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Pathology of carotid artery atherosclerotic disease

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Introduction

Stroke is the third leading cause of death in the United States, accounting for 600 000 cases each year, of which about 500 000 are first attacks (American Heart Association, 2001; Heart and Stroke Statistical Update. Dallas, TX, 2001). The pathologic events leading to stroke are complex, and involve atherosclerosis of the aorta and its branches, especially the carotid artery, obstruction of blood flow by increasing plaque burden, embolization of plaque components, especially of thrombotic material, and cerebrovascular factors. The importance of plaque components that predispose to plaque disruption, in addition to the degree of stenosis, has relatively recently been appreciated in relation to cerebral ischemic events. The purpose of this chapter is to characterize atherosclerotic carotid disease in light of our knowledge of coronary atherosclerosis and relate carotid plaque morphology to cerebral ischemic syndromes with special focus on features of plaque instability. A precise understanding of the histologic features of carotid atherosclerosis should help target specific treatments that are likely to be beneficial in the prevention of a subsequent event.

Pathologic features of atherosclerosis, lessons learned from aortic and coronary artery disease

The pathologic classification of atherosclerosis is in constant evolution, and should reflect in part variation based on the size of the artery involved. Two types of lesions were initially described based on gross examination of the aorta: the fatty streak and the atheromatous plaque. The fatty streak, as the less elevated and not prone to thrombosis, was considered a precursor lesion to the advanced atheromatous plaque. The fatty streak consists of smooth muscle cells, lipid-rich macrophages, and lymphocytes within a proteoglycan-collagenous matrix. The atheromatous or fibrofatty plaque is a raised lesion having a lipid-rich necrotic core containing cholesterol and cholesterol esters with an overlying fibrous cap. The atheromatous plaque, unlike the fatty streak, is prone to calcification, ulceration, thrombosis and hemorrhage.

The American Heart Association (Stary *et al.*, 1994, 1995) proposed a numeric classification that was intended to approximate the stages of plaque progression, especially in the aorta. We recently published a modification of the AHA classification based on examination of over 200 cases of sudden coronary death, tailored more to the coronary artery (Virmani *et al.*, 2000). A major modification includes the concept of *thin-cap atheroma*, which is thought to be a precursor lesion to plaque rupture, and hence a potentially more advanced lesion than the typical fibroatheroma (see Table 1.1). It is characterized by a necrotic core (~25% of plaque area), and a thin fibrous cap (<65 mm), heavily infiltrated by macrophages. A mechanistic term for the thin-cap atheroma is vulnerable plaque, based on the hypothetical

Carotid Disease: The Role of Imaging in Diagnosis and Management, ed. Jonathan Gillard, Martin Graves, Thomas Hatsukami and Chun Yuan. Published by Cambridge University Press.

propensity of this lesion to rupture. Although the importance of developing imaging modalities for the identification of thin-cap atheroma is well recognized in the coronary arteries, the concept of thin-cap atheroma in the carotid circulation is less developed.

In the coronary circulation, a less common form of thrombosis than plaque rupture is the *plaque erosion*. The precursor lesion for plaque erosion is less clearly defined than for plaque rupture, and, based on underlying plaque morphology of acute lesions, includes plaques with a developed necrotic core (fibroatheroma) and those without, i.e. *pathologic intimal thickening*. The concept of eroded plaques in the carotid artery has only been recently described; approximately 10% of carotid thrombi in patients with strokes or transient ischemic attacks demonstrated plaque erosion on detailed histologic examination of plaque removed following endarterectomy (Spagnoli *et al.*, 2004).

The ''calcified nodule'' represents the least frequent cause of luminal thrombus accounting for 2–5% of coronary thrombi (Virmani *et al.*, 2000). This lesion is least well understood and is always accompanied by an underlying calcified plate with or without bone formation and shows multiple pieces of calcified nodules admixed with the thrombus adjacent to the lumen. Although calcification with nodule formation is common in carotid plaques, thrombosis as a result of exposure of calcified material to the luminal circulation has not been clearly described in the carotid circulation, but is likely not uncommon.

Percent stenosis and risk of stroke

It is generally accepted that the degree of luminal compromised, as assessed by imaging, is important in determining response to surgical treatment. In the North American Symptomatic Carotid Endarterectomy Trial (NASCET) endarterectomy was efficacious in reducing the risk of stroke and death up to 2 years in patients with 70–99% stenosis of the ipsilateral carotid artery (North American Symptomatic Carotid Endarterectomy Trial Collaborators, 1991). The benefit of carotid endarterectomy is reduced for those with 50–69% stenosis; however, for patients with less than 50% stenosis the failure rate was similar for endarterectomy or medical therapy (Barnett *et al.*, 1998, 2002). Subsequent studies in asymptomatic carotid stenosis of 60% or greater among patients who are good surgical candidates have demonstrated a reduced 5-year risk of ipsilateral stroke after carotid endarterectomy versus medical therapy (Endarterectomy for asymptomatic carotid artery stenosis, 1995).

The optimal approach for managing patients with lower degrees of stenosis than 69% remains uncertain. The asymptomatic carotid atherosclerosis study (ACAS) showed that a reduction in the aggregate risk for stroke and perioperative stroke or death over 50 years was 53% for patients with 60% or more carotid narrowing treated surgically compared with those treated medically (Endarterectomy for asymptomatic carotid artery stenosis, 1995). Identification of asymptomatic individuals with low-grade narrowing who would benefit from surgical management depends on methods of determining high-risk plaques and stratification of carotid atherosclerosis by plaque composition. Addressing the needs of this large population requires an understanding of the pathology of carotid atherosclerosis in relation to plaque instability and thrombosis.

The NASCET study focused on luminal narrowing as a primary measure for evaluating the benefits of endarterectomy in stroke patients and currently guides the management for patients with symptomatic stenosis above 69% (North American Symptomatic Carotid Endarterectomy Trial, 1991). However, the degree of stenosis does not always accurately predict those patients who will develop symptomatic lesions, as low-grade stenosis may also result in cerebrovascular events (Wasserman *et al.*, 2005). Pathologic studies suggest that other factors such as atherosclerotic plaque composition may represent an independent risk factor for ischemic stroke.

Plaque morphology in carotid atherosclerosis

It is difficult to correlate carotid, aortic and cerebrovascular plaque morphology at autopsy, for technical reasons. As a result, the mechanisms by which carotid atherosclerosis results in cerebrovascular symptoms are less understood than those linking coronary disease and myocardial symptoms. From studies of surgically excised carotid plaques, it is apparent that occlusive thrombus triggered by plaque rupture is relatively uncommon in the carotid circulation (Carr *et al.*, 1996; Golledge *et al.*, 2000; Chu *et al.*, 2004; Spagnoli *et al.*, 2004). The relatively low incidence of carotid plaque rupture is probably related to high blood flow and tendency for ulceration and embolization of plaque contents and mural thrombus. Unlike the myocardial circulation, it is likely that ischemic damage in the brain is more dependent on embolization than static occlusion of the artery.

In the carotid artery, as in the coronary circulation, plaque rupture is much more frequent in symptomatic vs. asymptomatic patients, as are fibrous cap thinning and infiltration of the fibrous cap by macrophages and T cells (Carr *et al.*, 1996; Golledge *et al.*, 2000; Chu *et al.*, 2004; Spagnoli *et al.*, 2004). Studies in our laboratory showed that symptomatic carotid artery disease is more frequently associated with plaque rupture (74%) than is asymptomatic disease (32%) (Carr *et al.*, 1996). Our observations suggest critical differences in plaque morphology between patients with symptomatic and asymptomatic disease (Table 1.2).

There have been other attempts correlating plaque morphology, degree of stenosis, and symptoms in patients with carotid atherosclerosis. In a study comparing carotid endarterectomy specimens from symptomatic high-grade stenosis lesions to asymptomatic autopsy specimens without high-grade carotid artery stenosis, Bassiouny *et al.* came to the conclusion that high-grade lesions were more likely ulcerated and thrombosed, reflecting luminal irregularity, than less stenotic asymptomatic plaques (Bassiouny *et al.*, 1989). They were unable to demonstrate that plaque composition, including collagen, DNA, and lipid content, were associated with symptomatic lesions (Bassiouny *et al.*, 1989). However, in a subsequent report, Bassiouny's group studied 99 endarterectomy specimens from symptomatic and asymptomatic patients. Plaques from symptomatic patients had certain morphologic characteristics more frequently than those from asymptomatic patients. The necrotic core was twice as close to the lumen in symptomatic plaques when compared with asymptomatic plaques; the number of macrophages infiltrating the region of the fibrous cap was three times greater in the symptomatic plaques compared with the asymptomatic plaques; and regions of fibrous cap disruption or ulceration were more commonly observed in the symptomatic plaques than in the asymptomatic plaques (32% vs. 20%). The percent area of necrotic core or calcification was similar for both groups (22% vs. 26% and 7% vs. 6%, respectively) (Bassiouny *et al.*, 1997). These observations confirm the importance of histologic parameters, especially inflammation and features of thin-cap atheroma, in the evolution of symptoms associated with carotid stenoses.

A recent study by Spagnoli *et al.* demonstrated that there are significant differences in the types of surface disruption in patients with major stroke, transient ischemic attack, and no symptoms (Spagnoli *et al.*, 2004). Thrombosis was defined by the presence of platelets or fibrin on the plaque surface with or without interspersed red and white blood cells. A thrombotically active plaque was observed more frequently in patients with ipsilateral major stroke, compared to patients with transient ischemic attack and those without symptoms (Table 1.3). In addition, the type of thrombus differed by patient symptoms. In patients with major stroke, 90.1% were associated with plaque rupture and 9.9% with luminal surface erosion. However, erosion was seen in approximately twice as many patients with transient ischemic attack than with stroke. Moreover, the study demonstrated that ruptured plaques of patients affected by stroke were characterized by the presence of

Table 1.1. Atherosclerotic plaque classifications

		Virmani *et al.*	
	Stary *et al.*	Initial	Progression
Early plaques	Type I: microscopic detection of lipid droplets in intima and small groups of macrophage foam cells	Intimal thickening	None
	Type II: fatty streaks visible on gross inspection, layers of foam cells, occasional lymphocytes and mast cells	Intimal xanthoma	None
	Type III (intermediate): extracellular lipid pools present among layers of smooth muscle cells	Pathologic intimal thickening	Thrombus (Erosion)
Intermediate plaque	Type IV: well defined lipid core; may develop surface disruption (fissure)	Fibrous-cap atheroma	Thrombus (Erosion)[c]
Late lesions		Thin fibrous-cap atheroma	Thrombus (Rupture) Hemorrhage/fibrin[d]
	Type Va: new fibrous tissue overlying lipid core (multilayered fibroatheroma)[a]	Healed plaque rupture, erosion	Repeated rupture or erosion with or without total occlusion
	Type Vb: calcification[b]	Fibrocalcific plaque (with or without necrotic core)	
	Type Vc: fibrotic lesion with minimal lipid (could be result of organized thrombi)		
Miscellaneous/ complicated features	Type VIa: surface disruption Type VIb: intraplaque hemorrhage Type VIc: thrombosis		
		Calcified nodule	Thrombus (usually nonocclusive)

[a]May overlap with healed plaque ruptures; [b]occasionally referred to as type VII lesion; [c]may further progress with healing (healed erosion); [d]may further progress with healing (healed rupture).

a more severe inflammatory infiltrate, constituted by monocytes, macrophages, and T lymphocyte cells compared with that observed in the transient ischemic attack and asymptomatic groups ($p = 0.001$). These findings support other data implicating the involvement of inflammatory cells, cytokines, adhesion molecules, and other inflammatory mediators in the pathogenesis of ischemic cerebrovascular injury (Frijns and Kappelle, 2002) and demonstrate a major role of carotid thrombosis and inflammation in ischemic stroke in patients affected by carotid atherosclerotic disease.

Effect of high flow and carotid plaque morphology

Atherosclerosis begins near branch ostia, bifurcations and bends, suggesting that flow dynamics play an important role in its induction. Laminar

Table 1.2. Gross and microscopic plaque characteristics in symptomatic and asymptomatic patients undergoing carotid endarterectomy

Gross morphology	Symptomatic, % ($n=25$)	Asymptomatic, % ($n=17$)	p-value
% Stenosis(Duplex)	74±17	77±15	ns
Ulceration	94	64	0.02
Plaque hemorrhage	47	52	ns
Microscopic characteristics			
Plaque rupture	74	32	0.004
Thin fibrous cap	95	48	0.003
Cap foam cells	84	44	0.006
Intraplaque fibrin	100	68	0.008
Intraplaque hemo.	84	56	0.06
Necrotic core	84	72	ns
Ulceration	11	8	ns
Calcified nodule	7	7	ns
Thrombus	63	80	ns
SMC rich area	5	0	ns
Eccentric shape	68	64	ns

Abbreviations: hemo = hemorrhage; ns = non significant.
Modified from Carr *et al.*, 1996

Table 1.3. Thrombotically active plaques, cap rupture, and cap erosion by study groups

	No. of plaques %			p-value		
	Patients with major ipsilateral stroke ($n=96$) (%)	Patients with TIA ($n=91$) (%)	Asymptomatic patients ($n=82$) (%)	Stroke vs. TIA	Stroke vs. asymptomatic	TIA vs. asymptomatic
Thrombotically active plaque	71 (74)	32 (35.2)	12 (14.6)	<0.001	<0.001	0.002
Cap rupture	64 (66.7)	21 23.1)	11 (13.4)	<0.001	<0.001	0.004
Cap erosion	7 (7.3)	11 (12.1)	1 (1.2)	0.51	0.09	0.03

Abbreviation: TIA = transient ischemic attack.
Reproduced with permission from Spagnoli, L. G., *et al.* (2004). *Journal of the American Medical Association*, **292**:1845–52.

flow is disturbed at carotid bifurcation regions, resulting in decreased shear stress and atherosclerotic plaque accumulation on the outer wall of the proximal segment of the sinus of the internal carotid artery (Zarins *et al.*, 1983; Anayiotos *et al.*, 1994; Masawa *et al.*, 1994). The intimal thickness is the least on the flow divider side at the junction of the internal and external carotid arteries

where wall stress is the highest (Figure 1.1) (Glagov *et al.*, 1988).

High flow rates and the shear forces caused by the bifurcation of the common carotid artery into the internal and external carotids result in unique features of carotid plaque morphology as compared to the coronary circulation. Most importantly, the ulcerated plaque, which is uncommon in the coronary artery circulation, is relatively common in the carotid and other elastic arteries. Ulcerated plaque is a term used when the thrombus and a portion of the plaque have embolized, leaving an excavation in the remaining lesion (Figure 1.2). Another feature of carotid atherosclerosis is the infrequency of total occlusion relative to the coronary circulation. Occlusive carotid disease is reported in 3% of patients with posterior circulation infarcts, 14% in those with partial anterior circulation infarcts and 29% in patients with total anterior circulation infarcts; however, in coronary circulation the incidence of chronic total occlusion in patients dying suddenly is 40% (Golledge *et al.*, 2000; Burke *et al.*, 2001). The explanation for the low rate of total occlusions in carotid plaques is most likely related to high-flow rates that limit thrombotic occlusions unless there is severe luminal narrowing caused by repeated plaque ruptures.

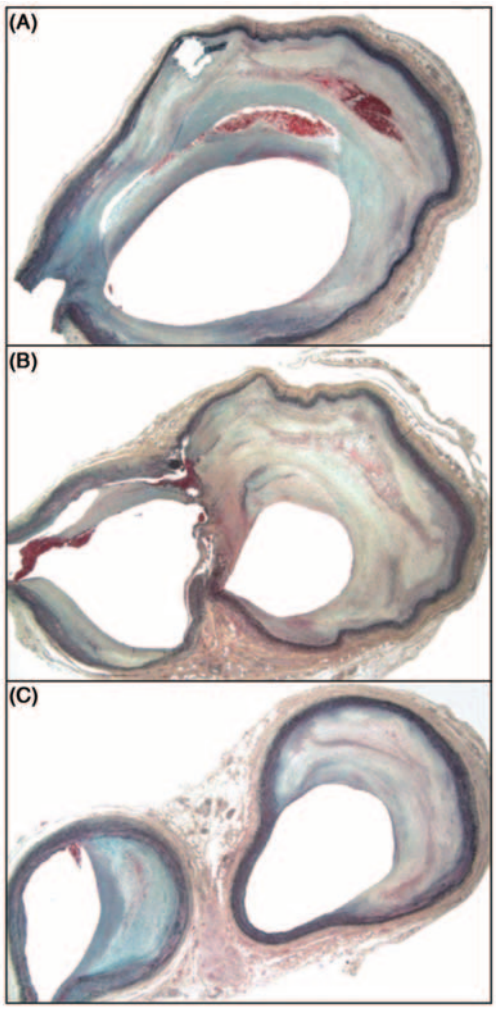

Figure 1.1 Carotid bifurcation, atherosclerotic disease. Panel A demonstrates the common carotid artery. There is moderate narrowing by atherosclerotic plaque, with two hemorrhagic necrotic cores. This layering indicates repeated surface disruption (rupture) and healing with smooth muscle cells. Panel B demonstrates the bifurcation, with the flow divider illustrated in the center. Note that the flow dividers on either side are relatively devoid of plaque, indicating the high shear stress in this site is relatively protective of accumulation of atherosclerotic material. Panel C shows the internal carotid artery (right), with the external carotid (left). Note the positive remodeling of the internal carotid artery at the site of atherosclerotic plaque.

Role of embolism and symptomatic carotid disease

The high rate of ulcerated plaques in the carotid circulation suggests that high-flow states result in relatively large amounts of embolized lipid material in cases of carotid plaque rupture. The reduction of stroke risk after carotid endarterectomy is attributed to removal of the cerebral embolic source in most patients. Transcranial Doppler-detected microembolic signals emanating from the ipsilateral middle cerebral artery have been associated with high-grade stenosis and recent stroke, and decrease after endarterectomy (Stork *et al.*, 2002). They have been associated with ulcerated plaques

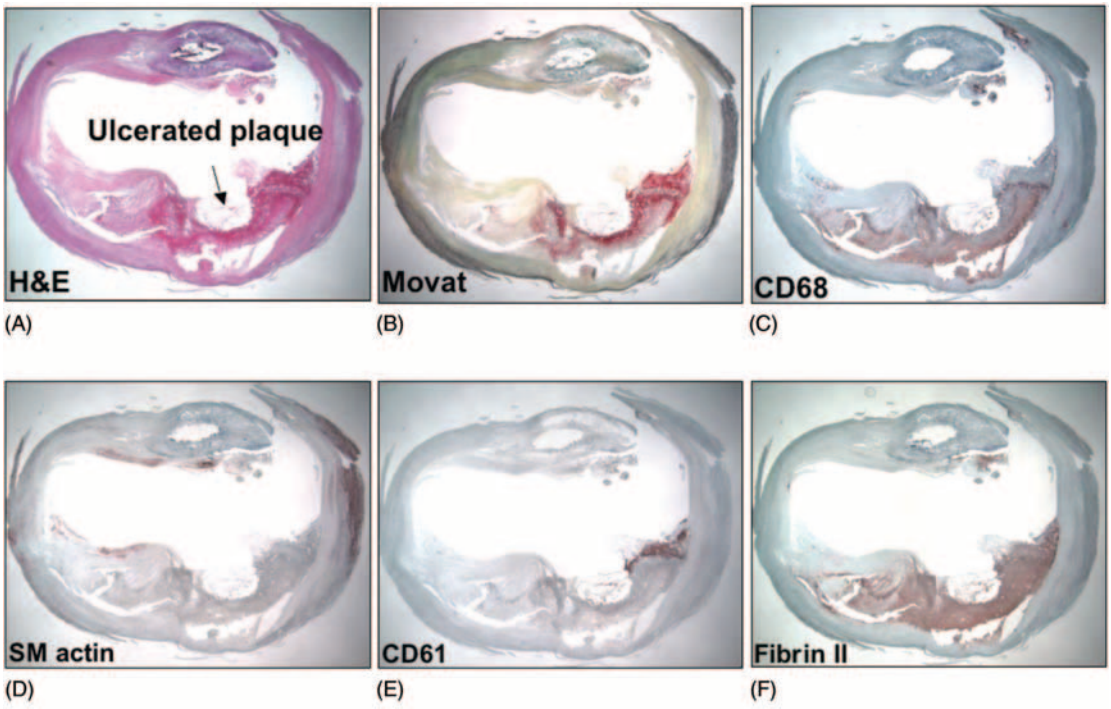

Figure 1.2 Plaque rupture with thrombosis and ulceration. Unlike coronary arteries, in which ulcers are unusual, plaque disruption in the carotid artery frequently results in embolization and crater formation. (A) demonstrates a routine hematoxylin eosin section of a carotid artery with thrombus and ulcer. (B) shows the corresponding Movat pentachrome stain, which highlights collagen (yellow) and elastic tissue (black). (C–F) are immunohistochemical stains for macrophages (Kp-1), smooth muscle cells (alpha actin), platelets (CD61) and fibrin (fibrin II). Note that at the ulcer crater, there are abundant macrophages (C) with few smooth muscle cells (D). The thrombus itself has largely embolized; there are residual platelets (E) and fibrin (E) at one edge of the crater.

as assessed by ultrasound (Valton *et al.*, 1998) and histologically disrupted plaques (Sitzer *et al.*, 1995). An association with plaque characteristics has not been uniformly demonstrated, however (Droste *et al.*, 1999; Stork *et al.*, 2002; Verhoeven *et al.*, 2005).

Comparison of coronary and carotid atherosclerosis

In our laboratory, we have compared the histomorphometric features of unstable coronary and carotid atherosclerotic plaques. The mean fibrous cap thickness in carotid plaque rupture was nearly three times greater than coronary plaque rupture (72 ± 15 microns vs. 23 ± 17 microns), respectively (Figure 1.3). We measured carotid vulnerable plaques (necrotic core with overlying thin cap and infiltration by macrophages, Figure 1.4) and found a mean cap thickness of 72 ± 24 microns, which is greater than the 65-micron upper limit of a thin-cap fibroatheroma in the coronary artery. In addition, there are fewer macrophages in the fibrous cap of carotid plaque ruptures than coronary plaque ruptures (13.5 ± 10.9% vs. 26 ± 20%). Similarly, in carotid-vulnerable plaques the number of macrophages

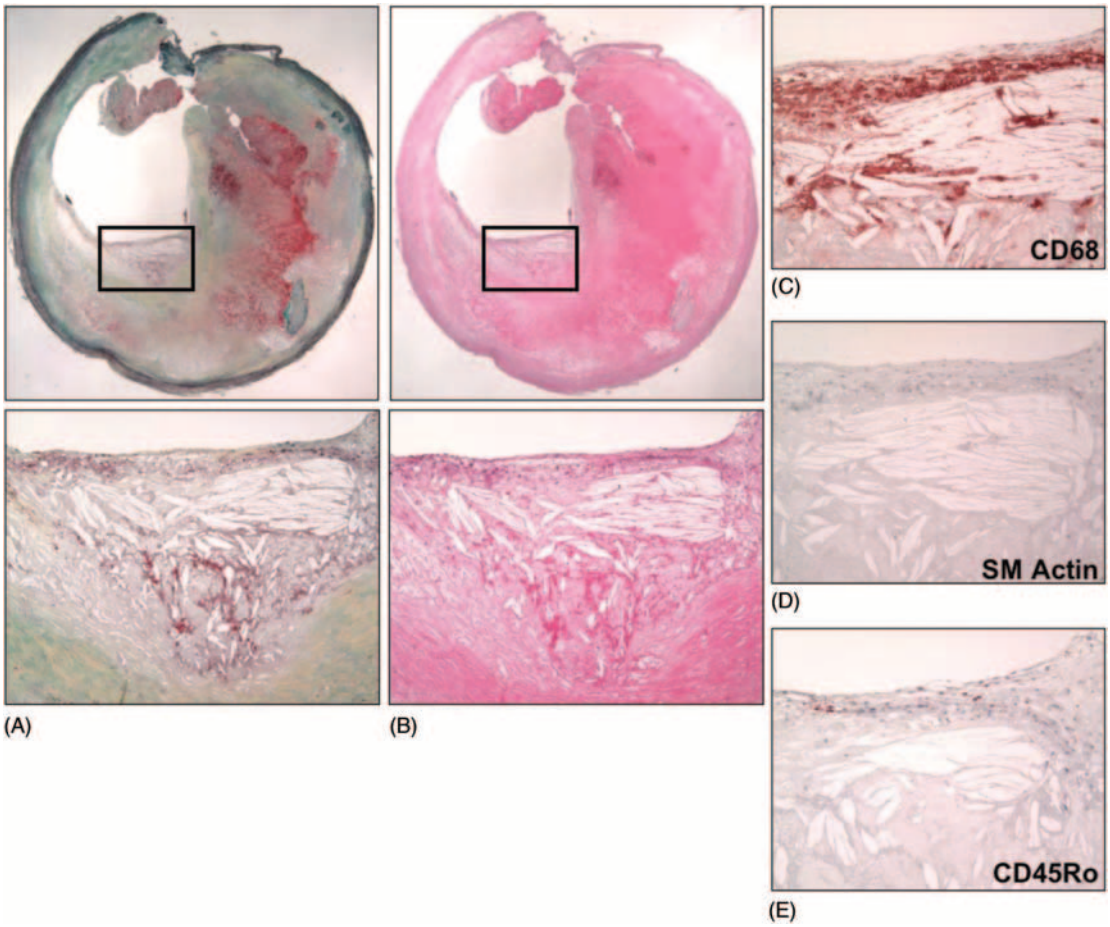

Figure 1.3 Vulnerable plaque with hemorrhage. Panel A (Movat stain) and panel B (hematoxylin-eosin stain) show carotid endarterectomy specimens with a thin fibrous cap (boxed areas, and insets below). Panels C (CD 68 for macrophages), D (alpha actin for smooth muscle cells) and E (CD45Ro for T-cells) demonstrate that, in the area of thinning of the cap, there are numerous macrophages, no smooth muscle cells, and a sprinkling of T lymphocytes.

is fewer than coronary-vulnerable plaques (10 ± 1.8% vs. 14 ± 10%). (Virmani *et al.*, 2000)

The role of angiogenesis in plaque progression has been studied in the coronary arteries (Kolodgie *et al.*, 2003). The role of vasa vasorum in precipitation of acute coronary syndromes and aortic plaque disruption is the focus of ongoing research. Plaque vascularity has, in addition, been shown to correlate with intraplaque hemorrhage and the presence of symptomatic carotid disease (Mofidi *et al.*, 2001). Imaging techniques for detection of vasa vasorum in carotid plaques may be important in future evaluation of carotid stenosis.

Plaque hemorrhage in the carotid artery (Figure 1.5) is far more frequent than in the coronary arteries and may be related to high-flow

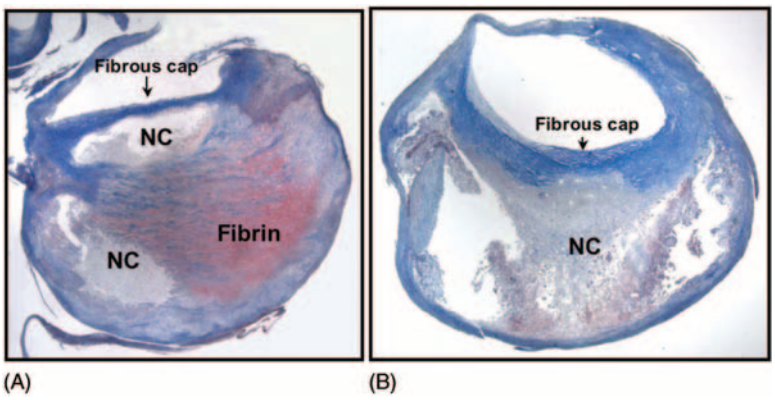

Figure 1.4 Fibrous cap, carotid atherosclerosis. These photographs of carotid plaques (Masson trichrome stain) demonstrate multiple necrotic cores (NC), with a fibrin-rich central area, and a thin fibrous cap (arrow) with collagen staining blue (A). Panel B shows a single large necrotic core, with a thicker fibrous cap than shown in panel A (arrow).

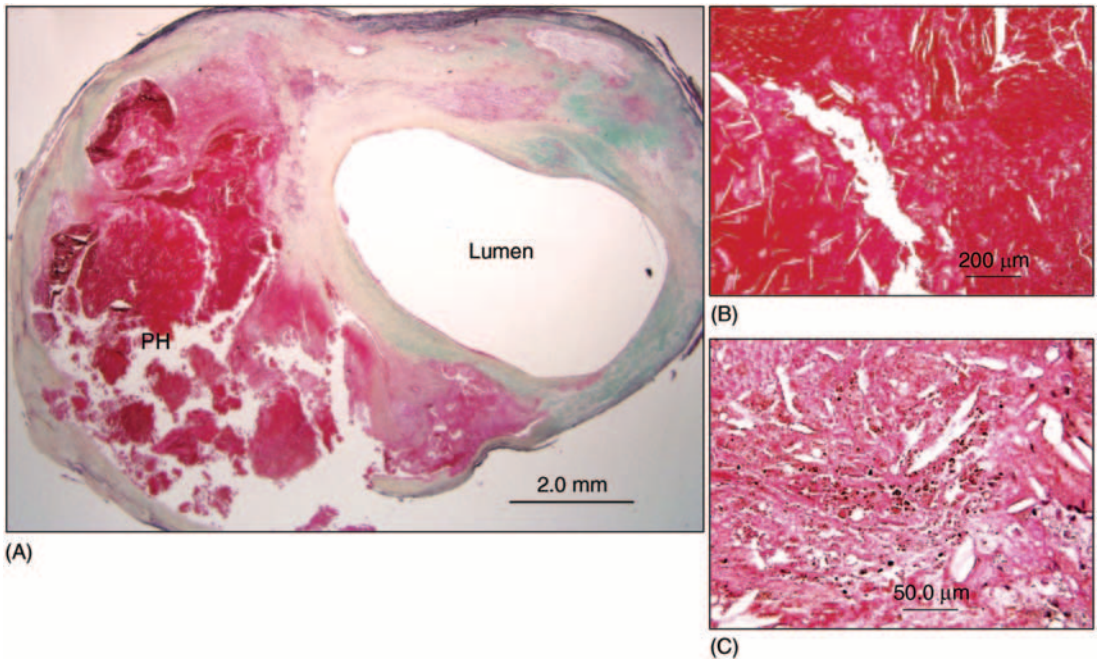

Figure 1.5 Plaque hemorrhage within a necrotic core. Photomicrograph of a carotid plaque (Movat stain) showing intraplaque hemorrhage with the necrotic core (nc), note the fibrous cap is thick (symptomatic patient). (B) shows a high power of the hemorrhage, which shows areas of well-formed red cells with interspersed free cholesterol crystals, and similar crystals are seen in areas where the red cells cannot be recognized and no foam cells are identified, suggestive that the free cholesterol may be derived from the red cell membranes that are rich in free cholesterol. (C) is high power showing hemosiderin (brown pigment) lying free as well as within macrophages.

rates and pressures in the lumen and the vasa vasorum. The maximum frequency of hemorrhage is observed in arteries with 50–75% cross-sectional area luminal narrowing. We have reported in coronary plaques that intraplaque hemorrhage is responsible for necrotic core enlargement and excessive foamy macrophages in the fibrous caps (Kolodgie *et al.*, 2003). Red blood cell membranes are the richest source of cholesterol as compared to any other cell in the body. The free cholesterol in the necrotic core is believed to arise from apoptotic cell death of foamy macrophages. However, we have shown that free cholesterol in fibroatheromas, thin-cap fibroatheromas and plaque ruptures is also derived from erythrocytes that become trapped in the necrotic core when intraplaque hemorrhages occur (Kolodgie *et al.*, 2003). Takaya *et al.* recently reported that patients with carotid intraplaque hemorrhage at 18 months follow-up had larger necrotic cores as well as accelerated plaque progression as compared to patients without intraplaque hemorrhage (Takaya *et al.*, 2005).

The frequency of calcification is similar in coronary and carotid arteries, with maximum calcification seen in carotid arteries narrowed greater than 70% cross-sectional area. Calcification in the carotid artery similar to coronary artery is at first speckled and occurs in areas rich in smooth muscle cells like pathologic intimal thickening and occurs at sites of smooth muscle cell loss. The next most frequent site is the base of the necrotic core, close to the media (Figure 1.6). Calcium in the carotid plaque is often fragmented and may be located deep in the plaque or close to the surface. However, the frequency of calcified nodules (with surface thrombus) (Figure 1.7), a form of calcification that results in irregular nodules of calcium, is higher in carotid disease (approximately 6–7%), as compared to coronary artery disease (1–2%). In contrast, plaque erosion, while common in the coronary circulation, is somewhat less frequent in the carotid artery. In carotid arteries, percent stenosis was highest in healed plaque ruptures and was greater than thin-cap atheromas and acute plaque ruptures.

Is luminal narrowing the only determinant of vulnerability of a plaque?

Vulnerable plaque is a concept well accepted in the coronary circulation but not so well established in the carotid plaque. In studies carried out in the coronary circulation it has been shown that plaque ruptures often occur at low degrees of luminal narrowing and that percent stenosis is a poor predictor of plaque rupture. Ambrose *et al.* showed in retrospective analysis of angiograms of patients with acute myocardial infarction, that the median stenosis of the initial angiogram in the artery that caused the infarction was 48% (Ambrose *et al.*, 1988). This concept subsequently has been now repeatedly proven to be correct; we have shown in sudden coronary death that at least 40% of patients dying suddenly with a luminal thrombus have underlying plaques <75% narrowed in the cross-sectional area (Farb *et al.*, 1995). Wasserman *et al.* have suggested that in the carotid artery it is time to look beyond stenosis (Figure 1.8). They state that ''although retrospective angiographic studies of extracranial carotid atherosclerosis and stroke have not been reported, the mechanism of plaque rupture may be similar to that seen in the coronary artery'' (Wasserman *et al.*, 2005).

Plaque progression through repeated silent ruptures (Figure 1.9)

Morphologic studies of coronary plaques have suggested that plaques beyond 50% cross-sectional area narrowing occur through repeated ruptures, which are most often clinically silent (Burke *et al.*, 2001). The sites of healed plaque ruptures can be recognized by the presence of a necrotic core with a discontinuous fibrous cap which is made up of type I collagen identified by either Movat and/or Sirius red-stained sections of the artery with an overlying neointimal tissue that is rich in smooth muscle cells in a proteoglycan-rich matrix and type III collagen (Figure 1.9). In the coronary circulation