

Vulnerability of atherosclerotic carotid disease: from laboratory to operating room – Part 2

Vulnerabilidade da doença aterosclerótica de carótidas: do laboratório à sala de cirurgia – Parte 2

Luciano Cabral ALBUQUERQUE¹, Luciane Barreneche NARVAES², João Rubião HOEFEL FILHO³, Maurício ANES⁴, Aluísio Antunes MACIEL⁵, Henrique STAUB⁶, Maurício FRIEDRICH⁷, Luis Eduardo ROHDE⁸

RBCCV 44205-826

Descriptors: Atherosclerosis. Carotid artery diseases. Vulnerability. Inflammation. Inflammatory markers.

Descritores: Aterosclerose. Doenças das artérias carótidas. Vulnerabilidade. Inflamação. Mediadores da inflamação.

1 - Member of the Brazilian Society of Cardiovascular Surgery. Master in Cardiology by the Federal University of RS. Cardiovascular Surgeon of Hospital São Lucas da PUCRS.

2 - Student of post graduation course in Cardiology at the Federal University of RS Cardiovascular Surgeon of Hospital São Lucas da PUCRS.

3 - Head of Imaging Diagnostic Center of Hospital São Lucas da PUCRS. Assistant Professor of Pathology Department of Medical School of PUCRS.

4 - Doctor of Magnetic Resonance and Computed Tomography Laboratory of Hospital São Lucas da PUCRS.

5 - Pathologist of Hospital São Lucas da PUCRS.

6 - PhD in rheumatology of Catholic Pontifical University of Rio Grande do Sul. Assistant Professor of Internal Medicine Department of Medical School of PUCRS. Rheumatologist of Hospital São Lucas da PUCRS.

7 - PhD in neurosciences by Catholic Pontifical University of Rio Grande do Sul. Head of Neurovascular Diseases Program of the Hospital São Lucas da PUCRS.

8 - PhD in Cardiology by the Federal University of RS. Adjunct Professor of Medicinal Internal Department of Medical School of PUCRS. Professor of post graduation course in Cardiology and Cardiovascular Sciences of Federal University of RS. Cardiologist and Echocardiographer of the Cardiology Service of the Hospital de Clínicas de Porto Alegre.

Work carried out in the Hospital São Lucas of Catholic Pontifical University of Rio Grande do Sul and in the post graduation course in Cardiology and Cardiovascular Sciences of the Federal University of Rio Grande do Sul, Porto Alegre, RS.

Correspondence address:

Luciano Cabral Albuquerque. Centro Clínico do Hospital da PUCRS. Av. Ipiranga 6690, conj. 615. Porto Alegre, RS. ECP 90610-000. Phone/Fax: (0XX51) 3336-8190. E-mail: alb.23@terra.com.br

INTRODUCTION

Atherosclerosis is a chronic predominantly silent process in which local and systemic factors interact to reduce or delay clinical events. In obstructive carotid artery disease, the catastrophic nature of strokes, currently a priority in public health worldwide, justifies the increasing concern with morphologic, inflammatory and biochemical characteristics of atheromatous plaque, which eventually may become vulnerability to injury.

Currently, it is well known that systemic aggression causes compensatory responses that alter endothelial homeostasis, in particular by the activation of leucocytes and platelets and changes in permeability. Endothelial cells secrete adhesion molecules, cytokines and growth factors among other substances which, if the aggression process is not blocked, continue to be produced indefinitely. Similarly, the inflammatory process induces the migration and proliferation of smooth muscle cells to the subintimal portion by means of chemical mediators released by macrophages modified by capture of lipids (foam cells), and by specific subtypes of T lymphocytes. In this phase, intraparietal growth in the area of inflammation occurs in the opposite direction to the lumen of the vessel, in the direction of the adventitial layer, continuously stimulating the release of proteolytic enzymes in the interstitial matrix, of cytokines and tumoral growth factor, which may eventually induce local necrosis [1,2]. Additionally, the macrophage accumulating process is related to an increase in the plasma concentration of interleukins, C-reactive protein and other inflammatory markers, currently proposed as markers of presence or clinical instability of atherosclerosis, both locally and systemically [3].

Also the possible role of infectious agents, such as chlamydia, has been investigated in the genesis of atheroma plaques or in triggering events but data is still inconclusive [4-6].

Another sign of the immuno-inflammatory activity in atherosclerotic disease relates to the production of autoantibodies that damage endothelial cells and/or smooth muscle cells of the arterial wall. Experimental and clinical studies have proposed that the increase in serum levels of antibodies against phospholipids of the cellular membrane or against thermal shock proteins are associated to acute events of atherosclerosis, although any causal relationship has not been elucidated yet [7-10].

Moreover, the refinement of noninvasive imaging methods, such as ultrasonography and angiography by nuclear magnetic resonance, has provided greater knowledge on the morphologic structure of carotid plaque and its possible association with clinical outcomes. The capacity of these methods to identify signs of instability

from an analysis of the plaque content and surface has recently been tested in several histological studies [11].

Part I of this review analysed current studies related to the vulnerability of carotid arteries in atherosclerotic disease, expressed in their epidemiological, clinical and inflammatory aspects. In Part II, currently used serum markers will be discussed as will histological aspects and diagnostic imaging in relation to the instability of carotid artery disease, including possible therapeutic implications and eventual changes of paradigms related to current indications of intervention.

Serological markers

Over the last years, particular interest has been shown in identifying circulating inflammatory mediators associated to atherosclerotic processes and in prediction of events. Possible therapeutic implications, such as the role of statins, acetylsalicylic acid and cyclooxygenase inhibitors, have been similarly studied [12,13].

In obstructive carotid artery disease, recent works have attempted to elucidate the role of several substances involved in its formation, growth and possibly in plaque stabilization. Although Waddington et al. [14] studying the oxidation of fatty acids in carotid artery disease, did not manage to demonstrate a direct correlation with clinical instability, Nishi et al. [15] observed that high levels of oxidized anti-LDL antibodies are associated with greater macrophage infiltration in a histological analysis of 44 specimens from carotid endarterectomies suggesting that this marker may confirm plaque vulnerability. However, this seems to be the protector role of HDL cholesterol in the progression of carotid artery disease. Johnsen et al. [16] accompanied 1952 patients with carotid artery disease diagnosed by ultrasonography for a period of seven years and demonstrated a lower growth rate of the lesions in the group with HDL levels in the upper quartile. This finding agrees with research related to the recurrence of coronary artery [17] and cerebral outcomes [18].

Another marker of interest in the immunological response that accompanies the atherosclerotic process is the CD40 and CD40 ligand interaction. Initially exclusively related to lymphocytic activity, these markers already have their expression documented in other cells present in atheroma plaque formation. There is evidence that the CD40 and CD40L interaction contributes to the instability of plaque through interactions with endothelial cells, smooth muscle cells, macrophages and platelets, causing the release of cytokines, alpha tumor necrosis factor, extracellular matrix metalloproteinases and procoagulant substances [19]. A sub-analysis of the Women's Health Study demonstrated significantly higher levels of CD40L in 130 women who developed acute myocardial infarction or strokes over a four-year period in comparison to smokers, matched in relation

to age, but with normal CD40L levels (p-value = 0.02) [20]. In another study, an analysis of 2908 cases of acute coronary syndromes demonstrated a higher risk of event recurrence over the 16-week period when the CD40L levels were over the 90th percentile [21]. The hypothesis that serum CD40L levels are related to morphological vulnerability characteristics was reported by Blake et al. [22] in 49 patients with carotid stenosis. Although the CD40L levels were not correlated to the percentage of stenosis, above average CD40L levels were associated to the presence of intraplaque acellular lipid-rich deposits.

Studies of histological associations of possible actions of extracellular matrix metalloproteinases on the atheromatous disease of the carotid artery have also been proposed. Sapienza et al. [23], analyzing 53 consecutive carotid endarterectomies, demonstrated that in patients with lesions classified as microscopically unstable, serum levels of several metalloproteinases were significantly higher than with stable lesions. However, using transcranial Doppler, Loftus et al. [24] observed that high levels of matrix metalloproteinase-9 were related to a higher occurrence of intraoperative microembolism during strokes, while Molloy et al. [25] demonstrated a correlation between high levels of matrix metalloproteinase-8, recent symptoms, silent embolization and histologic evidence of plaque rupture. Recently, Sluijter et al. [26] proposed that different MMP activity may vary according to the cellular predominance of the atheroma plaque. An analysis of 150 specimens from carotid endarterectomies revealed significantly greater activity of the matrix metalloproteinase 8 and 9 in macrophage-rich lesions and of matrix metalloproteinase-2 in plaques with predominance of smooth muscle cells. The correlation between the cellular elements, symptoms and matrix metalloproteinase-8 and 9 was verified by Verhoeven et al. [27], in 404 patients who underwent carotid artery surgery. In cases of transient ischemic attack (TIA) or recent strokes, the metalloproteinase levels were significantly higher than levels observed in asymptomatic patients (p-value < 0.001). However, although there seems to be a direct relationship between these markers and vulnerability of the plaque, there is no evidence for its use as a predictor for risk in the clinical practice.

Another group of substances with confirmed involvement in atheromatous disease, both in tissue and serum, are the interleukins [28]. Although the majority of the interleukins have a proinflammatory role and the most studied are interleukins-1, -2, -6, -7, -8 and -18, some subgroups seem to have anti-inflammatory qualities, determining a modulation of the resulting effect and producing oscillations during the course of the disease. The highest activity of interleukin-18 undoubtedly was observed by Mallat et al. [29] in symptomatic or ulcerated

carotid plaques in comparison to cases considered stable. Also its stimulating effect was demonstrated by the production of alpha tumoral necrosis factor, interferon and adhesion molecules, or cellular apoptosis induction from its interaction with interleukin-12 [30]. Recently, the relationship between elevated levels of circulating interleukin-18 and thinning of the fibrous cover of carotid plaques was demonstrated by Yamagami et al. [31] in 366 patients who had not suffered previous strokes, findings similar to those described by Elkind et al. [32] in relation to interleukin-2. Although with less consistency, the possible anti-inflammatory effect of some types of interleukins has also been investigated. In preliminary histochemical analysis of 21 specimens of carotid endarterectomies, an inverse relationship was demonstrated between the expression of interleukin-10 with the production of nitric oxide synthetase and the cellular apoptosis rate, suggesting a protector effect against excessive intraplaque necrosis and presumably, conferring stability to the injuries [33].

Among all the inflammatory substances associated to atherogenesis, C-reactive protein (CRP) has been affirmed as the most important biomarker of cardiovascular events, specifically after the discovery of an ultrasensitive method to measure it (US-CRP) [34,35]. CRP is a polypeptide secreted by the liver, mainly in response to elevations in serum interleukin-6 levels, which present a proven action in the differentiation of macrophages from foam cells [36], the production of adhesion molecules and selectins [37], local recruitment of monocytes [38], activation of complement [39], T-lymphocyte mediated cellular destruction [40], increase in the angiotensin II effects [40] and in the attenuation of nitric oxide production and action [41].

The association between elevated levels of US-CRP and cardiovascular prognosis was first demonstrated in acute coronary artery syndromes (ACS), from evidence that US-CRP levels = 0.3g/dL were related to more ischemic events and worst evolution, both over short and long terms [42,43]. In patients after acute myocardial infarction, the CARE study [44] revealed increased risk of recurrent events or death when high levels of US-CRP are present, a result in agreement with other authors [45,46].

When associated to the Framingham score and to LDL cholesterol levels, US-CRP may provide an additional predictive value in the evolution of coronary disease [47,48]. Moreover, US-CRP has been suggested in several studies as a predictor of sudden death, cerebral infarction and development of obstructive arterial disease of the lower limbs, even though the effect may be less accentuated [49-51].

The role of US-CRP in the prediction of strokes was

studied in a cohort of the Framingham study, correlating the incidence of the first episode of TIA or stroke to the US-CRP serum level over a 14-year period. In the 1462 cases evaluated, levels in the upper quartile doubled the relative risk of outcomes in men and tripled it in women, independent of age; although the sample consisted principally old patients (average of 70 years) [50]. Also it has been suggested that elevated US-CRP may be an independent predictor for death and recurrent events after the first stroke [52,53].

Without analyzing clinical outcomes, Wang et al. [54] demonstrated that the development of intimal-medial thickening of the internal carotid artery was more frequently seen with US-CRP in the upper quartile in 3173 patients with asymptomatic plaques. This result only continued significant in women after adjusting for other risk factors. In the Rotterdam study, the US-CRP, interleukin-6 and adhesion molecules levels were compared to the severity of atherosclerosis at specific arterial sites. In carotid bifurcation, only US-CRP levels = 0.28 g/dL (upper third) were associated to the intimal-medial thickening and doubled the risk of developing moderate or severe stenosis (= 50%) [55]. The association between US-CRP, intimal-medial thickening of the carotid artery and risk of strokes was prospectively investigated in 5417 patients in the *Cardiovascular Health Study* [56]. A strong positive correlation between the US-CRP levels and strokes was observed in cases that presented with thickening (p-value < 0.02).

The association between US-CRP serum concentrations, histological and immunocytological structure of the carotid plaque and presence of neurological symptoms was carefully investigated by Alvarez Garcia et al. [57] in 62 patients submitted to carotid endarterectomy. The average US-CRP was significantly higher in symptomatic cases and in cases histologically classified as unstable (p-value < 0.001), also demonstrating a positive correlation with the presence of macrophages and T lymphocytes in the plaque. These results confirm the usefulness of US-CRP as a marker of carotid artery disease vulnerability. However, as the group classified as clinically unstable also included patients with well established ischemic conditions, without recent symptoms, the results may be biased and interpretation difficult.

On the other hand, the association of US-CRP, interleukin-6 and silent cerebral infarction was prospectively assessed in 194 patients without evidence of carotid, coronary artery, or peripheral artery diseases over a 10-year period. In the 40 cases of mild silent cerebral infarction documented by encephalic resonance, the US-CRP and IL-6 levels were significantly higher, suggesting a proinflammatory action of these mediators in intracranial arteries [58].

Nevertheless, the behavior of US-CRP in patients with

coronary artery disease and carotid stenosis has been described as non-linear and controversial by some authors. Choi et al. [59] prospectively evaluated the development of coronary artery events and intimal-medial thickening of the internal carotid artery in a series of 122 hypertensive patients and compared this with interleukin-6 and US-CRP levels. US-CRP was significantly higher only in cases of coronary artery disease; this marker did not show any correlation with carotid artery disease, even when compared with normotensive controls.

Recently, the *American Heart Association* published a systematic review with recommendations on the clinical use of several inflammatory markers in atherosclerosis. In relation to US-CRP the report concluded that no justification was found to use this marker as an isolated predictor of events in the general population but as an adjuvant in individuals with risk of coronary disease estimated between 10% and 20%, over 10 years [60]. However, the extent to which these guidelines correspond to cerebrovascular diseases demonstrated there to be insufficient evidence for the systematic use of US-CRP in primary prevention of strokes. In secondary prevention, there is recognized prognostic value when associated to other factors but its role as a determinant of therapeutic decisions has not yet been established [61].

The influence of US-CRP levels associated with phospholipase A₂-lipoprotein complex serum levels, on the incidence of strokes was recently investigated in 12,819 patients in the ARIC study. After adjusting for other risk factors, when the US-CRP and phospholipase A₂-lipoprotein complex levels were in the upper third, the risk of strokes was 8 times higher (p-value < 0.001) [62]. Similar results were reported in the Rotterdam study, which analysed the relationship between the phospholipase A₂-lipoprotein complex and development of coronary disease and/or strokes [63].

The monocyte chemotactic factor 1 (MCF1) has been reported as being related to dyslipidemia and to the instability of coronary artery disease, by some authors [64,65]. In the *Dallas Heart Study* [66], the proportion of patients with detectable coronary artery disease increased linearly according to the quartiles of MCF1 (from 17% for the lowest quartile to 32% for the highest quartile – p-value < 0.0001). Increased expression of MCF1 was described in human atheromatous carotid arteries, when compared to healthy arteries however its relationship to acute outcomes remains unclear [67].

Infectious agents

The possible contribution of some infectious processes, by accelerating the growth of atheroma plaques, or facilitating ruptures and/or instability, has been studied, in

particular in relation to *Chlamydia pneumoniae* (chlamydia) infection. Antibodies against chlamydia have been detected in specimens of coronary plaques and of carotid arteries, sustaining the hypothesis of its involvement [68,69]. Fagerberg et al. [70] prospectively studied the association between chlamydia antibodies and cytomegalovirus with the incidence of strokes. Analyzing 152 men over 6.5 years, the authors demonstrated a eight-fold risk of suffering from strokes in patients with high titers of chlamydia antibodies, but not in relation to cytomegalovirus (p-value = 0.043). Subsequently, these researchers observed that seropositivity for chlamydia was associated with carotid artery intimal-medial thickening in 113 men with arterial hypertension [71].

The correlation between recent infections by chlamydia and clinical instability of carotid artery disease was investigated by Katsenis et al. [19], who studied 35 patients submitted to carotid endarterectomy. The positivity for antibodies was related to the presence of symptoms but not to ultrasonographic characteristics of vulnerability. Gibbs et al. [72], in more detailed studies, compared the presence of chlamydia in plaque, the degree of perioperative embolization (by transcranial Doppler) and the occurrence of cerebral infarction in 98 symptomatic patients. The finding that only 25% of the cases had evidence of chlamydia in the plaque and the absence of a correlation of this with the embolization rate or ipsilateral strokes suggest that other mechanisms are involved in the destabilization process. There is similar controversy in relation to the use of antibiotics against chlamydia as tertiary or secondary prevention strategies in cerebrovascular disease. A clinical trial performed by Sander et al. [73] investigated the effect of roxithromycin on the development of carotid artery plaque in 272 over 55-year-old patients who had suffered from cerebral infarction. After a three-year follow-up, there was a significant reduction in the intimal-medial thickness in cases with positive serology of the treated group, but no alterations in the non-reagent patients. However, Ieven et al. [74] did not observe any differences in the IgG antibodies against chlamydia assessed by immunofluorescence in 106 patients with coronary artery disease and 112 healthy controls, probably due to cross-reactions with other bacteria. On the other hand, Vainas et al. [75] described an association between IgA seropositivity and intraoperative thromboembolism in plaques considered histologically stable suggesting that infection by chlamydia can also have a thrombogenic effect in atherosclerosis.

A recent prospective study, analyzing the behavior of the antibodies against chlamydia, herpes virus and cytomegalovirus in 109 patients with carotid stenosis of more than 50%, did not identify differences in serum levels between groups, nor in a comparison between

asymptomatic and symptomatic patients, or even comparing the US-CRP levels, making the hypothesis that infectious processes directly determine the instability of existing lesions weaker [76].

Role of autoantibodies

The role of immune system activation by anti-endothelial antibodies is currently being researched in respect to atherosclerotic disease. Farsi et al. [7] measured anti- β 2 glycoprotein antibodies (IgG and IgM – ELISA) in 93 patients submitted to percutaneous coronary angioplasty and compared the results to 105 controls. The authors observed that the level of antibodies was 13% in the coronary artery disease group and only 1% in the control group (p-value < 0.001). Additionally, within the coronary disease group, the titers were significantly higher in patients with unstable angina than in patients with stable angina (p-value < 0.001) and the rate of angiographically identified restenosis was 66% in positive antibody cases and only 14% in negative patients (p-value < 0.0004). These findings suggest the presence of an immuno-active inflammatory state in the instable disease or in cases with endothelial injury due to the use of catheters.

The correlation between anticardiolipin antibody levels and the risk of acute myocardial infarction was studied in the *Helsinki Heart Study*, which followed-up 133 men with dyslipidemia out of a total of 19,000 patients over a period of five years. The IgG Class antibody level was significantly higher in the cases that subsequently developed infarction or sudden death than in control cases (0.417 versus 0.361; p-value < 0.005) [77]. Sherer et al. [78] also demonstrated elevation of anticardiolipin and anti- β 2 glycoprotein antibodies in patients with coronary artery disease both with and without arterial hypertension. Similar results in relation to the peripheral artery disease were reported by Godoy et al. [79] in 40 patients with ages between 45 and 84 years, in which the risk of developing claudication quadruplicated when high levels of anticardiolipin antibodies were observed (p-value < 0.0001). Studying the relationship between anti- β 2 glycoprotein antibodies and cerebral infarction, Staub et al. [80] compared 92 cases of strokes with 93 controls, demonstrating that high levels of IgA class antibodies gave a significantly higher risk of the occurrence of this outcome (p-value = 0.025). Nevertheless, a recent analysis of a series of 4974 patients in the *Framingham* study [81] demonstrated that the elevation of anticardiolipin antibody titers conferred a significant risk of developing strokes and/or TIA only in women.

In obstructive carotid artery disease George et al. [82] demonstrated a significant increase in anti- β 2 glycoprotein antibodies in subendothelial regions of the plaque, possibly indicating interactions with endothelial cells,

macrophages and lymphocytes. However, only future studies can confirm if anticardiolipin antibodies constitute an independent risk factor, or merely an incidental phenomenon in the atherosclerotic processes [10].

Also the cytoprotector role of thermal shock proteins has been investigated in respect to cardiovascular diseases. Thermal shock proteins constitute a family of proteins with molecular weights between 20 and 150 Daltons, which demonstrate highly homogeneous amino acid sequences among different species including bacteria and man. Their expression increases in stress situations, such as with infections, high temperatures, exposure to free ions and endothelial injury. Experimental and clinical evidence has associated the thermal shock proteins expression and even antibody titers, to the severity and instability of atherosclerotic disease. These analyses utilize recombinant thermal shock proteins of infectious agents, such as mycobacteria, *Helicobacter pylori* and *Chlamydia pneumoniae*, attributing a possible relationship of acute cardiovascular events to an auto-immune response triggered by exposure to the bacteria [9, 83-86].

Birnie et al. [87] researched a possible association of thermal shock anti-protein antibodies 65 with coronary atherosclerosis in 136 patients submitted to coronarography. These authors verified that the antibody titers were linked to the extent and severity of the disease and that patients with *Helicobacter pylori* infection that were effectively treated showed a drop in titers.

Zhu et al. [88] reported the possibility that antibodies against human thermal shock protein 60 conferred a risk for atherosclerosis. Of the 391 patients who underwent coronarography, 75% presented positive IgG fractions. The presence of antibodies was also associated to the severity of the disease, as high titers were associated to disease of a larger number of vessels, even after adjusting for other risk factors.

Prohaszka et al. [89] evaluated antibodies for human thermal shock protein 60 and 65 in a cohort involving three groups of patients: 1) Patients with severe coronary artery diseases submitted to revascularization surgery; 2) patients with risk factors for ischemic cardiopathy and normal coronarographies and 3) healthy controls. In the multivariate analysis, only the levels of thermal shock protein antibodies 60 were considerably higher in group I than in the other groups.

Mukherjee et al. [90] investigated the role of thermal shock anti-protein antibodies in patients who developed restenosis of coronary arteries after percutaneous angioplasty. The group of patients with restenosis coursed with sustained levels of the antibodies, while those with favorable evolution presented with drops in their titers suggesting that a reduction can indicate a better prognosis after revascularization.

In obstructive carotid artery disease, the correlation of high titers of thermal shock anti-protein antibodies 65 was first demonstrated by Xu et al. [91]. In a cross-sectional population study, the authors measured IgG antibody serum titles against recombinant thermal shock protein 65 of mycobacteria in 867 individuals with ages between 40 and 79 years and compared the results with assessments of the carotid bifurcation using ultra-sonography. There was a significant correlation between high antibody titers and the presence of plaques in over 60-year-old patients, even after adjusting for factors such as gender, smoking, diabetes and dyslipidemia (p-value < 0.003).

The clinical significance of this association was subsequently investigated by the same authors in a follow-up of 750 patients selected from the first study. The authors verified that the increase in these antibodies did not only present a strong correlation with carotid evolutive injuries (r = 0.78) but was also an independent predictor of five-year mortality (p-value < 0.001) [92].

The relevance of thermal shock anti-protein antibodies in atherogenesis and the possible diagnostic and therapeutic implications will only be better elucidated with further studies [93].

Morphological aspects of atheroma in carotid artery disease

Although the percentage of carotid stenosis currently defines therapeutic decisions, strokes are caused by vulnerable plaques, which in part, depend of distinct morphological characteristics. Thus, several studies have proposed improvements in the imaging techniques in order to identify high-risk plaques, the majority of which utilize histological composition as the gold standard [11,94]. However, the direct correlation between histology and instability of the plaque has not been consistently established yet. The classic study of Hatsukami et al. [95] analysed histological components of 43 specimens of carotid endarterectomy, in respect to intimal fibrosis, lipid pools, necrotic regions, intraplaque hemorrhage and calcification and compared this with the presence of preoperative symptoms; significant differences between the clinical classes were not found. Recently the systematic review of Lovett et al. [96] evaluated the quality and comparability of 73 studies correlating imaging and histology examinations of carotid plaque. Only 23% evaluated the reproducibility of the different imaging techniques and in only 12% the histological data were comparable; no studies reported possible limitations of the results. Apart from recommending important precautions when accessing and reading histological data, such as the utilization of representative samples with similar numbers of cases with or without symptoms, time of the symptoms and time between obtaining

the image and specimen, precaution in respect to the position of the image and inclusion of specimens, acquirement of imaging slices of at least 3 mm and blind reading, the authors reinforced the necessity of more homogeneous criteria for studies that aim at evaluating the association between imaging and histology.

Apart from having a defined role in the determination of the degree of stenosis and being considered sufficient for the decision to intervene, ultrasonography has contributed substantially to the characterization of the content and surface of carotid plaque [97-101]. Initial stages of atherosclerosis may be monitored by the ultrasonographic arterial wall thickness, called the intimal-medial thickness, which, although it does not define if infiltration is attributed to the intimal or to hypertrophy of the medial layer, is a recognized as a marker of evolution of early lesions [102]. On the other hand, there is evidence suggesting that thinning of the fibrous cover, the presence of “soft” content or of ulceration give instability to the plaque [103,104], and that the proximity of necrotic regions, specifically if associated to infiltration of macrophages, predispose the patient to clinical events [105]. Moreover, both in models utilizing surface ultrasonography and in more recent studies with computed reconstruction or intravascular ultrasonography, low echogenicity images have been related to necrotic or hemorrhagic content of the plaque [106-108], and the degree of calcification is considered to be inversely proportional to the stability of the lesions [75]. It is well known that, for any hemodynamically significant stenosis, patients with less echogenic plaques present with a higher incidence of cerebral infarction. However, the high intra- and inter-observer variability of conventional imaging and the morphological characterization criteria of plaque demand technical improvements [109]. Utilizing computed image reconstruction, with grayscale stratification, Gronholdt et al. [110] prospectively analyzed 146 patients with carotid artery disease over four years and observed that the low echogenicity associated to very low values on the scale was an independent predictor of strokes, but only in a symptomatic patient group (n=135). Recently, Sztajzel et al. [111] added the colored flow map to the stratified grayscale in 28 patients submitted to carotid endarterectomy and obtained a strong histological association in respect to determination of hemorrhagic regions, necrotic regions and surface irregularity. Also the codification of the B-mode image for a three-dimensional real time computed model has been proposed for the identification of high-risk plaques by reducing ultrasonographic artifacts [112].

High resolution nuclear magnetic resonance (NMR) represents another significant advance in the morphological analysis of atheroma plaque, initially by means of *ex vivo* developed models which proved to be ineffective to study

any association between histological and clinical aspects *in vivo*. Von Ingersleben et al. [113] compared images of the carotid bifurcation obtained using T1 weighted sequences and corresponding histological sections in patients submitted to carotid endarterectomy. After analyzing only eight specimens, the authors obtained a significant positive correlation of the identification of hemorrhage regions, lipid deposits, fibrosis and calcification. Subsequently, the same group tested the histological characteristics of fibrous cover - normal versus thinned versus ruptured - in 22 patients. Utilizing the same protocol of NMR and considering a thickness = 0.25 mm of fibrous cover as normal; the observed correlation coefficient was 0.88 (p-value = 0.01) [114]. In another study, rupture of the fibrous cover was strongly associated to a recent history of TIA or ipsilateral stroke which more frequently occurred in plaques with a thin layer of fibrous cover or with a great lipid core [115].

The composition of the plaque may be determined by NMR utilizing a combination of pulse sequences that vary according to the type of structure which will be identified; there are thus different proposed classifications. Models based on tissue measurement have been proved accurate and reproducible [11]. Saam et al. [116] studied candidates for carotid endarterectomy using NMR 31 utilizing sequences with weighted T1, T2 and time-of-flight (TOF). In the measurement of lipids or necrotic regions, extracellular matrix loss, calcification and fibrosis, the authors obtained a histologic correlation of between 73% and 95% of cases, suggesting the inclusion of the technique in prospective assessment of plaques before indication of intervention.

The detection using NMR of hemorrhagic regions or wall thrombi which are frequently associated to complex plaques and clinical events is of particular interest. Moody et al. [117] verified that the presence of methemoglobin produces an amplification of the sign in weighted T1 sequences, which confers to the region in which there is sub-acute hemorrhage or recent thrombi, an aspect of hyperintense signal. Among 63 plaques that presented intense brightness by NMR, 44 demonstrated at least one of four histological criteria of hemorrhage, bestowing a specificity of 84% and a positive predictive value of 93% to the method, with minimum inter-observer variability. In the subsequent study, these authors correlated the hyperintense signal of the plaque, to the presence of recent ipsilateral symptoms. Information from NMRs of 120 patients with TIA or strokes was compared to 14 normal controls and also to the contralateral carotid artery (asymptomatic) of the same patient. The hyperintense signal was present in 60% of symptomatic carotid arteries, in 36% of asymptomatic carotid arteries, and in none of the controls (p-value < 0.001) [118]. The hypothesis that intraplaque hemorrhages stimulate progression of the plaques was tested by Takaya et al. [119]

in 29 patients followed-up over 18 months. In this period, the mean parietal volume and increase in lipid core volume were 7% and 28% respectively in 14 cases with intraplaque hemorrhage at the start of the study and were 0.15% and 5%, respectively in controls without previous hemorrhages (p-value < 0.01). Moreover, new regions of hemorrhage were observed in 43% of the cases and in none of the controls (p-value = 0.006). Figure 1 demonstrates examples of angiography by NMR, with and without the hyperintense signal.

Determination of the evolution time of carotid plaque hemorrhages by high resolution NMR was recently proposed by Chu et al. [120]. In 27 patients submitted to NMR with weighted T1, T2 and proton density weighted sequences (PDw), the classification of fresh, recent or old hemorrhages demonstrated a strong histological correlation (coefficient kappa = 0.7) and good accuracy (specificity 90% and sensitivity 74%). Moreover, the authors suggest that the utilization of this sequence in NMR is capable of defining the exact local of the thrombus in advanced lesions, differentiating hemorrhage of the plaque of juxta-luminal

thrombi [121]. As intraplaque hemorrhage, without rupture of the fibrous cover, has not been directly associated with symptoms, similar to thrombi adjacent to the lumen in general indicate erosion, ulceration or rupture of the plaque, this capacity of differentiation by NMR, if confirmed in future studies, may have important therapeutic implications in the context of vulnerability.

Even in preliminary assessment, intravascular NMR seems to be a promising technique to characterize the plaque, mainly on the intimal surface, with a histological association described in *ex vivo* models [122]. Thus, recently, an improvement tested *in vitro* by Clarke et al. [123] recommends a validation of automatic algorithms of plaque classification by NMR, utilizing the histopathological scale of the American Heart Association [124].

In the future, techniques such as molecular imaging of macrophage activity, angiogenesis measurement, intraplaque enzymatic activity and cellular apoptosis must be included as imaging methods for atherosclerosis [9].

Currently used examinations for the characterization of carotid plaque are shown in Table 1

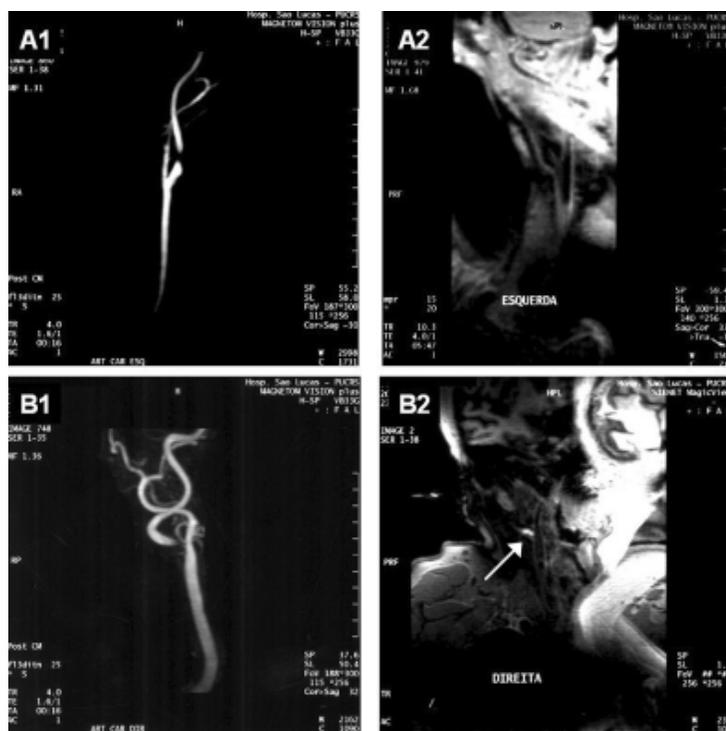


Fig. 1 – A1) Asymptomatic patient with 90% stenosis%; A2) Absence of hyperintense signal in weighted T1 sequence; B1) Patient with amaurosis fugax for one week and 90% stenosis; B2) hyperintense signal demonstrating intraplaque hemorrhage in the internal carotid (arrow)

Table 1. Imaging methods and vulnerability of carotid artery

Method	Characteristic
High resolution magnetic resonance	<ul style="list-style-type: none"> - Thinning/rupture of the fibrous cover - lipid core/necrotic - intraplaque hemorrhage - juxta-luminal thrombi - Neo vascularization - Imaging of macrophages (?) - Enzymatic activity (?)
Ultrasonography	<ul style="list-style-type: none"> - Thinning/rupture of the fibrous cover - Ulceration/erosion - Echo-lucent plaque content (hemorrhage or lipid core)

CONCLUSION

Atherosclerosis of carotid artery has acquired great epidemiological relevance due to its high prevalence in the aging population and as it is a frequent cause of strokes. Surgical endarterectomy constitutes the treatment of choice for severe lesions with its indication today only related to the percentage of stenosis.

However, the emerging concept of plaque vulnerability rising from knowledge of varying inflammatory, biochemical, metabolic and auto-immune mechanisms, observed in different stages of atherogenesis, has stimulated the study of markers capable of identifying the at-risk lesions. Among the systemically significant inflammatory mediators, US-CRP has been highlighted as a predictor of the presence and instability of carotid plaques, although there has been recent interest in relation to the behavior of CD40, extracellular matrix metalloproteinases and the A₂-lipoprotein phospholipase complex. The role of anticardiolipin antibodies and anti thermal shock proteins in carotid artery disease, in spite of to several experimental and clinical studies, remains unclear, as does the supposed causal relationship between chlamydia infection and atherosclerosis.

In respect to imaging methods, morphological plaque characterization has been a priority for the improvement of both ultrasonography and NMR. Intraplaque hemorrhage, hypocellularized lipid-rich regions and thinning and/or rupture of fibrosis cover have been reported as imminent risk markers of events, specifically when using high resolution NMR.

Because of all these factors, a new stratification of atherothrombotic risk in carotid artery disease may involve in the near future; a combination of systemic inflammatory markers, imaging by high resolution NMR, measurement of intraplaque molecular activity, with the widely used angiographic percentage of stenosis, which will possibly redefine the current intervention criteria.

REFERENCES

1. Kullo IJ, Edwards WD, Schwartz RS. Vulnerable plaque: pathobiology and clinical implications. *Ann Intern Med.* 1998;129(12):1050-60.
2. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med.* 1999;340(2):115-26.
3. Ballantyne CM, Nambi V. Markers of inflammation and their clinical significance. *Atheroscler Suppl.* 2005;6(2):21-9.
4. Leinonen M. Pathogenic mechanisms and epidemiology of Chlamydia pneumoniae. *Eur Heart J.* 1993;14(suppl. K):57-61.
5. Grayston JT. Chlamydia in atherosclerosis. *Circulation.* 1993;87(4):1408-9.
6. Katsenis C, Kouskouni E, Kolokotronis L, Rizos D, Dimakakos P. The significance of Chlamydia pneumoniae in symptomatic carotid stenosis. *Angiology.* 2001;52(9):615-9.
7. Farsi A, Domeneghetti MP, Brunelli T, Gori AM, Fedi S, Gensini GF et al. Activation of the immune system and coronary artery disease: the role of anti-endothelial cell antibodies. *Atherosclerosis.* 2001;154(2):429-36.
8. Wick G, Xu Q. Atherosclerosis: an autoimmune disease. *Exp Gerontol.* 1999;34(4):559-66.
9. Mandal K, Jahangiri M, Xu Q. Autoimmunity to heat shock proteins in atherosclerosis. *Autoimmun Rev.* 2004;3(2):31-7.
10. Sherer Y, Shoenfeld Y. Antiphospholipid antibodies: are they pro-atherogenic or an epiphenomenon of atherosclerosis? *Immunobiology.* 2003;207(1):13-6.
11. Nighoghossian N, Derex L, Douek P. The vulnerable carotid artery plaque. Current imaging methods and new perspectives. *Stroke.* 2005;36(12):2764-72.
12. Biasucci LM. CDC/AHA Workshop on markers of inflammation and cardiovascular disease. Application to clinical and public health practice. Clinical use of inflammatory markers in patients with cardiovascular diseases. A background paper. *Circulation.* 2004;110(25):e560-7.
13. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med.* 1997;336(14):973-9.
14. Waddington EI, Croft KD, Sienuarine K, Latham B, Puddey, IB. Fatty acid oxidation products in human atherosclerotic plaque: an analysis of clinical and histopathological correlates. *Atherosclerosis.* 2003;167(1):111-20.
15. Nishi K, Itabe H, Uno M, Kitazato KT, Horigushi H, Shinno K et al. Oxidized LDL in carotid plaques and plasma associates with plaque instability. *Arterioscler Thromb Vasc Biol.* 2002;22(10):1649-54.
16. Johnsen SH, Mathiesen EB, Fosse E, Joakimsen O, Stensland-Bugge E, Njolstad I et al. Elevated high-density lipoprotein cholesterol levels are protective against plaque

- progression: a follow-up study of 1952 persons with carotid atherosclerosis the Tromso study. *Circulation*. 2005;112(14):498-504.
17. Olsson AG, Schwartz GG, Szarek M, Sasiela WJ, Ezekowitz MD, Ganz P et al. High-density lipoprotein, but not low-density lipoprotein cholesterol levels influence short-term prognosis after acute coronary syndrome: results from MIRACL trial. *Eur Heart J*. 2005;26(9):890-6.
 18. Tirschwell DL, Smith NL, Heckbert SR, Lemaitre RN, Longstreth WT Jr., Psaty BM. Association of cholesterol with stroke risk varies in subtypes and patients subgroups. *Neurology*. 2004;63(10):1868-75.
 19. Schönbeck U, Libby P. CD40 signaling and plaque instability. *Circ Res*. 2001;89(12):1092-103.
 20. Schönbeck U, Varo N, Libby P, Buring J, Ridker PM. Soluble CD40L and cardiovascular risk in women. *Circulation*. 2001;104(19):2266-8.
 21. Kinlay S, Schwartz GG, Olsson AG, Rifai N, Sasiela WJ, Szarek M et al. Effect of atorvastatin on risk of recurrent cardiovascular events after an acute coronary syndrome associated with high soluble CD40 ligand in the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering MIRACL study. *Circulation*. 2004;110(4):386-91.
 22. Blake GJ, Ostfeld RJ, Yucel EK, Varo N, Schönbeck U, Blake MA et al. Soluble CD40 ligand levels indicate lipid accumulation in carotid atheroma: an in vivo study with high-resolution MRI. *Arterioscler Thromb Vasc Biol*. 2003;23(1):e11-4.
 23. Sapienza P, di Marzo L, Borrelli V, Sterpetti A, Mingoli A, Cresti S et al. Metalloproteinases and their inhibitors are markers of plaque instability. *Surgery*. 2005;137(3):355-63.
 24. Loftus IM, Naylor AR, Bell PR, Thompson MM. Plasma MMP-9: a marker of carotid plaque instability. *Eur J Vasc Endovasc Surg*. 2001;21(1):17-21.
 25. Molloy KJ, Thompson MM, Jones JL, Schwalbe EC, Bell PR, Naylor AR et al. Unstable carotid plaque exhibit raised matrix metalloproteinase-8 activity. *Circulation*. 2004;110(3):337-43.
 26. Sluijter JP, Pulskens WP, Schoneveld AH, Velema E, Stridjer CF, Moll F et al. Matrix metalloproteinase 2 is associated with stable and matrix metalloproteinases 8 and 9 with vulnerable carotid atherosclerotic lesions: a study in human endarterectomy specimen pointing to a role for different extracellular matrix metalloproteinase inducer glycosylation forms. *Stroke*. 2006;37(1):235-9.
 27. Verhoeven B, Hellings WE, Moll FL, de Vries JP, de Kleijn DP, de Bruin P et al. Carotid atherosclerotic plaque in patients with transient ischemic attacks and strokes have unstable characteristics compared with plaques in asymptomatic and amaurosis fugax patients. *J Vasc Surg*. 2005;42(6):1075-81.
 28. Von der Thüsen JH, Kuiper J, Van Berkel T, Biessen EA. Interleukins in atherosclerosis: molecular pathways and therapeutic potential. *Pharmacol Rev*. 2003;55(1):133-66.
 29. Mallat Z, Corbaz A, Scoazec A, Besnard S, Lesèche G, Chvatchko Y et al. Expression of Interleukin-18 in human atherosclerotic plaque and relation to plaque instability. *Circulation*. 2001;104(14):1598-603.
 30. Fantuzzi G, Reed DA, Dinarello CA. IL-12 induced IFN-gamma is dependent on caspase-1 processing of the IL-18 precursor. *J Clin Invest*. 1999;104(6):761-7.
 31. Yamagami H, Kitagawa K, Hoshi T, Furukado S, Hougaku H, Nagai Y et al. Association of serum IL-18 levels with carotid intima-media thickness. *Arterioscler Thromb Vasc Biol*. 2005;25(7):1458-62.
 32. Elkind MS, Rundek T, Sciacca RR, Ramas R, Chen HJ, Boden-Abdala B et al. Interleukin-2 levels are associated with carotid artery intima-media thickness. *Atherosclerosis*. 2005;180(1):181-7.
 33. Mallat Z, Heymes C, Ohan J, Faggin E, Lesèche G, Tedgui A. Expression of interleukin-10 in advanced human atherosclerotic plaques. Relation to inducible nitric oxide synthase expression and cell death. *Arterioscler Thromb Vasc Biol*. 1999;19(3):611-6.
 34. Jialal I, Devaraj S, Venugopal SK. C-reactive protein: risk marker or mediator in atherothrombosis? *Hypertension*. 2004;44(1):6-11.
 35. Koenig W. Predicting risk and treatment benefit in atherosclerosis: the role of C-reactive protein. *Int J Cardiol*. 2005;98(2):199-206.
 36. Fu T, Borensztajn J. Macrophage uptake of LDL bound to aggregated C-reactive protein: possible mechanism of foam-cell formation in atherosclerotic lesions. *Biochem J*. 2002;366(pt 1):195-201.
 37. Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation*. 2000;102(18):2165-8.
 38. Nakagomi A, Freedman SB, Geczy CL. Interferon-gamma and lipopolysaccharide potentiate monocyte tissue factor induction by C-reactive protein: relationship with age, sex and hormone replacement treatment. *Circulation*. 2000;101(15):1785-91.
 39. Mold C, Gewurz H, Du Clos TW. Regulation of complement activation by C-reactive protein. *Immunopharmacology*. 1999;42(1-3):23-30.

40. Nakajima T, Schulte S, Warrington KJ, Kopecky SL, Frye RL, Goronzy JJ et al. T-cell-mediated lysis of endothelial cells in acute coronary syndromes. *Circulation*. 2002;105(5):570-5.
41. Venugopal SK, Devaraj S, Yuhanna I, Shaul P, Jialal I. Demonstration that C-reactive protein decrease eNOS expression and bioactivity in human aortic endothelial cells. *Circulation*. 2002;106(12):1439-41.
42. Morrow DA, Rifai N, Antman EM, Weiner DL, McCabe CH, Cannon CP et al. C-reactive protein is a potent predictor of mortality independently of and in combination with troponin T in acute coronary syndromes: a TMI IIA substudy. *Thrombolysis in myocardial infarction. J Am Coll Cardiol*. 1998;31(7):1460-5.
43. Heeschen C, Hamm CW, Bruemmer J, Simoons ML. Predictive value of C-reactive protein and troponin T in patients with unstable angina: a comparative analysis. CAPTURE investigators. *J Am Coll Cardiol*. 2000;35(6):1535-42.
44. Ridker PM, Rifai N, Pfeffer MA, Sacks FM, Moye LA, Goldman S et al. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and recurrent events (CARE) investigators. *Circulation*. 1998;98(9):839-44.
45. Retterstol L, Eikvar L, Bohn M, Bakken A, Erikssen J, Berg K. C-reactive protein predicts death in patients with previous premature myocardial infarction – a 10 year follow-up study. *Atherosclerosis*. 2002;160(2):433-40.
46. Tomoda H, Aoki N. Prognostic value of C-reactive protein levels within six hour after the onset of acute myocardial infarction. *Am Heart J*. 2000;140(2):324-8.
47. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med*. 2002;347(20):1557-65.
48. Albert MA, Glynn RJ, Ridker PM. Plasma concentration of C-reactive protein and calculated Framingham Coronary Heart Disease Score. *Circulation*. 2003;108(2):161-5.
49. Albert CM, Ma J, Stampfer MJ, Ridker PM. Prospective study of C-reactive protein, homocysteine and plasma lipid levels as predictors of sudden cardiac death. *Circulation*. 2002;105(22):2574-6.
50. Rost NS, Wolf PA, Kase CS, Kelly-Haes M, Silbershatz H, Massaro JM et al. Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham study. *Stroke*. 2001;32(11):2575-9.
51. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation*. 1998;97(5):425-8.
52. Kocer A, Canbulat C, Gozke E, Ilhan A. C-reactive protein is an indicator for fatal outcomes in first-time stroke patients. *Med Sci Monit*. 2005;11(11):540-4.
53. Arenillas JF, Alvarez-Sabin J, Molina CA, Chacon P, Montaner J, Rovira A et al. C-reactive protein predicts further ischemic events in first-ever transient ischemic attack or stroke patients with intracranial large-artery occlusive disease. *Stroke*. 2003;34(10):2463-8.
54. Wang TJ, Nam BH, Wilson PW, Wolf PA, Levy D, Polak JF et al. Association of C-reactive protein with carotid atherosclerosis in men and women: the Framingham Heart Study. *Arterioscler Thromb Vasc Biol*. 2002;22(10):1662-7.
55. van der Meer IM, de Maat MP, Bots ML, Breteler MM, Meijer J, Kiliaan AJ et al. Inflammatory mediators and cell adhesion molecules as indicators of severity of atherosclerosis. The Rotterdam Study. *Arterioscler Thromb Vasc Biol*. 2002;22(5):838-42.
56. Cao JJ, Thach C, Manolio TA, Psaty BM, Kuller LH, Chaves PH et al. C-reactive protein, carotid intima-media thickness, and incidence of ischemic stroke in the elderly: the Cardiovascular Health Study. *Circulation*. 2003;108(2):166-70.
57. Alvarez-Garcia B, Ruiz C, Chacon P, Sabin JÁ, Matas M. High-sensitivity C-reactive protein in high-grade carotid stenosis: risk marker for unstable carotid plaque. *J Vasc Surg*. 2003;38(5):1018-24.
58. Hoshi T, Kitagawa K, Yamagami H, Furukado S, Hougaku H, Hori M. Relations of serum high-sensitivity C-reactive protein and interleukin-6 levels with silent brain infarction. *Stroke*. 2005;36(4):768-72.
59. Choi H, Cho DH, Shin HH, Park JP. Association of high-sensitivity C-reactive protein with coronary heart disease prediction, but not with carotid atherosclerosis, in patients with hypertension. *Circ J*. 2004;68(4):297-303.
60. Pearson TA, Mensah GA, Alexander RW, Anderson, JL, Cannon RO 3rd, Criqui M et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. 2003;107(3):499-511.
61. Di Napoli M, Schwaninger M, Cappelli R, Ceccarelli E, Gianfilippo G, Donati C et al. Evaluation of C-reactive protein measurement for assessing the risk and prognosis in ischemic stroke. A statement for health care professionals from the CRP Pooling Project Members. *Stroke*. 2005;36(6):1316-29.

62. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G et al. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Arterioscler Thromb Vasc Biol.* 2004;24:E-127-8.
63. Oei HH, van der Meer IM, Hofman A, Koudstaal PJ, Stijnen T, Breteler MM et al. Lipoprotein-associated phospholipase A2 activity is associated with risk of coronary heart disease and ischemic stroke. The Rotterdam study. *Circulation.* 2005;111(5):570-5.
64. Kowalski J, Okopien B, Madej A, Makowiecka K, Zielinski M, Kalina Z et al. Levels of sICAM-1, sVCAM-1 and MCP-1 in patients with hyperlipoproteinemia IIa and IIb. *Int J Clin Pharmacol Ther.* 2001;39(2):48-52.
65. Matsumori A, Furukawa Y, Hashimoto T, Yoshida A, Ono K, Shioi T et al. Plasma levels of the monocyte chemoattractant and activating factor/monocyte chemoattractant protein-1 are elevated in patients with acute myocardial infarction. *J Mol Cell Cardiol.* 1997;29(1):419-23.
66. Deo R, Khera A, McGuire DK, Murphy SA, Meo Neto JP, Morrow DA et al. Association among plasma levels of monocyte chemoattractant protein-1, traditional cardiovascular risk factors, and subclinical atherosclerosis. *J Am Coll Cardiol.* 2004;44(9):1812-8.
67. Nelken NA, Coughlin SR, Gordon D, Wilcox JN. Monocyte chemoattractant protein-1 in human atheromatous plaques. *J Clin Invest.* 1991;88(4):1121-7.
68. Linnanmäki E, Leinonen M, Mattila K, Neiminen MS, Valtonen V, Saikku P. Chlamydia pneumoniae-specific circulating immune complexes in patients with chronic coronary heart disease. *Circulation.* 1993;87(4):1130-4.
69. Cochrane M, Walker P, Gibbs H, Timms P. Multiple genotypes of Chlamydia pneumoniae identified in human carotid plaque. *Microbiology.* 2005;151(pt 7):2285-90.
70. Fagerberg B, Gnarpe J, Gnarpe H, Agewall S, Wikstrand J. Chlamydia pneumoniae but not cytomegalovirus antibodies are associated with future risk of stroke and cardiovascular disease: a prospective study in middle-aged to elderly patients with treated hypertension. *Stroke.* 1999;30(2):299-305.
71. Schmidt C, Hulthe J, Wikstrand J, Gnarpe H, Gnarpe J, Agewall S et al. Chlamydia pneumoniae seropositivity is associated with carotid artery intima-media thickness. *Stroke.* 2000;31(7):1526-31.
72. Gibbs RG, Sian M, Mitchell AW, Greenhalgh RM, Davies AH, Carey N. Chlamydia pneumoniae does not influence atherosclerotic plaque behavior in patients with established carotid artery stenosis. *Stroke.* 2000;31(12):2930-5.
73. Sander D, Winbeck K, Klingelhofer J, Etgen T, Conrad B. Reduced progression of early carotid atherosclerosis after antibiotic treatment and Chlamydia pneumoniae seropositivity. *Circulation.* 2002;106(19):2428-33.
74. Ieven MM, Hoymans VY, Bosmans JM. Early carotid atherosclerosis and Chlamydia pneumoniae seropositivity: are there arguments to treat with antibiotics? *Circulation.* 2004;110(7):e74-5.
75. Vainas T, Kurvers HA, Mess WH, de Graaf R, Ezzahiri R, Tordoir JH et al. Chlamydia pneumoniae serology is associated with thrombosis-related but not with plaque-related microembolization during carotid endarterectomy. *Stroke.* 2002;33(5):1249-54.
76. Muller BT, Huber R, Henrich B, Adams O, Berns G, Siebler M et al. Chlamydia pneumoniae, herpes simplex virus and cytomegalovirus in symptomatic and asymptomatic high-grade internal carotid artery stenosis. Does infection influence plaque stability? *Vasa.* 2005;34(3):163-9.
77. Vaarala O, Mänttari M, Manninen V, Tenkanen L, Puurunen M, Aho K et al. Anti-cardiolipin antibodies and risk of myocardial infarction in a prospective cohort of middle-aged men. *Circulation.* 1995; 91(1):23-7.
78. Sherer Y, Tenenbaum A, Praprotnik S, Shemesh J, Blank M, Fisman EZ et al. Autoantibodies to cardiolipin and beta2-glycoprotein I in coronary artery disease patients with and without hypertension. *Cardiology.* 2002;97(1):2-5.
79. Godoy JM, Batigália F, de Godoy MR, Brandão AC, Souza DR. Anticardiolipin antibodies as a risk factor of atherosclerosis in intermittent claudication. *Angiology.* 2004;55(4):357-9.
80. Staub HL, Norman GL, Crowther T, da Cunha VR, Polanczyk A, Bohn JM et al. Antibodies to the atherosclerotic plaque components beta2-glycoprotein I and heat-shock proteins as risk factors for acute cerebral ischemia. *Arq Neuropsiquiatr.* 2003;61(3B):757-63.
81. Janardhan V, Wolf PA, Kase CS, Massaro JM, D'Agostino RB, Franzblau C et al. Anticardiolipin antibodies and risk of ischemic stroke and transient ischemic attack: the Framingham cohort and off spring study. *Stroke.* 2004;35(3):736-41.
82. George J, Harats D, Gilburd B, Afek A, Levy Y, Schneiderman J et al. Immunolocalization of beta 2-glycoprotein I to human atherosclerotic plaque: potential implications for lesions progression. *Circulation.* 1999;99(17):2227-30.
83. Birmei DH, Holmet ER, McKay IC, Hood S, McColl KE, Hillis WS. Association between antibodies to heat shock protein 65 and coronary atherosclerosis: possible mechanism of action of Helicobacter pylori and other bacterial infections in increasing cardiovascular risk. *Eur Heart J.* 1998;19(3):387-94.

84. Fong IW, Chiu B, Viira E, Tucker W, Wood H, Peeling RW. Chlamydial Heat shock protein-60 antibody and correlation with *Chlamydia pneumoniae* in atherosclerotic plaques. *J Infect Dis*. 2002;186(10):1469-73.
85. Xu Q. Role of heat shock proteins in atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2002;22(10):1547-59.
86. Benjamin IJ, McMillan DR. Stress (heat shock) proteins: molecular chaperones in cardiovascular biology and disease. *Circ Res*. 1998;83(2):117-32.
87. Birnie DH, Holme ER, McKay IC, Hood S, McColl KE, Hillis WS. Association between antibodies to heat-shock protein 65 and coronary atherosclerosis. Possible mechanism of action of *Helicobacter pylori* and other bacterial infections in increasing cardiovascular risk. *Eur Heart J*. 1998;19(3):387-94.
88. Zhu J, Quyyumi AA, Rott D, Csako G, Wu H, Halcox J et al. Antibodies to human heat-shock protein 60 are associated with the presence and severity of coronary artery disease: evidence for an autoimmune component of atherogenesis. *Circulation*. 2001;103(8):1071-5.
89. Prohaszka Z, Duba J, Horvath L, Csaszar A, Karadi I, Skebeni A et al. Comparative study on antibodies to human and bacterial 60 kDa heat shock proteins in a large cohort of patients with coronary heart disease and healthy subjects. *Eur J Clin Invest*. 2001;31(4):285-92.
90. Mukherjee M, De Benedictis C, Jewit D, Kakkar VV. Association of antibodies to heat-shock protein-65 with percutaneous transluminal coronary angioplasty and subsequent restenosis. *Thromb Haemost*. 1996;75(2):258-60.
91. Xu Q, Willeit J, Marosi M, Kleindienst R, Oberhollechner F, Kiechl S et al. Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis. *Lancet*. 1993;341(8840):255-9.
92. Xu Q, Kiechl S, Mayr M, Metzler B, Egger G, Oberhollechner F et al. Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis: clinical significance determined in a follow-up study. *Circulation*. 1999;100(11):1169-74.
93. Mehta TA, Greenman J, Ettelaie C, Venkatasubramaniam A, Chetter IC, McCollum PT. Heat shock proteins in vascular disease: a review. *Eur J Vasc Endovasc Surg*. 2005;29(4):395-402.
94. Yuan C, Mitsumori LM, Beach KW, Maravilla KR. Carotid atherosclerotic plaque: noninvasive MR characterization and identification of vulnerable lesions. *Radiology*. 2001;221(2):285-99.
95. Hatsukami TS, Ferguson MS, Beach KW, Gordon D, Detmer P, Burns D et al. Carotid plaque morphology and clinical events. *Circulation*. 1997;28(1):95-100.
96. Lovett JK, Redgrave JN, Rothwell PM. A critical appraisal of the performance, reporting, and interpretation of studies comparing carotid plaque imaging with histology. *Stroke*. 2005;36(5):1091-7.
97. Reilly LM, Lusby RJ, Hughes L, Ferrell LD, Stoney RJ, Ehrenfeld WK. Carotid plaque histology using real-time ultrasonography: clinical and therapeutic implications. *Am J Surg*. 1983;146(2):188-93.
98. Langsfeld M, Gray-Weale AC, Lusby RJ. The role of plaque morphology and diameter reduction in the development of new symptoms in asymptomatic carotid arteries. *J Vasc Surg*. 1989;9(4):548-57.
99. Goes E, Janssens W, Maillet B, Freson M, Steyaert L, Osteaux M. Tissue characterization of atheromatous plaques: correlation between ultrasound image and histological findings. *J Clin Ultrasound*. 1990;18(8):611-7.
100. Droste DW, Karl M, Bohle RM, Kaps M. Comparison of ultrasonic and histopathological features of carotid artery stenosis. *Neurol Res*. 1997;19(4):380-4.
101. Holdsworth RJ, McCollum PT, Bryce JS, Harrinson DK. Symptoms, stenosis and carotid plaque morphology: is plaque morphology relevant? *Eur J Vasc Endovasc Surg*. 1995;9(1):80-5.
102. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation*. 1986;74(6):1399-406.
103. Golledge J, Greenhalgh RM, Davies AH. The symptomatic carotid plaque. *Stroke*. 2000;31(3):774-81.
104. Tegos TJ, Sohail M, Sabetai M, Robless P, Akbar N, Pare G et al. Echomorphologic and histopathologic characteristics of unstable carotid plaques. *Am J Neuroradiol*. 2000;21(10):1937-44.
105. Bassiouny HS, Davis H, Massawa N, Gewertz BL, Glasgow S, Zarins CK. Critical carotid stenoses: morphologic and chemical similarity between symptomatic and asymptomatic plaques. *J Vasc Surg*. 1989;9(2):202-12.
106. Wolverson MK, Bashiti HM, Peterson GJ. Ultrasonic tissue characterization of atheromatous plaques using a high resolution real time scanner. *Ultrasound Med Biol*. 1983;9(6):599-609.
107. Gronholdt ML. Ultrasound and lipoproteins as predictors of lipid-rich, rupture-prone plaques in the carotid artery. *Arterioscler Thromb Vasc Biol*. 1999;19(1):2-13.
108. Nissen SE, Yock P. Intravascular ultrasound: novel pathophysiological insights and current clinical applications. *Circulation*. 2001;103(4):604-16.

109. Arnold JA, Modaresi KB, Thomas N, Taylor PR, Padayachee TS. Carotid plaque characterization by duplex scanning. Observer error may undermine current clinical trials. *Stroke*. 1999;30(1):61-5.
110. Gronholdt ML, Nordestgaard BG, Schroeder TV, Vorstrup S, Sillesen H. Ultrasonic echolucent carotid plaques predict future strokes. *Circulation*. 2001;104(1):68-73.
111. Sztajzel R, Momjian S, Momjian-Mayor I, Murith N, Djebaili K, Boissard G et al. Stratified gray-scale median analysis and color mapping of the carotid plaque: correlation with endarterectomy specimen histology of 28 patients. *Stroke*. 2005;36(4):741-5.
112. Kern R, Szabo K, Hennerici M, Meairs S. Characterization of carotid artery plaques using real-time compound B-mode ultrasound. *Stroke*. 2004;35(4):870-5.
113. von Ingersleben G, Schmiedl UP, Hatsukami TS, Nelson JA, Subramaniam DS, Ferguson MS et al. Characterization of atherosclerotic plaques at the carotid bifurcation: correlation of high-resolution MR imaging with histologic analysis: preliminary study. *Radiographics*. 1997;17(6):1417-23.
114. Hatsukami TS, Ross R, Polissar NL, Yuan C. Visualization of fibrous cap thickness and rupture in human atherosclerotic carotid plaque in vivo with high-resolution magnetic resonance imaging. *Circulation*. 2000;102(9):959-64.
115. Yuan C, Zhang SX, Polissar NL, Echelard D, Ortiz G, Davis JW et al. Identification of fibrous cap rupture with magnetic resonance imaging is highly associated with recent transient ischemic attack or stroke. *Circulation*. 2002;105(2):181-5.
116. Saam T, Ferguson MS, Yarnykh VL, Takaya N, Xu D, Polissar NL et al. Quantitative evaluation of carotid plaque composition by in vivo MRI. *Arterioscler Thromb Vasc Biol*. 2005;25(1):234-9.
117. Moody AR, Murphy RE, Morgan PS, Martel AL, Delay GS, Allder S et al. Characterization of complicated carotid plaque with magnetic resonance direct thrombus imaging in patients with cerebral ischemia. *Circulation*. 2003;107(24):3047-52.
118. Murphy RE, Moody AR, Morgan PS, Martel AL, Delay GS, Allder S et al. Prevalence of complicated carotid atheroma as detected by magnetic resonance direct thrombus imaging in patients with suspected carotid artery stenosis and previous acute cerebral ischemia. *Circulation*. 2003;107(24):3053-8.
119. Takaya N, Yuan C, Chu B, Saam T, Polissar NL, Jarvik GP et al. Presence of intraplaque hemorrhage stimulates progression of carotid atherosclerotic plaques: a high-resolution magnetic resonance imaging study. *Circulation*. 2005;111(21):2768-75.
120. Chu B, Kampschulte A, Ferguson MS, Kerwin WS, Yarnykh VL, O'Brien KD et al. Hemorrhage in the atherosclerotic carotid plaque: a high-resolution MRI study. *Stroke*. 2004;35(5):1079-84.
121. Kampschulte A, Ferguson MS, Kerwin WS, Polissar NL, Chu B, Saam T et al. Differentiation of intraplaque versus juxtaluminal hemorrhage/thrombus in advanced human carotid atherosclerotic lesions by in vivo magnetic resonance imaging. *Circulation*. 2004;110(20):3239-44.
122. Larose E, Yeghiazarians Y, Libby P, Yucel EK, Aikawa M, Kacher DF et al. Characterization of human atherosclerotic plaques by intravascular magnetic resonance imaging. *Circulation*. 2005;112(15):2324-31.
123. Clarke SE, Beletsky V, Hammond RR, Hegele RA, Rutt BK. Validation of automatically classified magnetic resonance images for carotid plaque compositional analysis. *Stroke*. 2006;37(1):93-7.
124. Sary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W Jr et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Atherosclerosis, American Heart Association. *Circulation*. 1995;92(5):1355-74.