CHAPTER 7

From coronaries to carotids: shifting the paradigm of plaque vulnerability

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Introduction

Stroke is the third most common cause of death in North America, and approximately 600 000 new strokes are reported annually in the United States. Seventy-five per cent of these occur in the distribution of the carotid arteries. Among these, 400 000 affect previously asymptomatic patients and demonstrate a thromboembolic etiology due to carotid occlusive disease.

Carotid artery stenosis is a frequent finding in the general population, with a prevalence of 75% in men and 62% in women over 64 years as determined by ultrasonography in the Cardiovascular Health Study.[1] Stroke prevention has consequently become of utmost importance in modern healthcare. Since the introduction of catheter angiography, luminal narrowing has become the standard parameter used to report on the extent and severity of carotid artery stenosis. This is primarily related to several randomized clinical trials including the North American Symptomatic Carotid Endarterectomy Trial (NASCET) and the European Carotid Surgery Trial (ECST), which relied on the assessment of luminal narrowing. These trials demonstrated a significant reduction in stroke risk after carotid endarterectomy compared with medical treatment alone, in patients with luminal stenosis of $> 69\%$.[2,3]

Although the value of angiography is recognized for more advanced disease, its reliability for predicting the benefit of surgery for lower-grade narrowing remains less clear, particularly for those with 30–69% stenosis.[4] Indeed, today new detection imaging techniques such as pixel density analysis at Doppler ultrasound, intravascular ultrasound, elastography, and multisequence magnetic resonance imaging (MRI)[5–9] have been developed in order to document atherosclerotic lesion composition, which may help to investigate the association between plaque components and subsequent cerebrovascular events prospectively in asymptomatic patients.

Epidemiologically, it is also important to recognize that, although carotid artery disease is common in the general population, the prevalence of stenosis with luminal narrowing $> 50\%$ is rare (e.g. $< 8\%$ in the Cardiovascular Health Study). We know that the 5-year risk of stroke in asymptomatic patients with $< 60\%$ carotid artery stenosis is ~8% (and 1.6% annually).[10] Therefore, although the individual risk for cerebrovascular events with low- and moderate-grade luminal narrowing is relatively low, the implication for the overall risk of cerebrovascular events may be significant because of the high prevalence of this finding.

From these premises it is clear that parameters other than the simple concept of luminal narrowing are needed to predict the risk of cerebrovascular events more reliably and to assess the potential benefit of carotid endarterectomy, particularly in the group of asymptomatic patients with stenosis

of 30–69%.[9] Furthermore, as we know well from the coronary circulation, arterial remodeling is present even during early plaque formation. Angiography is not able to detect this early stage of atherosclerosis because luminal narrowing begins only when > 40% of the area of the vessel wall is occupied by plaque.[11] Coronary plaque rupture may occur in the presence of low-grade stenosis, and the degree of stenosis is a poor predictor of myocardial events.[12] We also know from pathologic studies in the coronary district that in > 75% of acute myocardial infarctions, thromboembolism arises from plaque rupture, and is more common in plaques with less luminal narrowing.[13,14] Although fewer data are available for the carotid arteries, plaque rupture even in low-grade disease seems to play a similar role in the development of cerebrovascular events.[15]

In other words, categorizing patients as candidates for either medical therapy or any carotid procedure on the basis of stenosis severity and the presence or absence of neurological symptoms alone does not identify sufficiently and accurately the real risk presented by the patient.

This chapter will focus on the similarities between coronary and carotid atherosclerotic disease with the intent of shifting the paradigm of plaque vulnerability from coronaries to carotids and to give a pathology-based rationale for the assessment, risk stratification, and decision-making therapeutic strategy in the treatment of carotid atherosclerotic disease.

Clinical correlation between coronary and carotid atherosclerotic disease

Different studies have demonstrated that patients with clinically manifested carotid disease (previous transient ischemic attack (TIA) or minor stroke) in the absence of known coronary disease are affected indeed by atherosclerotic disease of the coronary tree.[1,16–18] The tight association between carotid and coronary disease has been demonstrated not only in several autoptic studies[19–21] but also in different epidemiologic and clinical studies.[16,18,22–25] One of the most interesting, in terms of both the number of patients enrolled (5184 of both sexes with an age between 30 and 62 years) and follow-up duration, is the Framingham Study. In this study, Kannel and colleagues[26] demonstrated that stroke risk was low in subjects not affected by coronary disease while it increased in relation to disease onset severity (less for angina, greater for infarction), especially in the female sex. On the other hand, carotid atherosclerotic disease doubled the risk of death from coronary disease.[27] Eagle and associates[28] compared the survival rate of patients affected by stable coronary artery disease with (2296 subjects) and without (13 953 subjects) peripheral disease. At 12 years, survival curves showed significantly increased mortality in patients affected by peripheral disease compared to the others. In addition, at multivariate analysis, peripheral disease was the strongest predictor of mortality during follow-up. This concept is best expressed by the known notion of increased incidence of perioperative myocardial infarction in patients submitted to surgical carotid endarterectomy and increased cerebrovascular accidents in patients submitted to coronary artery bypass.[4,29] In this context, Lombardo and associates[30] have retrospectively evaluated the ultrasound characteristics of carotid plaques in patients scheduled for coronary bypass surgery, 181 with unstable and 92 with stable angina, and prospectively in a similar group of patients, 67 with unstable and 25 with stable angina, in whom serum C-reactive protein levels were also measured. The prevalence of carotid plaques was similar in the retrospective and prospective studies and > 64% in both unstable and stable coronary patients. The prevalence of complex, presumably unstable carotid plaques was 23.2% in unstable versus 3.2% in stable patients ($p < 0.001$) in the retrospective study and 41.8% versus 8.0% ($p = 0.002$) in the prospective study. C-reactive protein levels were higher in patients with complex (7.55 mg/l) than in those with simple (3.94 mg/l; $p < 0.05$) plaques or without plaques (2.45 mg/l; $p < 0.05$). On multivariate analysis, unstable angina and C-reactive protein levels > 3 mg/l were independently associated with complex carotid plaques (odds ratio (OR) 6.09, 95% confidence interval (CI) 1.01–33.72; $p = 0.039$, and OR 5.80, 95% CI 1.55–21.69; $p = 0.009$, respectively),

suggesting that in patients affected by unstable angina, plaque instability may not be confined to coronary arteries, and inflammation may be the common link with carotid plaque instability. We have recently confirmed and expanded these observations demonstrating that coronary instability may also influence carotid atherosclerotic plaque composition with a related increased risk to develop a cerebrovascular event.[31] In particular, we have demonstrated in patients affected by both coronary and carotid disease and undergoing surgical endarterectomy associated with coronary bypass a significantly greater amount of inflammatory infiltrate (macrophages and T lymphocytes) and a stronger expression of interleukin-6 and C-reactive protein in carotid plaques from patients affected by unstable angina compared to stable angina (Table 7.1 and Figures 7.1 and 7.2).

Similarities between coronary and carotid atherosclerotic disease: the grade of stenosis and risk of acute clinical events

The grade of stenosis due to plaque encroaching the lumen constitutes the most commonly utilized criterion to identify subgroups of patients at high risk for stroke.[4] Cumulative clinical evidence reported by different trials suggests that more severe stenosis correlates with greater severity of the clinical event.[32] In addition, the risk of stroke is increased in patients bearing symptomatic stenosis,[33] and for stenosis less than 50% the annual stroke risk is 1%. Conversely, stenosis greater than 50% has an annual risk of around 3%. As reported in the NASCET trial,[32] for patients who have already suffered a cerebrovascular event, the annual stroke risk is 13% in the presence of a carotid stenosis greater than 70%. Bilateral stenosis may heighten the risk, especially if the patient has an incomplete circle of Willis. In addition, asymptomatic patients with significant stenosis progress to have ipsilateral neurologic events (amaurosis fugax, TIA, and stroke) at a rate of 20.6% over 48 months, and progress to a detectable cerebral infarction at a rate of 11% over 2.7 years. Furthermore, plaque progression in asymptomatic patients seems to be common, occurring in 60% of patients, while rapid progression from minor to high-grade stenosis and symptoms occurs in 8% of cases.

In addition, from different pathologic studies in the coronary district it is now evident that plaque rupture occurs at low degrees of narrowing and the degree of narrowing poorly predicts events. Ambrose and associates[12] retrospectively assessed angiograms of 38 subjects, 23 of whom had a subsequent myocardial infarction (MI), and found that the median stenosis on the initial angiogram of an artery that later caused an infarction was 48%. This is in contradistinction to the median narrowing of vessels that subsequently occluded but did not lead to a MI which was around 73.5%. Only 22% of the lesions related to a subsequent infarction were narrowed > 70%, whereas 61% of lesions that occluded but did not lead to an infarction

Table 7.1 *Carotid plaque characterization in patients affected by stable or unstable angina submitted to combined carotid enderarterectomy and coronary artery bypass graft surgical intervention. Modified from reference 31*

Histologic variable	Patients with stable angina ($n = 29$)	Patients with unstable angina ($n = 23$)
Thrombosed plaques	10 (34.5%)	8 (34.8%)
Vulnerable plaques	7 (24.1%)	15 (65.2%)†
Stable plaques	12 (41.4%)	0†
Total inflammation*	23.10 ± 3.57	38.56 ± 4.22†
Cap inflammation*	22.45 ± 3.52	36.82 ± 3.94†
Shoulder inflammation*	28.35 ± 5.56	38.53 ± 5.83

*CD68 positive cells and CD3 positive cells × mm^2; †$p < 0.009$.

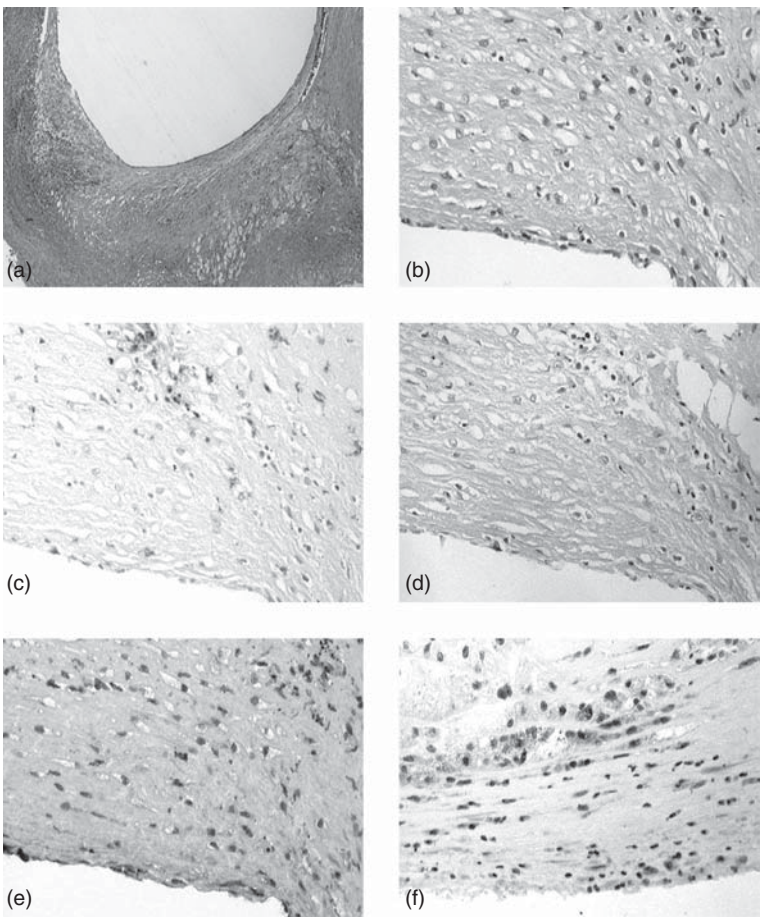

Figure 7.1 *Micrographs showing histopathologic carotid plaque segments from a patient affected by both carotid and coronary disease (stable angina) who underwent carotid endarterectomy. (a) A large, stable fibrotic plaque with small necrotic core and little area of hemorrhage (asterisk) is shown (Movat stain; original magnification ×2). (b) In the shoulder region of the plaque, numerous cellular types are present, and in particular a few macrophage foam cells (c, Kp1 staining; ×20) and T lymphocytes (d, CD3 staining; ×20). Note the weak positivity for C-reactive protein and interleukin 6 within the plaque (e, f, respectively, ×20).*

measured < 70%. Little et al[34] reviewed coronary angiograms both before and up to 1 month after an acute MI in 42 patients. The authors found that 65% of newly occluded vessels had > 50% stenosis on the initial angiogram. Plaque disruption may be more common at lower degrees of narrowing because of the higher shear stress experienced by the fibrous caps compared with more stenotic plaques. This can be explained by the greater tension created in the caps of plaques causing moderate stenosis compared with that created in caps of more severely stenotic plaques with the same cap thickness and at the same blood pressure, based on Laplace's law.[35]

Although angiographic studies of extracranial carotid atherosclerosis and stroke have not been reported, the mechanism of plaque rupture may be similar to that seen in coronary arteries. In this setting, despite additional factors such as slow flow or a hypercoagulable state that may contribute to the likelihood of symptoms for more advanced carotid disease, the initial event of stroke related

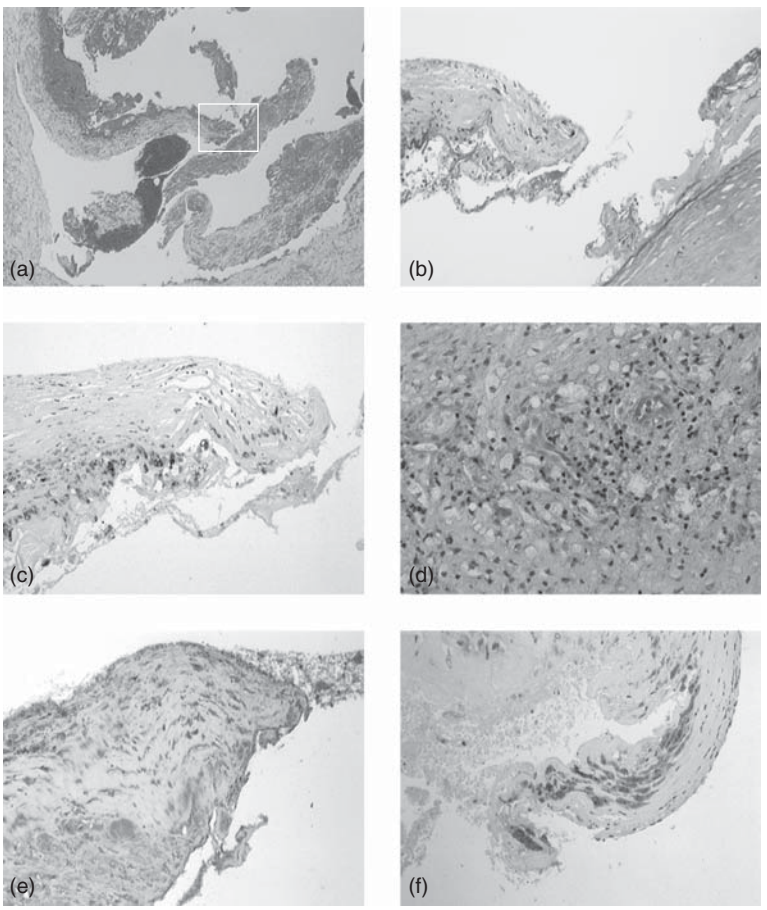

Figure 7.2 *Micrographs showing histological cross-section of a carotid plaque obtained from a patient affected by carotid disease and unstable angina. (a) The rupture site with thrombus apposition within the lumen (Movat stain; original magnification ×2). (b) High power view (box in a) in the cap region. Fibrin and platelet deposition at the rupture site are depicted (Movat stain; ×10). Cap is infiltrated by macrophage foam cells (c, Kp1, ×10), monocytes, and T lymphocytes (d, ×20). In addition a strong positivity for C-reactive protein (e, ×10) and IL-6 (f, ×10) is present, suggesting that the plaque is biologically active.*

to carotid atherosclerosis can be similar to that seen in coronary arteries.[36] In the case of high-grade carotid artery stenosis, it may be that plaque rupture results in vessel occlusion, but in low-grade carotid stenosis, plaque rupture theoretically may result in microembolism. In addition to plaque rupture, factors such as slow flow or a hypercoagulable state contribute to the likelihood of symptoms for more advanced carotid disease.[37]

The natural history of asymptomatic stenosis is different. In this setting, several prospective randomized studies have evaluated the results of surgical endarterectomy versus medical treatment for patients with asymptomatic carotid artery stenosis. A study conducted at the Mayo Clinic included 71 randomized patients and 87 non-randomized patients,[38] and demonstrated no differences in major strokes and deaths between the surgical and the medical arm. Surgically treated patients were not given aspirin, and myocardial infarction occurred in 9% of those in the medical arm versus 26% of those in the surgical arm ($p < 0.002$), suggesting

the importance of antiplatelet therapy in treating patients affected by cerebrovascular disease. A Veterans Affairs Cooperative Study included patients affected by > 50% asymptomatic stenosis[39] with a combined perioperative and angiographic risk of 4.7%. In this study a 38% risk reduction for the primary endpoint of ipsilateral TIA, transient monocular blindness, and stroke over 2 years was observed. Although the risk of stroke was reduced by 50% in the group treated by endarterectomy, the differences between groups were not statistically significant, but the study was not powered to detect such differences. The ACAS (Asymptomatic Carotid Atherosclerosis Study,[40]) the largest study in asymptomatic patients completed to date, evaluated the 5-year life expectancy in asymptomatic patients with > 60% carotid stenosis randomized between medical and surgical therapy. The event rate in surgically treated patients for the primary endpoint (ipsilateral stroke, perioperative stroke, or death) was 5.1% over 5 years. The corresponding rate in medically treated patients was 11% (with 55% risk reduction; 2%/year rate reduced to 1%/year, number needed to treat = 17; $p < 0.004$). Interestingly, in a subgroup analysis of the ACAS[40] there was no evidence of increasing efficacy with increasing degrees of stenosis. When applied to clinical practice the results of the ACAS are controversial. For instance, the lack of an identifiable higher-risk ACAS subgroup makes case selection difficult, and some authors have suggested that the rate of stroke may be higher in those patients with progressing stenosis compared with those with stable disease.[41] In addition, the ACAS included only asymptomatic individuals with carotid narrowing > 59%. The identification of asymptomatic individuals with low-grade narrowing who would benefit from surgical management would necessitate a highly specific method for stratifying risk not achievable by angiography, considering the high prevalence of low-grade disease. In support of this concept there is evidence that at 24-hour transcranial Doppler examination the embolization frequency is greater in patients affected by TIA, compared to patients bearing similar stenosis but asymptomatic.[42] Thus, these data suggest two types of carotid artery disease: one stable form, unlikely to produce symptomatic embolization or carotid occlusion, and a second unstable form, at high risk of producing symptomatic embolization or carotid occlusion, not necessarily being any more stenotic.

It is also important to recognize that a non-significant reduction in risk by surgery for low-grade (i.e. < 50%) symptomatic stenosis does not imply that these individuals are risk-free, but rather that the risk of surgery exceeds the stroke risk with medical management. These individuals had events included in these analyses despite their low-grade narrowing. Furthermore, the prevalence of low-grade carotid stenosis is very high. The Cardiovascular Health Study detected carotid stenosis in 75% of men and 62% of women over 64 years of age by ultrasound, although the prevalence of stenosis above 49% was only 7% in men and 5% in women.[1] As consequence, although the risk of stroke with < 50% carotid stenosis is low, the attributable risk for stroke resulting from < 50% carotid stenosis may be significant as a result of the high prevalence of this finding.

Therefore, it is now clear that the stenosis grade is no longer sufficient to identify patients at high risk to develop an acute cerebrovascular event, and that it is of great importance to identify other factors for correct risk stratification of ischemic complications in patients affected by atherosclerotic carotid disease. Research should thus focus on the concept of 'when to treat' rather than the concept of 'who should be treated'.

Similarities between coronary and carotid atherosclerotic disease: plaque morphology, composition, and risk of acute clinical events

Atherosclerosis is a diffuse, systemic disease process that typically begins many years before symptoms occur.[43] The atherosclerotic plaque at the carotid bifurcation is an example of the advanced fibrous plaque found at sites of predilection throughout the arterial system. Carotid atherosclerotic plaques, similarly to atherosclerotic plaques present in the coronary district, are composed of a dense cap of connective tissue embedded with smooth muscle cells, overlying a core of lipidic and necrotic debris.

The plaque contains monocyte-derived macrophages, smooth muscle cells, and T lymphocytes. Interaction between these cell types and the connective tissue appears to determine the development and progression of the plaque itself, including important complications such as thrombosis and rupture. Typically, the accumulating plaque burden is initially accommodated by an adaptive positive remodeling consisting of expansion of the vessel external elastic lamina with minimal changes in lumen size.[44] It is now generally accepted that sudden rupture, erosion, and embolization of such mildly stenotic but 'complex' lesions causes most coronary and cerebrovascular accidents. Animal and human studies demonstrate that such complex plaques also in peripheral and carotid districts are associated with positive remodeling and increased inflammatory infiltrate, which in turn may play a central role in destabilization of the atherosclerotic lesion, triggering plaque rupture and subsequent thrombosis.

Despite the many similarities demonstrated in plaque morphology between the carotid and coronary circulation, there are several unique features of carotid plaque morphology related to the high flow rates and shear forces caused by bifurcation of the common carotid artery into the internal and external carotids. One of the most important is ulcerated plaque, which is rare in the coronary artery circulation but relatively common in the carotid and other elastic arteries. While ulceration is associated with thrombotic lesions in symptomatic patients, thrombus is not always present at the ulcerative site, a phenomenon most likely related to embolic mechanisms in the carotid circulation.

Plaque hemorrhage in the carotid district is much more frequent than in the coronary arteries and may be related to high flow 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.[45] Kolodgie and co-authors have reported in coronary plaques that intraplaque hemorrhage is responsible for necrotic core enlargement and excessive foamy macrophages in fibrous caps.[46] Red blood cell membranes are the richest source of free 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. 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.[47] 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. However, the frequency of calcified nodules is slightly higher in carotid disease (approximately 6–7%) as compared to 2–5% in coronary artery disease. Finally, plaque erosion, which accounts for 30–35% of coronary thrombosis, is a distinctly rare entity in the carotid artery.

Pathologic investigations comparing endarterectomy tissue samples obtained from patients with or without neurological symptoms have clarified the instability determinants of carotid plaque, which are indeed similar to those characterizing coronary plaque.[48] The vulnerability of a plaque to rupture is mainly characterized by: decreased fibrous cap thickness, large lipidic–necrotic core, and increased inflammatory infiltrate (macrophages and T lymphocytes).[49] In addition, the likelihood of plaque rupture is due not only to the intrinsic plaque characteristics (vulnerability), but also to the tensile strength of the plaque and the stress exerted on it. Hemodynamic factors seem to influence also the cellular composition of the plaque. Indeed, Dirksen et al[50] demonstrated that different plaque areas have different cellular compositions. In particular, plaque areas distal to flow are more rich in smooth muscle cells, whereas proximal plaque areas are exposed to a greater shear stress and are therefore more rich in macrophages.

Histologic plaque composition modification seems to play a major role in the determination of cerebrovascular events due to complications, such as plaque hemorrhage, thrombosis, and embolization following destabilization of the plaque itself. Biasi and co-authors demonstrated a strong correlation between hypoechoic soft plaques with low

gray-scale median (GSM) values and the incidence of cerebrovascular events. In this setting, the rate of neurological complications following carotid stenting in patients with a GSM < 25 was statistically higher than that with GSM > 25 ($p << 0.001$).[51–53] Moreover, it has been demonstrated that particular risk factors, similar to those observed in the coronary district,[54] modifying histologic plaque composition may transform a stable plaque to an unstable one. In this setting, our group showed that histologic plaque composition strongly correlated with different cardiovascular risk factors. In particular, fibrous plaques were associated with diabetes, granulomatous plaques infiltrated by giant cells with hypertension, foam cell-rich plaques with hypercholesterolemia, and thrombotic plaques with smoking.[55] In addition, we demonstrated that patients affected by hyperfibrinogenemia (greater than 400 mg/dl) were characterized by a greater inflammatory infiltrate and thinner atherosclerotic plaque cap with consequent increased risk of thrombosis and rupture, compared to patients with lower fibrinogen levels, independent of other risk factors.[37]

More than 75% of thrombi responsible for acute coronary syndromes arise from plaque disruption.[14] Reports of the mean necrotic core size seen with plaque rupture related to sudden coronary death range from 34 to 50%.[56] These plaques are highly vascularized by first and second order vasa vasorum.[57] Carotid plaques follow a similar pattern of disruption, with fibrous cap foam cell infiltration and thinning and neovascularization also influencing the likelihood of rupture.[36,58] Morphologic studies of coronary arteries suggest that plaque progression beyond 50% cross-sectional luminal narrowing occurs secondary to repeated rupture, which may be clinically silent.[59,60] The sites of healed plaque rupture can be recognized by demonstrating a necrotic core with a discontinuous fibrous cap, which is rich in type I collagen, and an overlying intima formed by smooth muscle cells in a matrix rich in proteoglycans and type III collagen.[59] Few angiographic studies have demonstrated plaque progression, and short-term studies have suggested that thrombosis is the likely cause. Mann and Davies showed that the frequency of healed plaque rupture increases along with lumen narrowing.[60] Burke et al found healed plaque rupture in 61% of hearts from sudden coronary death victims.[59] Multiple healed plaque ruptures with layering are also common in carotid segments, and the percentage cross-sectional luminal narrowing is dependent on the number of healed repair sites. Therefore, it seems that the progression of atherosclerotic disease to severe stenosis is the result of repeated ruptures.

At least 40–50% of coronary rupture sites show $< 50\%$ diameter stenosis,[35,61] and the same may be true in carotid disease. In this setting, our group recently reported a higher incidence of thrombosis in patients with recent stroke as compared with asymptomatic individuals.[62] In particular, a total of 269 carotid plaques from patients submitted to carotid endarterectomy (96 from patients with ipsilateral major stroke, 91 from patients with transient ischemic attack, and 82 plaques from patients without symptoms) were evaluated. A thrombotically active carotid plaque associated with high inflammatory infiltrate was observed in 71 (74.0%) of 96 patients with ipsilateral major stroke (and in all 32 plaques from patients operated on within 2 months of symptom onset) compared with 32 (35.2%) of 91 patients with TIA ($p < 0.001$) or 12 (14.6%) of 82 patients who were without symptoms ($p < 0.001$). In addition, a fresh thrombus was observed in 53.8% of patients with stroke operated on 13–24 months after the cerebrovascular event. An acute thrombus was associated with cap rupture in 64 (90.1%) of 71 thrombosed plaques from patients with stroke and with cap erosion in the remaining seven cases (9.9%). Ruptured plaques of patients affected by stroke were characterized by the presence of a more severe inflammatory infiltrate, constituted by monocytes, macrophages, and T lymphocyte cells, compared with that observed in the TIA and asymptomatic groups ($p = 0.001$; Table 7.2).

Recently, Saam and co-authors[63] evaluated 23 patients with unilateral symptomatic carotid disease after examining both carotid arteries with 1.5-T time-of-flight magnetic resonance (MR) angiography and 1.5-T T1, intermediate, and T2 weighted MR imaging. Quantitative and morphologic plaque information were recorded. Compared with asymptomatic plaques, symptomatic plaques had a

Table 7.2 *Thrombotically active plaques, cap rupture, and cap erosion by patient clinical status. Modified from reference 62*

	Number of plaques (%)			*p* Value		
	Patients with stroke ($n = 96$)	Patients with TIA ($n = 82$)	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	< 0.001
Cap rupture	64 (66.7%)	21 (23.1%)	11 (13.4%)	< 0.001	< 0.001	< 0.001
Cap erosion	7 (7.3%)	11 (12.1%)	1 (1.2%)	0.51	0.09	0.03

higher incidence of fibrous cap rupture ($p = 0.007$), juxtaluminal hemorrhage or thrombus ($p = 0.039$), type I hemorrhage ($p = 0.021$), and complicated American Heart Association (AHA) type VI lesions ($p = 0.004$), and a lower incidence of uncomplicated AHA type IV and V lesions ($p = 0.005$). Symptomatic plaques also had larger hemorrhage ($p = 0.003$) and loose matrix ($p = 0.014$) areas and a smaller lumen area ($p = 0.008$). No significant differences between symptomatic and asymptomatic plaques were found for quantitative measurements of the lipid-rich necrotic core, calcification, and the vessel wall or for the occurrence of intraplaque hemorrhage or type II hemorrhage. Moreover, in an elegant study, Takaya and associates prospectively studied the association between plaque composition and cerebrovascular events in 154 asymptomatic patients with ultrasonographically proven 50–79% carotid artery stenosis over a time period of at least 12 months. They found that the presence of a thin or ruptured fibrous cap, intraplaque hemorrhage, larger mean intraplaque hemorrhage area, larger maximum percentage of lipid-rich/necrotic core, and larger maximum wall thickness accounted for an increased risk of subsequent ipsilateral cerebrovascular events. Thereby, they prospectively confirmed the well-established findings from retrospective trials that thin or ruptured fibrous caps and intraplaque hemorrhage are linked with cerebrovascular events.[47] It is noteworthy, however, that the presence of a lipid-rich/necrotic core without intraplaque hemorrhage was not significantly associated with subsequent ischemic events.

Finally, the relationship between plaque size and stroke is poorly understood in the carotid artery, although it is possible to suspect that there are features of low-grade disease that predispose to events. For example, Weinstein[15] found that hemorrhage and ulceration were strongly associated with symptoms, despite many having 50% or less internal carotid artery stenosis.

Emerging role of systemic biomarkers for evaluation of plaque vulnerability in coronary and carotid atherosclerotic disease

Inflammation plays a key role in the initiation, progression, and rupture of the atherosclerotic plaque.[64–67] Several systemic proteins have been proposed as markers of a chronic inflammatory state in the arterial wall, including high-sensitivity C-reactive protein (hsCRP), serum amyloid A protein, and soluble adhesion molecules,[68–71] and many of them have also been found in carotid atherosclerotic disease.[48] The Physicians' Health Study evaluated more than 14 000 healthy subjects demonstrating that ICAM 1 (intercellular adhesion molecule 1) correlated with cardiovascular risk, and that subjects in the highest quartile had a relative risk of developing an acute event 1.8 times greater than subjects in the lowest quartile.[68,72] Furthermore, soluble ICAM-1 and VCAM-1 (vascular adhesion molecule 1) correlate with the extent of peripheral vascular disease.[68] The proinflammatory cytokine

IL-18 (interleukin 18) is mainly produced by macrophage and monocyte cells, and stimulates interferon γ production, which in turn inhibits collagen production. Mallat and associates[73] demonstrated an elevated expression of IL-18 in unstable carotid plaques compared to stable plaques, and that the presence of this marker correlated with clinically symptomatic carotid plaques.

In addition, as largely demonstrated in the coronary district, also in the peripheral and carotid districts the presence of elevated inflammatory indices is considered an independent predictor for cerebrovascular disease. In the Physicians' Health Study the basal levels of C-reactive protein (CRP) were retrospectively evaluated in healthy subjects. The risk of developing a stroke was twice greater in subjects with CRP in the highest quartile compared to subjects in the lowest quartile. Such type of risk was not modified by smoking status, and it was independent of other risk factors.[65] Recently, Erren and colleagues[74] evaluated the plasma levels of different inflammatory markers in patients affected by both coronary and peripheral atherosclerosis. In 147 patients submitted to coronary angiography the levels of CRP, fibrinogen, serum amyloid A, and interleukin 6 were evaluated. All inflammatory indices were increased in patients with diffuse atherosclerosis compared to patients with only coronary disease or healthy controls, suggesting a higher degree of inflammatory activation in patients with multidistrict atherosclerotic disease.

Recently, pregnancy-associated protein A (PAPP-A) – a high molecular weight, zinc-binding metalloproteinase that is abundantly expressed in advanced atherosclerotic lesions – has been demonstrated in patients bearing coronary atherosclerosis.[75] In particular, this protease cleaves the bond between insulin-like growth factor 1 (IGF-1) and its natural inhibitor IGFBP-4 (IGF binding protein 4), increasing the levels of free IGF-1.[76] It is well known that IGF-1 stimulation of the chemotactic activity of monocytes–macrophages within the plaque, their activation with consequent release of circulating proinflammatory cytokines, and the endothelial cell migration and proliferation with neoangiogenesis, may be considered one of the most important mediators of transformation from a stable to an unstable plaque. In addition, macrophage stimulation by IGF-1 induces tumor necrosis factor (TNF) synthesis.[77] This cytokine amplifies the plaque destabilization mechanisms both directly and indirectly by other interleukins (IL-1, IL-6, and interferon γ (IFNγ) production. The latter induce apoptosis of the smooth muscle cells (principal producers of collagen, elastin, and metalloproteinase inhibitor within the plaque) and stimulate macrophages to produce metalloproteinases, with consequent digestion of the collagen and extracellular matrix of the fibrous cap.[66] Recently, Bayes-Genis et al found that PAPP-A was highly expressed in both ruptured and eroded coronary unstable plaques of eight patients who died suddenly from cardiac causes, but PAPP-A was absent or minimally expressed in stable plaques.[78] Beaudeux and associates,[79] found that elevated serum PAPP-A levels are associated not only with the echogenicity of atherosclerotic carotid lesions but also with an enhanced inflammatory state in asymptomatic hyperlipidemic subjects. In this context, our group has recently demonstrated that also in the carotid distribution there is an increased expression of PAPP-A related either to the presence of complex vulnerable plaques or ruptured plaques with thrombus.[80] Conversely, stable plaques or plaques with organized thrombus did not show PAPP-A expression. In addition, at confocal microscopic examination, PAPP-A expression was increased in macrophages compared to smooth muscle cells and T lymphocytes. PAPP-A serologic values were 4.02 ± 0.18 mIU/l in stable plaques, 7.43 ± 0.97 mIU/l in vulnerable plaques and 6.97 ± 0.75 mIU/l in ruptured plaques with thrombus (group 1 vs 3, $p = 0.01$; 1 vs 2, $p = 0.004$; 2 vs 3, $p = 0.71$, respectively), suggesting that this marker may represent a useful and simple method to detect high-risk subgroups of patients bearing vulnerable atherosclerotic carotid plaques before the onset of cerebrovascular events. In this context, a multicenter Italian registry study (SUBMARINE; Serum and Urinary Plaque Vulnerability Biomarkers Detection before and after Carotid Stent Implantation) currently in progress will assess whether patients presenting with recurrent transient ischemic attacks or minor stroke and submitted to early carotid stenting (2 days after TIA and 14 days after minor stroke) may have

mechanical plaque stabilization with concomitant reduction in the expression of different biomarkers of vulnerability.

Conclusions

Atherosclerotic lesions in the coronary and carotid arteries are morphologically similar, and the mean percentage luminal narrowing is similar when individual types of plaques are considered. In addition, patients affected by carotid atherosclerosis, are most probably also affected by coronary atherosclerosis and the presence of disease in one district may also influence the other in terms of plaque progression and destabilization. It is now clear that, similar to acute coronary syndromes, many cerebrovascular accidents are triggered by the rupture of a vulnerable carotid plaque, which in turn may not significantly encroach the lumen. Thus, to better stratify the risk of cerebrovascular events due to carotid atherosclerotic disease – similar to the coronary district – it is of utmost importance to define subgroups of patients characterized on the basis of different plaque features. Indeed, most probably an integration of different serologic profiles and either non-invasive or invasive morphologic and functional imaging modalities will be the method with a significant predictive role for correlation between complex plaque patterns and clinical outcomes. The opportunity created by the convergence of developments in these multiple fields will be impressive, and also coupled with a gain in patient quality of life and cost-saving generated when the downstream effects of acute coronary and cerebrovascular accidents are reduced.

References

1. O'Leary DH, Polak JF, Kronmal RA et al. Distribution and correlates of sonographically detected carotid artery disease in the Cardiovascular Health Study. Stroke 1992; 23: 1752–60.
2. North American Symptomatic Carotid Endarterectomy Trial Collaborators. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid artery stenosis. N Engl J Med 1991; 325: 445–53.
3. European Carotid Surgery Trialists' Collaborative Group. MRC European Carotid Surgery Trial: interim results for symptomatic patients with severe (70–99%) or with mild (0–29%) carotid stenosis. Lancet 1991; 337: 1235–43.
4. Biller J, Feinberg WM, Castaldo JE et al. Guidelines for carotid endarterectomy: a statement for helathcare professionals from a Special Writing Group of the Stroke Council, American Heart Association. Circulation 1998; 97: 501–9.
5. Aly S, Bishop CC. An objective characterization of atherosclerotic lesion. An alternative method to identify unstable plaque. Stroke 2000; 31: 1921–4.
6. Lal BK, Hobson RW, Pappas PJ et al. Pixel distribution analysis of B-mode ultrasound scan images predicts histologic features of atherosclerotic carotid plaque. J Vasc Surg 2002; 35: 1210–17.
7. MacNeill BD, Lowe HC, Takano M et al. Intravascular modalities for detection of vulnerable plaque: current status. Arterioscler Thromb Vasc Biol 2003; 23: 1333–42.
8. Kahn J. Beyond IVUS: new imaging methods zero in on vulnerable plaque – Part I. J Invasive Cardiol 2005; 18: 375–7.
9. Wassermann BA, Wityk RJ, Trout HH, Virmani R. Low-grade carotid stenosis: looking beyond the lumen with MRI. Stroke 2005; 36: 2504–13.
10. Inzitari D, Eliasziw M, Gates P 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: 1693–700.
11. Glagov S, Weisenberg E, Zarins CK et al. Compensatory enlargement of human artherosclerotic coronary arteries. N Engl J Med 1987; 316: 1371–5.
12. Ambrose JA, Tannenbaum MA, Alexopoulus D et al. Angiographic progression of coronary artery disease and the development of myocardial infarction. J Am Coll Cardiol 1988; 12: 56–62.
13. Fuster V, Stein B, Ambrose JA et al. Atherosclerotic plaque rupture and thrombosis. Evolving concepts. Circulation 1990; 82: 47–59.
14. Falk E. Coronary thrombosis: pathogenesis and clinical manifestations. Am J Cardiol 1991; 68: 28–35.
15. Weinstein R. Noninvasive carotid duplex ultrasound imaging for the evaluation and management of carotid atherosclerotic disease. Hematol Oncol Clin North Am 1992; 6: 1131–9.
16. Hertzer NR, Young GR, Bevent G et al. Coronary angiography in 506 patients with extracranial cerebrovascular disease. Arch Intern Med 1985; 145: 849–52.
17. Di Pasquale G, Andreoli A, Pinelli G et al. Cerebral ischemia and asymptomatic coronary artery disease: a prospective study of 83 patients. Stroke 1986; 17: 1098–101.
18. Craven T, Ryu J, Espeland M et al. Evaluation of the associations between carotid artery atherosclerosis and coronary artery stenosis. Circulation 1990; 82: 1230–42.
19. Youg W, Gofman JW, Tandy R. The quantification of atherosclerosis III. The extent of correlation of degrees of atherosclerosis within and between the coronary and cerebral vascular beds. Am J Cardiol 1960; 6: 300–8.
20. Mitchell JRA, Schwartz CJ. Relationship between arterial disease in different sites. A study of the aorta and coronary, carotid and iliac arteries. Br Med J 1962; 1: 1293–301.

21. Mathur KS, Kashyap SK, Kumar V. Correlation and the extent and severity of atherosclerosis in the coronary and cerebral arteries. Circulation 1963; 27: 929–34.
22. Howard G, Ryu JE, Evans GW. Extracranial carotid atherosclerosis in patients with and without transient ischemic attacks and coronary artery disease. Arteriosclerosis 1990; 10: 714–19.
23. Tanaka H, Nishino M, Ishida M. Progression of carotid atherosclerosis in Japanese patients with coronary artery disease. Stroke 1992; 23: 946–51.
24. Crouse J. Carotid and coronary atherosclerosis: what are the connections? Postgrad Med 1991; 90: 175–9.
25. Chimowitz M, Mancini G. Asymptomatic coronary artery disease in patients with stroke. Stroke 1991; 26: 23–7.
26. Kannel WB, Wolf PA, Verter J. Manifestations of coronary disease predisposing to stroke. The Framingham Study. JAMA 1983; 250: 2942–6.
27. Wolf PA, Kannel WB, Sorlie P, McNamara P. Asymptomatic carotid bruit and the risk of stroke. JAMA 1981; 245: 1442–5.
28. Eagle K, Riahal C, Foster E et al. Long-term survival in patients with coronary artery disease: importance of peripheral vascular disease. The Coronary Artery Surgery Study (CASS) Investigators. J Am Coll Cardiol 1994; 23: 1091–5.
29. Jones E, Craver J, Michalic R et al. Combined carotid and coronary operations: when are they necessary? J Thorac Cardiovasc Surg 1984; 87: 7–16.
30. Lombardo A, Biasucci LM, Lanza GA et al. Inflammation as a possible link between coronary and carotid plaque instability. Circulation 2004; 109: 3158–63.
31. Sangiorgi G, Mauriello A, Trimarchi S et al. Does carotid plaque inflammatory infiltrate differ between patients affected by stable or unstable angina? Eur Heart J 2000; 19(Suppl): (abstr p3569).
32. NASCET. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade stenosis. N Engl J Med 1991; 325: 445–53.
33. Bennett HJM, Taylor W, Eliasziw M et al. Benefit of carotid endarterectomy in patients with symptomatic moderate or severe stenosis. N Engl J Med 1998; 339: 1415–25.
34. Little WC, Constantinescu M, Applegate RJ et al. Can coronary angiography predict the site of a subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease? Circulation 1988; 78: 1157–66.
35. Falk E, Shah PK, Fuster V. Coronary plaque disruption. Circulation 1995; 92: 657–71.
36. Carr S, Farb A, Pearce WH et al. Atherosclerotic plaque rupture in symptomatic carotid artery stenosis. J Vasc Surg 1996; 23: 755–65; discussion 765–6.
37. Mauriello A, Sangiorgi G, Palmieri G et al. Hyperfibrinogenemia is associated with specific histological composition and complications of atherosclerotic carotid plaques in patients affected by transient ischemic attacks. Circulation 2000; 101: 744–50.
38. Mayo Asymptomatic Carotid Endarterectomy Study Group. Results of a randomized controlled trial of carotid endarterectomy for asymptomatic carotid stenosis. Mayo Clin Proc 1992; 67: 513–18.
39. Hobson RW 2nd, Weiss DG, Fields WS et al. Efficacy of carotid endarterectomy for asymptomatic carotid stenosis. The Veterans Affairs Cooperative Study Group. N Engl J Med 1993; 328: 221–7.
40. Executive Committee for the Asymptomatic Carotid Atherosclerosis Study. Endarterectomy for asymptomatic carotid artery stenosis. JAMA 1995; 273: 1421–8.
41. Holloway RG, Benesch CG, Rahilly CR, Courtright CE. A systematic review of cost-effectiveness research for stroke evaluation and treatment. Stroke 1999; 30: 1340–9.
42. Marcus HS, Thomson ND, Brown MM. Asymptomatic cerebral embolic signals in symptomatic and asymptomatic carotid artery disease. Brain 1995; 118: 1005–11.
43. Strong JP, Malcom GT, McMahan CA et al. Prevalence and extent of atherosclerosis in adolescent and young adults: implications for prevention from pathobiological determinants of atherosclerosis in youth study. JAMA 1999; 281: 727–35.
44. Schwartz RS, Topol EJ, Serruys PW et al. Artery size, neointima, and remodeling: time for some standards. J Am Coll Cardiol 1998; 32: 2087–94.
45. Burke AP, Farb A, Malcom GT et al. Plaque rupture and sudden death related to exertion in men with coronary artery disease. JAMA 1999; 281: 921–6.
46. Kolodgie FD, Gold HK, Burke AP et al. Intraplaque hemorrhage and progression of coronary atheroma. N Engl J Med 2003; 349: 2316–25.
47. Takaya N, Yuan C, Chu B et al. Presence of intraplaque hemorrhage stimulates progression of carotid atherosclerotic plaques: a high-resolution magnetic resonance imaging study. Circulation 2005; 111: 2768–75.
48. Carbone LG, Mauriello A, Christiansen M et al. Unstable carotid plaque biochemical and cellular marker of vulnerability. Ital Heart J 2003; 5(Suppl): 398–406.
49. Golledge J, Greenhalgh RM, Davies AH. The symptomatic carotid plaque. Stroke 2000; 31: 774–81.
50. Dirksen MT, van der Wal AC, van der Berg FM et al. Distribution of inflammatory cells in atherosclerotic plaques relates to the dirction of flow. Circulation 1998; 98: 200–3.
51. Biasi GM, Sampaolo A, Mingazzini P et al. Computer analysis of ultrasonic plaque echolucency in identifying high risk carotid bifurcation lesions. Eur J Vasc Surg 1999; 17: 476–9.
52. Geroulakos G, Hobson RW, Nicolaides A. Ultrasonographic carotid plaque morphology in predicting stroke risk. Br J Surg 1996; 83: 582–7.
53. Geroulakos G, Ramaswami G, Nicolaides A et al. Characterization of symptomatic and asymptomatic carotid plaques using high-resolution real-time ultrasonography. Br J Surg 1993; 80: 1274–7.
54. Virmani R, Burke AP, Farb AH, Kolodgie FD. Pathology of the unstable plaque. Prog Cardiovasc Dis 2002; 44: 349–56.
55. Spagnoli LG, Mauriello A, Palmieri G et al. Relationship between risk factors and morphological patterns of human carotid atherosclerotic plaques: a multivariate discriminant analysis. Atherosclerosis 1994; 108: 39–60.
56. Davies MJ. Anatomic features in victims of sudden coronary death. Coronary artery pathology. Circulation 1992; 85: I19–24.

57. Barger AC, Beeuwkes R. Rupture of vasa vasorum as a trigger of acute myocardial infarction. Am J Cardiol 1990; 66: 41G–3G.
58. McCarthy MJ, Loftus IM, Thompson MM et al. Angiogenesis and the atherosclerotic carotid plaque: an association between symptomatology and plaque morphology. J Vasc Surg 1999; 30: 261–8.
59. Burke AP, Kolodgie FD, Farb A et al. Healed plaque ruptures and sudden coronary death: evidence that subclinical rupture has a role in plaque progression. Circulation 2001; 103: 934–40.
60. Mann J, Davies MJ. Mechanisms of progression in native coronary artery disease: role of healed plaque disruption. Heart 1999; 82: 265–8.
61. Farb A, Burke AP, Tang AL et al. Coronary plaque erosion without rupture into a lipid core. A frequent cause of coronary thrombosis in sudden coronary death. Circulation 1996; 93: 1354–63.
62. Spagnoli LG, Mauriello A, Sangiorgi G et al. Extracranial thrombotically active carotid plaque as a risk factor for ischemic stroke. JAMA 2004; 292: 1845–52.
63. Saam T, Cai J, Ma L et al. Comparison of symptomatic and asymptomatic atherosclerotic carotid plaque features with in vivo MR imaging. Radiology 2006; 240: 464–72.
64. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999; 340: 115–26.
65. Ridker PM, Cushman M, Stampfer MJ et al. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997; 336: 973–9.
66. Libby P, Ganz P, Schoen FJ et al. Vascular biology of acute coronary syndromes. In Topol E (ed). Textbook of Interventional Cardiology. New York: Marcell Dekker, 2001: 33–57.
67. Libby P, Aikawa M. New insight into plaque stabilisation by lipid lowering. Drugs 1998; 56: 9–13; discussion 33.
68. Peter K, Nawroth P, Conradt C et al. Circulating vascular cell adhesion molecule-1 correlates with the extent of human atherosclerosis in contrast to circulating intercellular adhesion molecule-1, E-selectin, P-selectin and thrombomodulin. Arterioscler Thromb Vasc Biol 1997; 17: 505–12.
69. Blake GJ, Ridker PM. Inflammatory mechanisms in atherosclerosis: from laboratory evidence to clinical application. Ital Heart J 2001; 2: 796–800.
70. Davies MJ, Gordon JL, Gearing AJ et al. The expression of adhesion molecules ICAM-1, VCAM-1, PECAM, E-selectin in human atherosclerosis. J Pathol 1993; 171: 223–9.
71. De Michele M, Ascione L, Guarini P et al. Determinanti dell'instabilitá della placca carotidea:dall'istologia agli ultrasuoni. Ital Heart J 2001; 2: 606–13.
72. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction in apparently healthy men. Circulation 2000; 101: 1767–72.
73. Mallat Z, Corbaz A, Scoazec A et al. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. Circulation 2001; 104: 1598–603.
74. Erren M, Reinecke H, Junker R et al. Systemic inflammatory parameters in patients with atherosclerosis of the coronary and peripheral arteries. Arterioscler Thromb Vasc Biol 1999; 19: 2355–63.
75. Bayes-Genis A, Conover CA, Schwartz RS. The insulin-like growth factor axis. A rewiew of atherosclerosis and restenosis. Circ Res 2000; 86: 125–30.
76. Laursen LS, Overgaard MT, Soe R et al. Pregnancy-associated plasma protein-A (PAPP-A) cleaves insulin-like growth factor binding protein (IGFBP)-5 independent of IGF: implications for the mechanism of IGFBP-4 proteolysis by PAPP-A. FEBS Lett 2001; 504: 36–40.
77. Ranier G, Clement I, Desfaits AC, Lambert A. Direct stimulatory effect of insulin-like growth factor-I on monocyte and macrophage tumor necrosis factor-alpha production. Endocrinology 1996; 137: 4611–18.
78. Bayes-Genis A, Conover CA, Overgaard MT et al. Pregnancy-associated plasma protein A as a marker of acute coronary syndromes. N Engl J Med 2001; 345: 1022–9.
79. Beaudeux JL, Burc L, Imbert-Bismut F et al. Serum plasma pregnancy-associated protein A: a potential marker of echogenic carotid atherosclerotic plaques in asymptomatic hyperlipidemic subjects at high cardiovascular risk. Arterioscler Thromb Vasc Biol 2003; 23: e7–10.
80. Sangiorgi G, Mauriello A, Bonanno E et al. Pregnancy-associated plasma protein-a is markedly expressed by monocyte-macrophage cells in vulnerable and ruptured carotid atherosclerotic plaques: a link between inflammation and cerebrovascular events. J Am Coll Cardiol 2006; 47: 2201–11.