Increased serum neopterin: a marker of coronary artery disease activity in women

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Abstract

Objective—To assess whether neopterin concentrations in women with unstable angina differ from those in women with chronic stable angina.

Design—Prospective cohort study.

Setting—University hospital in south west London.

Patients—114 consecutive women with angina were studied: 82 had chronic stable angina (typical exertional chest pain, positive exercise ECG testing, and/or abnormal myocardial scintigraphy; symptoms stable for at least three months), and 32 had unstable angina (Braunwald class III). All patients with chronic stable angina (100%) and 18 with unstable angina (56.3%) underwent digital coronary angiography; neopterin concentrations were determined using a commercially available immunoassay.

Main outcome measures—Major clinical events during one year follow up were readmission with Braunwald's class IIIb unstable angina, non-fatal myocardial infarction, and cardiac death.

Results—Major events occurred in 12 women with chronic stable angina (14.6%) and nine women with unstable angina (28.1%). Mean (range) neopterin concentrations were significantly higher in women with unstable angina than in those with chronic stable angina (7.6 (5.1–11.5) nmol/l *v* 5.9 (4.4–7.5) nmol/l; *p* = 0.003), even after adjustment for variables which were significantly different on univariate analysis. In women with chronic stable angina, baseline neopterin concentrations were higher in those with cardiac events than in those without events (7.1 (5.9–9.1) nmol/l *v* 5.7 (3.9–7.3) nmol/l; *p* = 0.010), even after adjustment for variables with significant differences between both groups on univariate analysis.

Conclusions—On average, women with unstable angina had significantly higher neopterin concentrations than women with chronic stable angina. Women with chronic stable angina with events during follow up had higher neopterin concentrations than those without events. Neopterin concentrations were similar in patients with unstable angina and women with chronic stable angina who developed events. Neopterin concentrations may therefore be a marker of risk in women with coronary artery disease.

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Keywords: neopterin; women; coronary artery disease

Inflammation plays a key role in the pathogenesis of atherosclerosis.¹ Inflammatory mechanisms in the atherosclerotic plaque can be triggered, maintained, and enhanced by multiple factors, such as oxidised low density lipoprotein (LDL), increased concentration of superoxide species, activated macrophages and lymphocytes, and increases in interleukin 1, interleukin 6, interferon gamma (IFN γ), and Lp(a) lipoprotein.² There is increasing evidence that inflammation plays an important role in atherogenesis and may determine plaque vulnerability.¹ Recent observations suggest that vulnerable atherosclerotic plaques have an increased number of both macrophages and activated lymphocytes.³ Activated lymphocytes in atherosclerotic plaques produce a variety of cytokines such as IFN γ , a molecule that activates macrophages. Activated macrophages synthesise and release metalloproteinases,^{4,5} inducing an imbalance between synthesis and degradation of matrix components in areas of active inflammation within atherosclerotic plaques ("vulnerable sites"). Macrophages activated by IFN γ also synthesise neopterin, a pteridine derivative that has been used as an immune marker.⁶ Neopterin modulates the intracellular redox

state, probably through stimulation of the activity of both constitutive and inducible nitric oxide (NO) synthase.^{7,8} As a consequence of the modification of the redox state, neopterin activates the translocation of nuclear factor κ B (NF κ B) subunits to the nucleus,⁹ which in turn upregulates proinflammatory genes such as interleukin 6 and tumour necrosis factor α (TNF α).¹⁰ The overall result is an increase of the inflammatory tone within the vascular wall.¹¹

Studies have reported an association between neopterin and atherosclerosis.^{12,13} Taztbi and colleagues showed that neopterin concentrations were higher in patients with peripheral vascular occlusions,¹² and Weiss and colleagues showed that neopterin correlated with the extent of carotid atherosclerosis.¹³

There is little information about neopterin and coronary artery disease. To date only three reports have been published showing that neopterin is increased in patients with acute coronary syndromes compared to those with stable angina.^{14–16} These studies included a very limited number of women; overall there were only four women with myocardial infarction, 13 with either non-Q wave myocardial infarction or unstable angina, 12 with chronic stable angina, and 12 healthy women.

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Table 1 Baseline characteristics of 82 consecutive chronic stable angina female patients and 32 consecutive unstable angina female patients

	Chronic stable angina (n = 82)	Unstable angina (n = 32)	p Value
Demographics			
Age (years)	65.46 (8.83)	64.22 (12.26)	0.60
Diastolic blood pressure (mm Hg)	81.85 (10.70)	78.13 (12.10)	0.11
Systolic blood pressure (mm Hg)	139.14 (23.05)	142.19 (17.55)	0.50
Body mass index (kg/m ²)	27.32 (4.42)	25.83 (4.25)	0.16
Menopausal women, n (%)	76 (92.7)	29 (93.5)	0.87
Risk factors			
Diabetes mellitus, n (%)	4 (4.9)	4 (12.5)	0.22
Dislipidaemia, n (%)	46 (56.1)	13 (40.63)	0.14
Hypertension, n (%)	35 (42.7)	19 (59.4)	0.11
Peripheral vascular disease, n (%)	10 (12.2)	1 (3.1)	0.18
Previous myocardial infarction, n (%)	5 (6.1)	9 (29.0)	0.001
Smoking, n (%)	0 (0)	3 (9.4)	0.021
Angiography			
0 vessel disease, n (%)	25 (30.5)	6 (33.3)	
1 vessel disease, n (%)	31 (37.8)	4 (22.2)	
2 vessel disease, n (%)	16 (19.5)	4 (22.2)	
3 vessel disease, n (%)	10 (12.2)	4 (22.2)	0.46
Left ventricular ejection fraction (%)	68.22 (9.18)	63.93 (9.23)	0.10
Biochemistry			
Total cholesterol (mmol/l)	6.40 (1.30)	6.00 (1.26)	0.15
LDL cholesterol (mmol/l)	4.48 (1.30)	3.61 (0.92)	0.005
HDL cholesterol (mmol/l)	1.06 (0.27)	1.46 (0.34)	< 0.0005
LDL:HDL ratio	4.61 (2.21)	2.54 (0.71)	< 0.0005
Triglycerides (mmol/l)	1.79 (1.32)	2.17 (1.72)	0.32
Neopterin (nmol/l)	5.85 (4.40–7.50)	7.58 (5.14–11.50)	0.003
C reactive protein (mg/l)	3.30 (1.30–5.80)	7.64 (4.37–20.70)	< 0.0005
Baseline treatment			
Aspirin, n (%)	58 (70.7)	15 (46.9)	0.017
ACE inhibitor, n (%)	15 (18.3)	8 (25.0)	0.42
Lipid lowering, n (%)	19 (23.2)	6 (18.8)	0.61
Hormone replacement therapy, n (%)	17 (21.3)	2 (6.9)	0.094

Quantitative variables are expressed as mean (SD) or median (interquartile interval); qualitative variables are expressed as n (%); ACE, angiotensin converting enzyme.

Our study aimed to assess prospectively neopterin concentrations and their relation to coronary disease severity and coronary events in women with unstable angina and chronic stable angina pectoris.

Methods

PATIENTS

We studied 114 consecutive women admitted to our institution for the assessment of angina chest pain. Eighty two had chronic stable angina and 32 unstable angina. Stable angina was defined as typical exertional chest pain relieved by rest, glyceryl trinitrate administration, or both, with positive responses to exercise ECG stress testing, abnormal myocardial perfusion scintigraphy, or both. In all patients symptoms were stable for at least three months before study entry. None of the patients in this group had experienced a recent (< 12 weeks) myocardial infarction, previous coronary angioplasty or coronary bypass surgery, malignant arrhythmias, cardiac valve disease, acute or chronic liver disease, renal failure, inflammatory diseases, or other severe conditions likely to cause death within one year. Unstable angina was defined according to Braunwald,¹⁷ and only those individuals with class III unstable angina were included in the study. Thus, all unstable angina patients had angina at rest during the last 48 hours before admission and all had diagnostic ST segment changes, T wave, or both, and negative cardiac enzymes. These patients formed part of a larger cohort of 38 consecutive unstable angina women; five patients who developed Q wave myocardial infarction (as assessed by ECG criteria and raised creatine kinase (CK) and

CK-MB concentrations during the first 48 hours of admission) and one patient with hypertrophic cardiomyopathy were excluded. No women included in the study had ongoing systemic or cardiac inflammatory processes. All patients in the chronic stable angina group and 18 (56.3%) in the unstable angina group underwent coronary arteriography. The patients' baseline clinical and angiographic characteristics at initial presentation are summarised in table 1. All patients gave written informed consent before study entry and the study was approved by the local ethics committee.

FOLLOW UP AND STUDY END POINTS

After recruitment and baseline characterisation, patients were followed up for one year. Major clinical events during follow up, considered to represent study end points, were: readmission to the coronary care unit (CCU) with Braunwald's class IIIb unstable angina; non-fatal myocardial infarction, defined according to World Health Organisation criteria¹⁸ as raised cardiac enzymes, characteristic ECG changes, and prolonged typical chest pain; and cardiac death.

NEOPTERIN MEASUREMENTS

Neopterin serum concentrations were determined using a commercially available immunoassay (Elisa Kit, IBL, Hamburg, Germany). Limit of detection is 1.5 nmol/l. The within coefficient of variability in the 7.7 nmol/l range was below 3%, and was below 4% in the 20 nmol/l range. All other biochemistry measurements were carried out by the analytical unit of the biochemistry department of our institution using standard methods.

ANGIOGRAPHIC ANALYSIS

Coronary angiography was carried out according to the Judkins technique, and images of the coronary tree were obtained in routine standardised projections with the digital Phillips Integris 3000 system (Phillips, Holland) in all patients. Two experienced cardiologists, unaware of the patients' clinical characteristics and biochemical results, visually reviewed all angiographic images. The number of diseased coronary vessels was based on the number of coronary arteries showing stenosis of $\geq 75\%$ reduction in lumen diameter. When discrepancies arose, a third experienced observer was involved and the lesion classified by consensus.

STATISTICAL ANALYSIS

Results for normally distributed continuous variables are expressed as mean (SD), and continuous variables with non-normal distribution are presented as median (interquartile interval). Continuous variables were analysed using unpaired *t* tests and Mann-Whitney U tests where appropriate. The Spearman two way test was used to assess the correlation between two quantitative variables with non-normal distribution. Pearson's two way test was used to assess the correlation between two quantitative variables with normal distributions. As renal function is a major determinant

Table 2 Baseline characteristics of 82 chronic stable angina female patients with and without events during follow up

	No events (n = 71)	Events (n = 11)	p Value
Demographics			
Age (years)	65.1 (8.3)	68.0 (12.3)	0.46
Systolic blood pressure (mm Hg)	140.5 (23.3)	129.5 (19.8)	0.16
Diastolic blood pressure (mm Hg)	82.5 (10.3)	77.5 (13.2)	0.17
Body mass index (kg/m ²)	27.3 (4.5)	27.4 (4.4)	0.96
Menopausal women, n (%)	66 (93.0)	10 (90.9)	1.00
Risk factors			
Diabetes mellitus, n (%)	3 (4.2)	1 (9.1)	0.44
Dislipidaemia, n (%)	40 (56.3)	6 (54.6)	0.99
Hypertension, n (%)	29 (40.8)	6 (54.5)	0.39
Peripheral vascular disease, n (%)	9 (12.7)	1 (9.1)	1.00
Previous myocardial infarction, n (%)	3 (4.2)	2 (18.2)	0.13
Active smoking, n (%)	36 (50.7)	3 (27.3)	0.20
Baseline treatment			
Nitrates, n (%)	36 (50.7)	8 (72.7)	0.21
Aspirin, n (%)	50 (70.4)	8 (72.7)	1.00
β blocker, n (%)	43 (60.6)	6 (54.5)	0.71
ACE inhibitor, n (%)	12 (16.9)	3 (27.3)	0.41
Lipid lowering, n (%)	15 (21.1)	4 (36.4)	0.27
Diuretic, n (%)	17 (23.9)	5 (45.5)	0.13
Hormone replacement therapy, n (%)	17 (24.6)	0	0.11
Coronary angiography			
0 vessel disease, n (%)	25 (35.2)	0	
1 vessel disease, n (%)	26 (36.6)	5 (45.5)	
2 vessel disease, n (%)	13 (18.3)	3 (27.3)	
3 vessel disease, n (%)	7 (9.9)	3 (27.3)	0.014
Left ventricular ejection fraction (%)	69.1 (7.4)	62.5 (16.0)	0.20
Biochemistry			
Total cholesterol (mmol/l)	6.40 (1.25)	6.39 (1.40)	0.99
Triglycerides (mmol/l)	1.40 [1.10–2.00]	1.70 [1.15–3.15]	0.010
HDL cholesterol (mmol/l)	1.07 (0.27)	1.05 (0.27)	0.83
LDL cholesterol (mmol/l)	4.55 (1.28)	4.09 (1.39)	0.27
LDL/HDL ratio	4.69 (2.30)	4.11 (1.45)	0.43
Neopterin (nmol/l)	5.70 (3.90–7.25)	7.10 (6.95–9.10)	0.023*
C reactive protein (mg/l)	2.90 (1.25–5.65)	4.60 (3.20–9.90)	0.19
Haematology			
Whole blood cell count (× 10 ⁹ /l)	7.36 (1.48)	7.50 (1.08)	0.84

*Corrected by creatinine; quantitative variables are expressed as mean (SD) or median (interquartile interval); qualitative variables are expressed as n (%).

of neopterin concentrations in blood,¹⁹ neopterin concentrations have been adjusted for creatinine concentrations when correlated with other variables. Both neopterin and C reactive protein (CRP) were log transformed before being used in multiple regression as they had skewed distributions. Multiple regression analysis was used to obtain the adjusted independent contribution of the anginal type on both neopterin and CRP. Creatinine was included in the multiple regression analysis where the dependent variable was neopterin. Other independent variables introduced in multiple analysis were those with $p < 0.20$ on univariate analysis between women with unstable angina or chronic stable angina, and between chronic stable angina women with or without events. Logistic regression analysis was used to obtain unadjusted and adjusted risk assessment of the prognostic value of neopterin. Differences were considered to be significant if the null hypothesis could be rejected with $> 95\%$ confidence. The SPSS 7.0 statistical software package was used for all calculations.

Results

BASILINE CHARACTERISTICS OF WOMEN WITH UNSTABLE AND CHRONIC STABLE ANGINA

Baseline characteristics of women included in this study are shown in table 1. Women with unstable angina were more likely to have a positive history of previous myocardial infarction, and they also had a higher prevalence of smoking.

Figure 1 Neopterin concentrations in 82 women with chronic stable angina (11 events) and 32 women with unstable angina (nine events).

Baseline characteristics of women with chronic stable angina with and without events are shown in table 2. As in the unstable angina group, women with chronic stable angina with events were more likely to have a previous history of myocardial infarction. They also had more severe vessel disease, higher plasma triglycerides, and higher concentrations of neopterin.

Neopterin was not found to be associated with white blood cell count ($p = 0.94$), or the number of diseased coronary vessels ($r = 0.07$, $p = 0.52$).

NEOPTERIN AND TYPE OF ANGINA

Neopterin concentrations were significantly higher in patients with unstable angina than in those with stable angina ($p = 0.003$, table 1, fig 1). After adjustment for variables with $p < 0.20$ between groups of patients in the univariate analysis—that is, history of hypertension, prior myocardial infarction, body mass index, peripheral vessel disease, active smoking, left ventricular ejection fraction, total cholesterol, LDL:high density lipoprotein (HDL) ratio, aspirin treatment, and hormone replacement treatment—neopterin remained significantly higher in women with unstable angina than in those with stable angina (mean difference 4.50 nmol/l, 95% confidence interval (CI) 1.11 to 7.90 nmol/l; $p = 0.010$). CRP concentrations were also higher in women with unstable angina ($p < 0.0005$, table 1), and the difference remained significant after adjustment for the same variables (mean difference 2.89 mg/l, 95% CI 0.89 to 7.04 mg/l; $p < 0.0005$).

NEOPTERIN AND EVENTS IN STABLE ANGINA PATIENTS

Neopterin concentrations in women with chronic stable angina with cardiac events during follow up were higher than in those without events ($p = 0.036$, table 2, fig 1).

Table 3 Neopterin tertiles in 82 women with chronic stable angina

	Tertile 1	Tertile 2	Tertile 3	<i>p</i> for trends
No events	26	25	20	
Events, n (intraterile %)	2 (7)	1 (4)	8 (29)	0.019

When adjusted for variables that were different between both groups of patients at univariate analysis, neopterin concentrations remained significantly higher in women with events (mean difference 1.55, 95% CI 1.06 to 2.27; $p = 0.023$).

We observed an increased incidence of cardiac events with every increase in neopterin tertile distribution (table 3). After adjustment for other variables (see above), neopterin tertile distribution remained the independent variable most closely related to the incidence of events during follow up, together with the number of diseased vessels. Each increase in both neopterin tertile and the number of diseased coronary vessels yielded a similar increase in the risk of having a coronary event (3.0 fold, 95% CI 1.04 to 9.10 fold, $p = 0.041$; and 2.9 fold, 95% CI 1.26 to 6.59 fold, $p = 0.006$, respectively).

As events were observed mainly in the third tertile, we established a cut off point in the lower limit of the third tertile (neopterin 6.90 nmol/l). Patients above this neopterin serum concentration had a cardiovascular risk 2.5 fold higher than otherwise (95% CI 1.63 to 3.91 fold; $p = 0.0027$). When adjusting for variables that were different between women with and without events during follow up, the risk remained significantly higher (8.6, 95% CI 1.44 to 51.28; $p = 0.013$).

Discussion

Our study showed that, consistent with previous studies in men, concentrations of neopterin were significantly higher in women with unstable angina than in women with chronic stable angina. Importantly, we also observed that among women with chronic stable angina, those with cardiac events during follow up had significantly higher neopterin concentrations than those without events. Baseline neopterin concentrations were similar in women with unstable angina and in those with chronic stable angina who had events during follow up, suggesting that neopterin may be a valid marker of atherosclerotic disease activity in women. This is the first report to assess prospectively and systematically neopterin concentrations in women with coronary artery disease.

The results of the present study expand previous studies from our group¹⁴ and others¹⁵ which showed that neopterin is raised in patients with unstable angina and with acute myocardial infarction compared to patients with remote myocardial infarction and normal controls. Consistent with findings by Schumacher and colleagues,¹⁵ serum neopterin concentrations in our study did not correlate with the number of diseased coronary vessels. This marker was associated with coronary events during follow up, however.

There is growing evidence that inflammation markers are raised in unstable angina. CRP has been shown to be increased in acute coronary syndromes compared to controls or to stable patients.²⁰ Indeed, we observed that women with unstable angina have higher concentrations of both neopterin and CRP than women with stable angina, despite adjusting for confounding variables. Moreover, we found that neopterin concentrations were higher among women with chronic stable angina who develop coronary events during follow up than those without events, suggesting that disease "activity" is an important factor influencing neopterin serum concentration. This finding that a marker of inflammation or immune activation is raised in patients with stable angina with disease progression is consistent with previous reports that disease activity may be present despite clinical stability.²¹

Plaque vulnerability is a function of the increased number of macrophages and activated lymphocytes.⁵ Vulnerable coronary plaques have more extensive macrophage rich areas than stable plaques. These macrophages appear to play a key role in weakening the fibrous cap of atherosclerotic plaques by secreting proteases which contribute to the "active" phenomenon of plaque disruption.²² Vulnerable plaques also have activated T cells that produce the cytokine IFN γ and activate the macrophages.^{23 24} Importantly, IFN γ stimulated macrophages produce neopterin.⁶ The results of our study lend further support to the notion that inflammatory processes and immune activation may play a pathogenic role in acute coronary syndromes. In our study, no relation was found between white blood cell and monocyte count and neopterin concentration. This finding is not surprising as neopterin production is more likely to be an expression of monocyte activation rather than of increased monocyte number.⁶ Neopterin concentrations are most probably the result of immune activation related to the atherogenic process as well as the inflammatory mechanisms that lead to acute coronary events.

A possible explanation for the prognostic value of neopterin as observed in our study and others^{14 15} is that neopterin is a marker of coronary disease activity. Neopterin affects intracellular redox state and has been shown to be involved in the activation of both constitutive and inducible NO synthase.^{7 8} As a result, neopterin stimulates NF κ B translocation to the nucleus.²⁵ This nuclear factor has been associated with a variety of chronic inflammatory conditions,¹⁰ including coronary artery disease.²⁶ When activated, it promotes the expression of proinflammatory genes such as interleukins 1, 6, and 8, IFN γ , tumour necrosis factor, macrophage chemotactic protein 1, colony stimulating factors, c-myc, vascular cell adhesion molecule 1, intercellular adhesion molecule 1, E selectin, tissue factor, and other cell survival factors.^{27 28}

Previously, neopterin has been used as a marker of the stimulation of the cellular immune system. Our finding that neopterin is higher in unstable syndromes and in women

with stable angina with worse outcome is consistent with previous reports, which have shown that acute coronary syndromes are associated inflammatory mechanisms.^{23 24 29}

CONCLUSION

The present data extend prior observations concerning the potential clinical use of neopterin as a marker for disease activity in women with coronary artery disease. Chronic activation of immune cells may play a role in atherogenesis, atherosclerosis activity, or both. Our results confirm and expand the notion that acute coronary syndromes involve an activation of the immune system,³⁰ and that neopterin serum concentration may be a marker of disease activity³¹ rather than a measure of the anatomical extent of coronary artery disease. Studies are needed to ascertain the clinical usefulness of neopterin measurements as a marker of cardiovascular risk.

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