Neopterin: A potential marker in chronic peripheral arterial disease

SALVATORE SANTO SIGNORELLI¹, MASSIMILIANO ANZALDI¹, VALERIO FIORE¹, SAVERIO CANDIDO², ROBERTO DI MARCO², KATIA MANGANO², CINZIA QUATTROCCHI² and SERGIO NERI³

¹Department of Medical and Pediatric Science, Section of Internal and Vascular Medicine; Departments of ²Bio-Medical Sciences and ³Medical and Pediatric Science, Section of Internal Medicine, University of Catania, Catania I-95125, Italy

Received February 25, 2013; Accepted March 5, 2013

DOI: 10.3892/mmr.2013.1407

Abstract. Neopterin is a marker of macrophage activation that has exhibited high plasma levels in atherosclerotic diseases including coronary heart disease and critical limb ischemia. The role of neopterin in chronic peripheral arterial disease (PAD) has yet to be elucidated. In the present study, neopterin (N) serum concentrations were analyzed in asymptomatic (AsP) and symptomatic (SyP) patients with PAD as well as controls (C). In total 120 subjects, 40 AsP [ankle brachial index (ABI) ≤0.90], 40 SyP (ABI ≤0.90 plus pain in legs) and 40 controls (ABI >0.9) were enrolled. The results of the present study showed that neopterin plasma levels were statistically different among the groups. These findings demonstrated that activation of N-mediated monocyte-macrophage, was also observed in chronic PAD.

Introduction

Inflammation plays a key role in the atherosclerotic process (1-4) and experimental studies have shown the crucial role played by markers of the acute phase reaction, such as C-reactive protein (5,6), serum amyloid A (7-10) and fibrinogen in the atherosclerotic process. Activation of macrophages is a marker of chronic latent inflammation of the arterial wall, most likely as a result of the interaction between macrophages and oxidized lipoproteins. Activated macrophages are the principal source of proinflammatory cytokines such as IL-1β and TNF-α, and they contribute to the progression and instability of atherosclerotic plaques (12-14). Neopterin (N) is derived by specific monocyte-derived cells including macrophage-dendritic cells, and is considered to be a representative activation marker of macrophages on the stimulation of interferon γ (15). Elevated plasma levels of N were found in patients with coronary artery diseases including stable ischemic heart disease and acute coronary syndrome (16-18), and it was associated with the progression of coronary heart disease (19). N is considered an independent predictor of heart failure (19). However, a limited number of studies have focused on N in severe peripheral arterial disease (PAD) such as critical limb ischemia (21), whereas no study has focused on chronic PAD. PAD is considered a marker of the extent of the atheromatous process and it is known that PAD patients have a high risk of mortality for ischemic events (22-25). The present study focused on N plasma levels in symptomatic (SyP) and asymptomatic (AsP) PAD patients and compared them to the controls.

Materials and methods

Patients characteristics. In total, 120 subjects, attending an outpatient clinic of vascular medicine, were grouped as follows: 40 were SyP PAD patients, 40 were AsP patients and 40 were control subjects (C). SyP and AsP, as well as C patients were carefully selected to obtain a similar distribution of the main clinical parameters between the groups in order to reduce the impact of certain factors, such as diabetes, use of statins and smoking, on the serum concentrations of N (Table I). To diagnose PAD, the ankle brachial index (ABI) was taken as ≤0.9, 40 SyP (ABI ≤0.90 plus pain in legs) and 40 controls (ABI >0.9) were enrolled. The with in-coefficient of variability was <3% in the 7.5 nmol/l range.
Statistical analysis. The results are expressed as the means ± standard deviation (SD). The statistical analysis was performed using the ANOVA test and the Student's t-test to compare the values (mean ± SD) identified in the three groups. A correlation between the N and ABI values in patients with PAD was assessed by using the Pearson's Chi-square test or Fisher's exact test for categorical variables. A computerized statistical package (SPSS 10.1) for Windows was used. P<0.05 was considered to indicate a statistically significant difference.

Results

Mean plasma levels were higher in SyP (9.4±4.6 nmol/l) and AsP (7.4±4.0 nmol/l) patients as compared to C patients.

Table I. Clinical parameters of PAD patients (SyP, AsP and C).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sy PAD</th>
<th>As PAD</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>32M/8F</td>
<td>31M/9F</td>
<td>29M/11F</td>
</tr>
<tr>
<td>Age</td>
<td>66±7.1</td>
<td>64.5±8.3</td>
<td>65.3±7.2</td>
</tr>
<tr>
<td>Hypertension</td>
<td>62.5% (n=25)</td>
<td>52.5% (n=21)</td>
<td>55% (n=22)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>75% (n=30)</td>
<td>70% (n=28)</td>
<td>70% (n=28)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>50% (n=20)</td>
<td>45% (n=18)</td>
<td>42.5% (n=17)</td>
</tr>
<tr>
<td>Statins</td>
<td>40% (n=16)</td>
<td>35% (n=14)</td>
<td>35% (n=14)</td>
</tr>
<tr>
<td>Smoking</td>
<td>37.5% (n=15)</td>
<td>42.5% (n=17)</td>
<td>35% (n=14)</td>
</tr>
</tbody>
</table>

PAD, peripheral arterial disease; SyP, symptomatic; AsP, asymptomatic; C, controls.

Figure 1. Mean value of neopterin (N) and standard deviation in asymptomatic (AsP) and symptomatic (SyP) patients, as well as controls (C).

Figure 2. Inverse correlation observed between the ABI values and neopterin (N) concentration.
(5.3±3.2 nmol/l) (Fig. 1). Patients of the statistical analysis based on the ANOVA test demonstrated significant differences in N levels between the groups (P<0.0001). Moreover, an inverse correlation (Fig. 2) between the ABI values and the N concentration (r=−0.266, P<0.003) was observed.

Discussion

N is now considered a marker of macrophage activity in atherosclerosis. High serum levels of N were found in patients with chronic cardiac and coronary artery as well as acute coronary syndrome. A close correlation was observed between serum N concentration and angiographic evidence of multiple stenoses of coronary arteries in patients with stable angina (27). N is also considered a biomarker for atherosclerotic plaque instability both in coronary and carotid arteries (28). This biological product released from activated macrophages acts as a pro-oxidant (29,30). Consequently, N is crucial in the inflammatory process and pathophysiology of the atheromatous process as well as in cell death (28).

Concerning the peripheral arterial disorders, previously published data focused mainly on the high level of N in command-line interface (CLI) (20) and as previously reported (31) patients suffering from CLI have a poor diagnosis with regard to cardiovascular mortality. Those findings noted in the ischemic patients must be considered as relevant proof of the crucial pathophysiologic role played by the activated monocyte-macrophage.

The present study has focused on the effective role played by N in chronic AsP and SyP patients and the results showed higher blood concentrations of this marker compared to the C group. Our results must be considered useful in highlighting the activated process of macrophage cells involved both in severe or progressive stages of PAD as CLI, however, these cells are also deeply involved in chronic and stable PAD. Thus, we hypothesize that N is useful in identifying the activated process of macrophage cells in patients with chronic PAD. It is known that different biomarkers of inflammation, such as interleukins or metalloproteinases are elevated both in chronic and more severe PAD patients (32-38). Results of this study have shown the involvement of activated macrophages in chronic arterial disease of the lower limbs, albeit not symptomatic. This result may be associated with increased oxidative stress caused by chronic low blood perfusion. Furthermore, the inverse correlation between ABI values and levels of N demonstrates that there is a close association between the relative tissue ischemia as demonstrated by a lowered ABI value with the inflammatory process as marked by a higher plasma level of N. PAD patients suffer from relative ischemia as demonstrated by muscular effort when walking (e.g., intermittent claudication). Thus, a characteristic inflammatory pathway is present in the atherosclerotic diseases of coronary arteries and carotids, and of peripheral arteries (PAD).

In conclusion, neopterin is representative of the macrophage activation process and based on our findings, we hypothesize that the plasma level of N is useful to elicit the involvement of the activated macrophage, which, in turn, is able to promote oxidative stress. The plasma level of N may also be considered a new and diverse target for medical and interventional procedures in PAD patients. Therefore, new and original medical protocols are required to address this issue in order to protect against the inflammatory process, with N serving as a novel marker to monitor its efficacy.

References


1858

SIGNORELLI et al: NEOPTERIN IN PERIPHERAL ARTERIAL DISEASE