„GEORGE EMIL PALADE” UNIVERSITY OF MEDICINE, PHARMACY, SCIENCE AND TECHNOLOGY FROM TÎRGU MUREŞ

SCHOOL OF DOCTORAL STUDIES

The summary of doctoral thesis

Study of clinico-pathological features, morphological and immunohistochemical aspects of vulnerable atherosclerotic plaques obtained by carotid endarterectomy

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Introduction

Atherosclerosis is a chronic inflammatory disease of the artery wall that causes narrowing of the artery lumen. Numerous constitutional factors and multiple comorbidities have been identified as contributing to the development and progression of atherosclerosis. Carotid stenosis (CS), the consequence of carotid artery (CA) atherosclerosis, is a narrowing of the arterial lumen and is more common in the elderly and a frequent cause of stroke. Treatment of severe CS is conservative, combined with surgical treatment - carotid endarterectomy (CEA) with/without angioplasty, or with endovascular treatment - angioplasty and carotid stenting. The modified American Heart Association (AHA) classification of carotid atherosclerotic plaques allows, after histological identification of the plaque elements, their classification into one of eight categories. Although macrocalcification is classically associated with plaque stability, according to the latest published data on atherosclerosis, microcalcification does not confer plaque stability and is associated with morphological changes that also create plaque vulnerability - the presence of inflammatory infiltrate, lipid core and plaque surface ulceration. All morphological changes in the plaque are a consequence of long-term chronic inflammation in which monocytes and macrophages play an important role, and the degree of macrophage and mastocytes infiltration together with the microvascular density in the plaque are useful in predicting the risk of rupture of vulnerable carotid plaques.

Objectives

The studies performed aim at the complex characterization of symptomatic CS, both clinically and imaging, as well as using classical histological and immunohistochemical techniques, described by a high degree of interdisciplinarity (angiology, surgical techniques, imaging, histopathology, immunohistochemistry). We had specific objectives for each of the 4 studies. Thus, in the first study, considering the common characteristics of the patients enrolled in the study, we tried to map the risk factors, clinicopathological and paraclinical features of CA atherosclerosis based on the demographic information and clinical data of the patients. Since all patients enrolled in the study received open surgical treatment - CEA, in the second phase of the research (Study 2) we analyzed the incidence of immediate postoperative complications and risk factors that may influence their occurrence. In the third study we investigated histological correlations between patterns and location of intraplaque calcification, presence of inflammatory infiltrate and expression of osteopontin (OPN) with direct signs of vulnerability. Based on the results of Study 3, we selected plaques with active perilesional inflammatory infiltrate for immunohistochemical study. CD68+ infiltrate, iNOS2+, Arg1+ and CD31+ expressions were quantified around the lipid core by digital morphometry. These results were correlated with the presence of morphological changes leading to plaque instability (Study 4).

Study 1 – The study of epidemiological aspects, clinicopathological and paraclinical features of carotid artery atherosclerosis

This study included 119 symptomatic patients with a CS of more than 70%. Before surgery, imaging investigations were performed to diagnose, localize, and classify the stenosis. Of the 119 patients, 82 were males and 37 females, and the mean age was 67 years. Eighty-four individuals had unilateral lesions and 35 had bilateral CA lesion. A history of previous stroke was confirmed in 76 subjects. In most cases the patients were hypertensive (92.4%), and 27.7% were diabetic (type 1 and type 2). Eighty of them presented only carotid atherosclerosis; in 28 patients the involvement of two arterial beds (carotid and peripheral vessels or coronary arteries) was established, and in 11 cases the involvement of three arterial beds (carotid, peripheral vessels, and coronary arteries) was identified. 48.7% of the cohort population were smokers and except for 4 subjects, all of them presented hypercholesterolemia. Following distribution testing for continuous variables only for the age variable the data are normally distributed.

Study 2 – The study of the immediate postoperative outcome of patients with carotid endarterectomy

All 119 patients from Study 1 who received CEA were included in this prospective study. Patients were monitored for biological parameters and clinical status during the first two days postoperatively. Acute postoperative complications such as neurological disorders, medium to large cervical hematomas and facial nerve palsy requiring additional care or surgical reintervention were monitored. In our group, we identified 15 patients with complications after CEA and no deaths. We therefore sought to identify factors that might influence the occurrence of immediate postoperative complications. For example, cervical hematoma is

more common in patients with advanced coronary artery disease, smokers, or those who used anticoagulants preoperatively. Neurological complications occur when the duration of surgery is prolonged beyond 90 minutes, and hypertension may influence the occurrence of postoperative facial nerve palsy.

Study 3 – The study of the histological aspects of plaque vulnerability in patients with severe carotid stenosis

A total of 119 cases were selected for histopathological study. Atherosclerotic plaques were obtained by CEA from patients diagnosed with severe CA stenosis. All fragments were processed according to standard methods and stained with hematoxylin and eosin. Von Kossa staining and immunohistochemical staining with polyclonal OPN antibody were used to detect calcification. On microscopic examination, signs of plaque vulnerability were observed as follows: a large lipid-rich necrotic core was found in 58.8% of samples, an active mononuclear cell component (macrophages and lymphocytes) was found in 63.02% of cases, aggregated erythrocytes within the plaque structure - intraplaque hemorrhage (IPH) was present in 32.8% of cases. Many plaques showed neovascularization, by proliferation of thin-walled vessels with collapsed lumen (66.4%), in most cases coexisting with inflammation and IPH. Ulcerated plaques with irregular and discontinuous fibrous capsules (43.7%) led to thrombus formation in 16 cases. Microcalcifications and nodular calcifications were the most common types of calcification expression and calcification superficial position predominated in the plaques examined. High-level OPN expression in plaques was correlated with the presence of inflammatory infiltrate, lipid core and ulceration.

Study 4 - Inflammatory microenvironment and density of neoformed vessels in atherosclerotic plaques as predictors of vulnerability: immunohistochemical and morphometric study

In this study, we proposed to immunohistochemically characterize the heterogeneous inflammatory microenvironment and to map the architectural appearance of neovascularization within the plaque, by morphological characterization and determination of microvascular density in a series of 67 endarterectomy specimens scored for the presence of perilesional inflammatory infiltrate in Study 3. We used digital morphometry to assess CD68+ macrophages as major elements of the cellular infiltrate. We also quantified Arg1+ and iNOS2+ surface area within the "inflammatory hotspot" to determine the dominance of the M2-Arg1 positive or M1- iNOS2 positive subtype. We observed a predominance of M2 subtype in our group (55 cases) and in cases with M1 macrophage predominance this was associated with atherothrombosis. The CD68+ surface was almost the same in both groups (1.38%-Arg1+ vs. 1.33%-iNOS2+). Perilesional inflammation was associated with plaque neovascularization in only 41 cases. In these cases, neovascularization observed as the presence of newly formed microvessels within the plaque, was associated with a significantly higher CD31+ area than in plaques without this phenomenon (1.07 ± 0.14% vs. 0.05 ± 0.05%). In the 13 plaques with atherothrombosis, the CD31 positive area was also significantly higher than in plaques without atherothrombosis (1.02 ± 0.20% vs. 0.61 ± 0.12%).

Conclusions

From these studies, we observed that 63.9% of patients with severe CS who underwent surgical treatment-CEA had a preoperative stroke. Age, sex, hypertension, diabetes, and smoking were not associated with the presence of polyvascular disease, unilateral/bilateral carotid involvement, and degree of CA stenosis. CEA is a safe surgical procedure with few postoperative complications performed in patients with severe CA stenosis to prevent the occurrence or recurrence of stroke and to improve postoperative quality of life. Patients with carotid plaques with low/mild inflammatory components have a significantly lower incidence of cerebrovascular events (stroke) compared to those with abundant inflammation, suggesting the role of proinflammatory macrophages in atheroma vulnerability. Our results provide histological evidence for the critical role of microcalcification and chronic inflammation in the formation and destabilization of advanced carotid plaques. Considering only the macrophage population, without those that look like foam cells, although the M2 subtype of macrophages was dominant in endarterectomy specimens, atherothrombosis is only associated with inflammation in which M1 macrophages with proinflammatory character predominate.