学位論文内容の要旨

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学 位 論 文 題 名

Clinicopathological assessment of plaque instability in human carotid atherosclerosis

（ヒト頚動脈における粥状動脈硬化不安定性の臨床病理学的検証）

Background: Ischemic strokes and transient ischemic attacks (TIA) are usually caused by unstable carotid lesions, which result in thrombus formation and occlusion of the artery. The histological characteristics of unstable carotid plaques have been described in several studies. In studies of coronary arteries, unstable plaques have been identified as ruptured, inflammatory plaques with thin fibrous caps (TFC) that were often the cause of acute coronary syndromes rather than stable angina. In the cerebral circulation, however, most ischemic strokes are caused by distal embolization originating from atherosclerotic plaques, or by an acute, instead of chronic, occlusion of a carotid artery. The interest in the morphology and functional characteristics of carotid plaques, using various imaging techniques and biochemical markers, has been growing. The progression of carotid plaques accelerated by hemorrhages developing inside the plaque has been observed in several imaging studies, and has been associated with predictors of future ischemic cerebrovascular events (CVE). In previously asymptomatic patients presenting with 50-79% carotid artery stenoses, thin or ruptured fibrous caps, hemorrhages within a plaque, and large, lipid-rich and necrotic cores (NC) have been associated with the development of adverse CVE. However, there is no comprehensive, pathological measure of carotid plaque instability that can be used to predict the risk of ischemic CVE. We performed this study in patients who underwent carotid endarterectomy (CEA) to describe in detail the pathology of carotid atherosclerosis and develop a diagnostic pathological scoring of unstable plaques. **Methods:** We analyzed data from 74 men and 8 women aged >30 years who, between May 2015 and August 2016, consecutively underwent CEA in the departments of neurosurgery of Nakamura Memorial Hospital, Kashiwaba Neurosurgical Hospital and Hokkaido Neurosurgical Memorial Hospital in Japan. The indications for surgery were >70% asymptomatic or symptomatic carotid artery stenosis. Patients were considered symptomatic if they had experienced an ischemic stroke or a TIA. Atherosclerotic plaques associated with symptoms were referred to as symptomatic or unstable plaques. The mean age of the 67 symptomatic patients was 73.2±6.9 years and that of the 15 asymptomatic patients was 71.3±7.6 years (NS). CEA was performed using standard surgical techniques with minimal handling of the specimens. The plaques were removed en bloc, fixed in 10% buffered formalin, transected transversely in 5-mm specimens, and embedded in paraffin. After hematoxylin-eosin and elastica-Masson (which stains elastin black and collagen and proteoglycans green) staining, 3-4 sections per specimen were examined. The sections with ulcerated plaques or thrombi, the most stenotic segments, or both, were retained for further analysis. The sections were examined by 2 independent observers, 1 of whom was an experienced histopathologist unaware of the clinical status and identity of the patient. The sections were probed with anti-CD31, anti-CD34 and PDGFRβ antibodies, which recognize endothelial cells, to confirm the presence of microvessels in the plaque. Because one of the characteristics of an unstable plaque is the presence of ≥25 macrophages per high-power (0.3-mm diameter) field (HPF), and because immunohistochemical staining has revealed that most of the inflammatory cells at the site of plaque rupture are macrophages, we used ≥25 inflammatory cells/HPF as a threshold for plaque instability. Furthermore, because some studies have shown that (1) matrix metalloproteinase-12 (MMP-12) promotes atherosclerosis, (2) the main source of MMP-12 is a foamy cap macrophage, and (3) in patients undergoing CEA, the proportion of MMP-12 macrophages is approximately 10% of all macrophages, we used ≥3 foamy or hemosiderin-laden macrophages/HPF as a threshold for plaque instability.

Cellular infiltration (i.e., ≥25 inflammatory cells/HPF) was scored semiquantitatively as absent (=0), limited to the fibrous cap or the shoulder of the cap (=1), or extending to the fibrous cap and the shoulder of the cap (=2) in >1 cross-sectional image of the lesion. Similarly, infiltration by ≥3 foamy or hemosiderin-laden macrophages/HPF was scored semiquantitatively as absent (=0), limited to the fibrous cap or the shoulder of the cap (=1), or abundant (=2) in the fibrous cap and the shoulder of the cap in ≥1 cross-sectional image of the lesion. The distribution of calcification was scored semiquantitatively as absent (0), 1-90° (1), 91-180° (2), or >180° (3) in ≥1 cross-sectional image of the lesion. Intraplaque hemorrhages were scored semiquantitatively as ≤1 (0), 2 (1), or ≥3 (2) cross-sections of the lesion. **Results:** The 82 patients enrolled in the study comprised 67 symptomatic and 15 asymptomatic patients. Plaques were resected from all patients and examined histopathologically. The clinical characteristics of the 67 symptomatic and 15 asymptomatic patients and the pathological characteristics of the lesions analyzed in each group are compared. By single-variable analysis, several pathological characteristics were significantly associated with symptomatic manifestations: plaque rupture, minimum TFC overlying a NC, microcalcifications in the fibrous cap, intraplaque hemorrhage, intraplaque microvessels, hemosiderin-laden macrophages within the cap and an extensive NC. The prevalence of plaque rupture was highest (73%) in patients presenting with stroke, significantly higher than in patients presenting with TIA (33%) or no symptoms (13%). In contrast, the prevalence of a calcified nodule was significantly higher in patients presenting with a TIA (25%) than in patients presenting with stroke (2%) or no symptom (0%). By multiple-variable logistic regression analysis, the presence of plaque rupture, minimum TFC, the presence of microcalcifications in the fibrous cap and intraplaque microvessels were independent correlates of symptomatic plaques. We used receiver-operating characteristic statistics to examine the diagnostic performance of these indices of plaque instability. Among all correlates of symptomatic cerebral ischemic event, minimum TFC had the highest diagnostic performance (area under the curve [AUC] 0.78, optimal cutoff value 70.2 μm, sensitivity 90%, specificity 60%, PPV 91%, NPV 56%, diagnostic accuracy 84%). However, combining the 4 independent correlates into a single score, using the equation derived from the multiple-variable logistic regression model (Logit(Score)=0.179+2.277* (insert 1 if plaque rupture present; otherwise 0)−0.355* (insert minimum TFC in multiples of 10 μm)+2.057* (insert 1 if microcalcification in the fibrous cap present; otherwise 0)+0.646* (insert intraplaque microvessels/mm^2), increased the diagnostic performance to an AUC of 0.92 (95% CI 0.85-0.99; optimal cutoff value 0.814; sensitivity 90%; specificity 87%; PPV 97%; NPV 65%; diagnostic accuracy 89%).**Discussion:** This pathological study is the first to show that the presence of microcalcifications in the fibrous cap is an important sign of symptomatic carotid plaque. Microcalcifications typically 10 μm in diameter within a TFC promote plaque rupture by increasing the local stress and causing interfacial debonding. Therefore, microcalcifications in the fibrous cap represent an additional factor of risk of carotid plaque instability. The individual and combined power of these plaque characteristics to measure plaque instability is incompletely understood. In this study, we combined these previously described individual characteristics of plaque instability into a single score. From our comprehensive pathological study and multiple-variable analysis, we identified the factors correlating with plaque instability. Among all the pathological indices, plaque rupture, minimum TFC, presence of microcalcifications and intraplaque microvessels were the only independent correlates of symptomatic plaque in this sample of patients with carotid artery stenosis. **Conclusions:** This pathological analysis of carotid artery plaques suggested that our diagnostic scoring may facilitate understanding of plaque instability. Pathological quantification by this scoring simplified the assessment of carotid artery plaques, instead of requiring the wide variety of pathological characteristics reflecting plaque instability.