Leukocyte activation and inflammation in cardiovascular disease
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Elevated neopterin and C-reactive protein levels predict cardiovascular events in patients with non Q-wave myocardial infarction


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Inflammation is a hallmark of non Q-wave myocardial infarction. Monocytes are major contributors to plaque vulnerability. In the present study we aimed to determine if neopterin, a marker of monocyte/macrophage activity predicts recurrent cardiovascular events in patients with non Q-wave myocardial infarction (NQMI). Therefore we conducted a prospective cohort study in a subgroup of patients participating in a clinical trial. In 210 patients, admitted because of a NQMI, levels of neopterin and C-reactive protein (CRP) were determined. Follow-up duration was one year and a combined cardiovascular end point (death and recurrent myocardial infarction) was recorded. It was found that patients with elevated neopterin more often met the combined end point: 11 out of 64 (15.9%) vs. 9 out of 141 (6.4%). Also patients with C-reactive protein levels above 5 mg/L had more end points: 9 out of 54 (16.7%) vs. 12 out of 156 (7.1%). Patients with both inflammatory markers elevated had the highest risk for death or recurrent myocardial infarction: 5 out of 22 (22.7%) vs. 15 out of 188 (8.0%) with an adjusted RR of 4.9 (95% CI 1.4-17.3), P <0.01. We conclude that neopterin alone or in combination with CRP is predictive for an adverse outcome following a NQMI. Our findings suggest that activation of monocytes/macrophages in patients with NQMI is associated with an adverse outcome and requires special attention.

INTRODUCTION

Atherosclerosis is nowadays recognized as a chronic inflammatory disease. The acute coronary syndromes (ACS) e.g. unstable angina pectoris (UAP) and myocardial infarction are often the result of rupture of an unstable plaque in the coronary artery [1]. Monocytes play a crucial role in the pathogenesis of the acute coronary syndromes. The atheromatous plaque in ACS is characterised by the presence of T cells and a large amount of monocytes/macrophages in the so-called shoulder region of the plaque [2]. Activated monocytes/macrophages produce pro-inflammatory cytokines and matrix-degrading products such as matrix-metalloproteinases giving rise to an unstable, rupture prone plaque [3]. Activation of monocytes/macrophages may be the result of interferon-γ produced by activated T-lymphocytes and is reflected systemically by levels of neopterin [4]. Neopterin levels are elevated in patients with acute coronary syndromes [5] and are associated with more complex forms of coronary artery disease [6]. Systemic inflammatory activity, as reflected by levels of CRP, has proven to predict an adverse short-term outcome in patients with unstable angina and non Q-wave myocardial infarction (NQMI)[7].

The value of monocyte/macrophage activity as a prognostic factor in patients with NQMI has not yet been investigated. Moreover, only scarce data are available on the long-term predictive value of markers of inflammation in patients suffering from NQMI. The present study aimed to determine the predictive value of neopterin, alone and in combination with levels of C-reactive protein, on the recurrence of major clinical cardiovascular events in patients who suffered a NQMI.

MATERIALS AND METHODS

Patients
The present study was undertaken in patients with NQMI who participated in the FLuvastatin On Risk Diminishment after Acute myocardial infarction (FLORIDA) study. This multi-center study is described in detail elsewhere [8]. Figure 1 depicts the selection of the current study population. In brief, patients of either sex and at least 18 years of age, who were admitted to the coronary care unit for the diagnosis of myocardial infarction, were included in the study. Myocardial
Neopterin in non-Q-wave myocardial infarction was defined as an elevation of the myocardial band of creatinine phosphokinase (CK-MB) \( \geq \) two times the upper limit in combination with the presence of new of markedly increased chest pain lasting longer than 30 minutes. If CK-MB was not available, then CK levels \( \geq \) two times upper limit of the normal local laboratory range were permitted. A Q-wave myocardial infarction was defined as a new pathological Q wave of \( \geq 0.04 \) seconds duration or \( \geq 25\% \) of the corresponding R wave amplitude, or both in at least two contiguous leads combined with elevated CK-MB or CK levels as mentioned above. Consequently, patients with a MI who did not meet the ECG criteria of QMI were considered to have a NQMI. Main exclusion criteria were: use of lipid-lowering agents within the previous three months, triglyceride levels over 4.5 mmol/L, a scheduled percutaneous coronary intervention or coronary artery bypass operation and co-medication known to influence the ST-segment. All patients provided written informed consent. The medical ethical committee approved the study. The current study focused on the patients of the original cohort who suffered a NQMI and of whom a baseline blood sample was available.

**Laboratory assessments**

Assessments were performed in venous blood obtained within 48 hours from admission and one year after discharge. The samples had been stored at -20°C directly after sampling.

Levels of C-reactive protein were measured using a high-sensitivity C-reactive protein ELISA assay using the anti-CRP polyclonal antibodies A073 and P227, DAKO A/S (Glostrup, DK) as reported [9]. Both samples have been analyzed at the same time in duplicate using the same batch of reagents. Levels of neopterin were determined using a commercially available ELISA-kit (Neopterin ELISA, RE 593 21, IBL, Hamburg, Germany).

**Study endpoints**

For this study, the primary endpoint was a composite endpoint of all cause mortality and recurrent myocardial infarction. An Independent Monitoring Committee, blinded for treatment groups, reviewed all major clinical events.

**Statistics**

All randomized patients with available neopterin level assessments were included in the analysis of time to composite endpoint event. Continuous data are presented as mean and standard deviation (SD). Group comparisons were made using

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**Figure 1.** Flow chart of patient selection for current study

The grey boxes represent the studied population, QMI=Q-wave myocardial infarction, NQMI=non-Q-wave myocardial infarction.
Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>non Q-wave MI (N=210)</th>
<th>Total population (N=540)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years±SD)*</td>
<td>63±0.7</td>
<td>61±0.5</td>
</tr>
<tr>
<td>Male</td>
<td>82%</td>
<td>83%</td>
</tr>
<tr>
<td>Current smoker</td>
<td>49%</td>
<td>52%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>28%</td>
<td>24%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>10%</td>
<td>11%</td>
</tr>
<tr>
<td>Family history for CHD</td>
<td>43%</td>
<td>43%</td>
</tr>
<tr>
<td>Previous MI</td>
<td>13%</td>
<td>11%</td>
</tr>
</tbody>
</table>

Medication on admission
(before any intervention occurred)

<table>
<thead>
<tr>
<th></th>
<th>non Q-wave MI (N=210)</th>
<th>Total population (N=540)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylates</td>
<td>17%</td>
<td>16%</td>
</tr>
<tr>
<td>Beta blockers</td>
<td>17%</td>
<td>16%</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>7%</td>
<td>8%</td>
</tr>
<tr>
<td>long-acting nitrates</td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>calcium antagonists</td>
<td>10%</td>
<td>9%</td>
</tr>
</tbody>
</table>
| Therapeutic measures for MI
| Fibrinolysis             | 42%                    | 50%                      |
| PCI                      | 2%                     | 2%                       |
| Both                     | 0%                     | 1%                       |
| None                     | 55%                    | 47%                      |
| AMI related total CK (mmol/L±SEM)# | 1325±120               | 1676±90                  |

* P < 0.05, # P < 0.01, MI= myocardial infarction, ACE= angiotensin converting enzyme, PCI= percutaneous coronary intervention, CHD= coronary heart disease, SEM= standard error of the mean.

one-way analysis of variance if normally distributed, or by (Mann-Whitney) Wilcoxon's two sample test if the distribution was skewed. Categorical data are presented by percentage and count of each category. Group comparisons were made using Fisher exact or Chi-square tests. CRP and neopterin were treated as dichotomous variables. The cut off value for CRP was set at 5 mg/L according to previous reports [10]. Neopterin levels have not yet been evaluated in this setting, therefore, we choose to set the cut off value at the upper third tertile, which was 9.7 nmol/L. The associations between inflammatory parameters and relevant baseline characteristics were measured by the correlation coefficient. With normally distributed responses, the Pearson correlation coefficient was calculated. In case of a skewed distribution the Spearman rank-correlation coefficient was used (p-value Shapiro-Wilk test for normality < 0.05). Time-to-event analysis was performed using log rank tests and these data are presented as Kaplan-Meier survival curves. Adjusted relative risks (RR) and 95% CI) are given for the results of multivariate Cox regression analyses. Potential confounders, univariately significant at the level of P < 0.20 were included in the multivariate Cox regression analysis. A P-value of <0.05 was considered statistically significant. All analyses were performed using commercially available computer software (Statistical Analysis System version 6.12, SAS Institute, Cary, NC).

RESULTS

Patients
Blood samples from 210 patients who suffered a NQMI (figure 1) were available. Patient characteristics are given in table 1.

Correlations
Correlations of neopterin levels, CRP levels at baseline and after one year are displayed in table 2. There was a strong correlation between levels of neopterin at baseline and neopterin levels after one year. No such correlation was found for C-reactive protein.
Inflammation and cardiovascular events
Seven (10.1%) of 69 patients with neopterin levels in the upper tertile (> 9.7 nmol/L) died and 4 (5.8%) of 69 suffered a recurrent MI. Two (1.4%) of 141 patients with lower neopterin levels died and 7 (5.0%) of 141 patients suffered a recurrent MI. In patients with CRP levels above 5 mg/L, death occurred in 5/54 (9.3%) and 4/54 (7.4%) had a myocardial infarction as compared to 4/156 (2.6%) patients with CRP < 5 mg/L that died and 7/156 (4.5%) of these who suffered a recurrent myocardial infarction. There were 22 patients who had both elevated neopterin and CRP levels. Of these, 3 died (13.6%) and 2 (9.1%) had recurrent MI.

Table 2. Correlations of CRP and neopterin with clinical parameters

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neopterin baseline and CRP</td>
<td>0.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Neopterin baseline and</td>
<td>0.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>neopterin 1 year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP baseline and CRP 1 year</td>
<td>0.06</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

*Correlations are calculated as Spearman’s Correlation Coefficients. N.s.=not significant.

DISCUSSION
The major finding of this study is that neopterin, a marker for monocyte/macrophage activation is predictive for adverse events (death and recurrent myocardial infarction) in patients who suffered a non Q-wave myocardial infarction. When levels of CRP were added, the predictive value proved even stronger. Remarkably, and in contrast to

Figure 2. Survival curves of patients displaying different levels of inflammation

Multivariate analysis
A multivariate model was applied to calculate risk ratios corrected for possible confounding factors. When corrected for age, sex, smoking, previous myocardial infarction and therapeutic measures applied at baseline, the relative risk in patients with elevation of both inflammatory markers was 4.9 (95% confidence interval, 1.4-17.3, \( P = 0.01 \)). For patients with one of both markers elevated the risk ratio was 2.9 (95%CI 1.0-8.6), \( P <0.05 \), see figure 2.

The overall risk ratio for patients with one or both markers elevated as compared to patients with no elevated markers was 3.6 (95%CI 1.3-10.0), \( P =0.01 \).
CRP levels, we found a strong correlation between levels of neopterin at baseline and after one year.

Inflammation in acute coronary syndromes
It is nowadays accepted that prognosis in NQMI equals that in QMI [11]. However, there are still many unresolved issues with regard to the ideal treatment modality in many categories of patients with UAP, non ST elevation MI or NQMI [12]. Extensive research in recent years has led to the understanding that inflammation, as reflected by levels of several biomarkers, is a major determinant of short- and long term outcome in patients with unstable angina or NQMI [7;13-16]. Enhanced inflammatory activity is frequently encountered in these conditions and, often present at presentation, it is predictive for recurrent cardiovascular events [17]. It is hypothesized that a sustained inflammatory response in unstable angina and NQMI is present up to six months after the initial clinical event [18] and it was recently reported that it is probably the result of activity in the complete coronary vasculature instead of the site where the culprit lesion is located [19].

Monocyte/macrophage activation in the acute coronary syndromes
A central role in the pathogenesis of the acute coronary syndromes is attributed to monocytes/macrophages. By producing numerous pro-inflammatory mediators, activated monocytes/macrophages together with T lymphocytes give rise to the variety of changes finally resulting in the rupture-prone vulnerable plaque [1;20]. Regardless of the fact that these mechanisms have been extensively studied in vitro and in animal models, it is remarkable how little is known about monocyte/macrophage activation in vivo.

Neopterin, a pteridine derivative, is synthesised by monocytes/macrophages upon stimulation with interferon-γ, a cytokine originating mainly from activated T helper type 1-lymphocytes [4]. Neopterin levels are a useful tool to monitor monocyte/macrophage activation in atherosclerotic vascular disease [5;21; 22]. Recently, a small series of patients has been described, in which patients with adverse outcomes after an ACS had higher serum levels of neopterin shortly after the event as compared to patients without adverse events [23].

Interestingly, we found that there was a rather strong correlation between levels of neopterin determined within 48 hours from admission and levels of neopterin determined after one year. No such correlation could be found for levels of C-reactive protein, suggesting that neopterin reflects another inflammatory process than C-reactive protein. However, this issue remains speculative.

Study limitations
This study has some limitations. At first it is a post-hoc analysis in patients who participated in a clinical trial. Therefore, a selection bias in the patients studied cannot be ruled out, although clinical characteristics were comparable (table 1). The original trial was a placebo-controlled trial with a HMG CoA inhibitor in patients with cholesterol levels below 6.5 mmol/L. Importantly, the medication tested did not influence the occurrence of adverse events, nor did it affect levels of C-reactive protein or neopterin in this patient group. The latter is not surprising since levels of neopterin and CRP were taken shortly after the event and were repeated after one year. This implies that a rigorous ‘natural’ decrease in biomarkers will probably have disguised a possible treatment effect. Also, in our subset of patients, the use of medication or placebo was equal in both patients with elevated and inflammatory markers. No correlations with markers of inflammation and lipid levels were found.

Conclusions
In this study we demonstrate for the first time that monocyte/macrophage activation reflected by levels of neopterin is
predictive for adverse events in patients with NQMI. We confirm that elevated levels of C-reactive protein predict adverse events in the same patient group. When combined, the two markers of inflammation were even stronger predictors for adverse outcome following NQMI. Our findings demonstrate that monocyte/macrophage activity is an important determinant of morbidity and mortality in patients suffering from NQMI. Therefore, we feel that more aggressive therapeutic measures should be instituted in patients with NQMI with evidence for monocyte/macrophage activity. We speculate that activated monocytes may prove a future therapeutic target in patients with UAP and NQMI.

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