

# Vulnerability of atherosclerotic carotid disease: from laboratory to operating room - Part 1

*Vulnerabilidade da doença aterosclerótica de carótidas: do laboratório à sala de cirurgia - Parte 1*

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## INTRODUCTION

Cerebrovascular obstructive disease (COD) is currently a priority for public healthcare in developed countries, due to its significant prevalence in the adult population, the severity of ischemic events and the high potential to negatively affect productive life [1,2]. Almost 30% of all cases of strokes may be attributed to atherosclerotic disease of the carotid bifurcation, acute thrombotic mechanisms or more commonly to distal embolization [3, 4].

In spite of significant advances that occurred in the medicinal treatment of COD, carotid endarterectomy (CE) remains an important measure in the prophylaxis of strokes and the elective treatment in severe obstructive lesions, even when ipsilateral hemispherical symptoms are not present [5,6]. Although in the 1970s CE became the favorite method to treat strokes, only in the 1980s and 1990s, with the results of several clinical trials, the precise identification of subgroups of patients who really benefited from the surgical strategy was possible. These findings resulted in establishing the consensual indications for CE used today, based exclusively on the percentage of stenosis [7, 8].

However, the concept of vulnerability of atherosclerotic plaque, initially introduced for coronary artery obstructive disease, reveals that susceptible injuries are characterized by the presence of necrosis in the nucleus of the plaque, by locations with great lipid concentrations but little cellularized, by areas of intraplaque hemorrhage and by eccentric growth, a phenomenon denominated as positive remodeling [9,10]. Ischemic events are dependent on the rupture of the fibrous cover, allowing the thrombogenic content of the plaque to come in contact with the blood, and not on the degree of stenosis or the hemodynamic significance of the lesions [11, 12].

Evidence on the involvement of inflammatory mechanisms in atherosclerosis have contributed to changing of etiopathogenic paradigms, substituting the traditional model of progressive and concentric accumulation of lipids on the arterial wall for the concept that the inflammation plays a central role in the formation and progression of the atheroma.

Part I of this review aims at analyzing recent studies and relevance related to the vulnerability of atherosclerotic disease of carotid arteries, expressed by its epidemiological, clinical and inflammatory aspects. In Part II currently used serum markers are discussed, as are histological aspects and diagnosis by imaging, related to the instability of carotid artery disease, together with possible therapeutic implications or possible changes of criteria related to the current indications for intervention.

### **Epidemiological aspects**

In developed countries, strokes constitute the third

commonest cause of death in the adult population and the greatest determinant of permanent invalidity [13]. The incidence has been calculated as from 0.2% to 0.3% in the general population, with approximately 700,000 new cases each year in the United States, of which one-third of the patients die during the first year, one-third become unable to lead a productive life and only the remaining one-third are rehabilitated [14]. The in-hospital mortality rate has been described as approximately 25% and is significantly higher in female and over 75-year-old patients [15].

Among survivors, the reported recurrence of strokes is as high as 9% per year, and the survival over 5 years is not greater than 50% [16]. While mortality after the first recurrent stroke is similar to the initial event, the observed mortality in the hospital period of subsequent recurrent episodes is greater than 60%. Data from the Framingham study [17] show that the incidence of brain infarction doubles with each decade in the over 55-year-old population, affecting 1700 individuals per year in every 100,000 over 75-year-olds, with a two times higher mortality rate in women compared to men. The great clinical impact of these epidemiological data makes this condition a world-wide priority in government healthcare policies.

Atheromatosis of the carotid bifurcation is the cause of 25% of the cases of brain infarction and an important factor in primary and secondary prevention of strokes [18]. Furthermore, the reported prevalence of asymptomatic stenosis of 50% or more in over 65-year-olds is between 7% and 10% in men and between 5% and 7% in women [19], although the occurrence of stenosis of less than 49% is 10 times more frequent [20].

CE has been chosen as the first-line surgical procedure in the prevention of recurrent strokes, specifically in high risk subgroups [21]. From 1980 to 1993, the mean number annually of CE performed in more of 6,000 American hospitals, registered in the National Hospital Discharge Survey, was 81,000, with the highest number of surgeries (107,000) in 1985. In the over 65-year-old population, 196 procedures in each 100,000 inhabitants/year were performed making CE the most commonly major surgery performed in the USA over that period [22]. Bravata et al. [23] studied the long-term evolution of 5,123 patients treated for cerebrovascular disease and demonstrated that, in the patients who were submitted to CE, the mortality rate over 5 years was 38%, significantly lower than the observed mortality rate in cases of stroke patients who did not operate (60%).

The impact on hospital costs due to cerebrovascular events has been the target of worldwide concern as, for example, hospitalizations for strokes use 4% of the healthcare budget in the USA, 5.5% in Scotland and 3% in Holland [24-27]. In England, although only 55% of patients with cerebral

ischemia in effect stay in hospital, strokes involve 2% of all patients released from hospital, 7% of beds available for adults and are responsible for 6% of hospital costs [2]. Smurawska et al. [28] studying 285 consecutive stroke cases treated over 24 months, ignored recurrent episodes, calculated a mean cost of US\$21,150 per patient and a total cost of US\$6.6 millions. An analysis of intra-hospital costs, made in 137 community hospitals in the USA, with 18,740 brain infarction and 7,861 transitory ischemic cases, showed mean costs of US\$5,837 and US\$3,350 per hospital stay, respectively [29].

In an epidemiological context, the measures already accepted that aim to reduce the impact of cerebrovascular diseases include: optimized treatment of acute strokes; the control of risk factors for atherosclerosis; effective medicinal or surgical primary prevention of high risk subgroups and prevention of recurrent events. However, studies testing laboratorial or imaging methods that are potentially able to detect instability of the carotid plaque and its susceptibility to clinical events prior to established infarction, may also contribute to an improvement in the current state of affairs.

#### **Clinical aspects and indications for intervention**

In carotid obstructive diseases, the symptoms are generally related to the occurrence of macro- or micro-embolizations, which indicate resulting regional ischemia. The size of ischemic states will depend on compensatory mechanisms, related to the general hemodynamical state of the patient and on the collateral branches of the Willis polygon. The event may be abrupt and catastrophic or tenuous and subtle, which makes recognition of the symptoms difficult even for the patient. Characteristically, the thromboembolic phenomena deriving from carotid bifurcation occlusion cause focal hemispherical deficits, emerging with ipsilateral amaurosis, hemiparesis or contralateral hemiplegia, predominantly brachial, or by difficulties of expression, such as aphasia, dysphasia or dysarthria, when the dominant cerebral hemisphere is accessed. Although rare, symptoms such as giddiness, vertigo, postural alterations or syncope may occur which generally suggests concomitant compromise of the basilar-vertebral region. At the extremes of the wide clinical range of cerebrovascular disease it is possible to have complaints such as slight and relapsing pins and needles in the hand, to sudden and irreversible hemiplegia states [30].

Classically, the nomenclature of cerebral ischemic syndromes takes into account the reversibility and the time of evolution of the neurological deficit. When the clinical syndrome presents total reversal within 24 hours without leaving sequels, it is named a transient ischemic attack (TIA), with a risk of recurrence or evolution to a stroke of 30%. If

there is no reversal within this period, it may be characterized as a brain infarction, independently of future recovery or of the degree of neurological sequels. However, nowadays, this division has been made less rigid because of the possibility of identifying small regions of injured tissue. In the USA, it is estimated that the use of magnetic nuclear resonance in the initial investigation of cerebrovascular events, should result in a reduction in the annual rate of TIA of about 33%, and an increase of 7% in the recognition of cerebral infarctions [31]. Whilst in cases of TIA, endarterectomy may be performed at any time after diagnosis, in strokes there is a necessity to wait at least 2 or 3 weeks for interventions, due to the risk of severe intracerebral hemorrhage; the reason that surgery in acute stroke cases is not normally indicated [32].

The efficacy of CE to prevent new cerebral events in patients with symptomatic or asymptomatic stenosis has been confirmed in several clinical trials, with current indications for interventions being well-defined and mainly based on the percentage of stenosis.

#### **A. Symptomatic patients**

In symptomatic patients, CE may be performed with the aim of impeding strokes when the clinical diagnosis is transient ischemic attack (TIA) or with the aim of saving cerebral parenchyma at risk of new infarctions and improving the quality of life in pre-established cases of strokes. Of the clinical trials that have attempted to compare the results of surgical interventions with pharmacological treatment, two currently consolidate the international consensus, the North American Symptomatic Carotid Endarterectomy Trial (NASCET) and the European Carotid Surgery Trial (ECST).

In the NASCET study, published in 1991, patients with carotid stenosis = 70% by angiography, who had suffered transient ischemia or non-disabling strokes in the previous 120 days, were randomized to receive platelet anti-aggregation therapy associated or not to carotid endarterectomy. In the 659 cases analyzed, the incidence over two years of disabling ipsilateral strokes was significantly greater in the clinically treated group (26%) than in the surgically treated group (9%), with a reduction in the relative risk of 65% [7]. Recently, NASCET researchers published the results of CE in patients with carotid stenosis considered moderate (between 30% and 69%). In cases of obstruction between 30% and 49%, no benefit was reported when comparing the surgical intervention to the medicinal therapy but, in patients with stenosis between 50% and 69%, there was a statistically significant benefit for the EC group, although lower than that observed in individuals with severe stenosis. The reduction of absolute risk of ipsilateral strokes with CE was 4.4% over 90 days (p-value = 0.045), and this benefit was maintained over 8 years of follow-

up, justifying the procedure, as the accumulated rate of strokes and intra-hospital death did not exceed the 3% described [33].

In the ECST study, which also included symptomatic patients with stenosis = 70%, 1811 cases referred to surgical treatment presented with a reduction of 84% in the relative risk for major strokes compared to 1213 clinically treated patients (p-value <0.001), with greater benefits for patients with stenosis of more than 80% and in men compared to women. However, the method of measuring the percentage of stenosis was different to that utilized in the NASCET trial, overestimating the severity of the lesions. Obstructions of 80% by ECST criteria were approximately equal to 60% according to NASCET [34]. In addition, the final analysis of the ECST study also demonstrated the benefit of CE in the group of patients with stenosis estimated by angiography of between 50% and 69% and results, in truth worse than antiplatelet therapy in lesions of less than 30%, and thus it should not be indicated for these cases [35,36].

Interestingly, the results of the NASCET and ECST trials confirmed the findings of the Veterans Affairs Cooperative Study, which, studying 189 symptomatic cases with stenosis equal to or greater than 50%, observed an absolute reduction in risk of about 11% in patients who were submitted to CE. This benefit was increased to 17% when only the subgroup with stenosis = 70% was analyzed (p-value = 0.004) [37].

### **B. Asymptomatic patients**

In cases of asymptomatic carotid obstructive disease, it is difficult to specify the exact number of strokes that may be avoided by CE in the general population. It is estimated that approximately 4% of the American population between 50 and 75 years old has carotid stenosis of from 60% to 99% without symptoms of ischemia. If these individuals could be identified and submitted to CE, the rate of strokes could potentially be reduced from 11% to 5% over 5 years, preventing around 120 cases of strokes for each 10,000 of the population every year, a beneficial prevention measure even though a higher number of patients would undergo surgery to prevent one stroke [3].

The most important clinical trials testing the results of CE in asymptomatic patients are the Asymptomatic Carotid Atherosclerosis Study (ACAS) and the Asymptomatic Carotid Surgery Trial (ACST).

In the ACAS study, 1662 patients with stenosis of at least 60% demonstrated by ultrasound or arteriography (with similar stenosis measurement criterion to NASCET), were randomized to receive antithrombotic therapy with or without CE and were followed-up over 5 years. In the clinical group, the ipsilateral stroke rate was 11%, a little higher than two times the rate in operated patients (5%), indicating

an annual incidence of 2.2% strokes. The reduction in absolute risk was 5.9% and the reduction in relative risk was 55% (p-value < 0.004) with this benefit being more significant for men because of the high rate of complications observed in women (3.6% versus 1.2%) [8]. Maybe, the size of the difference between groups in the ACAS study was prejudiced owing to the high rate of major complications (2.3%), most of which were related to the arteriography (1.2%), with more incidents affecting women [38]. Currently, the ACAS study supports the recommendations of the American Heart Association; indicating CE for patients with stenosis of more than 60% as the morbidity and mortality rates are no more than 3% [5].

Recently concluded, the ACST European trial enrolled more than 3000 asymptomatic cases with carotid stenosis = 70% by ultrasound or angiography using magnetic nuclear resonance. With more liberal criteria of eligibility than the criteria utilized in the ACAS study so as to increase the applicability of their conclusions, the authors observed that there was reduction of 50% in the relative risk of ipsilateral strokes or death in the group referred to surgical treatment during a 5-year follow-up period (p-value < 0.001). These results already include hospital morbidity and mortality [39].

Before the ACAS and ACST studies, other trials had demonstrated conflicting results. The Mayo Asymptomatic Carotid Endarterectomy Study, which randomized 158 patients in two groups for therapy with AAS or CE, was interrupted because of the high occurrence of acute myocardial infarction in the surgical group [40]. The Veterans Affairs Cooperative Study involved 444 patients with stenosis = 50% but did not demonstrate any significant difference in the number of strokes or death over 30 days between the clinical and surgical groups [41]. In the CASANOVA trial of 410 cases, CE did not show benefits when compared to antithrombotic treatment, although the exclusion of patients with lesions = 90% significantly limited the validity of the study [42].

Recently, the Asymptomatic Carotid Stenosis and Risk of Ipsilateral Hemispheric Ischaemic Events (ACSRS) study [43], accompanied the evolution of 1115 asymptomatic patients with stenosis = 50% according to the ECST criteria over 5 years in respect to ischemic phenomena. Elevation of serum creatinine levels, history of TIAs in the contralateral hemisphere and a high percentage of stenosis (=70%) were independent predictors of risk with a threefold rate of events (7.3% versus 2.3%), findings that suggest the possible involvement of other variables in the genesis of ischemic events in pre-existent plaque apart from the percentage of stenosis.

Table 1 summarizes the indications currently accepted for CE according to the percentage of stenosis and the correspondent clinical trials

Table 1. Current indications for carotid endarterectomy

Clinical class	Indication (*)	Trials
Symptomatic	Lesions $\geq$ 70%	NASCET [7], ECST [46]
	Lesions $\geq$ 50 and $<$ 70%	NASCET [45], ECST [48], VACS [49]
Asymptomatic	Lesions $\geq$ 60%	ACAS [8]
	Lesions $\geq$ 70%	ACST [51]

(\*) Considering the risk of death or perioperative strokes  $\leq$  3%  
 NASCET: North American Symptomatic Carotid Endarterectomy Trial; ECST: European Carotid Surgery Trial; VACS: Veterans Affairs Cooperative Study; ACAS: Asymptomatic Carotid Atherosclerosis Study; ACST: Asymptomatic Carotid Surgery Trial.

### Atherosclerosis and Inflammation

Faced with the frightening number of more than 19 millions de deaths due to cardiovascular events annually, the early detection of so-called vulnerable or high-risk plaque using local or systemic markers has been a worldwide priority of scientific investigation [44, 10].

Historically atherosclerosis was considered a condition resulting from the progressive accumulation of lipoproteins of cholesterol, but nowadays it is known that it is a chronic inflammatory disease of the arterial system.

The fact that only 50% of atherosclerotic patients have dyslipidemia [45] and that the prevalence of traditional risk factors, such as smoking, arterial hypertension and diabetes, in over 65-year-olds, does not differ substantially from the results with normal individuals [46], suggests the involvement of other mechanisms.

Rothwell et al. [47], studying 3007 symptomatic cases of carotid artery disease, observed that patients with angiographic abnormalities of both carotid arteries, had higher rates of prior acute myocardial infarction and sudden death, maybe due to a systemic pattern in the genesis of more aggressive atherothrombotic phenomena. Recently, Rossi et al. [48] studied the correlation between carotid ultrasonography data of 164 patients, with or without acute myocardial infarction. In 69 cases with infarction, the plaques presented with softer and less fibrocalcic content, when compared to the 95 patients who had not suffered cardiac events, suggesting that the instability is triggered or

aggravated as a response to vascular injury, a hypothesis shared by several other authors [49-54].

In the current model of atherogenesis, the alteration of the endothelial homeostasis due to antagonists with local or systemic action, such as the accumulation of lipoprotein, mechanical stress (arterial hypertension, percutaneous interventions), toxins from smoking or oxidant substances, infectious agents, autoimmune diseases, homocysteinemia, constituted the initial event of the formation and progression of the plaque [55]. Initially, there is a loss in the anti-aggregation nature of circulating cells due to the production of adhesion, integrin and selectin molecules by the endothelial cells. These substances modulate the recruitment and the aggregation of monocytes, macrophages, lymphocytes and platelets that cause alterations in the permeability of the endothelial layer. The migration of white blood cells into the arterial wall is followed by the release of proinflammatory agents, in particular interleukins, tumoral necrosis factor, CD40 ligand, cyclooxygenase-2 and metalloproteinases. The inflammation state undergoes positively feedback with systemic effects, with additional releases of cytokines by the leucocytes, greater aggregation of oxidized LDL particles on the surface endothelial layer, hepatic production and release of inflammatory substances, such as reactive C protein (RCP), activation of the systemic inflammatory cascade and of the complement system [56]. The presence of macrophages modified by phagocytosis of antigenic lipid molecules, denominated foam cells, in the subendothelial space, and its coalescence in the interstitial matrix, histologically typifies the plaque in this phase [57], possibly having a direct relationship with the degree of destabilization of the disease [58].

With the continuation of proinflammatory stimuli, the proliferative phase is initiated, at first with marked synthesis of collagen, starting with activation of fibroblasts, thus forming the fibrotic cover [59]. This phase is notable due to the intense production of interleukins, alpha and beta tumoral necrosis factor, platelet growth factor, fibroblastic growth factor and by the formation of mature plaque, which generally has excentric growth and suffers a dynamical process of positive remodeling [10, 60]. Alterations in the intracellular matrix composition, mainly by the action of metalloprotein proteolytic enzymes of the extracellular matrix, determine degradation of the collagen, an increase of the elastin synthesis and lower levels of hydroxyapatite, which have been associated to vulnerable lesions [61]. Variable degrees of calcification may occur, which it has been suggested, give stability to the plaque [62, 63], formation of lipid or necrotic areas with few cells that seem to be prone to events [64], mainly when they are associated to thinning of the fibrous cover [65]. On the other hand, researchers such as Trostorf et al. [66] showed that the thinning of the

fibrous cover, although not an essential prerequisite for the rupture of the plaque, is related to the degree of apoptosis of the smooth muscle cells and associated to the presence of symptoms (Figure 1).

The development of atheroma plaque also progressively compromises the anticoagulant property of the endothelial surface. Local inflammatory activity stimulating platelet aggregation, increases in the A2 phospholipase, vasoconstriction mediated by angiotensin II, endothelins and serotonin, thrombogenic action of thromboxane and leukotrienes, as well as toxin from tobacco, and the action of extracellular matrix metalloproteinases, are the main factors that cause prothrombotic states, which, in advanced or vulnerable lesions, are associated to intraplaque hemorrhage, fibrous cover rupture, thrombosis and embolization [57].

Table 2 succinctly illustrates the inflammatory processes involved in endothelial dysfunction and the biological effects.

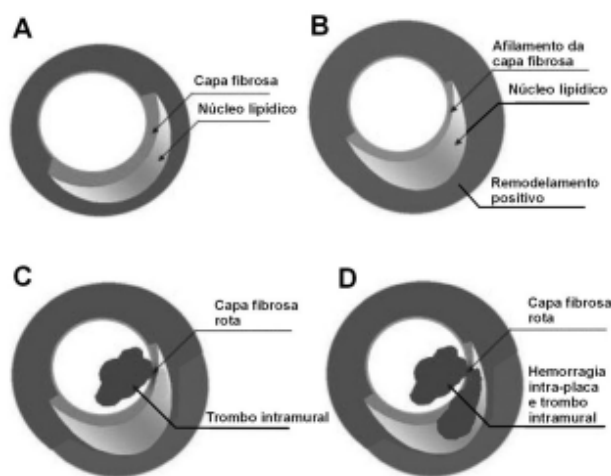


Fig. 1 – Pathogenesis of the atherosclerotic plaque: A) initial lesion; B) positive remodeling and thinning of the fibrous cover; C) fibrous cover rupture without intraplaque hemorrhage; D) intraplaque hemorrhage determining fibrous cover. (Adapted from Rohde & Lee [60])

Table 2. Inflammatory processes of the endothelial dysfunction, atherosclerotic plaque formation and main biological effects

Alteration of the function	Main direct mediators	Cellular elements	Inflammatory response	Biologic effect
Adhesiveness Permeability	Adhesion molecules Integrins Selectins LDL-OX	Endothelial cells Monocytes Macrophages T Lymphocytes Platelets	↑ Cytokines ↑ IL 1,2,6,7,8,18 ↑ Interferon ↑ CD40L ↑ MMP ↑ COX2	- Additional recruitment of cell elements - LDL-OX aggregation - Activation of the inflammatory cascade - complementary activation - macrophage Sequestration (foam cells) - Proliferation of CML
Cellular proliferation	Alpha-TNF IL-1 PDGF FGF MMP	Smooth muscle cells	- CML Proliferation - Collagen synthesis (fibrous cover) - Extracellular matrix degradation	- Formation of mature plaque - Positive remodeling
Thrombogenesis	A2 Phospholipase Angiotensin II Endothelins Serotonin Thromboxane Leukotrienes toxins from Cigarettes Oxidants	Platelets Endothelial cells	Loss of anticoagulant properties - thrombogenic state	- intraplaque hemorrhage - Rupture of the fibrous cover - Thrombosis - Embolization

LDL-ox: oxidized LDL; IL: interleukin; CD40L: CD40 ligand; MMP: metalloproteinases; COX: cyclooxygenase; TNF: tumoral growth factor; PDGF: platelet growth factor; FGF: Fibroblast growth factor; CML: smooth muscle cells.

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