Fibrinogen and Neopterin Is Associated with Future Myocardial Infarction and Total Mortality in Patients with Stable Coronary Artery Disease

Øistein Rønneberg Mjelva[1,2] Gard F.T. Svingen[1] Eva K.R. Pedersen[1] Reinhard Seifert[3] Jan Terje Kvaløy[4,5] Øivind Midttun[6] Per M. Ueland[1,7] Jan E. Nordrehaug[1,8] Ottar Nygård[1,3] Dennis W.T. Nilsen[1,8]

[1] Department of Clinical Science, University of Bergen, Bergen, Norway
[2] Department of Medicine, Stavanger University Hospital, Stavanger, Norway
[3] Department of Heart Disease, Haukeland University Hospital, Bergen, Norway
[4] Department of Research, Stavanger University Hospital, Stavanger, Norway
[5] Department of Mathematics and Natural Sciences, University of Stavanger, Stavanger, Norway
[6] Bevital AS, Bergen, Norway
[7] Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway
[8] Department of Cardiology, Stavanger University Hospital, Stavanger, Norway

Address for correspondence Øistein Rønneberg Mjelva, MD, Department of Medicine, Stavanger University Hospital, PO Box 8100, 4068 Stavanger, Norway (e-mail: oisteinm@broadpark.no).

Thromb Haemost 2018;118:778–790.

Abstract

Systemic fibrinogen and neopterin are related to inflammation. We investigated the prognostic utility and possible interactions of these biomarkers in stable coronary artery disease (SCAD) patients undergoing coronary angiography. We included 3,545 patients with suspected stable angina with a median follow-up of 7.3 and 10.2 years for incident acute myocardial infarction (AMI) and all-cause mortality, respectively. Prospective associations were explored by Cox regression. Potential effect modifications were investigated according to strata of fibrinogen, neopterin or high-sensitivity troponin T (hsTnT) below and above the median, as well as gender and smoking habits. During follow-up, 543 patients experienced an AMI and 769 patients died. In a multivariable model, the hazard ratios (HRs; 95% confidence interval [CI]) per 1 SD increase for fibrinogen in relation to these endpoints were 1.30 (1.20, 1.42; $p < 0.001$) and 1.22 (1.13, 1.31; $p < 0.001$), respectively. For neopterin, the HRs (95% CI) were 1.31 (1.23, 1.40; $p < 0.001$) and 1.24 (1.15, 1.34; $p < 0.001$), respectively. No significant interaction between fibrinogen and neopterin was observed. The prognostic utility of neopterin for incident AMI was improved in patients with an hsTnT above the median, for total mortality in non-smokers, and for both total mortality and AMI in females. In conclusion, both fibrinogen and neopterin were associated with future AMI and total mortality, but had low discriminatory impact. No interaction was observed between these two biomarkers. The prognostic utility of neopterin was improved in patients with hsTnT levels above the median, and in females and non-smokers.

Keywords
- fibrinogen
- neopterin
- hsTnT
- prognosis
- stable coronary artery disease

received
May 9, 2017
accepted after revision
December 29, 2017

DOI https://doi.org/10.1055/s-0038-1629912.
ISSN 0340-6245.

Introduction

In patients with stable coronary artery disease (SCAD), systemic biomarkers may improve risk classification and should be selected according to their pathogenetic significance. In the present study, we selected fibrinogen and neopterin, which both respond to inflammation, known to be involved in thromboatherogenesis.

Fibrinogen is a large, soluble glycoprotein made up of three different polypeptides (Aα, Bβ and γ-chains), and is mainly synthesized by hepatocytes.[1] It forms bridges between platelets during thrombus formation and is a precursor of fibrin in which they are trapped, but it also regulates plasma viscosity by promoting red blood cell aggregation.[2] Fibrin contributes to the growth of and is abundantly present in atheromatous lesions. Subintimal fibrin attracts leukocytes, stimulates smooth muscle cell proliferation and affects endothelial permeability and vascular tone.[2] Fibrinogen is an acute-phase protein and a marker of ongoing inflammation. Its synthesis is stimulated by IL-6 and other proinflammatory cytokines,[1] and elevated levels have been reported in a variety of thrombotic and inflammatory conditions, including cardiovascular disease.[1] Fibrinogen levels have been found to be independently associated with coronary artery disease (CAD) among both healthy subjects and cardiovascular patients.[2–5] Its increase is usually considered to be secondary, but causality is also being debated.[6]

Neopterin is an aromatic pteridine which is produced by the enzyme GTP cyclohydrolase I in activated monocytes or macrophages within atherosclerotic plaques.[7] It is regarded as a sensitive marker of activation of cell-mediated immunity and phagocytic activity,[7,8] which constitute important parts in the transformation from relatively stable to more vulnerable lesions and unstable atheromatous plaques.[9,10] Furthermore, neopterin and its derivatives act as pro-oxidants and may play a role in the inflammatory process related to atherosclerosis.[11] They also stimulate the expression of thromboplastin (CD 142),[12–14] and thereby promote thromboatheromatous growth. Higher levels of neopterin have been found in many clinical conditions involving increased activity of monocytes or macrophages, such as infectious diseases; malignancies and autoimmune-, renal- and cardiovascular diseases.[15] Several reports link neopterin to the acute coronary syndrome (ACS),[16–20] more complex and extensive atherosclerotic lesions,[21,22] and to a higher probability of major adverse cardiac events.[23–27] In patients with SCAD, it has been found to be of some prognostic value.[28,29]

Neopterin's pro-oxidant properties and its association with inflammation, together with that of fibrinogen, led us to assess the prognostic utility of these biomarkers in an established SCAD population. Furthermore, their prognostic utility with respect to future events was also assessed in patient groups stratified according to the level of high-sensitivity troponin T (hsTnT), considered to be a risk factor in chronic CAD patients,[30] and in relation to gender and smoking habits. Whether these markers interact or act independently among patients with suspected SCAD has previously not been investigated. We hypothesize that fibrinogen and neopterin or the two combined may correlate with the long-term outcome in patients with SCAD.

Materials and Methods

Study Design and Patient Population

Patients were collected from the BECAC (Bergen Coronary Angiography Cohort) and the WENBIT trials (Western Norway B Vitamin Intervention Trial, ClinicalTrials.gov Identifier: NCT00354081).[31] Participants ($n = 4{,}166$) were included between 2000 and 2004, as previously described,[31,32] and all patients underwent coronary angiography. We lacked fibrinogen measurements in 621 patients (mostly due to administrative reasons), leaving 3,545 SCAD patients to be enrolled in the present study. However, patients without available fibrinogen were statistically described as similar to the investigated cohort with respect to baseline characteristics and clinical endpoints. Among the 3,545 SCAD patients, there were 18 missing neopterin measurements. Written informed consent was obtained from all participants. The study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate, and conducted in accordance with the Declaration of Helsinki.

Primary and secondary endpoints in the present study were acute myocardial infarction (AMI; both fatal and nonfatal) and total mortality. AMI was classified according to the revised definition of myocardial infarction from 2000.[33] Procedure-related nonfatal AMI occurring within the first 24 hours was excluded. Follow-up of incident AMI lasted throughout 2009 and of total mortality throughout 2012. Information on AMI was collected from the Cardiovascular Disease in Norway project (www.cvdnor.no)[34] and death from the Cause of Death Registry at Statistics Norway (www.ssb.no) and from the Western Norway Cardiovascular Registry.

Baseline Parameters

The patients underwent interview, clinical examination and blood sampling at baseline. Clinical and laboratory parameters, as presented in the baseline tables and elsewhere,[32] were collected by study personnel. In addition, hospital journals were searched for confirmation of reported data. Smoking was classified based on self-reports where non-smokers include both ex-smokers and never-smokers. Left ventricular ejection fraction (LVEF) was estimated either by echocardiography or by ventriculography during cardiac catheterization. Coronary angiography was performed and evaluated by trained invasive cardiologists. The angiograms were analysed in orthogonal views, and a significant stenosis was defined as greater than 50% luminal diameter narrowing, and the extent of CAD was described by adding the number of vessels with significant stenosis (0–3 vessels).

Blood Sampling and Laboratory Measurements

Blood samples were drawn at baseline (before angiography) and were collected and processed by study personnel. Routine blood analyses were performed by the hospital

laboratories.[31] Aliquots of serum and citrated plasma were immediately frozen at −80°C for further analysis.

Fibrinogen was measured using quantitative determination according to Clauss, utilizing the STA—Liquid Fib kit cat. number 00673 (Diagnostica Stago S.A.S., France), with a lower detection limit of 0.4 g/L and an intra-assay and inter-assay coefficients of variations (CVs) of 2.1 to 4.9% and 2.1 to 3.2%, respectively. Plasma concentrations of neopterin were analysed by liquid chromatography-tandem mass spectrometry at Bevital A/S (www.bevital.no, Bergen, Norway).[35] The cardiac troponin T was analysed using a high-sensitivity cardiac troponin T (hsTnT) assay on Modular E170 from Roche Diagnostics, with a lower detection limit of 3 ng/L.[36] High-sensitivity C-reactive protein (hsCRP) was determined in serum by an ultrasensitive immunoassay, using the Behring nephelometer II system (CV: 8.1–11.4%; N Latex CRP mono; Behring Diagnostic, Germany).

Statistics

Baseline characteristics are reported for all and for quartile (Q) subsets of patients arranged according to fibrinogen and neopterin values. Normally distributed variables were summarized as means and standard deviations (SD), whereas variables with skewed distributions were reported as medians and quartiles. The chi-square and the Kruskal–Wallis tests were applied to test for differences in categorical variables and for differences in continuous variables across quartile groups, respectively. The Student *t*-test or Mann–Whitney test was used to test for differences in fibrinogen and neopterin levels in subgroups according to gender and smoking habits depending on whether the variable was normally distributed or not. Differences in logarithmically transformed levels of the biomarkers in these subgroups were further evaluated in a linear regression model including age.

Kaplan–Meier plots according to quartiles of fibrinogen and neopterin were used for estimating the survival curves for times to event. The log-rank test was used to test for differences in the survival curves. Cox proportional hazard regression analysis was used to obtain hazard ratios (HRs; 95% confidence intervals [CI]) for incident AMI per 1 SD increase of logarithmically transformed fibrinogen and neopterin levels, in a crude model, a model adjusted for age and gender (multivariable Model 1), in addition to a model adjusted for age, gender, diabetes, current smoking, hypertension, estimated glomerular filtration rate (eGFR), serum levels of triglycerides, apolipoprotein A1 and apolipoprotein B (multivariable Model 2). Potential effect modifications were investigated according to strata (below or above the median) of fibrinogen, neopterin or hsTnT, as well as according to gender and smoking status. Assumption for the proportional hazard was confirmed by investigating the Schoenfeld residuals. Differences of the area under the curve (AUC) based on Cox regression models were assessed using the DeLong's test for two correlated receiver operator curves (ROC). A *p*-value <0.05 was considered significant. The statistical analysis were performed using the statistical package SPSS version 23.0 and R version 3.3.1.[37]

Results

Baseline Characteristics

Baseline patient characteristics across quartiles of fibrinogen and neopterin are presented in ►**Tables 1** and **2**, respectively, and evaluation of correlates between these and the baseline variables are shown in ►**Supplementary Tables S1** and **S2** (online only). Patients with higher fibrinogen levels had higher levels of neopterin, hsTnT, hsCRP, leucocytes and thrombocytes, whereas eGFR, HDL cholesterol and apolipoprotein A1 were lower. Furthermore, these patients were older, and a higher proportion were current smokers, had known hypertension, diabetes mellitus, hypercholesterolemia, known history of vascular disease (CAD, cerebrovascular disease and peripheral artery disease) and heart failure, and a higher proportion used medication such as warfarin and angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and diuretics. Similar associations were observed across quartiles of neopterin, except that neopterin was inversely associated with smoking and hypercholesterolemia.

Baseline fibrinogen and neopterin levels were modestly positively correlated (Spearman's rho = 0.18, *p*-value < 0.001). Fibrinogen was strongly correlated with hsCRP (Spearman's rho = 0.50, *p*-value < 0.001), and modestly to age ($r = 0.18$), hsTnT ($r = 0.17$), thrombocyte count ($r = 0.14$). Fibrinogen correlated negatively to eGFR ($r = -0.13$). Neopterin was strongly positively associated with eGFR ($r = 0.52$, *p*-value < 0.001), and modestly also to age ($r = 0.36$), hsTnT ($r = 0.26$) and hsCRP ($r = 0.19$).

Patients with higher serum hsTnT had more extensive CAD, as assessed by baseline coronary angiography, when compared with those with lower hsTnT (►**Supplementary Table S3** [online only]). In Q1 of hsTnT, 36.5% ($n = 542$) had no significant lesions and 18.4% ($n = 274$) had three vessel disease, in contrast to 14.1% ($n = 115$) versus 43.2% ($n = 353$), respectively, in the upper quartile ($p < 0.001$). A similar relationship with CAD was noted across fibrinogen quartiles. These data are displayed in ►**Supplementary Tables S4** and **S5** (online only).

Supplementary Subgroup Baseline Information

Differences according to gender and smoking status are depicted in ►**Supplementary Table S6** (online only). Females had higher levels of fibrinogen ($p = 0.008$) and neopterin ($p < 0.001$), and lower levels of hsTnT level ($p < 0.001$), as compared with males. Smokers had a higher median concentration of fibrinogen ($p < 0.001$) but lower levels of neopterin ($p < 0.001$) and hsTnT ($p < 0.001$), as compared with non-smokers. The proportion of female and male smokers was not statistically different, but current smokers were younger than non-smokers.

In females, fibrinogen and neopterin levels were statistically higher in the presence of significant CAD (≥1 vessel disease, as compared with patients with no significant lesions; $p < 0.001$ and $p = 0.001$, respectively), whereas in men such difference was noted only for fibrinogen ($p < 0.001$). Median concentrations of fibrinogen were also higher, irrespective of smoking habits, when comparing

Table 1 Baseline characteristics according to fibrinogen quartiles

Variable	*n*	Total	Quartiles of fibrinogen				
		Cohort	Q1	Q2	Q3	Q4	*p*-Value[a]
Age, y	3,545	62.0 (55.0, 70.0)	59.0 (53.0, 67.0)	61.0 (54.5, 69.0)	64.0 (56.0, 71.0)	64.0 (57.0, 71.0)	<0.001
Male gender, *n* (%)	3,545	2,503 (70.6)	632 (74.5)	579 (70.9)	669 (69.5)	623 (67.9)	0.017
Risk markers at baseline							
Fibrinogen (g/L)	3,545	3.60 (3.20, 4.10)	2.90 (2.70, 3.00)	3.30 (3.20, 3.50)	3.80 (3.70, 3.90)	4.40 (4.20, 4.80)	<0.001
Neopterin (nmol/L)	3,527	8.22 (6.69, 10.49)	7.62 (6.41, 9.40)	8.03 (6.71, 10.05)	8.33 (6.72, 10.57)	9.04 (6.96, 12.20)	<0.001
hsTnT (ng/L)	3,475	5.00 (3.00, 10.00)	3.00 (3.00, 7.00)	4.00 (3.00, 9.00)	5.00 (3.00, 11.00)	6.00 (3.00, 13.00)	<0.001
hsCRP (mg/L)	3,542	1.79 (0.87, 3.69)	0.92 (0.53, 1.79)	1.44 (0.77, 2.64)	1.88 (1.03, 3.62)	3.93 (2.05, 8.22)	<0.001
Leucocytes ($\times 10^6$/mL)	3,543	6.8 (5.7, 8.2)	6.3 (5.2, 7.4)	6.5 (5.5, 7.7)	7.0 (5.8, 8.2)	7.7 (6.4, 9.3)	<0.001
Monocyte fraction	3,438	0.08 (0.06, 0.09)	0.08 (0.06, 0.09)	0.08 (0.06, 0.09)	0.08 (0.06, 0.09)	0.07 (0.06, 0.09)	0.308
TRC (10^9/L)	3,521	239 (204, 281)	233 (201, 270)	234 (198, 274)	239 (203, 277)	255 (215, 302)	<0.001
Risk factors							
Current smoking, *n* (%)	3,538	816 (23.1)	149 (17.6)	165 (20.2)	212 (22.1)	290 (31.7)	<0.001
Hypertension, *n* (%)	3,545	1,677 (47.3)	331 (39.0)	358 (43.8)	476 (49.5)	512 (55.8)	<0.001
IDDM, *n* (%)	3,545	36 (1.0)	10 (1.2)	8 (1.0)	10 (1.0)	8 (0.9)	<0.001[b]
NIDDM, *n* (%)	3,545	394 (11.1)	73 (8.6)	81 (9.9)	99 (10.3)	141 (15.4)	<0.001[b]
eGFR (mL/min per 1.73 m^2)	3,539	90.0 (78.0, 99.0)	92.0 (82.0, 101.0)	90.0 (80.0, 99.0)	89.0 (77.0, 98.0)	88.0 (73.0, 98.0)	<0.001
Hypercholesterolemia, *n* (%)	3,545	1,892 (57.4)	424 (53.3)	432 (55.5)	530 (59.9)	506 (60.5)	0.007
BMI, kg/m^2	3,542	26.0 (24.0, 29.0)	25.0 (23.0, 28.0)	26.0 (24.0, 28.0)	26.0 (24.0,29.0)	26.0 (24.0,29.0)	<0.001
Serum lipids and apolipoproteins							
Total cholesterol (mmol/L)	3,544	4.90 (4.30, 5.70)	5.00 (4.23, 5.80)	5.00 (4.30, 5.70)	4.90 (4.30, 5.70)	4.90 (4.28, 5.70)	0.690
LDL (mmol/L)	3,543	2.97 (2.40, 3.70)	2.93 (2.30, 3.70)	3.00 (2.40, 3.70)	2.95 (2.40,3.70)	2.90 (2.40, 3.70)	0.865
HDL (mmol/L)	3,545	1.20 (1.00, 1.50)	1.30 (1.10, 1.50)	1.30 (1.10, 1.50)	1.20 (1.00, 1.50)	1.20 (1.00, 1.50)	<0.001
Triglycerides (mmol/L)	3,542	1.49 (1.07, 2.11)	1.51 (1.05, 2.20)	1.42 (1.06, 2.19)	1.51 (1.10, 2.08)	1.46 (1.05, 1.98)	0.545
ApoB, g/L	3,543	0.87 (0.73, 1.05)	0.85 (0.71, 1.04)	0.86 (0.73, 1.03)	0.87 (0.74, 1.04)	0.88 (0.75, 1.07)	0.023
ApoA1, g/L	3,542	1.31 (1.14, 1.50)	1.34 (1.18, 1.52)	1.34 (1.18, 1.51)	1.29 (1.14, 1.49)	1.27 (1.11, 1.47)	<0.001
History of cardiovascular disease							
Previous AMI, *n* (%)	3,545	1,386 (39.1)	306 (36.1)	299 (36.6)	382 (39.7)	399 (43.5)	0.005
Previous CBV, *n* (%)	3,545	254 (7.2)	43 (5.1)	55 (6.7)	59 (6.1)	97 (10.6)	<0.001
Previous PAD, *n* (%)	3,545	312 (8.8)	51 (6.0)	47 (5.8)	96 (10.0)	118 (12.9)	<0.001
Previous PCI, *n* (%)	3,545	665 (18.8)	162 (19.1)	152 (18.6)	169 (17.6)	182 (19.8)	0.646
Previous CABG, *n* (%)	3,545	399 (11.3)	90 (10.6)	90 (11.0)	111 (11.5)	108 (11.8)	0.870

(*Continued*)

Table 1 (*Continued*)

Variable	*n*	Total	Quartiles of fibrinogen				
		Cohort	Q1	Q2	Q3	Q4	*p*-Value[a]
Known CHF, *n* (%)	3,545	242 (6.8)	37 (4.4)	38 (4.7)	63 (6.5)	104 (11.3)	<0.001
LVEF, %	3,545	66 (60, 70)	69 (60, 70)	68 (60, 70)	67 (60, 70)	65 (59, 70)	<0.001
Extent of CAD at baseline							
No stenotic vessels, *n* (%)	3,545	949 (26.8)	284 (33.5)	239 (29.3)	229 (23.8)	197 (21.5)	<0.001[c]
One-vessel disease, *n* (%)	3,545	811 (22.9)	192 (22.6)	178 (21.8)	227 (23.6)	214 (23.3)	<0.001[c]
Two-vessel disease, *n* (%)	3,545	773 (21.8)	168 (19.8)	175 (21.4)	231 (24.0)	199 (21.7)	<0.001[c]
Three-vessel disease, *n* (%)	3,545	1,012(28.5)	204 (24.1)	225 (27.5)	275 (28.6)	308 (33.6)	<0.001[c]
Treatment following baseline coronary angiography							
No specific treatment	3,544	237 (6.7)	91 (10.7)	80 (9.8)	44 (4.6)	22 (2.4)	<0.001[d]
Medical therapy only	3,544	1,432 (40.4)	346 (40.8)	315 (38.6)	376 (39.1)	395 (43.1)	<0.001[d]
PCI	3,544	1,120 (31.6)	256 (30.2)	246 (30.1)	329 (34.2)	289 (31.5)	<0.001[d]
CABG	3,544	671 (18.9)	141 (16.6)	159 (19.5)	189 (19.6)	182 (19.8)	<0.001[d]
Combination of surgical treatments	3,544	84 (2.4)	14 (1.7)	17 (2.1)	24 (2.5)	29 (3.2)	<0.001[d]
Medication prior to inclusion							
Acetylsalicylic acid, *n* (%)	3,545	2,869 (80.9)	668 (78.8)	656 (80.3)	799 (83.1)	746 (81.3)	0.130
ADP receptor antagonist, *n* (%)	3,545	551 (15.5)	134 (15.8)	126 (15.4)	155 (16.1)	136 (14.8)	0.882
Warfarin, *n* (%)	3,545	196 (5.5)	25 (2.9)	36 (4.4)	49 (5.1)	86 (9.4)	<0.001
Statin, *n* (%)	3,534	2,806 (79.4)	642 (75.9)	631 (77.4)	783 (81.5)	750 (82.2)	0.016
Beta-blocker, *n* (%)	3,545	2,548 (71.9)	578 (68.2)	567 (69.4)	729 (75.8)	674 (73.4)	0.001
ACE-inhibitor, *n* (%)	3,545	725 (20.5)	141 (16.6)	154 (18.8)	187 (19.4)	243 (26.5)	<0.001
ARB, *n* (%)	3,545	413 (11.7)	81 (9.6)	90 (11.0)	112 (11.6)	130 (14.2)	0.023
Loop diuretics, *n* (%)	3,545	394 (11.1)	49 (5.8)	69 (8.4)	108 (11.2)	168 (18.3)	<0.001
Thiazide diuretic, *n* (%)	3,545	253 (7.1)	44 (5.2)	56 (6.9)	63 (6.5)	90 (9.8)	0.002

Abbreviations: ACE, angiotensin-converting enzyme; ADP, adenosine diphosphate; AMI, acute myocardial infarction; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; ARB angiotensin receptor blocker; BMI, body mass index; BNP, B-type natriuretic peptide; CABG, coronary artery bypass graft; CBV, cerebrovascular disease; CHF, congestive heart failure; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein cholesterol; IDDM, insulin-dependent diabetes mellitus; LDL, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; NIDDM, non–insulin-dependent diabetes mellitus; PAD, peripheral artery disease; PCI, percutaneous coronary intervention; TNT, troponin T; TRC, platelets.

[a]*p*-Values for chi-square tests of difference in categorical variables across quartile groups, and for Kruskal–Wallis tests for differences in continuous variables across quartile groups.

[b]*p*-Value for chi-square test comparing IDDM, NIDM and no history of diabetes mellitus.

[c]*p*-Value for chi-square test comparing no stenotic vessels—one, two and three-vessel disease.

[d]*p*-Value for chi-square test comparing the different treatments given following baseline coronary angiography.

Note: Continuous variables are reported as median (25^{th}–75th percentile), and categorical variables as counts (percentage of total).

Table 2 Baseline characteristics according to neopterin quartiles

Variable	*n*	Total	Quartiles of neopterin[a]				*p*-Value[b]
		Cohort	Q1	Q2	Q3	Q4	
Age, y	3,545	62.0 (55.0, 70.0)	58.0 (51.0, 64.0)	59.5 (53.0, 66.0)	64.0 (57.0, 71.0)	69.0 (60.0, 75.0)	<0.001
Male gender, *n* (%)	3,545	2503 (70.6)	699 (79.3)	649 (73.6)	598 (67.8)	547 (62.0)	<0.001
Risk markers at baseline							
Neopterin (nmol/L)	3,527	8.22 (6.69, 10.49)	5.91 (5.30, 6.32)	7.41 (7.02, 7.80)	9.19 (8.65, 9.69)	12.72 (11.45, 15.38)	<0.001
Fibrinogen (g/L)	3,545	3.60 (3.20, 4.10)	3.50 (3.10, 4.00)	3.50 (3.10, 4.00)	3.60 (3.20, 4.00)	3.80 (3.30, 4.40)	<0.001
hsTnT (ng/L)	3,475	5.00 (3.00, 10.00)	3.00 (3.00, 7.00)	3.00 (3.00, 8.00)	5.00 (3.00, 10.00)	8.00 (3.00, 17.00)	<0.001
hsCRP (mg/L)	3,542	1.79 (0.87, 3.69)	1.46 (0.76, 2.69)	1.69 (0.79, 3.26)	1.82 (0.91, 3.70)	2.48 (1.16, 13.22)	<0.001
Leucocytes ($\times 10^6$/mL)	3,543	6.8 (5.7, 8.2)	6.8 (5.7, 8.2)	6.7 (5.6, 7.9)	6.8 (5.8, 8.0)	6.9 (5.8, 8.4)	0.044
Monocyte fraction	3,438	0.08 (0.06, 0.09)	0.08 (0.06, 0.09)	0.07 (0.06, 0.09)	0.08 (0.06, 0.09)	0.08 (0.06, 0.09)	0.010
TRC (10^9/L)	3,521	239 (204, 281)	242 (206, 282)	243 (207, 281)	238 (202, 282)	238 (201, 280)	0.476
Risk factors							
Current smoking, *n* (%)	3,538	816 (23.1)	268 (30.5)	212 (24.1)	184 (20.9)	149 (16.9)	<0.001
Hypertension, *n* (%)	3,545	1677 (47.3)	381 (43.2)	384 (43.5)	404 (45.8)	499 (56.6)	<0.001
IDDM, *n* (%)	3,545	36 (1.0)	13 (1.5)	7 (0.8)	5 (0.6)	11 (1.2)	0.328[c]
NIDDM, *n* (%)	3,545	394 (11.1)	98 (11.1)	94 (10.7)	89 (10.1)	108 (12.2)	0.328[c]
eGFR (mL/min per 1.73 m^2)	3,539	90.0 (78.0, 99.0)	97.0 (90.0, 105.0)	93.0 (86.0, 101.0)	87.0 (76.0, 96.0)	75.0 (61.0, 88.0)	<0.001
Hypercholesterolemia, *n* (%)	3,545	1892 (57.4)	519 (61.8)	474 (57.2)	453 (55.4)	439 (55.2)	0.023
BMI, kg/m^2	3,542	26.0 (24.0, 29.0)	26.0 (24.0, 29.0)	26.0 (24.0, 29.0)	26.0 (23.0,28.0)	26.0 (23.0,28.0)	0.003
Serum lipids and apolipoproteins							
Total cholesterol (mmol/L)	3,544	4.90 (4.30, 5.70)	5.00 (4.30, 5.80)	4.90 (4.30, 5.70)	4.90 (4.30, 5.70)	4.80 (4.20, 5.70)	0.139
LDL (mmol/L)	3,543	2.97 (2.40, 3.70)	3.00 (2.48, 3.70)	2.97 (2.40, 3.70)	3.00 (2.40,3.70)	2.90 (2.30, 3.70)	0.152
HDL (mmol/L)	3,545	1.20 (1.00, 1.50)	1.20 (1.10, 1.50)	1.20 (1.00, 1.50)	1.30 (1.10, 1.50)	1.20 (1.00, 1.50)	0.004
Triglycerides (mmol/L)	3,542	1.49 (1.07, 2.33)	1.60 (1.12, 2.33)	1.53 (1.09, 2.19)	1.40 (1.05, 1.95)	1.43 (1.04, 1.98)	<0.001
ApoB, g/L	3,543	0.87 (0.73, 1.05)	0.88 (0.74, 1.05)	0.86 (0.74, 1.05)	0.87 (0.72, 1.04)	0.87 (0.74, 1.06)	0.777
ApoA1, g/L	3,542	1.31 (1.14, 1.50)	1.31 (1.16, 1.48)	1.31 (1.15, 1.48)	1.33 (1.15, 1.53)	1.28 (1.12, 1.50)	0.005
History of cardiovascular disease							
Previous AMI, *n* (%)	3,545	1,386 (39.1)	338 (38.4)	343 (38.9)	316 (35.8)	383 (43.4)	0.011
Previous CBV *n* (%)	3,545	254 (7.2)	45 (5.1)	42 (4.8)	59 (6.7)	107 (12.1)	<0.001
Previous PAD *n* (%)	3,545	312 (8.8)	64 (7.3)	57 (6,5)	88 (10.0)	102 (11.6)	<0.001
Previous PCI, *n* (%)	3,545	665 (18.8)	174 (19.8)	174 (19.7)	147 (16.7)	170 (19.3)	0.288
Previous CABG, *n* (%)	3,545	399 (11.3)	80 (9.1)	96 (10.9)	108 (12.2)	113 (12.8)	0.063

(*Continued*)

Table 2 (*Continued*)

Variable	*n*	Total	Quartiles of neopterin[a]				*p*-Value[b]
		Cohort	Q1	Q2	Q3	Q4	
Known CHF, *n* (%)	3,545	242 (6.8)	33 (3.7)	44 (5.0)	69 (7.8)	95 (10.8)	<0.001
LVEF, %	3545	66 (60, 70)	69 (60, 70)	66 (60, 70)	66 (60, 70)	65 (56, 70)	<0.001
Extent of CAD at baseline							
No stenotic vessels, *n* (%)	3,545	949 (26.8)	235 (26.7)	232 (26.3)	258 (29.3)	221 (25.1)	0.010[d]
One-vessel disease, *n* (%)	3,545	811 (22.9)	218 (24.7)	217 (24.6)	187 (21.2)	182 (20.6)	0.010[d]
Two-vessel disease, *n* (%)	3,545	773 (21.8)	206 (23.4)	188 (21.3)	193 (21.9)	183 (20.7)	0.010[d]
Three-vessel disease, *n* (%)	3,545	1,012(28.5)	222 (25.2)	245 (27.8)	244 (27.7)	296 (33.6)	0.010[d]
Treatment following baseline coronary angiography							
No specific treatment	3,544	237 (6.7)	63 (7.2)	61 (6.9)	62 (7.0)	50 (5.7)	0.134[e]
Medical therapy only	3,544	1,432 (40.4)	339 (38.5)	344 (39.0)	365 (41.4)	378 (42.9)	0.134[e]
PCI	3,544	1,120 (31.6)	283 (32.1)	280 (31.7)	286 (32.4)	263 (29.9)	0.134[e]
CABG	3,544	671 (18.9)	173 (19.6)	186 (21.1)	147 (16.7)	163 (18.5)	0.134[e]
Combination of surgical treatments	3,544	84 (2.4)	23 (2.6)	11 (1.2)	22 (2.5)	27 (3.1)	0.134[e]
Medication prior to inclusion							
Acetylsalicylic acid, *n* (%)	3,545	2,869 (80.9)	733 (83.2)	742 (84.1)	702 (79.6)	677 (76.8)	<0.001
ADP receptor antagonist, *n* (%)	3,545	551 (15.5)	138 (15.7)	137 (15.5)	134 (15.2)	137 (15.5)	0.994
Warfarin, *n* (%)	3,545	196 (5.5)	42 (4.8)	26 (2.9)	53 (6.0)	75 (8.5)	<0.001
Statin, *n* (%)	3,534	2,806 (79.4)	710 (80.7)	721 (81.8)	679 (77.3)	680 (77.5)	0.165
Beta-blocker, *n* (%)	3,545	2,548 (71.9)	627 (71.2)	637 (72.2)	609 (69.0)	660 (74.8)	0.056
ACE inhibitor, *n* (%)	3,545	725 (20.5)	157 (17.8)	148 (16.8)	171 (19.4)	246 (27.9)	<0.001
ARB, *n* (%)	3,545	413 (11.7)	86 (9.8)	102 (11.6)	107 (12.1)	117 (13.3)	0.140
Loop diuretics, *n* (%)	3,545	394 (11.1)	45 (5.1)	56 (6.3)	93 (10.5)	200 (22.7)	<0.001
Thiazide diuretic, *n* (%)	3,545	253 (7.1)	40 (4.5)	60 (6.8)	69 (7.8)	82 (9.3)	0.001

Abbreviations: ACE inhibitor, angiotensin-converting enzyme; ADP, adenosine diphosphate; AMI, acute myocardial infarction; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; ARB angiotensin receptor blocker; BMI, body mass index; BNP, B-type natriuretic peptide; CABG, coronary artery bypass graft; CBV, cerebrovascular disease; CHF, congestive heart failure; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein cholesterol; IDDM, insulin-dependent diabetes mellitus; LDL, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; NIDDM, non–insulin-dependent diabetes mellitus; PAD, peripheral artery disease; PCI, percutaneous coronary intervention; TNT, troponin T; TRC, platelets.

[a]Patients with valid measurements of neopterin.

[b]*p*-Values for chi-square tests of difference in categorical variables across quartile groups, and for Kruskal–Wallis tests for differences in continuous variables across quartile groups.

[c]*p*-Value for chi-square test comparing IDDM, NIDM and no history of diabetes mellitus.

[d]*p*-Value for chi-square test comparing no stenotic vessels—one-, two- and three-vessel disease.

[e]*p*-Value for chi-square test comparing the different treatments given following baseline coronary angiography.

Note: Continuous variables are reported as median (25^{th}–75th percentile), and categorical variables as counts (percentage of total).

patients with no stenotic vessels to those with one- to two-vessel disease, whereas a similar relationship was not found for neopterin.

Acute Myocardial Infarction

A total of 543 patients suffered an AMI during a median (25th and 75th percentiles) follow-up time of 7.3 (6.3, 8.6) years. Kaplan–Meier plots for patient groups divided according to quartiles of fibrinogen and neopterin are presented in ►**Fig. 1**. Patients with fibrinogen or neopterin levels in the upper quartile had a shorter time to incident AMI (p-value for log-rank test for survival curves is < 0.001 for both biomarkers).

In the univariable analysis, fibrinogen (►**Table 3**) and neopterin (►**Table 4**) were strongly associated with the primary endpoint AMI (HRs [95% CIs] per 1 SD increment of logarithmically transformed values: 1.30 [1.20, 1.42], $p < 0.001$ and 1.31 [1.23, 1.40], $p < 0.001$, respectively). This association remained statistically significant for both biomarkers after adjusting for possible confounders in our multivariable models (►**Tables 3** and **4**). For both biomarkers, the association with AMI risk was essentially linear across the

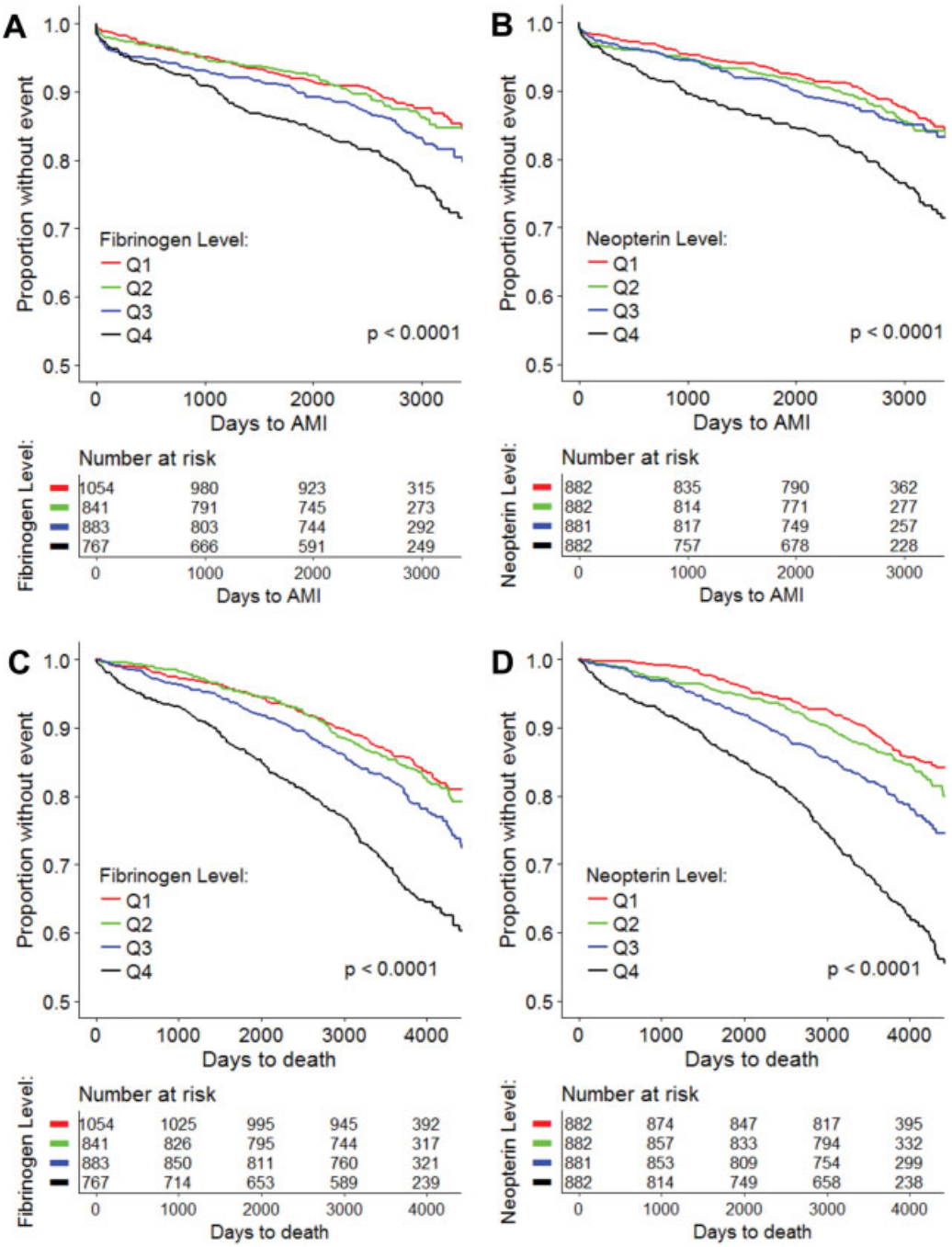

Fig. 1 Kaplan–Meier plots for the cumulative risk of AMI (**A and B**) and total mortality (**C and D**) during follow-up stratified by fibrinogen and neopterin quartiles. Numbers at risk displayed for each time point. p-Value represents log-rank.

Table 3 Association of fibrinogen and covariates with incident acute myocardial infarction

	Univariable[a]		Multivariable Model 1[a]		Multivariable Model 2[a]	
	HR (95% CI)	*p*-Value	HR (95% CI)	*P*-value	HR (95% CI)	*p*-Value
Fibrinogen[b]	1.30 (1.20, 1.42)	<0.001	1.26 (1.15, 1.37)	<0.001	1.18 (1.08, 1.29)	<0.001
Age[c]			1.40 (1.28, 1.54)	<0.001	1.40 (1.25, 1.57)	<0.001
Gender, male			1.41 (1.16, 1.72)	<0.001	1.34 (1.09, 1.65)	0.006
IDDM					2.64 (1.37, 5.08)	0.004
NIDDM					1.45 (1.15, 1.84)	0.002
Current smoker					1.65 (1.35, 2.01)	<0.001
Hypertension					1.11 (0.93, 1.32)	0.266
TG[c]					1.05 (0.97, 1.14)	0.185
ApoB[c]					1.01 (0.92, 1.10)	0.861
ApoA1[c]					0.91 (0.83, 1.01)	0.064
eGFR[c]					0.89 (0.80, 0.98)	0.016

Abbreviations: ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; eGFR, estimated glomerular filtration rate; IDDM, insulin-dependent diabetes mellitus; NIDDM, non–insulin-dependent diabetes mellitus; SD, standard deviation; TG, triglycerides.
[a]Estimated by Cox proportional hazard regression analysis.
[b]Per 1 SD increase in logarithmically transformed fibrinogen levels.
[c]Per 1 SD increase.

whole distribution (►**Fig. 2**). The AUC for the ROC for covariates applied to multivariable Model 2, without fibrinogen and neopterin, was 0.650, which increased to 0.665 after inclusion of both fibrinogen and neopterin ($p = 0.007$; ►**Table 5**; ►**Supplementary Figs. S1** and **S2** [online only]).

There were no statistically significant interactions across subgroups of fibrinogen and neopterin dichotomized at median levels. However, there was a statistically significant interaction between neopterin and hsTnT groups (below/above the median), such that the association for neopterin with AMI was greater when hsTnT levels were above median ($p = 0.032$). This interaction remained statistically significant in Model 1 adjusting for age and gender, but not after adjusting for all covariates in our multivariable Model 2 (*p*-value for interaction is 0.056).

Acute Myocardial Infarction According to Gender and Smoking Status

In both males and females, fibrinogen and neopterin were associated with incident AMI in univariable and

Table 4 Association of neopterin and covariates with incident acute myocardial infarction

	Univariable[a]		Multivariable Model 1[a]		Multivariable Model 2[a]	
	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Neopterin[b]	1.31 (1.23, 1.40)	<0.001	1.24 (1.16, 1.34)	<0.001	1.21 (1.10, 1.33)	<0.001
Age[c]			1.35 (1.23, 1.48)	<0.001	1.44 (1.29, 1.62)	<0.001
Sex, male			1.44 (1.18, 1.76)	<0.001	1.33 (1.08, 1.65)	0.007
IDDM					2.30 (1.18, 4.49)	0.014
NIDDM					1.51 (1.19, 1.92)	<0.001
Current smoker					1.73 (1.42, 2.10)	<0.001
Hypertension					1.13 (0.94, 1.34)	0.189
TG[c]					1.04 (0.96, 1.13)	0.318
ApoB[c]					1.01 (0.92, 1.10)	0.847
ApoA1[c]					0.91 (0.83, 1.00)	0.059
eGFR[c]					1.00 (0.89, 1.13)	0.988

Abbreviations: ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; eGFR, estimated glomerular filtration rate; IDDM, insulin-dependent diabetes mellitus; NIDDM, non–insulin-dependent diabetes mellitus; SD, standard deviation; TG, triglycerides.
[a]Estimated by Cox proportional hazard regression analysis.
[b]Per 1 SD increase in logarithmically transformed neopterin levels.
[c]Per 1 SD increase.

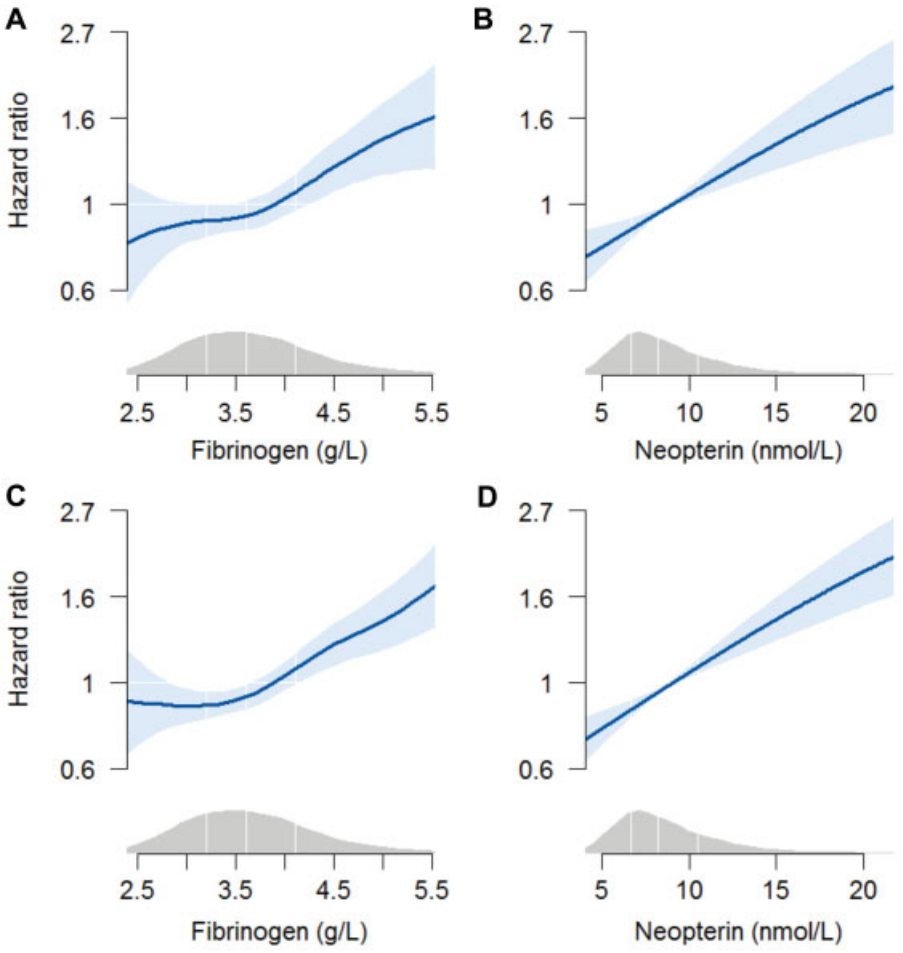

Fig. 2 Dose–response relationship between risk of acute myocardial infarction (**A and B**) or all-cause mortality (**C and D**) and fibrinogen or neopterin. Estimated by generalized additive Cox regression with penalized smoothing spline (multivariable model 2). The solid lines represent hazard ratios, and the shaded areas represent the 95% confidence intervals. The *x*-axes are trimmed, excluding the lower and upper 2.5 percentile. Density plots are superimposed along the *x*-axes, with vertical lines displaying (from left) the 25th, 50th and 75th percentile of the distribution.

multivariable models (►**Supplementary Table S7** [online only]). The association for neopterin was stronger among females than among males ($p = 0.006$ for interaction). Both fibrinogen and neopterin were associated with incident AMI in the simple model, irrespective of smoking habits, remaining statistically significant in the fully adjusted model for non-smokers (►**Supplementary Table S7** [online only]). However, no statistically significant interactions were found between fibrinogen or neopterin and smoking status with respect to future AMI.

All-Cause Mortality

Median (25th and 75th percentiles) follow-up time with respect to the secondary outcome measure of total mortality

Table 5 Model discrimination

Model[a]	AMI		Total mortality	
	AUC (95% CI)	*p*-Value[b]	AUC (95% CI)	*p*-Value[b]
Model 2	0.650 (0.584, 0.643)		0.750 (0.724, 0.776)	
Model 2 + fibrinogen	0.657 (0.629, 0.685)	0.155	0.755 (0.729, 0.780)	0.141
Model 2 + neopterin	0.661 (0.661, 0.689)	0.006	0.757 (0.732, 0.782)	0.011
Model 2 + fibrinogen and neopterin	0.665 (0.637, 0.693)	0.007	0.760 (0.735, 0.785)	0.008

Abbreviations: AMI, acute myocardial infarction; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; eGFR, estimated glomerular filtration rate.
[a]Model 2 includes age, gender, diabetes mellitus, smoking status, hypertension, triglycerides, ApoA1, ApoB and eGFR.
[b]*p*-Value represents comparison of gains in AUC between Model 2 and Model 2 + fibrinogen, Model 2 and Model 2 + neopterin, and Model 2 and Model 2 + both fibrinogen and neopterin.

was 10.2 (9.2, 11.4) years, during which a total of 769 patients died. Kaplan–Meier plots for quartile of fibrinogen and neopterin are presented in ►**Fig. 1**. Patients in the upper quartile of both fibrinogen and neopterin had a worse outcome, as compared with patients of the lower quartile (log-rank test, $p < 0.001$). In the univariable and multivariable Cox regression models,[1,2] both fibrinogen and neopterin were associated with all-cause death during follow-up. The HR (95% CI) per 1 SD (logarithmically transformed) was 1.22 (1.13, 1.31) for fibrinogen and 1.24 (1.15, 1.34) for neopterin in the fully adjusted Model 2 ($p < 0.001$ for both; ►**Supplementary Tables S8** and **S9** [online only]; ►**Fig. 2**). The AUC for the ROC curve with respect to all-cause mortality was 0.750, based on multivariable Model 2, not including fibrinogen and neopterin, increasing to 0.760 when adding both these biomarkers to the model ($p = 0.008$; ►**Table 5**, ►**Supplementary Figs. 3** and **4** [online only]).

For all-cause mortality, there was a statistically significant interaction between neopterin and fibrinogen groups (arranged according to below and above the median, respectively) (p-value for interaction is 0.019). We observed a reduced association of neopterin with risk among patients with fibrinogen levels above the median (HR: 1.39 [95% CI: 1.31, 1.46], $p < 0.001$), below the median (HR: 1.61 [95% CI: 1.44, 1.80], $p < 0.001$). However, this interaction was no longer significant after multivariable adjustment. Furthermore, no significant interaction was found between fibrinogen and neopterin across subgroups of hsTnT below or above median (p-value for interaction is 0.126–0.725).

All-Cause Mortality According to Gender and Smoking Status

For gender, higher levels of both fibrinogen and neopterin correlated with increased risk of total mortality (►**Supplementary Table S7** [online only]). Neopterin was more strongly related to death in women ($p = 0.021$ for interaction).

Fibrinogen and neopterin were associated with death in both smokers and non-smokers in our univariable model, and this relationship remained statistically significant after adjusting for possible confounding factors in Model 2 (►**Supplementary Table S7** [online only]). In addition, a significant interaction ($p = 0.027$) was found between neopterin and smoking status, where the prognostic utility of neopterin was greater among non-smokers.

Discussion

Principal Findings

We found that higher values of fibrinogen and neopterin were independently associated with increased risk of AMI and total mortality in a population of SCAD patients, supporting our hypothesis. We also observed that the association with total mortality for neopterin was higher at hsTnT values above the median, in women and in non-smokers.

Fibrinogen

In the total cohort, fibrinogen was related to both incident AMI and all-cause mortality, but it did not add additional significant prognostic information, as compared with traditional risk parameters, based on comparison of AUC in the ROC analysis. The extent of CAD as judged angiographically increased across the quartiles of fibrinogen, from Q1 to Q4, which is in accordance with a recent study by Tabacki et al.[38] Fibrinogen is a marker of inflammation, and showed expected positive correlation with hsCRP. Our findings with respect to future AMI and mortality are essentially in accordance with a report on patients with angiographically significant CAD by Sinning et al.[39]

Neopterin

We found that neopterin was a significant risk marker for both incident AMI and total mortality. In the ROC analysis, this biomarker significantly improved the AUC for both AMI and total mortality, as compared with traditional risk markers (Model 2). Neopterin has previously been shown to yield prognostic information related to both incident AMI and total mortality.[29] In a mixed angiographic population consisting of 1,083 SCAD and 718 ACS patients with a median follow-up of 8.0 years, neopterin levels were associated with both total and cardiovascular mortality.[40] The results of our study, which included only SCAD patients, are essentially in agreement with that study. Neopterin may also serve as a useful risk marker for death or recurrent acute coronary events among ACS patients, as demonstrated in the TIMI 22 study by Ray et al,[25] in which blood samples were collected 1 week after an acute coronary event.

Interactions

Both fibrinogen and neopterin were independently associated with outcome in patients with SCAD, but no interactions were observed between the two biomarkers with respect to both incident AMI and total mortality.

Small increases in troponins may reflect active plaques generating microembolisms associated with asymptomatic minor myocardial injury in SCAD.[41] Lyngbæk et al have previously reported an association between increasing hsTnT for both total mortality and AMI, evaluated as separate endpoints.[30] In the present study, we noted that the association of neopterin with incident AMI was enhanced in patients with hsTnT above the median (limited to Model 1), whereas this interaction was not observed for total mortality. Thus, the prognostic utility of neopterin may be improved among high-risk SCAD patients, as defined by their hsTnT levels. Notably, to the best of our knowledge, a potential effect modification between neopterin and hsTnT has not been evaluated previously.

Subgroups

Circulating concentrations of both fibrinogen and neopterin were higher in females than in males, also after adjustment for age. In a previous report on healthy subjects, the level of neopterin was similar for men and women,[42] whereas in one study with unstable angina pectoris patients the level was higher in females.[22] We also observed that neopterin in women was associated with the presence of significant CAD, whereas this association was not noted in males. To our knowledge,

similar findings have previously not been reported. Our main findings related to prognosis were valid in both genders. According to an interaction analysis, the risk associations for neopterin were more pronounced in the female subgroup.

Current smokers had higher levels of fibrinogen but lower levels of neopterin as compared with non-smokers. These findings for fibrinogen are consistent with previous reports,[43] whereas data on the influence of smoking on neopterin are inconsistent.[29,40,44,45] In our study, the association with respect to total mortality for neopterin was stronger among non-smokers, but not modified by smoking status for fibrinogen. These observations on effect modification by smoking have not previously been reported.

Strengths and Limitations

Strengths of the present study are its prospective design, large sample size and long-term follow-up. Furthermore, the cohort is well described and includes information on the angiographic extent of CAD. Information regarding the clinical endpoints was obtained through population-based and patients-administrative registries, and we cannot entirely rule out some underreporting or misclassifications. Any misclassification is likely to be independent of biomarker status, and will weaken rather than strengthen the obtained associations with risk. A single blood sample at admission may represent a possible limitation in our study, although neopterin has been found to have fair-to-good within-person reproducibility, allowing a one-exposure assessment of biomarker status,[46] which would suggest only moderate regression dilution bias. The present multivariable model has previously been used in several publications related to this patient population, and did not include LVEF. For consistency, the same model was applied in this report. However, to assess the impact of LVEF as a cofactor, we redid the analyses and observed that the association of fibrinogen or neopterin with respect to both endpoints was only slightly attenuated and still highly statistically significant. As this study was based on coronary events, we did not consider cardiac-related mortality, which also includes arrhythmia and heart insufficiency as modes of death. Furthermore, we excluded patients with active infectious diseases that may increase inflammation and immune activation.

Conclusion

Both fibrinogen and neopterin were found to be of independent long-term prognostic importance in patients with SCAD, but did not add much additional information to traditional risk factors. No interaction was observed between these two biomarkers, suggesting different mechanism of action in relation to progression of atherothrombosis. The prognostic utility of neopterin was improved in females and non-smokers, and in patients with above median levels of hsTnT, which may serve as a valuable risk stratifier in SCAD patients, and should be evaluated in future studies.

What is known about this topic?

- Fibrinogen and neopterin are related to inflammation known to be involved in thromboatherogenesis, and may yield prognostic information among patients with coronary artery disease.

What does this paper add?

- Fibrinogen and neopterin are strong, independent markers of long-term future risk of incident acute myocardial infarction and total mortality among 3,545 patients with angiographically characterized stable coronary artery disease.
- No interaction between fibrinogen and neopterin was observed after multivariable adjustment.
- The prognostic utility of neopterin was improved among patients with above the median level of high sensitivity troponin T, in addition to among women and non-smokers.
- This emphasizes the prognostic significance of especially neopterin among high-risk and female patients with stable coronary artery disease.

Conflict of Interest
None.

Acknowledgements
We thank the Bergen Coronary Angiography Cohort and Western Norway B Vitamin Intervention Trial participants, recruiting physicians and nurses, laboratory personnel at Bevital A/S and at the Department of Clinical Chemistry at Haukeland University Hospital, and other coworkers and co-investigators at Haukeland University Hospital and at the Department of Clinical Science, University of Bergen, Bergen, Norway.

References

1 Bridge KI, Philippou H, Ariëns R. Clot properties and cardiovascular disease. Thromb Haemost 2014;112(05):901–908
2 Tousoulis D, Papageorgiou N, Androulakis E, Briasoulis A, Antoniades C, Stefanadis C. Fibrinogen and cardiovascular disease: genetics and biomarkers. Blood Rev 2011;25(06):239–245
3 Espinola-Klein C, Rupprecht HJ, Bickel C, et al; AtheroGene Investigators. Inflammation, atherosclerotic burden and cardiovascular prognosis. Atherosclerosis 2007;195(02):e126–e134
4 Coppola G, Rizzo M, Abrignani MG, et al. Fibrinogen as a predictor of mortality after acute myocardial infarction: a forty-two-month follow-up study. Ital Heart J 2005;6(04):315–322
5 Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease. The Framingham Study. JAMA 1987;258(09):1183–1186
6 Wolberg AS. Primed to understand fibrinogen in cardiovascular disease. Arterioscler Thromb Vasc Biol 2016;36(01):4–6
7 Vrecko K, Staedtler P, Mischak I, Maresch L, Reibnegger G. Periodontitis and concentrations of the cellular immune activation marker neopterin in saliva and urine. Clin Chim Acta 1997;268(1-2):31–40
8 Müller TF, Vogl M, Neumann MC, Lange H, Grimm M, Müller MM. Noninvasive monitoring using serum amyloid A and serum neopterin in cardiac transplantation. Clin Chim Acta 1998;276(01):63–74

9 Konstantino Y, Wolk R, Terra SG, Nguyen TT, Fryburg DA. Non-traditional biomarkers of atherosclerosis in stable and unstable coronary artery disease, do they differ? Acute Card Care 2007;9 (04):197–206

10 Adachi T, Naruko T, Itoh A, et al. Neopterin is associated with plaque inflammation and destabilisation in human coronary atherosclerotic lesions. Heart 2007;93(12):1537–1541

11 Gieseg SP, Crone EM, Flavall EA, Amit Z. Potential to inhibit growth of atherosclerotic plaque development through modulation of macrophage neopterin/7,8-dihydroneopterin synthesis. Br J Pharmacol 2008;153(04):627–635

12 Pawashe AB, Golino P, Ambrosio G, et al. A monoclonal antibody against rabbit tissue factor inhibits thrombus formation in stenotic injured rabbit carotid arteries. Circ Res 1994;74(01):56–63

13 Ragni M, Cirillo P, Pascucci I, et al. Monoclonal antibody against tissue factor shortens tissue plasminogen activator lysis time and prevents reocclusion in a rabbit model of carotid artery thrombosis. Circulation 1996;93(10):1913–1918

14 De Rosa S, Cirillo P, Pacileo M, et al. Neopterin: from forgotten biomarker to leading actor in cardiovascular pathophysiology. Curr Vasc Pharmacol 2011;9(02):188–199

15 Berdowska A, Zwirska-Korczala K. Neopterin measurement in clinical diagnosis. J Clin Pharm Ther 2001;26(05):319–329

16 Schumacher M, Halwachs G, Tatzber F, et al. Increased neopterin in patients with chronic and acute coronary syndromes. J Am Coll Cardiol 1997;30(03):703–707

17 Garcia-Moll X, Cole D, Zouridakis E, Kaski JC. Increased serum neopterin: a marker of coronary artery disease activity in women. Heart 2000;83(03):346–350

18 Auer J, Berent R, Labetanig E, Eber B. Serum neopterin and activity of coronary artery disease. Heart Dis 2001;3(05):297–301

19 Melichar B, Gregor J, Solichová D, Lukes J, Tichý M, Pidrman V. Increased urinary neopterin in acute myocardial infarction. Clin Chem 1994;40(02):338–339

20 Gupta S, Fredericks S, Schwartzman RA, Holt DW, Kaski JC. Serum neopterin in acute coronary syndromes. Lancet 1997;349(9060): 1252–1253

21 Gurfinkel EP, Scirica BM, Bozovich G, Macchia A, Manos E, Mautner B. Serum neopterin levels and the angiographic extent of coronary arterial narrowing in unstable angina pectoris and in non-Q-wave acute myocardial infarction. Am J Cardiol 1999;83 (04):515–518

22 Garcia-Moll X, Coccolo F, Cole D, Kaski JC. Serum neopterin and complex stenosis morphology in patients with unstable angina. J Am Coll Cardiol 2000;35(04):956–962

23 Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez M. Usefulness of neopterin levels and left ventricular function for risk assessment in survivors of acute myocardial infarction. Int J Cardiol 2006;111(02):318–320

24 van Haelst PL, Liem A, van Boven AJ, et al. Usefulness of elevated neopterin and C-reactive protein levels in predicting cardiovascular events in patients with non-Q-wave myocardial infarction. Am J Cardiol 2003;92(10):1201–1203

25 Ray KK, Morrow DA, Sabatine MS, et al. Long-term prognostic value of neopterin: a novel marker of monocyte activation in patients with acute coronary syndrome. Circulation 2007;115(24):3071–3078

26 Kaski JC, Consuegra-Sanchez L, Fernandez-Berges DJ, et al; SIESTA Investigators. Elevated serum neopterin levels and adverse cardiac events at 6 months follow-up in Mediterranean patients with non-ST-segment elevation acute coronary syndrome. Atherosclerosis 2008;201(01):176–183

27 Nazer B, Ray KK, Sloan S, et al. Prognostic utility of neopterin and risk of heart failure hospitalization after an acute coronary syndrome. Eur Heart J 2011;32(11):1390–1397

28 Avanzas P, Arroyo-Espliguero R, Quiles J, Roy D, Kaski JC. Elevated serum neopterin predicts future adverse cardiac events in patients with chronic stable angina pectoris. Eur Heart J 2005; 26(05):457–463

29 Pedersen ER, Midttun Ø, Ueland PM, et al. Systemic markers of interferon-γ-mediated immune activation and long-term prognosis in patients with stable coronary artery disease. Arterioscler Thromb Vasc Biol 2011;31(03):698–704

30 Lyngbæk S, Winkel P, Gøtze JP, et al; CLARICOR Trial Group. Risk stratification in stable coronary artery disease is possible at cardiac troponin levels below conventional detection and is improved by use of N-terminal pro-B-type natriuretic peptide. Eur J Prev Cardiol 2014;21(10):1275–1284

31 Ebbing M, Bleie Ø, Ueland PM, et al. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: a randomized controlled trial. JAMA 2008;300(07):795–804

32 Svingen GF, Ueland PM, Pedersen EK, et al. Plasma dimethylglycine and risk of incident acute myocardial infarction in patients with stable angina pectoris. Arterioscler Thromb Vasc Biol 2013; 33(08):2041–2048

33 Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined–a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000;36 (03):959–969

34 Sulo G, Igland J, Vollset SE, et al. Cardiovascular disease and diabetes mellitus in Norway during 1994 -2009; CVDNOR - nationwide research project. Nor Epidemiol 2013;23(01):101–107 (DOI: http://dx.doi.org/10.5324/nje.v23i1.1609)

35 Midttun Ø, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2009;23 (09):1371–1379

36 Jarolim P. High sensitivity cardiac troponin assays in the clinical laboratories. Clin Chem Lab Med 2015;53(05):635–652

37 Team RCR. A Language and Environment for Statistical Computing. 3.3.1 ed. Vienna, Austria: R Foundation for Statistical Computing; 2016

38 Tabakci MM, Gerin F, Sunbul M, et al. Relation of plasma fibrinogen level with the presence, severity, and complexity of coronary artery disease. Clin Appl Thromb Hemost 2017;23(06):638–644

39 Sinning JM, Bickel C, Messow CM, et al; AtheroGene Investigators. Impact of C-reactive protein and fibrinogen on cardiovascular prognosis in patients with stable angina pectoris: the AtheroGene study. Eur Heart J 2006;27(24):2962–2968

40 Grammer TB, Fuchs D, Boehm BO, Winkelmann BR, Maerz W. Neopterin as a predictor of total and cardiovascular mortality in individuals undergoing angiography in the Ludwigshafen Risk and Cardiovascular Health study. Clin Chem 2009;55(06):1135–1146

41 Rabbani R, Topol EJ. Strategies to achieve coronary arterial plaque stabilization. Cardiovasc Res 1999;41(02):402–417

42 Diamondstone LS, Tollerud DJ, Fuchs D, et al. Factors influencing serum neopterin and beta 2-microglobulin levels in a healthy diverse population. J Clin Immunol 1994;14(06):368–374

43 Woodward M, Lowe GD, Rumley A, et al. Epidemiology of coagulation factors, inhibitors and activation markers: The Third Glasgow MONICA Survey. II. Relationships to cardiovascular risk factors and prevalent cardiovascular disease. Br J Haematol 1997;97(04):785–797

44 Schennach H, Murr C, Gächter E, Mayersbach P, Schönitzer D, Fuchs D. Factors influencing serum neopterin concentrations in a population of blood donors. Clin Chem 2002;48(04):643–645

45 Djordjevic VB, Stojanovic I, Cosic V, et al. Serum neopterin, nitric oxide, inducible nitric oxide synthase and tumor necrosis factor-alpha levels in patients with ischemic heart disease. Clin Chem Lab Med 2008;46(08):1149–1155

46 Midttun O, Townsend MK, Nygård O, et al. Most blood biomarkers related to vitamin status, one-carbon metabolism, and the kynurenine pathway show adequate preanalytical stability and within-person reproducibility to allow assessment of exposure or nutritional status in healthy women and cardiovascular patients. J Nutr 2014;144(05):784–790