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Neopterin as a Predictor of Total and Cardiovascular Mortality in Individuals Undergoing Angiography in the Ludwigshafen Risk and Cardiovascular Health Study

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BACKGROUND: Neopterin is produced upon activation of the cell-mediated immune response, and may be a novel risk marker for adverse outcomes resulting from coronary artery disease.

METHODS: We measured neopterin in 1801 study participants with and 511 without angiographic coronary artery disease. Rates of death were determined after a median follow-up of 8.0 years.

RESULTS: Estimated glomerular filtration rate and N-terminal pro-B–type natriuretic peptide (NT-proBNP) were the strongest predictors of neopterin. Neopterin was positively related to age and inversely related to LDL cholesterol, HDL cholesterol, and triglycerides. Use of lipid-lowering drugs lowered neopterin. Sex, body mass index, diabetes mellitus, hypertension, smoking status, Friesinger coronary score, and clinical instability at presentation were not associated with neopterin. Unlike C-reactive protein, neopterin was not increased in unstable angina pectoris, non–ST–elevation myocardial infarction, or ST-elevation myocardial infarction. In the third and fourth quartiles of neopterin, unadjusted hazard ratios for death from any cause were 1.94 (95% CI 1.44–2.61) and 3.32 (95% CI 2.53–4.30) compared to individuals in the first quartile, whereas hazard ratios for death from cardiovascular causes were 2.14 (95% CI 1.44–3.18) and 3.84 (95% CI 2.67–5.52), respectively. Neopterin remained predictive of total and cardiovascular mortality after adjusting for sex, age, body mass index, type 2 diabetes, hypertension, smoking status, LDL cholesterol, HDL cholesterol, triglycerides, estimated glomerular filtration rate, NT-proBNP, and clinical status at presentation, but NT-proBNP substantially weakened this association.

CONCLUSIONS: Neopterin is an independent predictor of all-cause and cardiovascular mortality in individuals with or without stable coronary artery disease.

Neopterin is produced by monocytes and macrophages in response to stimulation by interferon γ, a cytokine originating mainly from activated T-helper type-1 lymphocytes and natural killer cells *(1)*. Neopterin is formed by hydrolysis and oxidation of 7,8-dihydroneopterin triphosphate, which originates from the conversion of guanosine triphosphate by the action of guanosine triphosphate–cyclohydrolase I *(2–4)*. The amount of neopterin secreted is strongly related to the release of reactive oxygen radicals by cells, reflecting the level of oxidative stress caused by activation of the immune system *(5)*. Neopterin has been found to be increased in infectious diseases, malignancies, autoimmune diseases, anemia, heart and kidney failure, coronary artery disease (CAD)[7] and allograft rejection *(6)*. In renal disease, neopterin concentrations are increased because of impaired excretion as well as increased production of the compound due to systemic inflammation *(7)*. Furthermore, increased neopterin has been shown to be predictive for risk of mortality in HIV-1 infection *(8, 9)* and malignancies *(10, 11)* and of allograft rejection *(12)*; to indicate the activity of autoimmune diseases *(13)*; and to predict adverse outcomes in patients with myocardial infarction (MI) as well as in individuals with chronic stable angina pecto-

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[7] Nonstandard abbreviations: CAD, coronary artery disease; MI, myocardial infarction; CRP, C-reactive protein; NT-proBNP, N-terminal pro-B–type natriuretic peptide; NSTEMI, non–ST-elevation MI; C, cholesterol; eGFR, estimated glomerular filtration rate.

ris *(14–19)*. In addition statins were shown to lower the neopterin production that downregulates the immunoreactivity of T cells and of macrophages in vitro as well as in a case-control study of patients undergoing coronary angiography *(20, 21)*. It also has been postulated that cellular immunity might be activated and thus neopterin concentrations might be increased in patients with congestive heart failure *(22–24)*.

The objective of this investigation was to examine the importance of neopterin as a prognostic factor for long-term mortality in individuals scheduled for coronary angiography.

Material and Methods

STUDY DESIGN AND PARTICIPANTS

We studied participants of the Ludwigshafen Risk and Cardiovascular Health study *(25)*. Inclusion criteria were: German ancestry, clinical stability except for acute coronary syndromes, and the availability of a coronary angiogram. The indications for angiography in individuals in a clinically stable condition were chest pain and/or noninvasive test results consistent with myocardial ischemia. Individuals who were suffering from acute illness other than acute coronary syndromes or who had chronic noncardiac diseases or malignancy within the 5 past years and those unable to understand the purpose of the study were excluded. The study was approved by the ethics committee at the "Ärztekammer Rheinland-Pfalz". Informed written consent was obtained from all participants. CAD was assessed by angiography, with maximum luminal narrowing estimated by visual analysis. Clinically relevant CAD was defined as the occurrence of $\geq$1 stenosis of $\geq$20% in $\geq$1 of 15 coronary segments. Individuals with stenoses $<$20% were considered as not having CAD. Diabetes mellitus was diagnosed if plasma glucose was $>$1.25 g/L (6.9 mmol/L) in the fasting state or $>$2.00 g/L (11.1 mmol/L) 2 h after an oral glucose load *(26)* or if individuals were receiving antidiabetic treatment. Hypertension was diagnosed if the systolic and/or diastolic blood pressure exceeded 140 and/or 90 mm Hg or if there was a history of hypertension, evident through the use of antihypertensive drugs.

Measurements of neopterin along with other relevant biomarkers such as lipoproteins, C-reactive protein (CRP), and N-terminal pro-B–type natriuretic peptide (NT-proBNP) were complete in 2312 individuals with coronary angiograms. The severity of CAD was quantified with the Friesinger Score *(27)* The study participants included 1801 patients who presented within 14 days after onset of unstable angina, non–ST-elevation MI (NSTEMI, troponin T $>$0.1 μg/L), or ST-elevation MI (STEMI, troponin T $>$0.1 μg/L). The mean concentration of neopterin in these patients was close to that in the stable CAD patients. We therefore included all patient groups into the analysis. Stable CAD was defined in asymptomatic patients with at least 20% luminal narrowing of a major coronary artery in the angiogram. Unstable angina was defined as new-onset chest pain at rest, new-onset crescendo angina, or post-MI unstable angina within the first 2 weeks of MI. Unstable angina may be associated with reversible changes in the electrocardiogram (mostly decreases of ST-segment and negative T-waves, but no ST-segment elevation); Troponin T had to be negative in persons classified as unstable angina. STEMI was based on the criteria of ST elevation in the electrocardiogram plus typical symptoms of chest pain and/or typical enzyme changes (elevation of creatinine phosphokinase, creatinine phosphokinase MB, troponin T above 0.1 μg/L), whereas NSTEMI was characterized by typical symptoms of chest pain and typical enzyme changes (elevation of creatinine phosphokinase, creatinine phosphokinase MB, troponin T above 0.1 μg/L) but without signs of ST-segment elevation in the electrocardiogram (only atypical new and persistent ST-segment changes or T-wave changes).

Mortality information was obtained from local registries. No patients were lost to follow-up. Of the 2312 persons studied, 531 (23%) died during a median follow-up of 8.0 years. Death certificates were missing for 16 decedents who were included in the total mortality analysis but excluded from the cardiovascular mortality analysis. Cardiovascular death included the following: sudden death, fatal MI, death due to congestive heart failure, death immediately following intervention to treat CAD, fatal stroke, and other causes of death due to CAD.

LABORATORY PROCEDURES

For neopterin measurement and all other analyses, we collected fasting blood samples immediately upon patient admission to the hospital and before angiography. The standard laboratory methods have been described *(25)*. LDL cholesterol (LDL-C) and HDL-C were quantified by use of a combined ultracentrifugation–precipitation method. Neopterin was measured in frozen serum (−80 °C) by RIA (BRAHMS Diagnostica). NT-proBNP was measured by electrochemiluminescence on an Elecsys 2010 (Roche Diagnostics). High-sensitivity CRP was measured by immunonephelometry on a Behring Nephelometer II (N High Sensitivity CRP, Dade Behring). Estimated glomerular filtration rate (eGFR) was calculated as eGFR $[\text{mL} \cdot \text{min}^{-1} \cdot (1.73\ \text{m}^2)^{-1}] = 186 \cdot \text{creatinine}^{-1.154} \cdot \text{age}^{-0.203}$ and eGFR $[\text{mL} \cdot \text{min}^{-1} \cdot (1.73\ \text{m}^2)^{-1}] = 138 \cdot \text{creatinine}^{-1.154} \cdot \text{age}^{-0.203}$ in males and in females, respectively *(28)*.

Table 1. Clinical and biochemical characteristics of study participants at baseline.[a]

	No CAD, (n = 511)	CAD, (n = 1801)	P[b]
Age, y	59 ± 12	64 ± 10	<0.001[c]
Male sex	50	75	<0.001[d]
Body mass index, kg/m^2	27 ± 4	27 ± 4	0.916
Diabetes mellitus	18	35	<0.001
Systemic hypertension	64	75	0.002
Smoking			
Never	51	32	
Past	31	47	
Current	18	21	<0.001
Previous MI	—	52	—
Peripheral vascular disease	2	11	<0.001
Cerebrovascular disease	6	10	0.061
Systolic blood pressure, mmHg	137 ± 23	142 ± 24	0.045[e]
Diastolic blood pressure, mmHg	80 ± 11	81 ± 12	0.438[e]
Fasting blood glucose, g/L	1.04 ± 0.26[f]	1.16 ± 0.37[f]	<0.001
LDL-C, g/L	1.21 ± 0.31[f]	1.17 ± 0.35[f]	
LDL-C, g/L, estimated marginal means (95% CI adjusted for lipid-lowering drugs)	1.13[f] (1.10; 1.16)	1.20[f] (1.18; 1.21)	0.001[g]
HDL-C, g/L	0.43 ± 0.12[f]	0.38 ± 0.10[f]	<0.001[g]
Triglycerides, g/L	1.33[f] (0.95; 2.00)	1.49[f] (1.13; 2.02)	<0.001[g,h]
NT-proBNP, ng/L	162 (69; 559)	328 (116; 925)	<0.001[h]
eGFR, mL · min^{-1} · (1.73 m^2)$^{-1}$	83 ± 18	82 ± 19	0.908
CRP, mg/L	2.22 (0.96; 5.81)	3.70 (1.42; 9.09)	<0.001[h]
Use of lipid-lowering drugs	20	58	<0.001

[a] Results are expressed as mean ± SD, %, or median (25th; 75th percentile), unless otherwise indicated.
[b] Analysis of variance or logistic regression, respectively, adjusted for age and sex.
[c] Logistic regression, adjusted for sex only.
[d] Logistic regression, adjusted for age only.
[e] Adjusted for use of beta blockers, ACE inhibitors, AT1 receptor antagonists, calcium channel blockers, diuretics, and lipid-lowering agents.
[f] To convert LDL-C, HDL-C from g/L to mmol/L, multiply by 2.59. To convert triglycerides from g/L to mmol/L, multiply by 1.13. To convert glucose from g/L to mmol/L, multiply by 5.55.
[g] Adjusted for use of lipid-lowering agents.
[h] ANOVA of logarithmically transformed values.

STATISTICAL ANALYSIS

Data for triglycerides, neopterin, CRP, and NT-proBNP were transformed logarithmically. We established quartiles of neopterin and other continuous variables according to their values in individuals without CAD, and in the case of variables known to be affected by the use of lipid-lowering drugs (triglycerides, LDL-C, HDL-C, and CRP) according to the values of individuals without CAD who were not taking such drugs. Characteristics of individuals with and without CAD are presented as percentages for categorical variables and as mean (SD) or median and 25th and 75th percentiles for continuous variables. Associations of categorical and continuous variables were analyzed by logistic regression and univariate ANOVA, respectively, with covariables as indicated (Table 1). ANOVA analysis with covariables was used to study the relationships of neopterin concentrations with sex, age, Friesinger score, CAD status, and cardiovascular risk factors (Table 2), and to evaluate the relationships between presenting clinical conditions and concentrations of neopterin and CRP (Table 3). Cox proportional hazards modeling with multivariable adjustment was used to examine the relationship between neopterin and mortality adjusted for sex, age, CAD status, and cardiovascular risk factors (Tables 4 and 5; also see Tables 1 and 2 in the Data Supplement that accompanies the online version of this article at http://www.

Table 2. Effect of confounding factors and clinical presentation on the neopterin concentration.

	n = 2312	Neopterin, nmol/L[a]	Difference, %[b]	*P*[c]
Sex				
Men	1610	7.2 (7.0–7.3)		
Women	702	7.0 (6.8–7.2)	−2.6	0.209
Age (years)				
<60	876	6.8 (6.6–7.0)		
60–70	825	7.1 (6.9–7.3)	+4.3	0.041
>70	611	7.6 (7.3–7.8)	+10.8	<0.001
CAD				
None	511	7.0 (6.6–7.3)		
Stable CAD	1083	7.1 (6.9–7.3)	+2.1	0.454
Unstable CAD (troponin T−)	437	7.2 (6.9–7.4)	−3.1	0.335
NSTEMI (troponin T+)	82	7.7 (7.0–8.4)	+10.2	0.062
STEMI (troponin T+)	199	7.2 (6.8–7.7)	+3.8	0.358
Friesinger score				
0–1	486	6.8 (6.5–7.1)		
2–4	520	7.3 (7.1–7.6)	+6.9	0.015
5–8	756	7.1 (6.9–7.4)	+4.5	0.134
9–15	550	7.1 (6.9–7.4)	+4.2	0.195
Body mass index, kg/m^2				
≤26 or 27[d]	1087	7.1 (7.9–7.3)		
>26 or 27[d]	1225	7.1 (7.9–7.3)	0.0	0.973
Diabetes mellitus				
No	1582	7.1 (7.0–7.3)		
Yes	730	7.0 (6.8–7.2)	−1.8	0.343
Hypertension				
No	638	7.0 (6.8–7.3)		
Yes	1674	7.1 (7.0–7.3)	+1.1	0.592
Smoking				
Never	838	7.1 (6.9–7.3)		
Former	1012	7.2 (7.0–7.4)	+2.1	0.284
Current	462	6.9 (6.7–7.2)	−2.0	0.438
LDL-C, g/L[e]				
1st quartile (<1.00)	700	7.5 (7.3–7.8)		
2nd quartile (1.00–1.19)	550	7.1 (6.9–7.4)	−5.4	0.016
3rd quartile (1.20–1.40)	525	6.8 (6.6–7.0)	−9.7	0.001
4th quartile (≥1.41)	537	6.9 (6.7–7.1)	−8.8	<0.001
HDL-C, g/L[e]				
1st quartile (<0.34)	874	7.7 (7.5–7.9)		
2nd quartile (0.34–0.41)	632	7.0 (6.8–7.2)	−9.0	<0.001
3rd quartile (0.42–0.49)	462	6.7 (6.5–7.0)	−12.8	<0.001
4th quartile (≥0.50)	344	6.3 (6.0–6.6)	−18.3	<0.001

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Table 2. Effect of confounding factors and clinical presentation on the neopterin concentration. (*Continued from page 1138*)

	n = 2312	Neopterin, nmol/L[a]	Difference, %[b]	*P*[c]
Triglycerides, g/L[e]				
1st quartile (<0.97)	405	7.5 (7.2–7.8)		
2nd quartile (0.97–1.32)	552	7.1 (6.8–7.3)	−6.0	0.019
3rd quartile (1.33–1.94)	723	7.1 (6.9–7.3)	−5.6	0.024
4th quartile (≥1.95)	632	6.9 (6.7–7.1)	−8.7	<0.001
NT-proBNP, ng/L				
1st quartile (<68)	357	6.4 (6.2–6.7)		
2nd quartile (68–161)	478	6.6 (6.4–6.9)	+2.9	0.322
3rd quartile (162–532)	670	6.8 (6.6–7.0)	+5.0	0.079
4th quartile (≥533)	807	8.0 (7.8–8.3)	+24.6	<0.001
eGFR, $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$				
≥90	778	6.2 (6.0–6.4)		
60–89	1274	7.1 (6.9–7.3)	14.2	<0.001
<60	260	10.8 (10.3–11.4)	74.7	<0.001
Use of lipid-lowering drugs				
No	1176	7.3 (6.8–7.3)		
Yes	1136	6.9 (7.0–7.3)	−6.5	<0.001

[a] Estimated marginal means and 95% CIs obtained in a general linear model (ANOVA) adjusted for each of the other factors, whereby age, body mass index, LDL-C, HDL-C, triglycerides (log transformed), NT-proBNP (log transformed), and eGFR were included as continuous rather than categorical covariables. The estimated marginal means and CIs of logarithmically transformed neopterin have been back-transformed onto the original scale.
[b] Difference compared to the first category of each variable.
[c] *P* values compared to the first category of each variable.
[d] Thresholds of 26 and 27 kg/m2 apply to females and males, respectively.
[e] To convert LDL-C, HDL-C from g/L to mmol/L, multiply by 2.59. To convert triglycerides from g/L to mmol/L, multiply by 1.13.

clinchem.org/content/vol55/issue6). Log-minus-log diagnostic plots were used to determine whether the proportional hazards assumption was met. All statistical tests were 2-sided; $P < 0.05$ was considered significant. The SPSS 16.0 statistical package (SPSS Inc.) was used for statistical analyses.

Table 3. Neopterin and CRP according to different clinical conditions at presentation.

		Neopterin			CRP		
	n	Concentration, nmol/L[a]	Difference, %[b]	*P*[c]	Concentration, mg/L[a]	Difference, %[b]	*P*[c]
CAD							
None	511	7.0 (6.6–7.3)			2.8 (2.4–3.2)		
Stable CAD	1083	7.1 (6.9–7.3)	+2.1	0.454	2.8 (2.6–3.0)	+0.8	0.923
Unstable CAD (troponin T−)	437	7.2 (6.9–7.4)	−3.1	0.335	4.0 (3.6–4.5)	+43.6	<0.001
NSTEMI (troponin T+)	82	7.7 (7.0–8.4)	+10.2	0.062	7.6 (5.9–9.7)	+172.1	<0.001
STEMI (troponin T+)	199	7.2 (6.8–7.7)	+3.8	0.358	9.0 (7.6–10.6)	+222.2	<0.001

[a] Estimated marginal means and 95% CIs obtained in a general linear model (ANOVA) adjusted for sex, age, Friesinger score, body mass index, diabetes mellitus, hypertension, LDL-C, HDL-C, triglycerides (log transformed), and eGFR and use of lipid-lowering drugs. The continuous variables were used in the models as such. The estimated marginal means and CIs of logarithmically transformed neopterin have been back-transformed onto the original scale.
[b] Difference compared to the first category of each variable.
[c] *P* values compared to the first category of each variable.

Table 4. Hazard ratios (HR) for death from all causes according to neopterin.

Variable	Model[a] 1 HR (95% CI)	*P*	Model[a] 2 HR (95% CI)	*P*	Model[a] 3 HR (95% CI)	*P*	Model[a] 4 HR (95% CI)	*P*
All individuals (n = 2312)								
Neopterin, nmol/L								
1st quartile, <5.28 (n = 497)	1.0[reference]		1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile, 5.28–6.44 (n = 512)	0.98 (0.69–1.38)	0.979	0.85 (0.60–1.20)	0.365	0.84 (0.59–1.18)	0.308	0.81 (0.57–1.15)	0.234
3rd quartile, 6.45–7.98 (n = 567)	1.94 (1.44–2.61)	<0.001	1.45 (1.07–1.96)	0.016	1.41 (1.04–1.92)	0.028	1.35 (0.99–1.83)	0.056
4th quartile, ≥7.99 (n = 736)	3.32 (2.53–4.30)	<0.001	2.07 (1.56–2.74)	<0.001	1.81 (1.33–2.46)	<0.001	1.42 (1.04–1.95)	0.027
Per quartile[b]	1.60 (1.47–1.74)	<0.001	1.36 (1.24–1.48)	<0.001	1.29 (1.17–1.42)	<0.001	1.18 (1.07–1.30)	0.001
CRP (per quartile)[c]	1.34 (1.24–1.46)	<0.001	1.29 (1.19–1.40)	<0.001	1.16 (1.06–1.27)	0.001	1.09 (1.00–1.19)	0.063
Age (per year)			1.06 (1.05–1.08)	<0.001	1.06 (1.05–1.07)	<0.001	1.05 (1.04–1.06)	<0.001
Sex (female)			0.70 (0.57–0.84)	<0.001	0.77 (0.62–0.95)	0.015	0.73 (0.59–0.91)	0.004
Diabetes mellitus					1.81 (1.51–2.17)	<0.001	1.71 (1.43–2.06)	<0.001
Smoking (never/former/current)					1.28 (1.12–1.47)	<0.001	1.21 (1.05–1.39)	0.007
Use of lipid-lowering drugs					0.84 (0.70–1.02)	0.076	0.87 (0.72–1.05)	0.133
ln NT-proBNP (per ng/L)							1.50 (1.40–1.62)	<0.001
No angiographic CAD (n = 511)								
Neopterin (nmol/L)								
1st quartile, <5.28 (n = 134)	1.0[reference]		1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile, 5.28–6.44 (n = 139)	0.97 (0.34–2.76)	0.752	0.93 (0.33–2.66)	0.932	0.81 (0.28–2.34)[d]	0.694	0.76 (0.26–2.20)[d]	0.614
3rd quartile, 6.45–7.98 (n = 114)	2.28 (0.91–5.72)	0.0079	1.86 (0.74–4.72)	0.189	1.71 (0.66–4.40)[d]	0.266	1.64 (0.63–4.23)[d]	0.311
4th quartile, ≥7.99 (n = 124)	6.29 (2.79–14.21)	<0.001	3.80 (1.61–8.96)	0.002	2.69 (1.06–6.87)[d]	0.038	1.97 (0.74–5.25)[d]	0.174
Per quartile[b]	2.10 (1.61–2.73)	<0.001	1.71 (1.30–2.25)	<0.001	1.51 (1.12–2.05)[d]	0.007	1.37 (1.00–1.87)[d]	0.052
CRP (per quartile)[c]	1.66 (1.31–2.11)	<0.001	1.50 (1.17–1.92)	0.001	1.43 (1.09–1.87)[d]	0.010	1.34 (1.02–1.76)[d]	0.034
Angiographic CAD (n = 1801)								
Neopterin (nmol/L)								
1st quartile, <5.28 (n = 363)	1.0[reference]		1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile, 5.28–6.44 (n = 373)	0.98 (0.68–1.41)	0.920	0.83 (0.58–1.20)	0.333	0.83 (0.57–1.20)	0.312	0.83 (0.57–1.19)	0.826
3rd quartile, 6.45–7.98 (n = 453)	1.79 (1.31–2.46)	<0.001	1.36 (0.99–1.87)	0.059	1.33 (0.96–1.84)	0.086	1.27 (0.92–1.76)	0.143
4th quartile, ≥7.99 (n = 612)	2.82 (2.11–3.77)	<0.001	1.85 (1.38–2.50)	<0.001	1.65 (1.19–2.28)	0.003	1.31 (0.941–1.82)	0.109
Per quartile[b]	1.50 (1.37–1.64)	<0.001	1.31 (1.19–1.43)	<0.001	1.24 (1.12–1.38)	<0.001	1.14 (1.02–1.26)	0.016

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Table 4. Hazard ratios (HR) for death from all causes according to neopterin. (*Continued from page 1140*)

Variable	Model[a] 1 HR (95% CI)	*P*	Model[a] 2 HR (95% CI)	*P*	Model[a] 3 HR (95% CI)	*P*	Model[a] 4 HR (95% CI)	*P*
CRP (per quartile)[c]	1.26 (1.16–1.37)	<0.001	1.25 (1.15–1.36)	<0.001	1.15 (1.05–1.27)	0.004	1.08 (0.98–1.19)	0.102
Stable CAD (n = 1083)								
Neopterin, nmol/L								
1st quartile, <5.28 (n = 231)	1.0[reference]		1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile, 5.28–6.44 (n = 230)	1.11 (0.72–1.72)	0.626	0.94 (0.61–1.46)	0.793	0.90 (0.58–1.40)[d]	0.645	0.90 (0.58–1.39)[d]	0.633
3rd quartile, 6.45–7.98 (n = 282)	1.72 (1.17–2.53)	0.006	1.36 (0.92–2.00)	0.125	1.24 (0.83–1.84)[d]	0.451	1.19 (0.80–1.77)[d]	0.401
4th quartile, ≥7.99 (n = 340)	3.04 (2.14–4.33)	<0.001	2.04 (1.42–2.94)	0.001	1.61 (1.07–2.41)[d]	0.022	1.23 (0.81–1.86)[d]	0.327
Per quartile[b]	1.51 (1.36–1.69)	<0.001	1.33 (1.19–1.49)	<0.001	1.22 (1.07–1.38)[d]	0.003	1.10 (0.96–1.25)[d]	0.159
CRP (per quartile)[c]	1.38 (1.24–1.53)	<0.001	1.36 (1.22–1.51)	<0.001	1.20 (1.07–1.34)[d]	0.002	1.10 (0.98–1.30)[d]	0.126
Unstable CAD (n = 718)								
Neopterin, nmol/L								
1st quartile, <5.28 (n = 132)	1.0[reference]		1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile, 5.28–6.44 (n = 143)	0.74 (0.37–1.47)	0.392	0.62 (0.31–1.24)	0.178	0.64 (0.32–1.28)[d]	0.204	0.63 (0.32–1.27)[d]	0.196
3rd quartile, 6.45–7.98 (n = 171)	1.94 (1.12–3.37)	0.018	1.39 (0.80–2.43)	0.248	1.39 (0.79–2.46)[d]	0.258	1.36 (0.78–2.40)[d]	0.281
4th quartile, ≥7.99 (n = 272)	2.65 (1.60–4.39)	<0.001	1.66 (0.99–2.80)	0.055	1.56 (0.89–2.75)[d]	0.120	1.33 (0.76–2.33)[d]	0.325
Per quartile[b]	1.51 (1.29–1.77)	<0.001	1.30 (1.10–1.53)	0.001	1.26 (1.05–1.51)[d]	0.012	1.18 (0.99–1.41)[d]	0.072
CRP (per quartile)[c]	1.19 (1.02–1.40)	0.032	1.19 (1.02–1.39)	0.030	1.05 (0.88–1.25)[d]	0.608	1.05 (0.88–1.25)[d]	0.627

[a] Model 1: unadjusted; Model 2: adjusted for age and sex; Model 3: in addition adjusted for body mass index, type 2 diabetes, hypertension, smoking status, LDL-C, HDL-C, triglycerides, CRP, eGFR, clinical status at presentation, and use of lipid lowering drugs. All continuous variables were treated as such in the modeling; Model 4: in addition adjusted for NT-proBNP.
[b] The hazard ratios are the ratios associated with an increase of one quartile in neopterin.
[c] Models correspond to those used for neopterin with the exception that CRP was stratified in quartiles and neopterin was used as continuous predictor.
[d] Status at presentation not included as a covariable.

Results

STUDY PARTICIPANTS

Compared to the group without CAD, CAD patients were significantly older, more likely to be male, and current or past smokers; CAD patients also had higher prevalence rates of diabetes mellitus, hypertension, and cerebrovascular and peripheral artery disease. A history of MI occurred in 52% of the CAD patients. CAD patients had higher systolic blood pressure, fasting glucose, triglycerides, NT-proBNP, and CRP and lower HDL-C. Unadjusted LDL-C was lower in individuals with CAD than those without CAD; 58% of the CAD patients received lipid-lowering drugs compared to 20% of those without CAD. After statistical adjustment for the use of lipid-lowering drugs we found that LDL-C was significantly higher ($P < 0.001$) in CAD patients (adjusted mean: 1.20 g/L) than in individuals without CAD (adjusted mean, 1.13 g/L). Body mass index, diastolic blood pressure, and eGFR were similar in both groups (Table 1).

ASSOCIATION OF NEOPTERIN WITH CARDIOVASCULAR RISK FACTORS AND CAD STATUS

The relationships of neopterin concentrations with potential confounding factors, sex, age, cardiovascular risk factors (body mass index, diabetes mellitus, hypertension, smoking, lipoproteins, NT-proBNP) and of the clinical and angiographic findings at presentation were examined in a general linear model that included other factors not under examination as covariables. Neopterin was significantly and positively associated with age, NT-proBNP, and eGFR. Patients with stable CAD had, on average, identical neopterin concentra-

Table 5. Hazard ratios (HR) for death from all causes according to neopterin and CRP.

Variable	Model[a] 1 HR (95% CI)	P	Model[a] 2 HR (95% CI)	P	Model[a] 3 HR (95% CI)	P	Model[a] 4 HR (95% CI)	P
CRP low, <3 mg/L (n = 1089)								
Neopterin (nmol/L)								
1st quartile, <5.28 (n = 303)	1.0[reference]		1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile, 5.28–6.44 (n = 290)	1.01 (0.62–1.64)	0.977	0.92 (0.57–1.50)	0.742	0.90 (0.55–1.46)	0.657	0.89 (0.54–1.45)	0.628
3rd quartile, 6.45–7.98 (n = 275)	2.13 (1.39–3.25)	0.001	1.52 (1.00–2.35)	0.057	1.68 (1.08–2.60)	0.021	1.70 (1.10–2.63)	0.017
4th quartile, ≥7.99 (n = 221)	2.61 (1.70–4.01)	<0.001	1.50 (0.96–2.35)	0.078	1.72 (1.08–2.74)	0.022	1.36 (0.85–2.18)	0.198
Per quartile[b]	1.45 (1.26–1.66)	<0.001	1.19 (1.03–1.37)	0.018	1.25 (1.08–1.45)	0.003	1.16 (1.00–1.35)	0.044
CRP high, ≥3 mg/L (n = 1223)								
Neopterin (nmol/L)								
1st quartile, <5.28 (n = 194)	1.0[reference]		1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile, 5.28–6.44 (n = 222)	0.92 (0.56–1.50)	0.731	0.74 (0.45–1.20)	0.221	0.75 (0.46–1.23)	0.254	0.71 (0.43–1.17)	0.177
3rd quartile, 6.45–7.98 (n = 292)	1.65 (1.08–2.50)	0.020	1.23 (0.80–1.88)	0.343	1.24 (0.80–1.90)	0.336	1.13 (0.73–1.73)	0.587
4th quartile, ≥7.99 (n = 515)	3.01 (2.06–4.39)	<0.001	1.87 (1.27–2.77)	0.002	1.75 (1.16–2.65)	0.008	1.41 (0.93–2.14)	0.111
Per quartile[b]	1.58 (1.41–1.77)	<0.001	1.37 (1.22–1.54)	<0.001	1.31 (1.15–1.49)	<0.001	1.81 (1.04–1.35)	0.013

[a] Model 1: unadjusted; Model 2: adjusted for age and sex; Model 3: in addition adjusted for body mass index, type 2 diabetes, hypertension, smoking status, LDL-C, HDL-C, triglycerides, eGFR, and use of lipid lowering drugs. All continuous variables were treated as such in the modeling. Model 4: in addition adjusted for NT-proBNP.

[b] The hazard ratios are the ratios associated with an increase of one quartile in neopterin.

tions compared to those without CAD. Further, there was no increase of neopterin in patients with acute coronary syndromes compared to patients with stable CAD. Of note, there was a slight elevation of neopterin in patients with intermediate degrees of coronary atherosclerosis (Friesinger score 2 through 4) which was not seen in patients with more advanced disease (Friesinger scores of 5 and more). Thus, there was no consistent link between neopterin and changes of the vessel wall seen by angiography. Neopterin was significantly and inversely related to LDL-C, HDL-C, and triglycerides, and it was slightly but significantly lower in the users of lipid-lowering drugs (among whom 97% were taking statins). Body mass index, diabetes mellitus, hypertension, and smoking status revealed no significant association with neopterin. Multiple linear regression using forward selection of independent variables showed that the relative importance of predictors of the neopterin concentration decreased in the order of eGFR > NT-proBNP > CRP > HDL-C > triglycerides > LDL-C > use of lipid-lowering drugs > age > body mass index (R^2 = 0.216). Sex, diabetes mellitus, hypertension, smoking status, and clinical status at presentation (owing to redundancy with CRP) were not selected for inclusion in that particular model.

We were interested in the question of whether neopterin and CRP demonstrated similar relationships with clinical status at presentation as those seen for inflammatory markers. For this purpose, we examined the changes in neopterin and CRP according to the clinical status at presentation (Table 3). Compared with persons without CAD, CRP was found to be significantly increased in persons with unstable CAD, NSTEMI, or STEMI; it was not increased in individuals with stable CAD. Neopterin was virtually identical in all of these groups (Table 3).

NEOPTERIN AND MORTALITY FROM ALL CAUSES

Among the 2312 persons studied, 531 deaths (23.0%) occurred. Compared to individuals in the lowest quartile of neopterin, the unadjusted hazard ratios and 95% CI for death at neopterin concentrations in the third and fourth quartile were 1.94 (95% CI, 1.44–2.61) and 3.32 (95% CI, 2.53–4.30), respectively (Model 1, Table 4).

Inclusion of age and sex as covariables decreased these estimates to 1.45 (95% CI, 1.07–1.96) and 2.07 (95% CI, 1.56–2.74), respectively (Model 2, Table 4). Although the hazard ratios showed further slight decreases after additional adjustment for established cardiovascular risk factors as well as for CRP and CAD status at presentation, neopterin retained its prognostic importance in the third and fourth quartiles (Model 3, Table 4). Final adjustment with addition of NT-proBNP as a covariable appreciably decreased the hazard ratios (Model 4, Table 4), but, neopterin in the fourth quartile was still significantly associated with mortality from all causes (hazard ratio 1.42, 95% CI 1.04–1.95).

Among the 1801 patients with CAD, 470 (26.0%) died. In this subgroup, hazard ratios of neopterin for death were essentially equal to those in the entire cohort (Table 4). Neopterin was also independently and significantly associated with mortality from all causes in patients classified as having no CAD (n = 511), although only 61 deaths (12%) occurred in this subgroup (Table 4). It was only in the fully adjusted model (including NT-proBNP) that the association between neopterin and all-cause mortality became statistically insignificant in the subgroups. Among the patients with CAD, 1083 were studied more than 14 days following the onset of symptoms of an acute coronary event or did not have a history of such an event. These patients were considered as having stable CAD. 725 patients underwent angiography within 14 days after the onset of symptoms of acute coronary syndromes (unstable angina, NSTEMI, or STEMI) and were classified as having unstable CAD; 304 (28%) and 166 (23%) of deaths, respectively, occurred in the stable and unstable CAD patients. In both groups, we found consistent and robust associations of neopterin with mortality from all causes, with the exception of the models incorporating NT-proBNP (Table 4).

CRP was also independently related to mortality from all causes. By means of the hazard ratio of death per quartile, neopterin (unadjusted hazard ratio 1.60, 95% CI 1.47–1.74) was a slightly better predictor of death than CRP (unadjusted hazard ratio 1.34, 95% CI 1.47–1.74). The association of CRP and all-cause mortality was, however, not statistically significant once NT-proBNP was included in the model (Table 4). This finding applied essentially to all subgroups of study participants.

We further wished to examine whether neopterin added predictive power above and beyond CRP, which is currently the most frequently used marker of systemic inflammation in the assessment of cardiovascular risk. For this purpose we stratified the cohort into persons with CRP <3 mg/L (n = 1089) and CRP ≥3 mg/L (n = 1223). At both low and high CRP, neopterin was found to be significantly associated with mortality. Of interest, the association appeared stronger at high than at low CRP concentrations.

NEOPTERIN AND MORTALITY FROM CARDIOVASCULAR CAUSES

Death certificates were not available for 16 deceased persons. Therefore the analysis of cardiovascular mortality included 2296 individuals before stratification and 511, 1785, 1076, and 713 individuals without angiographic CAD, with angiographic CAD, with stable CAD, or with unstable CAD, respectively. Cardiovascular mortality rates in these groups were 32 (6.2%), 293 (16.3%), 196 (18.1%), and 97 (13.5%), respectively. For death from cardiovascular causes hazard ratios calculated according to neopterin were similar to those obtained for mortality from all causes in all models and across all subgroups examined (see online Supplemental Tables 1 and 2).

Discussion

Our study findings suggested that neopterin was a strong predictor of all-cause and cardiovascular mortality in patients undergoing coronary angiography, regardless of the angiographic findings and regardless of whether or not individuals presented in a stable or unstable (unstable angina, NSTEMI, or STEMI) condition. There was also an increased risk of death at high neopterin concentrations in individuals without CAD. These associations were largely maintained following adjustment for well-established risk factors and for CRP, a marker of systemic inflammation widely recommended for application in cardiovascular risk assessment.

Only limited information has been available so far with regard to the utility of neopterin as a diagnostic and prognostic marker in cardiovascular diseases. We first analyzed the relationships of traditional cardiovascular risk factors and of emerging biomarkers with neopterin. After controlling for other confounding factors, sex and body mass index were not found to have a relationship with neopterin concentrations. Neopterin was positively related to age, as repeatedly shown previously *(29)*. Intriguingly, despite the occurrence of oxidative stress in smokers, neopterin was not found to be significantly related to the smoking habit. This finding is in line with previous reports *(30, 31)* and may reflect the fact that activated T-helper–type 1 lymphocytes, which produce interferon-γ, the most important effector of neopterin secretion, are suppressed in smokers *(32)*. However, in a study by Diamondstone et al. *(31)* neopterin was found to be even lower in smokers than in nonsmokers. Statistically, the most important predictor of neopterin was renal function, closely followed by NT-proBNP. Neopterin increased as the

eGFR declined, most likely owing to a combination of impaired renal excretion and enhanced endogenous production in a condition of high inflammatory burden, as seen in atherosclerosis *(7, 33, 34)*. To our knowledge, this is the first report of a strong association between NT-proBNP and neopterin. This finding confirms and extends the observation by Samsonov et al. who saw that neopterin increased in parallel with the severity of congestive heart failure *(23)*. LDL-C and triglycerides were low in persons with high neopterin. It is unlikely that this finding is attributable to the fact that lipid-lowering therapy also lowers neopterin, because the inverse association between LDL-C and neopterin was still found after adjustment for drug intake (Table 2). Rather, and more likely, it may be an effect of acute and/or chronic activation of the immune system, which is indicated by high neopterin, attended by the release of interleukin 6, up-regulation of LDL receptors, and consequentially low LDL-C and triglycerides *(35)*. Similarly, in patients with HIV infection the increase in neopterin concentrations was found to be correlated with the decrease of LDL-C and HDL-C *(36)*. Therefore, the combination of high neopterin concentrations and low LDL-C and triglycerides was not completely unexpected and may in fact reflect a high inflammatory activity of the atherosclerotic process.

Remarkably, neopterin maintained its association with all-cause mortality after adjustment for conventional cardiovascular risk factors, angiographic CAD, and even CRP. It is of particular importance that in most analyses the hazard ratios for adverse outcomes associated with an increase of 1 quartile were greater with neopterin than with CRP, which is currently the most widely used marker of systemic inflammation for estimating cardiovascular risk. Furthermore, neopterin remained predictive of adverse outcomes at both low and high CRP concentrations (Table 5 and online Supplemental Table 2). Neopterin was only slightly higher in patients with unstable compared to those with stable CAD. This result stands in contrast to CRP which, as expected, significantly increased in the following conditions in the order they are listed: unstable angina, NSTEMI, and STEMI (Table 3). Neopterin may remain relatively stable for years, which is in line with previous data from the Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22 trial, in which no statistical differences were seen in absolute neopterin concentrations measured at 30 d, 4 months, and the end of the study (at 2 years) *(18)*. Neopterin thus appears to be a marker of long-term prognosis indicating the probable clinical course even years after the onset of the first symptoms. Such a marker would add substantial clinical information to the determination of CRP, which is highly affected by acute coronary events.

Interestingly, inclusion of NT-proBNP as a confounder diminished the association of neopterin and adverse outcomes in multiple models. Neopterin and NT-proBNP were tightly correlated, so this finding is not surprising. Currently, however, it is not known if neopterin is pathobiochemically related to left ventricular function or if this correlation is merely a chance coincidence of 2 unrelated factors or pathways.

So far, our study is the second largest study of neopterin (following the study by Ray et al. *(18)*) to address the role of neopterin as a long-term prognostic parameter in a clinical setting. Notably, our study has the longest follow-up time, namely 8 years, in contrast to the 2-year follow-up period of the Pravastatin or Atorvastatin Evaluation and Infection Therapy trial. Additionally, we included the largest population of clinically stable patients with angiographically proven CAD.

Avanzas et al. *(16)* showed that increased serum neopterin predicts future adverse cardiac events in patients with chronic stable angina pectoris. They carried out a 1-year prospective study in 297 patients with chronic stable angina pectoris undergoing diagnostic coronary angiography. The primary end-point was the composite of nonfatal MI, unstable angina, and cardiac death. Their data are in accord with our results, but we have been able to extend their observation in a much larger set of patients and rely on the strongest endpoint, namely death from any cause. In our cohort, total mortality was evidently driven by mortality from cardiovascular causes, but we also observed an association of neopterin with noncardiovascular mortality. Because the number of noncardiovascular deaths was low in our study, this finding has to be investigated further in other cohorts.

Only recently another study has shown that increased serum neopterin concentrations predict adverse cardiac events at 6 months of follow-up in Mediterranean patients with non–ST-segment elevation acute coronary syndromes *(19)*. This study involved 397 patients and showed that increased neopterin concentrations at admission predicted the combined endpoint of MI, unstable angina, and cardiac death already after 6 months of follow-up.

So far, the very few studies available to investigate the prognostic value of neopterin for cardiovascular outcomes have been carried out in small groups of patients who have been followed up only for very short periods of time. When combined with the findings of Ray et al. *(18)*, our study provides strong evidence that neopterin is an important prognostic parameter in pa-

tients with cardiovascular diseases and is independent of acute-phase markers such as CRP. Thus, measurement of neopterin should be given more attention in patients presenting to chest pain units. In addition, we are convinced that the joint determination of biomarkers including neopterin might improve the stratification of patients at risk for cardiovascular disease above and beyond that available from clinical and angiographic findings.

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