Inflammation plays a key role in atherosclerosis and plaque vulnerability, and monocyte/macrophage activation contributes to these processes. Neopterin, a by-product of the guanosine triphosphate pathway, is produced by activated macrophages on stimulation with interferon-γ released from T lymphocytes, and is an activation marker for monocytes/macrophages. Coronary angiographic studies have shown a relationship between increased circulating levels of neopterin and the presence of complex coronary lesions in patients with unstable angina pectoris (UAP). Furthermore, in an immunohistochemical study performed using coronary atherectomy specimens, a significantly higher prevalence of neopterin-positive macrophages was found in culprit lesions in patients with UAP than in those with stable angina pectoris (SAP). We recently clarified that the presence of complex carotid plaques detected by carotid ultrasound was related to increased circulating levels of neopterin, and immunohistochemical localization of neopterin was observed in complex carotid lesions obtained from carotid endarterectomy in patients with SAP. These findings suggest that neopterin is an important biomarker of plaque instability in both coronary and carotid atherosclerotic lesions.


Key words: Neopterin, Complex plaques, Coronary artery, Carotid artery

Introduction

Inflammatory processes play an important role in the pathogenesis of atherosclerotic plaque and its complications, and monocyte/macrophage activation contributes to the processes associated with atherosclerosis and plaque vulnerability. Neopterin is considered an activation marker of the monocyte/macrophage system in various inflammatory diseases. In vulnerable plaques, interferon-γ, which is released by activated T lymphocytes, stimulates macrophages to produce neopterin. So far, there is increasing evidence of an association between neopterin and plaque instability. In this review, we focus on the relationship between neopterin and plaque instability in coronary and carotid arteries, and also introduce the results of our recent studies.

Neopterin Synthesis and Inflammation

In inflammatory processes, activated T lymphocytes of the Th1 subtype produce interferon-γ, a molecule that reduces collagen synthesis and stimulates macrophages. Neopterin is produced by these activated macrophages on stimulation with interferon-γ via the guanosine triphosphate (GTP) pathway (Fig. 1). Inside macrophages, GTP-cyclohydrolase, one of the enzymes upregulated by interferon-γ, catalyses the breakdown of GTP to form 7, 8-dihydronopterin. 7, 8-Dihydronopterin can diffuse out of activated macrophages into the plasma and is oxidized to neopterin by HOCl released from neutrophils during inflammation. Although the exact role of

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neopterin synthesis and released by activated macrophages is unknown, several studies suggest that neopterin can act as a pro-oxidant, enhancing oxidant production, and promoting cell death or atherosclerotic plaque instability\textsuperscript{3, 21, 22}. Furthermore, because neopterin is biologically and chemically stable and relatively easy to measure by high-performance liquid chromatography (HPLC)\textsuperscript{3}, it has been investigated as a marker of immune cell activation in a wide range of diseases, such as infection\textsuperscript{23}, autoimmune disease\textsuperscript{24}, cancer\textsuperscript{25} and allograft rejection\textsuperscript{26}. Recently, assays for neopterin have been performed with a commercially available ELISA kit.

Neopterin and Plaque Instability in Coronary Arteries

Previous clinical studies have demonstrated that neopterin is associated with atherosclerotic plaque instability in coronary artery disease (CAD)\textsuperscript{6-17}. Circulating levels of neopterin have been reported to be higher in patients with acute coronary syndrome (ACS) than in control subjects and in patients with stable angina pectoris (SAP)\textsuperscript{6, 7}. Furthermore, recent studies have shown a direct association between neopterin and plaque instability in coronary arteries\textsuperscript{8-11} (Table 1). Several coronary angiographic studies have shown a relationship between increased neopterin levels and complex coronary lesions in patients with unstable angina pectoris (UAP)\textsuperscript{8, 9}; however, neopterin levels did not correlate with the number of diseased coronary vessels, suggesting that neopterin may be a marker of inflammatory CAD activity rather than a measure of the anatomical extent of the coronary atheromatous process\textsuperscript{8, 9}. Moreover, Zouridakis et al.\textsuperscript{10} have demonstrated that increased serum neopterin levels were associated with the rapid progression of CAD diagnosed by coronary angiography in patients with SAP and UAP.

To elucidate the role of neopterin in coronary plaque destabilization, we previously performed immunohistochemical analysis to detect neopterin in coronary atherectomy specimens obtained from 25 patients with SAP and 25 patients with UAP\textsuperscript{11}. In 22 of 25 patients with UAP, abundant neopterin-positive macrophages were found at the sites of coronary culprit lesions; however, in patients with SAP, only 11 of 25 lesions showed neopterin positivity. Additionally, we quantified the tissue area occupied by immunostained macrophages and neopterin using computer-
aided planimetry and expressed it as a percentage of the total surface area of the tissue section. On the basis of these quantifications, a “neopterin-positive macrophage score” was calculated as follows: neopterin-positive macrophage score = neopterin-positive area/macrophage-positive area. Quantitatively, the neopterin-positive macrophage score in patients with UAP was significantly higher than that in patients with SAP (p < 0.001). In addition, the neopterin-positive macrophage score was significantly higher in patients with angiographic complex lesions than in patients with angiographic smooth lesions (p < 0.01). Furthermore, the neopterin-positive macrophage score showed a significant positive correlation with the number of neutrophils or T lymphocytes, respectively (neutrophils, r = 0.55, p < 0.001; T lymphocytes, r = 0.70, p < 0.001).

Studies in vitro have shown that neopterin can enhance the oxidative potential of reactive oxygen species produced from immunocompetent cells. Neopterin has been shown to have significant effects on oxidized low-density lipoprotein (ox-LDL) formation. Previously, we have reported that plasma levels of ox-LDL related to the severity of ACS, and ox-LDL-positive macrophages were significantly higher in patients with UAP than in those with SAP in coronary atherectomy specimens. Moreover, our previous study demonstrated that infiltration of myeloperoxidase (MPO), a strong oxidant enzyme released from activated neutrophils, occurs in the culprit lesions of patients with UAP. In our immuno- histochemical analysis to detect neopterin in coronary atherectomy specimens, the neopterin-positive macrophage score showed a significant positive correlation with the number of MPO-positive neutrophils. In addition, it has been shown that the reaction generating neopterin from 7,8-dihydroneopterin is oxidation by HOCl, mainly produced by MPO from neutrophils. These findings suggest that the interactions between macrophage-derived neopterin and neutrophil-derived products, such as MPO and HOCl, may lead to plaque instability in coronary arteries; however, the precise molecular mechanisms remain unknown, and further studies are thus needed to confirm these potential associations.

### Neopterin and Plaque Instability in Carotid Arteries

Cerebral embolism from atherosclerotic plaques or thrombosis at the site of plaque rupture frequently causes ischemic stroke. Vascular ultrasound imaging, including carotid ultrasound and transesophageal echocardiography, can noninvasively identify several characteristic morphological features of atherosclerotic plaques. Complex plaques exhibiting irregular morphology, ulcerations and mobile components in the carotid arteries or aortic arch have been shown to be associated with the risk of stroke; therefore, plaque complexities assessed by vascular ultrasound should be considered in the evaluation of plaque destabilization.

On the other hand, recent studies have consistently shown that several inflammatory biomarkers are associated with plaque destabilization. Many large population-based studies have demonstrated that C-reactive protein (CRP), as a representative inflammatory marker, can predict future cardiovascular events. Lombardo et al. performed carotid ultra-

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**Table 1.** Summary of recent studies examining the direct relationship between neopterin and plaque instability

<table>
<thead>
<tr>
<th>Authors</th>
<th>Type of arteries</th>
<th>Method for evaluating plaque instability</th>
<th>N</th>
<th>Type of patients</th>
<th>Result</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garcia-Moll et al.</td>
<td>Coronary</td>
<td>Angiographic complex lesions</td>
<td>50</td>
<td>UAP</td>
<td>Multivariate β = 0.30*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Avanzas et al.</td>
<td>Coronary</td>
<td>Angiographic complex lesions</td>
<td>55</td>
<td>NSTE-ACS</td>
<td>Multivariate β = 0.48*</td>
<td>0.002</td>
</tr>
<tr>
<td>Zouridakis et al.</td>
<td>Coronary</td>
<td>Angiographic progression of stenosis</td>
<td>124</td>
<td>SAP</td>
<td>Multivariate OR = 5.5**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adachi et al.</td>
<td>Coronary</td>
<td>Angiographic complex lesions</td>
<td>50</td>
<td>SAP, UAP</td>
<td>SAP vs. UAP Smooth vs. complex</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sugioka et al.</td>
<td>Carotid</td>
<td>Complex plaques by US Immunohistochemical analysis</td>
<td>102</td>
<td>SAP</td>
<td>Multivariate OR = 2.2***</td>
<td>0.02</td>
</tr>
</tbody>
</table>

ACS = acute coronary syndrome; NSTE = non-ST segment elevation; OR = odds ratio; SAP = stable angina pectoris; UAP = unstable angina pectoris; US = ultrasound imaging. *β is the increment of the number of complex lesions with every unit of neopterin. **OR using the median of neopterin as the cutoff point. ***OR per SD increment of neopterin.
sound in patients scheduled for coronary bypass surgery and evaluated the relationship between serum levels of CRP and the presence of complex carotid plaques in these patients. In multivariate analysis, the presence of complex carotid plaques was independently associated with high sensitivity CRP (hs-CRP) >3 mg/L (p = 0.009) as well as UAP (p = 0.039); however, there are few reports on the relationship between neopterin and carotid atherosclerosis. Although Weiss et al. investigated the association between increased serum concentrations of neopterin and the extent of carotid atherosclerosis in a community-based study, there are no findings on the association between neopterin and characteristics of carotid plaques, which may be closely associated with the risk of stroke. We hypothesized that the increased plasma neopterin levels in patients with SAP may be related to carotid plaque instability, which is characteristic of complex plaques as well as coronary lesions. Our recent study was designed to assess this hypothesis.

The plasma levels of neopterin were measured, and carotid ultrasound was performed in 102 patients with SAP (1-vessel CAD, 62; multi-vessel CAD, 40). We evaluated the presence of carotid plaques and categorized the plaque surface characteristics as complex or noncomplex. Plaques with an irregular surface, including ulcerations or mobile components, were classified as complex plaques. There were no significant differences in the plasma neopterin levels (median interquartile range): 1-vessel CAD, 20.4 [14.9–29.0] nmol/L vs. multi-vessel CAD, 20.6 [14.7–29.1] nmol/L; p = 0.89) and hs-CRP levels (1-vessel CAD, 0.63 [0.54–1.4] mg/L vs. multi-vessel CAD, 0.87 [0.45–2.7] mg/L; p = 0.13) between patients with 1-vessel CAD and those with multi-vessel CAD. In the carotid ultrasound examination, at least 1 atherosclerotic plaque was detected in the carotid arteries in 77 of 102 patients (noncomplex plaques, 43; complex plaques, 34). The plasma neopterin levels in patients with complex plaques were significantly higher than in patients with noncomplex plaques or those without any plaques (complex plaques, 24.2 [19.2–39.3] nmol/L vs. noncomplex plaques, 19.4 [11.9–25.1]; p = 0.01 and vs. no plaques, 18.8 [14.9–23.6] nmol/L; p = 0.001); however, no difference was observed between the values for patients with noncomplex plaques and those without any plaques (p = 0.46). The frequency of detecting of complex plaques in the highest tertile of plasma neopterin levels (>24.2 nmol/L) was higher than in the intermediate (16.6–24.2 nmol/L) or lowest tertiles (<16.6 nmol/L) (56% vs. 26%; p = 0.04 and vs. 18%; p = 0.007). Univariate logistic analyses revealed that age (odds ratio [OR], 1.07 per year increase, 95% CI 1.02–1.13; p = 0.006), multi-vessel CAD (OR 2.84, 95% CI 1.21–6.64; p = 0.02), hs-CRP >3.0 mg/L (OR 2.35, 95% CI 1.15–6.40; p = 0.03) and plasma neopterin levels (OR 2.47 per SD increase, 95% CI 1.45–4.20; p < 0.001) were significantly associated with complex carotid plaques. In multivariate logistic analyses performed after adjusting for traditional atherosclerotic risk factors, multi-vessel CAD, and hs-CRP, increased plasma neopterin levels were found to be independently associated with the presence of complex plaques (adjusted OR 2.21 per SD increase, 95% CI 1.13–4.33; p = 0.02).

Further, we obtained endarterectomy specimens of extracranial high-grade carotid stenosis with complex plaques from 5 patients with SAP and performed immunohistochemical examination with antibodies to smooth muscle cells, endothelial cells, platelets, macrophages, and T lymphocytes. All 5 carotid atherosclerotic lesions exhibited distinct accumulation of macrophages and T lymphocytes, and 4 of the 5 carotid lesions exhibited platelet thrombi on the luminal surface. Moreover, all 5 carotid lesions contained abundant neopterin-positive cells. Double-immunostaining for neopterin and macrophages revealed that neopterin was strongly expressed in the vast majority of macrophages.

In conclusion, this study demonstrated that the presence of carotid plaques with complex morphology causes increased plasma neopterin levels and immunohistochemical localization of neopterin in patients with SAP. Neopterin can be considered an important biomarker of plaque destabilization in carotid atherosclerotic lesions in this population.

**Prognostic Values of Neopterin Associated with Plaque Instability**

There is increasing evidence indicating that neopterin is a significant predictor of coronary events and mortality. Representative studies focused on plaque instability and prognostic values of neopterin are shown in Table 2. Avanzas et al. carried out a 1-year follow-up prospective study in 297 patients with SAP who were undergoing diagnostic coronary angiography. In multiple regression analysis, neopterin (p = 0.021), the number of diseased coronary vessels (p = 0.001), and history of previous myocardial infarction (p = 0.009) were independent predictors of nonfatal myocardial infarction, UAP, and cardiac death from adverse events. In their study population, there was no significant difference between hs-CRP levels in patients with and without events. Additionally, serum neopterin levels in this study did not correlate with...
the severity or extent of CAD, confirming that neopterin is not a marker of the anatomical extent of the coronary atheromatous process but of the inflammatory activity of CAD. In 3946 ACS patients, Ray et al.\(^\text{15}\) assessed the relationship between the plasma neopterin levels and the risk of death and that between the plasma neopterin levels and death or acute coronary events (nonfatal myocardial infarction or UAP) over 2 years after the onset of ACS. The plasma neopterin levels were measured at hospital discharge (mean, 7 days) and at 4 months after an ACS. After multivariable adjustment for confounding coronary risk factors and hs-CRP levels, higher neopterin levels (≥12.11 nmol/L) both at discharge and at 4 months after an ACS event were associated with an increased risk of death (\(\text{p}=0.003\) and \(\text{p}=0.001\), respectively) and an increased risk of death or acute coronary events (\(\text{p}=0.006\) and \(\text{p}=0.001\), respectively). Kaski et al.\(^\text{15}\) demonstrated the relationship between plasma neopterin levels and adverse clinical outcomes in 397 Mediterranean patients with non-ST segment elevation ACS. Increased neopterin levels (>9.56 nmol/L) yielded a 76% adjusted increase of risk in the 180-day combined outcome. Finally, a recent population-based study by Vengen et al.\(^\text{17}\) has shown that neopterin levels can predict the risk of fatal CAD in 200 patients with diabetes mellitus.

The findings of our recent study\(^\text{18}\) suggest that elevated neopterin levels may be an important marker of the long-term risk of cerebrovascular events as well as coronary events. To date, however, there are few studies on the relationship between neopterin and ischemic stroke. Only a case-control study\(^\text{40}\) has evaluated the association of incident ischemic stroke with plasma levels of neopterin in a limited population of postmenopausal women, but the results showed borderline significance (\(\text{p}=0.05\)). Because there has been no prospective study and no data from men or premenopausal women, the association between neopterin levels and stroke risk should be confirmed in future studies.

### Limitation of Neopterin as a Biomarker for Cardiovascular Diseases

Increased neopterin production by inflammatory cells in coronary and carotid complex plaques may contribute to the higher circulating neopterin levels observed; however, whether neopterin is derived from complex plaques alone or from systemic atherosclerotic instability as well cannot be determined. As mentioned above, in addition to cardiovascular diseases, increased neopterin production occurs in non-cardiovascular diseases, such as infections\(^\text{23}\), autoimmune diseases\(^\text{24}\), cancer\(^\text{25}\) and allograft rejection\(^\text{26}\); there-

### Table 2. Summary of recent studies examining the prognostic values of neopterin associated with plaque instability

<table>
<thead>
<tr>
<th>Authors</th>
<th>Clinical events</th>
<th>Follow-up</th>
<th>N</th>
<th>Type of patients</th>
<th>Result</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garcia-Moll et al.(^\text{12})</td>
<td>UAP, AMI, cardiac death</td>
<td>1 year</td>
<td>114</td>
<td>Women with UAP</td>
<td>Multivariate HR = 8.6</td>
<td>0.013</td>
</tr>
<tr>
<td>Van Haelst et al.(^\text{13})</td>
<td>AMI, all-cause death</td>
<td>1 year</td>
<td>210</td>
<td>NQMI</td>
<td>Multivariate HR = 2.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Avanzas et al.(^\text{14})</td>
<td>UAP, AMI, cardiac death</td>
<td>1 year</td>
<td>297</td>
<td>SAP</td>
<td>Multivariate HR = 3</td>
<td>0.015</td>
</tr>
<tr>
<td>Kaski et al.(^\text{15})</td>
<td>UAP, AMI, cardiac death</td>
<td>6 months</td>
<td>397</td>
<td>NSTE-ACS</td>
<td>Multivariate HR = 1.76</td>
<td>0.04</td>
</tr>
<tr>
<td>Ray et al.(^\text{16})</td>
<td>All-cause death</td>
<td>2 years</td>
<td>3946</td>
<td>ACS</td>
<td>Multivariate HR = 1.86</td>
<td>0.003</td>
</tr>
<tr>
<td>Vengen et al.(^\text{17})</td>
<td>Fatal IHD</td>
<td>12.6 years</td>
<td>200</td>
<td>Diabetes mellitus</td>
<td>Multivariate HR = 2.59</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

ACS = acute coronary syndrome; AMI = acute myocardial infarction; HR = hazard ratio; IHD = ischemic heart disease; NQMI = non-Q wave myocardial infarction; NSTE = non-ST segment elevation; SAP = stable angina pectoris; UAP = unstable angina pectoris.
fore, it should be noted that increased neopterin production may occur in these non-cardiovascular diseases.

**Conclusion**

Recent studies have shown a close relationship between neopterin and plaque instability in coronary and carotid arteries; however, further studies may be required to clarify the precise role of neopterin in the pathogenesis of atherosclerotic plaque instability and in clinical practice. Furthermore, a combination of inflammatory biomarkers, such as neopterin and cardiovascular imaging, may contribute to the risk stratification of atherosclerosis, including coronary and cerebral events.

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**Conflicts of Interests**

None.

**References**