plaque can be objectively assessed using image analysis techniques through the measurement of median grey scale (GS) value of the ultrasound image, percentage of echo-lucent pixels and entropy in GS characteristics of the lesion(figure-4). [99-103] In order to remove variability associated with acquisition of the ultrasound image, the US images are normalised using linear scaling so that the adventitia would have a grey scale median value of 185-195 and blood 0-5. Plaques with a low GS median were associated with a significantly higher annual risk of stroke. [99-104]

Interestingly, although characterisation of the internal structure of the plaque assessed by image analysis correlates closely with clinical symptoms, the correlation between computerised assessment of plaque morphology and histological features of the lesion is less strong. [94] This indicates that values such as GS median represent a median value of the whole atherosclerotic area and do not necessarily reflect the presence of particular regional components.

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The use of stratified GS median measurements which create a profile of the regional GS median as a function of distance from plaque surface combined with colour mapping correlates better with the presence of various histopathological components and identify determinants of plaque instability with a high degree of accuracy. [94]

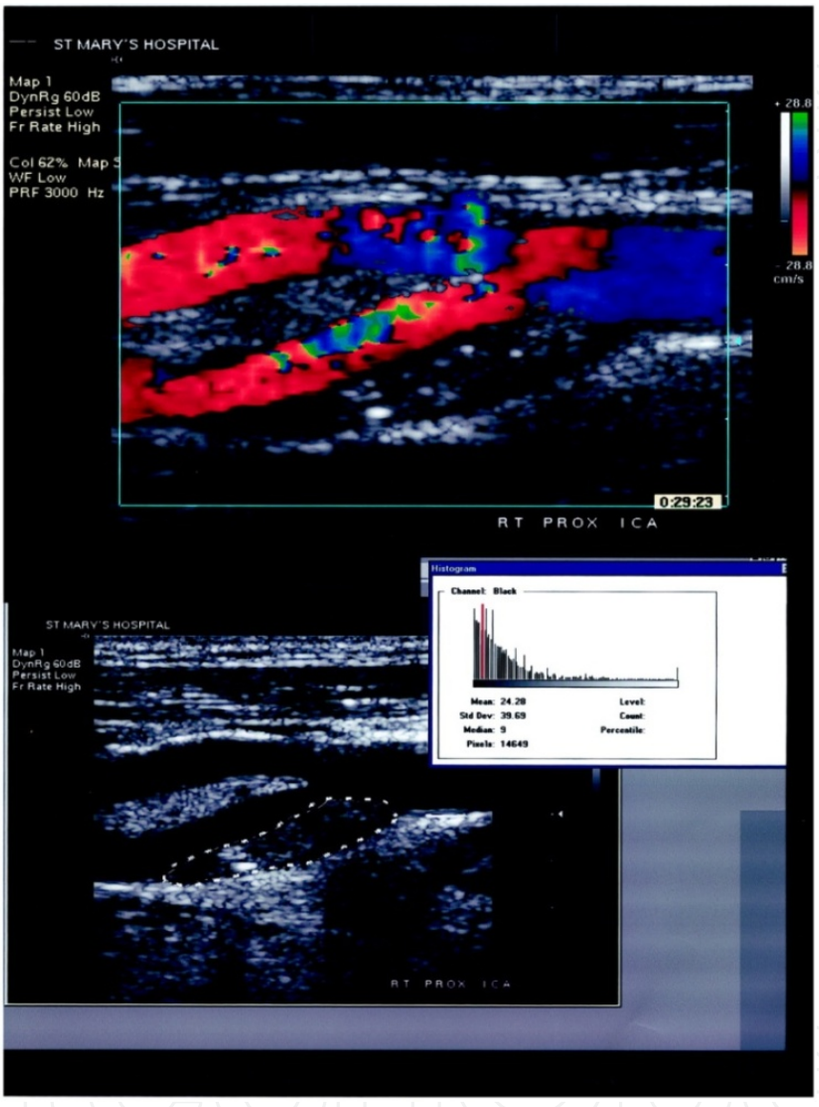

Figure 4. Calculation of Grey Scale Median of a hypo-echoic plaque. (*Swiss Med* 2005; 135:635–643.)

9. Magnetic resonance imaging assessment of plaque morphology

Magnetic resonance imaging (MRI) is a promising modality for characterisation of carotid plaque morphology and assessment of composition of atherosclerotic plaques. It can accurately identify the presence of ulcerated or thin plaque cap, [105-107] quantify intra-plaque haemorrhage [105-107], or the presence of a large necrotic plaque core [105-107]. Serial MRI

examinations in asymptomatic patients with moderate (50-70-percent) ICA stenosis have revealed correlation between these plaque findings and development of subsequent ipsilateral ocular and ischaemic neurological events [108] (Figure-5).

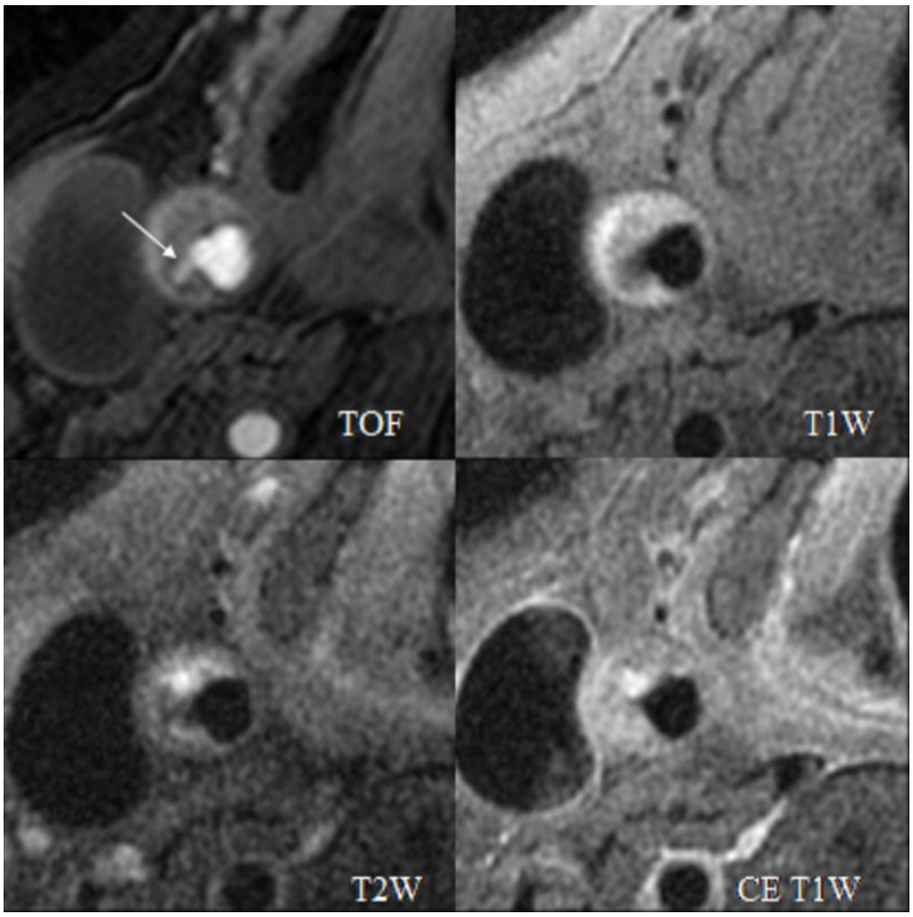

Figure 5. Identification of intra-plaque haemorrhage using high spatial resolution, multi-contrast MRI image. (*JACC Cardiovasc Imaging.* 2009; 2:883-96.)

One of the strengths of MRI is the availability of multi-contrast weighted protocols. The most common application of carotid MRI remains the acquisition of an angiogram which uses a bright blood sequence using a 3-dimensional time of flight sequence. This attenuates the signal from stationary (plaque) tissues. Black blood sequences eliminate the luminal signal and help to characterise plaque morphology. [105-109] Combining the information, multiple-contrast weightings can be used to identify all plaque components. [105-110] Plaque compositional characteristics can be assessed using automatic classifiers such as morphology enhanced probabilistic plaque segmentation (MEPPS) algorithms with a high degree of accuracy (Figure-6). [111, 112] Administration of gadolinium-DTPA together with T1-weighted sequences in addition to bright blood time of flight sequence can be used to create maximal intensity projection (MIP) images for measurement of the degree of ICA stenosis [105-107] and accurately measure the thickness of plaque cap in relation to the necrotic core volume. [105-107]

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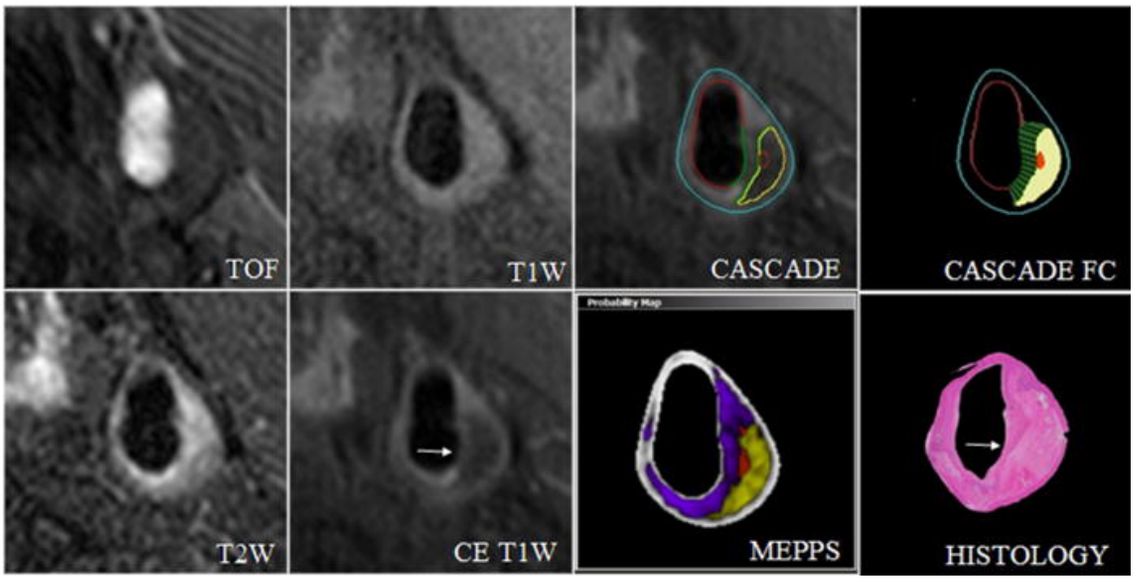

Figure 6. Automated segmentation of bright–and black-blood, high-spatial resolution, Multi-contrast in vivo MR images compared with histological characteristics of plaque. (*JACC Cardiovasc Imaging.* 2009; 2:883-96.)

High resolution MRI can identify and age intra-plaque haemorrhage. [113-115] Prospective serial MRI studies have demonstrated that haemorrhage in atherosclerotic plaques is associated with sudden increases in plaque volume, necrotic core, and progression of degree of stenosis. [115]

In addition to Duplex and MRI, other modalities such as fludeoxyglucose (FDG) positron emission tomography (PET) CT scanning has been used to assess the level of metabolic activity in carotid atherosclerotic plaque. This is used in turn as a surrogate marker of plaque instability.

PET CT scanning has shown some promise as a tool for assessment of plaque instability. [116] However it is unlikely to gain mainstream applicability due to its limited availability, expense, and the significant exposure to ionising radiation (meaning serial assessments are not possible) and availability of non-invasive accurate imaging modalities to assess plaque morphology.

10. Conclusion

Over the last 20 years the advances in technology have led to the evolution of non-invasive imaging modalities with high spatial resolution. The application of this technology in the assessment of carotid plaque morphology has advanced our understanding of the natural history of atherosclerotic lesions more than the assessment of histological characteristics of atherosclerotic plaques. Consequently for the first time, plaque morphology could be assessed against the two functions that ultimately matter the most: time and occurrence of future embolic ischaemic events.

New and continuing advances in MRI technology such as higher field strength, phased-array coils, and the application of 3-dimensional and contrast enhanced ultrasound will provide even more tools for assessment of carotid plaque morphology. Gradual application of these modalities in clinical practice will help clinicians select patients with significant ICA stenosis who are likely to benefit from carotid intervention prior to occurrence of ischaemic neurological events.

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