Macrophage Activation and Coronary Atherosclerosis in Systemic Lupus Erythematosus and Rheumatoid Arthritis

Young Hee Rho, MD, PhD[1], **Joseph Solus, PhD**[2], **Paolo Raggi, MD**[3], **Annette Oeser, BS**[1], **Tebeb Gebretsadik, MPH**[4], **Ayumi Shintani, PhD, MPH**[4], and **C. Michael Stein, MBChB**[1]

[1]Divisions of Clinical Pharmacology and Rheumatology, Departments of Medicine and Pharmacology, Vanderbilt University, Nashville, Tennessee

[2]Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee

[3]Division of Cardiology, Emory University, Atlanta, Georgia

[4]Department of Biostatistics, School of Medicine, Vanderbilt University, Nashville, Tennessee

Abstract

Objectives—Activation of macrophages may contribute to increased atherosclerosis and coronary artery disease in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Neopterin, a pteridine derivative, is a novel marker of monocyte and macrophage activation that is associated with atherosclerosis and cardiovascular risk in the general population. We examined the hypothesis that macrophage activation is associated with accelerated atherosclerosis in SLE and RA.

Methods—We compared serum neopterin concentrations, adjusted for age, race, sex and serum creatinine concentration, in patients with SLE (n=148), RA (n=166) and control subjects (n=177). In patients with SLE or RA, serum neopterin concentrations were then tested for association (adjusted for age, race, sex, serum creatinine and medication use) with measures of disease activity or damage, inflammatory markers and mediators, and coronary artery calcium measured by electron beam computed tomography.

Results—Neopterin concentrations were significantly higher in patients with SLE (median 8.0, IQR [6.5–9.8] nmol/l) and RA (6.7[5.3–8.9] nmol/l) than controls (5.7[4.8–7.1] nmol/l), and were higher in SLE than RA (all p<0.001). In SLE, neopterin was significantly correlated with higher ESR (p=0.001), TNF-α (p<0.001), MCP-1 (p=0.005) and homocysteine concentrations (p=0.01), but in RA only with ESR (p=0.01). Neopterin was not associated with coronary calcium in either SLE (p=0.65) or RA (p=0.21).

Conclusion—Macrophage activation, reflected by increased serum neopterin concentrations, was increased in both SLE and RA. Neopterin was more robustly associated with atherogenic mediators of inflammation and homocysteine in SLE than RA but was not associated with coronary atherosclerosis in either disease.

Keywords

Systemic Lupus Erythematosus; Rheumatoid Arthritis; Neopterin; Homocysteine; Atherosclerosis

Correspondence to: C. Michael Stein, MBChB, 560 RRB, Department of Clinical Pharmacology, School of Medicine, Vanderbilt University, 23rd Ave. S at Pierce Avenue, Nashville, TN, 37232-6602, michael.stein@vanderbilt.edu, Tel: 615-936-3420, FAX: 615-936-2746.

Introduction

Morbidity and mortality due to coronary artery disease are increased in both systemic lupus erythematosus (SLE) (1) and rheumatoid arthritis (RA) (2). The mechanisms underlying these increases are unclear, but inflammation is thought to play a role. The involvement of macrophages in the atherosclerosis associated with SLE and RA has not been studied extensively, but is important since monocytes and macrophages play a key role in the early pathogenesis of atherosclerosis by ingesting oxidized LDL, transforming into foam cells, and recruiting additional monocytes and macrophages to the vessel wall (3).

Neopterin, a pteridine derivative, is a marker of macrophage activation. It is formed by GTP cyclohydrolase I that catabolizes GTP into 7,8-dihydroneopterintriphosphate, which is further metabolized to neopterin (4). Interferon-gamma (IFN-γ) is the most important inducer of GTP cyclohydrolase I, and therefore of neopterin formation. Thus, neopterin is considered a marker of T_H1 immune activation (4). Atherosclerosis has been described as a T_H1 mediated disease (3), and higher concentrations of neopterin are associated with increased cardiovascular risk and atherosclerosis in the general population (5–8).

Several small studies indicate that concentrations of neopterin are elevated, or correlated with disease activity, in patients with SLE (9–13) or RA (14–18), but the association between neopterin and quantitative measures of atherosclerosis such as coronary artery calcification or carotid artery intima-media thickness (IMT) has not been tested in these diseases. Thus, we examined the hypothesis that macrophage activation is associated with atherosclerosis in SLE and RA by assessing whether neopterin concentrations are: 1) higher in patients with SLE or RA compared to controls; 2) associated with disease activity, inflammatory mediators of cardiovascular risk, and coronary calcium in patients with SLE or RA.

Methods

Study Population

We studied patients with SLE (n=148), RA (n=166) and control subjects (n=177). All subjects were 18 years of age or older and patients fulfilled the ACR classification criteria for SLE (19) or RA (20), respectively. The subjects are participants in ongoing studies of cardiovascular risk in cohorts of patients with SLE or RA; details regarding recruitment and methodological procedures have been described (1,2). By design, there were two separate groups of control subjects without inflammatory disease that were frequency-matched for age, race and sex with patients with SLE or RA, respectively (1,2). In the present study we combined these to form a single control group to allow comparison of neopterin concentrations between the three study groups with statistical adjustment for demographic variables. The study was approved by the Vanderbilt University Institutional Review Board and subjects gave written informed consent.

Measurements

Clinical information, laboratory data, current and past medication use and Agatston coronary calcium scores were obtained as described previously (1,2,21,22). In brief, coronary calcium was measured by electron beam computed tomography (EBCT) scanning with an Imatron C-150 scanner (GE/Imatron) except for 37 patients with SLE and 5 controls in whom a 64-row mulitdetector CT (LightSpeed VCT, General Electric, Milwaukee, WI) was used. For logistic reasons scans were not performed in 3 patients. Coronary calcium was quantified as described by Agatston (23) by a single reviewer (PR) blinded to the clinical status of the

subjects. Clinical indices of disease activity and damage including the DAS28 (24), SLEDAI (25) and SLICC (26) scores were measured. Medication use was classified according to whether or not patients were currently taking corticosteroids, methotrexate (MTX) or antimalarials (both SLE and RA), or anti-TNF agents (only RA). Erythrocyte sedimentation rate (ESR), plasma homocysteine and serum C-reactive protein (CRP) concentrations were determined in the Vanderbilt University Hospital clinical laboratory. Before 2003, the laboratory did not use a high-sensitivity CRP assay, and low concentrations were reported as <3 mg/l; in 40 patients with SLE and 40 with RA who had CRP concentrations <3 mg/l, CRP concentrations were measured by multiplex ELISA (Lincoplex® Multiplex Immunoassay Kit, Millipore). Serum concentrations of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) were measured by multiplex ELISA (Millipore). Serum neopterin was measured by ELISA (ALPCO Diagnostics).

Statistical Analysis

Descriptive statistics were calculated as frequencies and proportion, mean with standard deviation (mean ± SD) or median with interquartile range (median [IQR]) according to the distribution of the variables. Demographic and clinical factors were compared by disease group using a Kruskal-Wallis test or Wilcoxon rank-sum test, or a Pearson chi-square test as appropriate.

Serum neopterin concentrations were compared between patients with SLE, RA and control subjects using the Kruskal-Wallis test or Wilcoxon rank-sum test for comparisons. A multivariable linear regression model was used to adjust for age, sex, race, and also serum creatinine as renal function can affect neopterin concentrations (27).

Drugs used for the treatment of SLE and RA affect inflammatory activity, thus we compared neopterin concentrations separately in patients with SLE or RA who were currently receiving corticosteroids, MTX, antimalarials (both SLE and RA) and anti-TNF agents (only RA), and those who were not. Each comparison was performed using multivariable linear regression adjusting for age, race, sex, serum creatinine, and use of the other drugs. For example, in SLE, when neopterin concentrations were compared in patients taking or not taking corticosteroids, the model was adjusted for use of MTX and antimalarials. In RA, the analysis for corticosteroids was adjusted for use of MTX, antimalarials and anti-TNF agents.

Neopterin concentrations were examined for correlation with indices of disease activity and damage (SLE: SLEDAI and SLICC, RA: DAS28), inflammatory mediators (TNF-α, IL-6, MCP-1, ESR and CRP), homocysteine, and coronary calcium score using Spearman's correlation coefficient in patients with SLE or RA. The relationships were further tested with adjustment for age, sex, race, serum creatinine, current corticosteroid, MTX and antimalarial use in SLE, and additionally for anti-TNF agent use in RA, using either multiple linear regression (inflammatory mediators, DAS28 and homocysteine as the outcome variables) or proportional odds logistic regression (28). The proportional odds model was applied both to skewed continuous variables by using ranks for cut-points and also to skewed ordinal variables including SLEDAI, SLICC, and coronary calcium.

Values for concentrations of inflammatory mediators, neopterin, homocysteine and creatinine were $\log_{10}$-transformed when used in multivariate regression analysis in order to normalize the distribution. Statistical analysis was performed using R 2.9.2 (www.r-project.org). All tests were two-sided and a p value of <0.05 was considered significant.

Results

The clinical characteristics of patients with SLE or RA and control subjects are shown in Table 1. As expected, patients with SLE were younger and more likely to be female than patients with RA. As we have reported previously, concentrations of inflammatory mediators were higher in patients with RA or SLE than control subjects (22,29), and serum IL-6 concentrations were higher in RA than SLE (21). Only 1.7% of controls, 2.7% of SLE and 1.8% of RA patients had a serum creatinine concentration >1.5 mg/dl.

Serum neopterin concentrations were significantly higher in patients with SLE or RA than in controls, and were higher in SLE than in RA (all p values <0.001, Table 1, Figure 1). These differences remained significant after adjusting for age, race, sex and serum creatinine (all p values <0.001). Serum creatinine was significantly associated with higher neopterin values (p <0.001) in this model.

Medications were associated with neopterin concentration (Table 2). Current antimalarial therapy in SLE and current MTX use in RA were associated with lower neopterin concentrations. Anti-TNF agents did not affect neopterin levels in RA (p=0.18).

The correlations between neopterin and disease indices, inflammatory mediators, homocysteine and coronary calcium score in patients with SLE or RA are shown in Tables 3 and 4, respectively. In SLE neopterin correlated significantly with TNF-α (rho=0.42, adjusted p<0.001), MCP-1 (rho=0.31, adjusted p=0.005), ESR (rho=0.33, adjusted p=0.001) and homocysteine (rho=0.20, adjusted p=0.01), but in RA neopterin correlated only with ESR (rho=0.21, adjusted p=0.01). There were marginal correlations between neopterin and SLICC in SLE (Table 3), and DAS28 in RA (Table 4). The association between SLICC score and neopterin in SLE was attenuated by statistical adjustment that included serum creatinine in the model. Similarly, the univariate correlation between homocysteine and neopterin in RA (rho=0.24, p=0.002), was attenuated by adjusting for serum creatinine (p=0.11). Neopterin was not associated with coronary calcium in either SLE (p=0.65) or RA (p=0.21) in adjusted analyses. When we additionally adjusted the results from Table 3 and 4 for disease activity (SLEDAI and DAS28, respectively) the statistical significance of association between neopterin and coronary calcification did not change (SLE, p=0.70, RA, p=0.22).

Discussion

The major finding of this study is that concentrations of neopterin, a marker of macrophage activation and IFN-γ activity, are increased in large cohorts of patients with SLE or RA, and have a more robust association with mediators of inflammation and homocysteine in SLE than in RA.

Previous studies of neopterin in SLE (9–13) and RA (14–17) have been limited largely to defining the relationship between neopterin and disease activity. Generally, they found that neopterin correlated with disease activity in both SLE and RA, and decreased with treatment. Thus, neopterin has been considered as a candidate biomarker for monitoring disease activity. However, these studies most often used the urinary neopterin : creatinine ratio as a biomarker. This ratio adjusts neopterin excretion for variation in urine concentration rather than renal function (30,31). We found that neopterin correlated marginally with DAS28 in RA, but not with SLEDAI or SLICC in SLE. The SLICC score measures damage in SLE and encompasses renal damage. The lack of association between neopterin and the SLICC score after adjustment that included serum creatinine was likely due to the fact that both were positively associated with serum creatinine concentrations.

The influence of renal function, as well as medications, on neopterin concentrations suggests that its usefulness as a biomarker for disease activity is limited.

The lower concentrations of neopterin found in patients with SLE taking antimalarials and in those with RA taking MTX suggest that these drugs may inhibit monocyte and macrophage activation. Such associations are interesting, but should be interpreted cautiously given the cross-sectional design of the study.

Higher neopterin concentrations in patients with SLE compared to those with RA have also been observed by others (9). This observation is counterintuitive since current thinking is that SLE is a T_H2-mediated disease (32), RA a T_H1-mediated (or more recently, T_H17) disease (33), and neopterin a marker of T_H1 immune activation (4). However, several recent lines of evidence implicate IFN-γ in the pathogenesis of SLE (34), and thus our finding of higher neopterin concentrations in patients with SLE is concordant with those observations.

We have recently reported that TNF-α and IL-6 were independently associated with coronary calcification in SLE and RA (22,29,35). The stronger association of neopterin with TNF-α, IL-6 and MCP-1 (a mediator of monocyte activation) (36) in SLE than in RA, along with the higher neopterin concentrations in SLE than RA, suggests that the activation pathways that drive neopterin production may differ in the two diseases, and that neopterin is associated more closely with atherogenic pathways in SLE than in RA.

Neopterin has been associated with atherosclerosis and coronary risk in the general population. Higher concentrations of neopterin were associated with increased carotid (5) and coronary atherosclerosis (6), and were of prognostic value in patients with acute coronary syndrome (7). These clinical observations suggested a role for monocyte and macrophage activation in the pathogenesis of atherosclerosis and its complications, and were concordant with histological studies in which monocytes and macrophages were prominent in atherosclerotic lesions (37).

Although neopterin concentrations were correlated with some important atherogenic inflammatory mediators in SLE, they were not independently correlated with coronary calcium in either SLE or RA. It is possible that in studies finding an association between neopterin and increased cardiovascular risk (5–7) or carotid artery plaque (8) in the general population, this may have occurred indirectly. For example, if concentrations neopterin and inflammatory mediators were correlated, the observed association between neopterin and atherosclerosis could actually have been due to the relationship between atherosclerosis and inflammatory mediators that were not measured. Alternatively, in chronic inflammatory diseases such as RA and SLE, the increase in neopterin concentration could be driven by generalized inflammation, and this could act to obscure any smaller neopterin signal produced by macrophages associated with atherosclerosis. Finally, some studies suggest that neopterin may identify a patient group with increased cardiovascular risk that is not related to the amount of atherosclerosis present (38). We cannot exclude this possibility in our study.

Homocysteine is a strong predictor of cardiovascular risk (39) and others have also noted an association between homocysteine and neopterin concentrations in a range of populations (17,40,41), including a study of 33 patients with RA (17). The association between neopterin and homocysteine concentrations is thought to occur because immune activation results not only in higher neopterin concentrations but also in oxidative stress, consumption of folate, and impaired metabolism of homocysteine (42). Thus, neopterin and homocysteine levels may both reflect immune activation. Also, because both neopterin (27) and homocysteine (43) are cleared by the kidney, their concentrations would both tend to track with renal function. Our observation that the association between neopterin and homocysteine was

attenuated when creatinine was included in the model in RA, but not in SLE, is compatible with the idea that neopterin has a stronger association with inflammation in SLE than in RA.

Our study has several limitations. It was cross-sectional in design, and therefore causal inferences cannot be drawn. Also, we measured coronary calcium, a technique that provides an excellent measure of the atherosclerotic burden, but does not discriminate between stable and unstable lesions. The prognostic association between neopterin concentrations and acute coronary syndrome (7) suggests that additional studies will be required to determine whether the increased concentrations of neopterin in patients with SLE and RA could reflect a greater burden of unstable atheromatous plaque, or an increase in the processes by which stable plaque becomes unstable.

In conclusion, macrophage activation, reflected by increased serum neopterin concentrations, is increased in both SLE and RA compared to control subjects. Neopterin is more robustly associated with atherogenic mediators of inflammation and homocysteine in SLE than in RA, but is not associated with the severity of coronary atherosclerosis in either disease.

Acknowledgments

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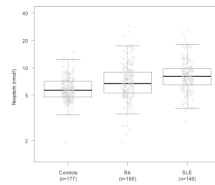
Figure 1. Distribution of Neopterin Concentrations in Control Subjects and Patients with SLE and RA

In the boxplot the horizontal bold line and the upper and lower hinges of the box represent the median and the interquartile range. The whiskers extends to 1.5 times the upper and lower interquartile range. Neopterin concentrations were significantly higher in patients with SLE or RA compared to controls, and in patients with SLE compared to those with RA (all p<0.001 with or without adjustment).

Table 1

Characteristics of the Study Population

	SLE (N=148)	RA (N=166)	Controls (N=177)	P-value[*]
Age (Years)	40[30–48][§†]	54[45–63][†]	47[39–55]	<0.001
Sex (Males %)	9.5%[§†]	31.3%	25.4%	<0.001
Race (Caucasian %)	66.9%[§†]	88.6%[†]	78.5%	<0.001
Hypertension (%)	44.6%[†]	53.0%[†]	28.8%	<0.001
Diabetes (%)	4.7%	11.4%[†]	2.8%	0.003
Current Smoking (%)	22.3%	23.5%[†]	13.6%	0.04
HDL (mg/dl)	48[36–56]	43[37–54]	45[38–46]	0.35
LDL (mg/dl)	94[76–126][§†]	111[88–134]	117[95–138]	<0.001
Triglycerides (mg/dl)	99.5[73.2–147.0]	112.0[80.0–158.0][†]	93.0[65.0–127.0]	0.003
Creatinine (mg/dl)	0.8[0.7–0.9]	0.8[0.7–0.9]	0.8[0.7–0.9]	0.81
Agatston Score	0 [0–0.7][¶]	2.7[0–150.4][¶]	0 [0–0]	<0.001
SLEDAI	4 [0–6]	-	-	-
SLICC	1 [0–2]	-	-	-
DAS28	-	3.89[2.66–4.90]	-	-
TNF-α (pg/ml)	4.8[3.1–7.4][†]	5.6[2.8–11.0][†]	2.7[2.0–3.9]	<0.001
IL-6 (pg/ml)	5.5[1.9–26.2][§†]	13.9[4.4–43.6][†]	2.5[1.0–10.0]	<0.001
MCP-1 (pg/ml)	188.2[124.7–294.6][†]	201.8[145.1–289.3][†]	144.1[98.8–206.1]	<0.001
CRP (mg/l)	3.6[0.8–7.0][§]	4.0[1.2–10.8]	-	
ESR (mm/hr)	20.0[9.0–34.0]	16.0[7.0–36.0]	-	
Homocysteine (umol/l)	9.2[7.7–11.2][§†]	10.2[8.1–11.9][†]	8.1[6.9–9.5]	<0.001
Neopterin (nmol/l)	8.0[6.5–9.8][§†]	6.7[5.3–8.9][†]	5.7[4.8–7.1]	<0.001

Data are shown as median [IQR] or percentages(%).

TNF-α, IL-6 and MCP-1 were available in 109 of 148 subjects with SLE, 163 of 166 with RA, and 172 of 177 Controls.

[*] Continuous variables compared with Kruskal-Wallis test and categorical variables with Pearson Chi-Square Test (across all 3 groups)

[§] p<0.05, Wilcoxon rank-sum test comparing SLE and RA.

[†] p<0.05, Wilcoxon rank-sum test comparing SLE and controls, or RA and controls.

[¶] P<0.001, proportional odds logistic regression model comparing SLE vs. controls, or RA vs. controls adjusted for age, race and sex.

Table 2

Neopterin Concentrations in Patients with SLE and RA and Current Medications

Current Medication Use		Neopterin (SLE)		Neopterin (RA)		
	n	(nmol/l)	P*	n	(nmol/l)	P*
Corticosteroids						
No	63	7.6 [5.9–9.8]	0.21	76	6.6 [5.4–8.6]	0.94
Yes	85	8.5 [7.1–9.8]		90	7.0 [4.9–8.9]	
Methotrexate						
No	134	8.0 [6.5–9.8]	0.87	48	7.6 [6.0–10.7]	0.01
Yes	14	8.6 [7.1–11.7]		118	6.4 [4.9–8.3]	
Antimalarials						
No	49	8.7 [7.3–12.2]	0.03	124	7.1 [5.5–9.3]	0.12
Yes	99	7.7 [6.3–9.3]		42	5.8 [4.8–7.5]	
Anti-TNF agents						
No	-	-	-	132	6.9 [5.3–9.2]	0.18
Yes	-	-	-	34	6.2 [5.0–8.0]	

*Adjusted P value for age, sex, race, serum creatinine and the current use of other drugs.

Table 3

Correlations between Neopterin and Disease Indices, Inflammatory Mediators and Coronary Calcium in SLE

Factor	Rho	p	Adjusted p[†]
SLEDAI	0.08	0.34	0.49[*]
SLICC	0.19	0.02	0.08[*]
TNF-α	0.42	<0.001	<0.001
IL-6	0.22	0.02	0.22
MCP-1	0.31	0.001	0.005
CRP	0.06	0.48	0.21
ESR	0.33	<0.001	0.001
Homocysteine	0.20	0.01	0.01
Agatston Score	0.02	0.77	0.65[*]

Rho is the Spearman correlation coefficient

[†]Adjusted for age, sex, race and creatinine and current corticosteroid, MTX and antimalarial use with multivariable linear regression.

[*]Used proportional odds logistic regression.

Table 4

Correlations between Neopterin and Disease Indices, Inflammatory Mediators and Coronary Calcium in RA

Factor	Rho	p	Adjusted p[†]
DAS28	0.14	0.07	0.08
TNF-α	0.17	0.03	0.22
IL-6	0.04	0.63	0.75
MCP-1	0.12	0.13	0.24
CRP	0.13	0.10	0.07
ESR	0.21	0.01	0.01
Homocysteine	0.24	0.002	0.11
Agatston Score	0.20	0.01	0.21[*]

Rho is the Spearman correlation coefficient

[†] Adjusted for age, sex, race and creatinine and current corticosteroid, MTX, antimalarial use and anti-TNF agent use with multivariable linear regression.

[*] Used proportional odds logistic regression.