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Elevated levels of neopterin are associated with carotid plaques with complex morphology in patients with stable angina pectoris

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ABSTRACT

Objective: Neopterin is an activation marker for monocytes/macrophages, and circulating levels of neopterin are elevated in patients with coronary complex lesions in unstable angina pectoris. We investigated the possible association between neopterin and complex carotid plaques which may be associated with the risk of ischemic stroke in patients with stable angina pectoris (SAP).
Methods: We measured plasma levels of neopterin in 102 patients with SAP and carotid ultrasound was performed for evaluation of the presence of carotid plaques and plaque surface characteristics categorized as complex or noncomplex. In addition, endarterectomy specimens of extracranial high-grade carotid stenosis with complex plaques from five patients with SAP were immunohistochemically examined with antibodies to smooth muscle cells, endothelial cells, platelets, macrophages, and T cells.
Results: Plasma neopterin levels were significantly higher in patients with complex carotid plaques than in those with noncomplex plaques (median [interquartile range]: 24.2 [19.2–39.3] nmol/L vs. 19.4 [11.9–25.1] nmol/L; $P = 0.01$) or without any plaques (18.8 [14.9–23.6] nmol/L; $P = 0.001$). On multivariate logistic analyses after adjustment for traditional atherosclerotic risk factors, multi-vessel coronary disease and high sensitivity C-reactive protein, neopterin levels were independently associated with the presence of complex carotid plaques (adjusted OR 2.21 per SD increase, 95%CI 1.13–4.33, $P = 0.02$). Immunohistochemical staining revealed abundant neopterin-positive macrophages in carotid complex lesions.
Conclusion: These findings demonstrate that carotid plaques with complex morphology have increased circulating neopterin levels and immunohistochemical localization of neopterin in patients with SAP. Neopterin can be considered an important biomarker of plaque destabilization in carotid artery atherosclerotic lesions in this population.

1. Introduction

Recent studies have shown that complex plaques in the carotid arteries, such as lesions exhibiting irregularity or ulcerated surface morphology, detected on carotid ultrasound independently predict ischemic stroke [1–4]. Although the reason for the relationship between complex carotid plaques and ischemic stroke is unclear in detail, complex carotid plaques may be not only a potential source of emboli but also a marker of generalized atherosclerosis and indicate systemic atherosclerotic instability [3,4]. Detection of carotid complex lesions by carotid ultrasound might thus help identify patients at high risk for ischemic stroke [3,4].

Inflammatory processes play roles in the pathogenesis of atherosclerotic plaque and its thrombotic complications. Monocyte/macrophage activation plays a significant role in the inflammatory process associated with atherosclerosis and plaque vulnerability [5]. Neopterin, a by-product of the guanosine triphosphate pathway, is produced by activated macrophages after stimulation by interferon-γ released by T cells, and is an activation marker for monocytes/macrophages [6,7]. The previous

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studies have reported that serum levels of neopterin are elevated in patients with unstable angina pectoris (UAP) compared with control subjects and patients with stable angina pectoris (SAP) [8,9]. Furthermore, several coronary angiographic studies have shown a relationship between increased neopterin levels and coronary complex lesions in patients with UAP [10,11]. Recently, our immunohistochemical study using coronary atherectomy specimens confirmed a significantly higher prevalence of neopterin-positive macrophages in culprit lesions in patients with UAP than in those with SAP [12].

On the other hand, even in patients with SAP, mean levels of serum neopterin have been shown to be higher than in healthy volunteers [9]. In this context, Schumacher et al. speculated that pronounced atherosclerosis in large vessels like the carotid artery might induce higher mean levels of neopterin in SAP patients [9]. We hypothesized that increased plasma neopterin levels in patients with SAP may be related to carotid plaque instability such as characteristic of complex plaques. In this study, we measured circulating plasma neopterin levels in patients with SAP and evaluated the relationship between plasma neopterin levels and carotid plaque surface morphology assessed by carotid ultrasound (group I). Additionally, we immunohistochemically examined the expression of neopterin in specimens of carotid plaque complex lesions obtained from SAP patients undergoing carotid endarterectomy (CEA) (group II).

2. Methods

2.1. Study patients: group I (plasma neopterin)

We enrolled 106 patients (81 men, mean age, 68 ± 10 years) with SAP who were admitted to Osaka City General Hospital to undergo coronary angiography. SAP was defined as effort-related angina without any change in clinical pattern in the preceding 2 months. All patients in the study had angiographically documented coronary disease and underwent carotid ultrasound. Patients were excluded from the study if they had previous carotid endarterectomy/angioplasty, or intercurrent inflammatory, infectious diseases and neoplastic diseases likely to be associated with an acute-phase response. We excluded four patients due to renal dysfunction (serum creatinine levels >1.2 mg/dL) because renal function is a major determinant of blood neopterin concentration [11].

For each study patient, clinical data and history regarding risk factors such as age, diabetes mellitus, hypertension, hypercholesterolemia, and smoking were obtained. Diabetes mellitus was determined by the presence of an existing diagnosis, fasting blood glucose >126 mg/dL, glycohemoglobinA1c >5.8%, or use of antidiabetic medication or insulin. Hypertension was defined as a systolic blood pressure >140 mmHg and a diastolic blood pressure >90 mmHg or use of antihypertensive medications. Hypercholesterolemia was determined by a serum cholesterol value of >220 mg/dL or by use of cholesterol-lowering medications. Patients were classified as nonsmokers if they had never smoked or if they had stopped smoking ≥1 year before the study. All other patients were classified as smokers. Glomerular filtration rate was estimated with the Modification of Diet in Renal Disease (MDRD) Study equation [13] modified by a Japanese coefficient [14]. The study protocol was approved by the hospital ethics committee, and written informed consent was obtained from each patient.

Coronary angiography was performed in all patients using standard techniques within a week of carotid ultrasound. In all patients, off-line quantitative coronary angiography was performed with the view revealing the highest degree of stenosis. Calculations were performed using the Cardiovascular Measurement System (CMS-MEDIS Medical Imaging System, Leiden, The Netherlands) by an investigator who was unaware of the study design. The number of diseased coronary vessels was scored as 1, 2 or 3 according to the number of major coronary vessels with luminal stenosis ≥70%, with left main stenosis ≥50% scored as 2-vessel coronary disease. Multi-vessel coronary disease was defined as 2- or 3-vessel coronary disease.

2.2. Assessment of carotid arteries

Carotid scans were acquired by high-resolution ultrasound (SSA-700A Aplio, Toshiba, Tochigi, Japan) with a 7.5 or 12 Hz linear array transducer. The common carotid arteries, bifurcation, and internal and external carotid arteries were examined bilaterally in all patients. Carotid plaque was defined as any focal protrusion into the lumen of a vessel for an intima-media thickness greater than 1.1 mm [1,15]. We evaluated the presence of carotid plaques and carotid plaque morphologic characteristics. The presence of an irregular surface [2,16] including ulcerations or mobile components was classified as complex plaque (Fig. 1). Interpretation of the carotid studies was performed by two experienced investigators unaware of the study design. Discrepancies were resolved by agreement between two.

2.3. Measurements of high sensitivity C-reactive protein and neopterin

Venous blood samples were obtained from all patients after an overnight fast. The following measurements were performed: high sensitivity C-reactive protein (hs-CRP) level, leukocyte count, neutrophil count, and plasma neopterin levels. Serum hs-CRP levels were assayed with the use of latex-enhanced immunonephelometric assays on a BN II analyzer (Dade Behring, Newark, DE, USA). Plasma neopterin levels were determined by the method described by Fukushima and Nixon [17] using high performance liquid chromatography with fluorimetric detection. Intra-assay coefficient of variation for the measurement of plasma neopterin levels was <6.3% and inter-assay coefficient of variation was <7.9%.

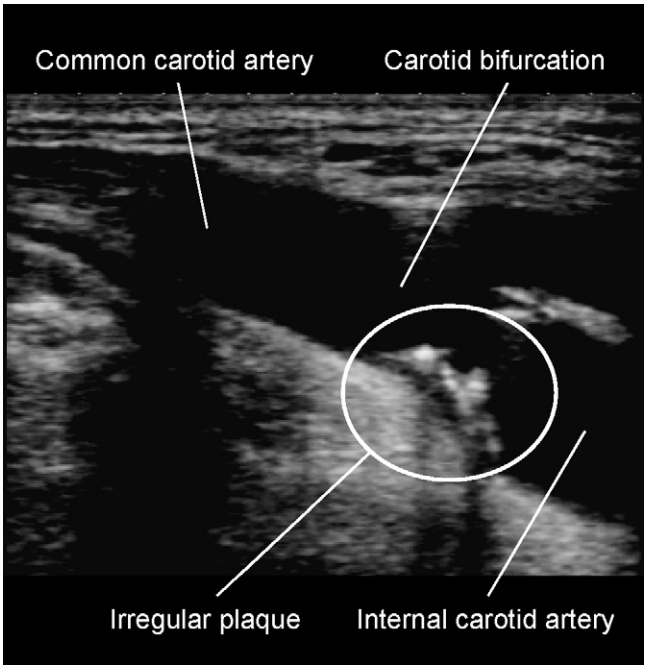

Fig. 1. Carotid ultrasound image of carotid complex plaque.