Systemic Markers of Interferon-γ–Mediated Immune Activation and Long-Term Prognosis in Patients With Stable Coronary Artery Disease

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Objective—Interferon γ (IFN-γ) is centrally involved in atherosclerosis-related inflammation, but its activity cannot be reliably assessed by systemic measurements. In activated macrophages, IFN-γ stimulates production of neopterin and conversion of tryptophan to kynurenine. We evaluated the relationships of plasma neopterin and plasma kyunernine:tryptophan ratio (KTR) to long-term prognosis in patients with stable angina pectoris and angiographically verified significant coronary artery disease.

Methods and Results—Samples were obtained from 2380 patients with a mean age of 63.7 years; 77.3% were men. During a median follow-up of 56 months, 10.8% of patients experienced a major coronary event (MCE), and 9.5% died. For MCE, each SD increment of neopterin and KTR (logarithmically transformed) was associated with multivariable adjusted hazard ratios and 95% CIs of 1.28 (1.10 to 1.48) and 1.28 (1.12 to 1.48), respectively. The corresponding hazard ratios (95% CIs) for all-cause mortality were 1.40 (1.21 to 1.62) (neopterin) and 1.23 (1.06 to 1.43) (KTR).

Conclusion—In patients with stable angina pectoris, systemic markers of IFN-γ activity, plasma neopterin, and plasma KTR provide similar risk estimates for MCE and mortality. Our results support experimental data linking IFN-γ to acute atherosclerotic complications. (***Arterioscler Thromb Vasc Biol***. 2011;31:698-704.)

Key Words: angina pectoris ∎ epidemiology ∎ immune system ∎ risk factors

Atherogenesis is a chronic and generalized inflammatory process that may lead to cardiovascular disease (CVD). Inflammation is initiated by the trapping of cholesterol-rich lipoproteins into the intima of arteries, but it does not merely represent an epiphenomenon of lipid accumulation.[1] Macrophages and lymphocytes are present in all stages of plaque development and contribute actively to its progression.[2] These inflammatory cells also release cytokines that stimulate production of C-reactive protein (CRP) from the liver. Elevated serum CRP levels predict adverse prognosis in patients with CVD,[3] but Mendelian randomization studies indicate that CRP per se is not causally related to atherosclerotic events.[4]

Interferon γ (IFN-γ) is synthesized by CD4 + helper T (Th1) cells. This cytokine is highly expressed within atherosclerotic lesions and may increase the recruitment of inflammatory cells, impair reverse cholesterol transport, and enhance production of matrix metalloproteinases and other proinflammatory cytokines.[5] Hence, an active role of IFN-γ in atherogenesis has been hypothesized.[5] After release into the circulation, however, the cytokine becomes rapidly neutralized by soluble receptors or binds to target structures.[6] Consequently, the half-life of circulating IFN-γ is short, and its activity cannot be reliably assessed by systemic measurements.[6]

IFN-γ stimulates production of neopterin by activated macrophages. Neopterin is a biochemically inert by-product of the guanosine triphosphate–biopterin pathway.[6] Neopterin levels are shown to predict adverse prognosis in coronary artery disease (CAD) patients[7–9] and in patients with chest pain without obstructive CAD.[10] However, it is not clear whether this biomarker provides incremental prognostic information when added to a model of conventional CVD risk indicators.[11]

IFN-γ also induces the enzyme indoleamine 2,3-dioxygenase, which converts the essential amino acid tryptophan into kynurenine, thereby increasing the kynurenine:tryptophan ratio (KTR).[12] Tryptophan metabolism has been explored only to a limited extent in atherosclerotic disorders[13,14] and, to the best of our knowledge, has not been related to clinical outcomes.

We evaluated the associations of circulating neopterin and KTR levels with long-term prognosis in patients undergoing elective coronary angiography for stable angina pectoris (SAP) and compared their incremental prognostic values with that of CRP.

Methods

Study Population

The Bergen Coronary Angiography Cohort includes 3314 patients examined because of SAP. Among these, 2380 had angiographically verified significant CAD and were included in the current study. The participants were consecutively recruited in the period of January 2000 to April 2004, at the Department of Heart Disease, Haukeland University Hospital, Bergen, Norway. Participants represent ≈99% of all individuals diagnosed with stable CAD after elective coronary angiography (excluding only those who declined to participate). The study was approved by the regional Committee for Medical and Health Research Ethics and the Norwegian Data Inspectorate. Written informed consent was obtained from all subjects.

Baseline Data

Patients completed a self-administered questionnaire that provided information about medical history, risk factors, and medications. History of hypertension refers to subjects currently being treated with antihypertensive drugs, according to clinical criteria. Diabetes mellitus includes both type 1 and 2. Smokers include current smokers and those reporting having quit within the last 4 weeks, because relapse rates usually are very high the first month after smoking cessation.[15] For all patients, information from the questionnaires was checked against medical records. Left ventricular ejection fraction (LVEF) (%) was determined by ventriculography or echocardiography.

Angiographic Evidence of CAD

Coronary angiograms were performed by cardiologists. Angiographically verified CAD was defined by the presence of any lesion with ≥50% diameter stenosis in the main coronary arteries, ie, left ascending artery, circumflex artery, or right coronary artery, including their major side branches. The extent was scored 1 to 3 according to the number of main vessels with stenosis. Presence of left main-stem artery stenosis was classified as double vessel disease if no right coronary artery stenosis was present or as triple vessel disease if right coronary artery was stenotic or hypoplastic. Lesions ≥20% and <50% were classified as nonobstructive. Patients with only such lesions and those with angiographically normal coronary arteries were excluded from the study.

Follow-Up and Clinical End Points

The patients were followed from the time of first angiography in 2000 to 2004 and throughout the year in 2006. Information on clinical events was collected from the Cause of Death Registry at Statistics Norway and from the Western Norway Cardiovascular Registry, which contains all CVD discharge diagnoses from the patient administrative systems at the hospitals in Western Norway. Data from the registries were checked against hospital medical records.

Primary end points were major coronary events (MCE) and all-cause mortality. MCE included fatal and nonfatal acute myocardial infarction, sudden cardiac death, and sudden death (International Statistical Classification of Disease Tenth Revision [ICD-10] codes I46 and R96 for sudden cardiac death and sudden death, respectively). Myocardial infarctions were classified according to the diagnostic criteria of the revised definition published in 2000.[16] Events occurring within 24 hours after percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) were considered procedure-related and were not included. CVD mortality was analyzed as a secondary end point and included causes of death coded I00 to I99 or R96 according to the ICD-10 system. All events were adjudicated by members of the end-points committee, who were unaware of the patients' levels of inflammation markers.

Biochemical Analyses

Blood samples were collected before coronary angiography. Aliquots of serum and plasma were immediately frozen at −80°C, with a single freeze-thaw cycle before analysis in 2007. Plasma concentrations of neopterin, kynurenine and tryptophan were analyzed by liquid chromatography/tandem mass spectrometry[17] at Bevital A/S (Bergen, Norway). CRP was determined in serum by an ultrasensitive immunoassay, using the Behring nephelometer II system N Latex CRP mono (Behring Diagnostics, Marburg, Germany). Serum levels of apolipoprotein A1 (apoA1) and apolipoprotein B (apoB) were measured on Hitachi 917 and 912 analyzers, respectively, and serum creatinine was measured on the Hitachi 917 analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical Analyses

Continuous variables are reported as means (SD) or medians (25th to 75th percentiles) and categorical variables as counts (percentages). For subgroups of patients, age and gender-adjusted differences in biomarker levels were explored using analyses of covariance. Neopterin and KTR showed right skewed distributions and were therefore logarithmically transformed before analyses and back transformed to the original scale when presented. Associations between continuous variables were assessed with Spearman rank correlation adjusted for age and gender.

In Cox regression analyses, hazard ratios (HR) are reported for 1 SD increments of logarithmically transformed values of neopterin, KTR, and CRP. The multivariable model includes age (years), gender, body mass index (kg/m^2), smoking status (current smoking; yes versus no), hypertension (yes versus no), diabetes mellitus (yes versus no), LVEF (%), angiographic extent of CAD (1 to 3), treatment following baseline coronary angiography (medications only, PCI, CABG), apoB:apoA1 ratio, creatinine (μmol/L), and use of statin medications (yes versus no). We performed log-log plots and plotted Schoenfeld residuals[18] to ensure that assumptions of proportional hazards were not violated.

Kaplan-Meier curves show unadjusted cumulative survivals according to quartiles of neopterin and KTR levels. Multivariable adjusted associations between biomarker levels and risk of events are visualized by generalized additive regression plots. In these plots, neopterin and KTR values (log transformed) are modeled with a 4-degrees of freedom smoothing spline fit in Cox proportional hazard models[19] including the same variables as described above.

The incremental prognostic values of biomarkers were tested by calculation of areas under receiver operating characteristics curves and by the determination of net reclassification improvement (NRI),[20] using a follow-up time of 32 months. Multivariable models including the conventional risk markers were compared with models that also included neopterin, KTR, or CRP. For the NRI analyses, participants were classified into 3 risk categories (<5%, 5% to <15%, and 15% or greater risk of events during follow-up).

Reported probability values are 2-tailed and were considered significant when <0.05. We used the statistical packages R (version 2.10 for Windows)[21] and SPSS (version 17 for Windows, Chicago, IL).

Results

Baseline characteristics are presented in Table 1. For the 2380 patients, mean (SD) age was 63.7 (10.1) years and 77.3% (n=1840) were men. At inclusion, 30.2% (n=719) of patients had single vessel disease, 29.5% (n=701) had double vessel disease, and 40.3% (n=960) had triple vessel disease.

Biomarkers According to Baseline Characteristics

Median levels of neopterin and KTR were higher in women than in men (neopterin, 9.23 versus 7.83 nmol/L; KTR, 25.9

Table 1. Baseline Characteristics of the Study Population (n=2380)

Characteristic	Value
Age (years)	63.7 (10.1)
Gender (male)	1840 (77.3)
Body mass index (kg/m^2)	26.7 (3.9)
LVEF (%)	63 (12)
ApoB (g/L)	0.92 (0.25)
ApoA1 (g/L)	1.31 (0.25)
ApoB:apoA1 ratio	0.73 (0.26)
Creatinine (μmol/L)	90 (82, 100)
Kynurenine (nmol/L)	1.76 (1.47 to 2.08)
Tryptophan (μmol/L)	72.4 (63.0 to 81.6)
KTR (nmol/μmol)	24.2 (20.0 to 29.5)
Neopterin (nmol/L)	8.1 (6.6 to 10.5)
CRP (mg/L)	1.84 (0.92 to 3.78)
Cardiovascular history and risk factors	
Prior acute myocardial infarction	1155 (48.5)
Prior PCI	513 (21.6)
Prior CABG	349 (14.7)
Hypertension	1192 (50.1)
Diabetes mellitus	333 (14.0)
Current smoking	620 (26.1)
Hypercholesterolemia*	1326 (55.7)
Family history of premature CAD‡	696 (29.2)
Baseline coronary angiography‡	
Single-vessel disease	719 (30.2)
Double-vessel disease	701 (29.5)
Triple-vessel disease	960 (40.3)
Treatment following baseline coronary angiography	
Medication only	686 (28.8)
PCI	985 (41.4)
CABG	709 (29.8)
Medications at discharge	
Aspirin	2166 (91)
Statins	2110 (88, 7)
Beta blockers	1908 (80.2)
Angiotensin-converting enzyme inhibitors	563 (23.7)
Loop diuretics	298 (12.5)
Clinical end points during follow-up	
MCE	257 (10.8)
All-cause mortality	226 (9.5)
CVD mortality	127 (5.3)

Values are given as means (SD), medians (25th to 75th percentiles), or numbers (percentages).

*Untreated total cholesterol, ≥6.5 mmol/L.

†Diagnosis of CAD in first-degree relative before 55 (men) or 65 (women) years of age.

‡No. of main coronary arteries with ≥50% diameter stenosis.
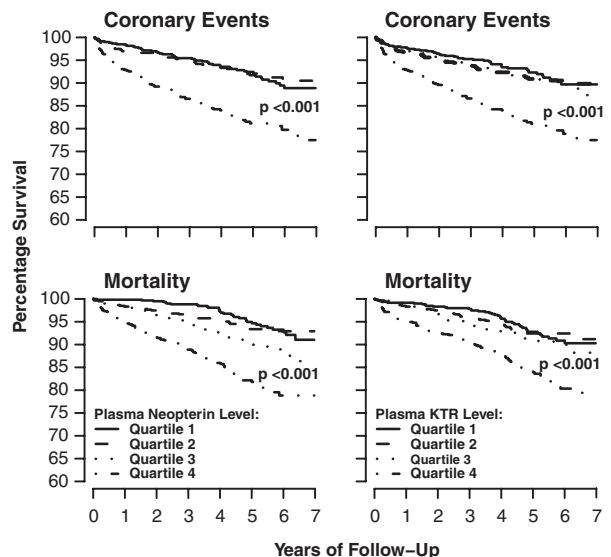

Figure 1. Kaplan-Meier curves showing unadjusted cumulative incidence of MCE and all-cause mortality according to quartiles of neopterin or KTR levels. Reported probability values are for the corresponding log-rank tests.

versus 23.8 nmol/μmol, both $P<0.001$). Adjusting for age and gender, mean neopterin and KTR values were higher in hypertensive than in normotensive patients (neopterin, 8.92 versus 8.39 nmol/L, $P<0.001$; KTR, 25.3 versus 24.3 nmol/μmol, $P=0.01$). Neopterin levels were similar in smokers and nonsmokers (8.47 versus 8.71 nmol/L, respectively, $P=0.13$), whereas KTR values were somewhat lower in smokers (23.5 versus 25.3 nmol/μmol, $P<0.001$). Mean values of neither biomarker differed significantly between patients with and without diabetes mellitus (neopterin, 8.78 versus 8.63 nmol/L, $P=0.45$; KTR, 24.9, versus 24.8 nmol/μmol, $P=0.84$).

Neopterin and KTR values were strongly correlated ($r=0.52$). They were moderately correlated with age (neopterin, $r=0.40$; KTR, $r=0.38$) and creatinine (neopterin, $r=0.38$; KTR, $r=0.38$), whereas weak positive associations were observed with CRP (neopterin, $r=0.21$; KTR, $r=0.18$) and apoB:apoA1 ratio (neopterin, $r=0.009$; KTR, $r=0.009$, all $P<0.001$). Both were weakly negatively related to LVEF (neopterin, $r=-0.09$; KTR, $r=-0.12$, both $P<0.001$) but showed essentially no relationship with the angiographic extent of CAD at inclusion (neopterin, $r=0.01$, $P=0.56$; KTR, $r=0.04$, $P=0.06$).

The association between plasma levels of kynurenine and tryptophan was moderate ($r=0.22$). Kynurenine was also moderately positively related to neopterin ($r=0.40$) and weakly related to CRP ($r=0.17$). Tryptophan showed a moderate negative correlation to neopterin ($r=-0.23$) (all $P<0.001$), whereas the negative relation to CRP was only modest ($r=-0.06$, $P=0.01$).

Inflammation Markers and MCE

During a median (25th to 75th percentiles) follow-up time of 56 (43 to 69) months, 10.8% (n=257) of patients experienced an MCE. Figure 1 shows crude survival curves and Figure 2, the multivariable adjusted associations of neopterin and KTR to risk of MCE. The biomarkers provided consistent risk estimates across a range of baseline characteristics (Figure 3). For each SD increase (log transformed), multivariable HRs (95% CI) were similar for neopterin (1.28 [1.10 to 1.48]) and KTR (1.28 [1.12 to 1.48], both $P=0.001$). In comparison,
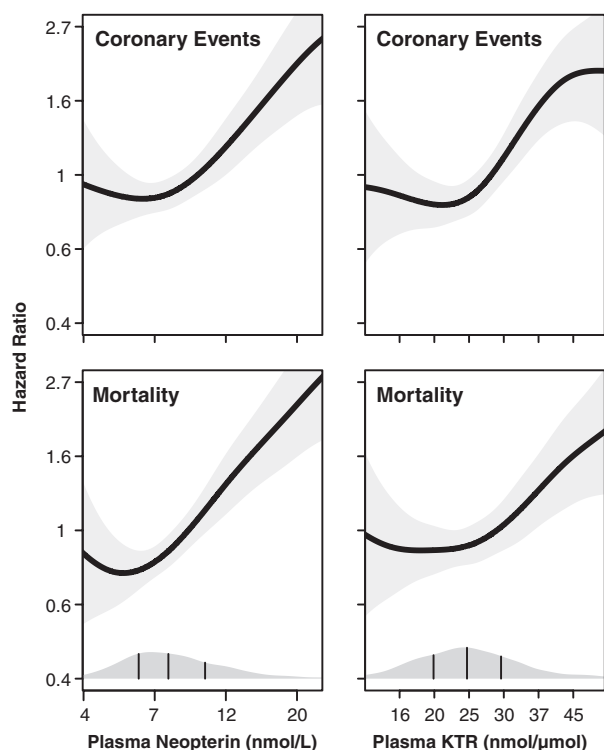
Figure 2. Dose-response relationships between risk of MCE or all-cause mortality and plasma neopterin or KTR obtained by generalized additive regression. The regression models are adjusted for age, gender, body mass index, smoking status, hypertension, diabetes mellitus, LVEF, angiographic extent of CAD, treatment following baseline coronary angiography (medications only, PCI, CABG), apoB:apoA1 ratio, creatinine, and statin therapy. The solid lines show HRs, and the shaded areas show 95% CIs. Ranges from the 2.5th to 97.5th percentiles of exposure variables are included. Density plots show the distribution of biomarkers, and vertical lines denote the 25th, 50th, and 75th percentiles.

Figure 3. Unadjusted hazards for MCE across a range of baseline characteristics. HR, represented by squares, are reported per SD (for the total study population) increment of (log-transformed) neopterin or KTR levels. Areas are proportional to the number of patients in each group. Horizontal lines indicate 95% CIs.

individual components of the KTR showed far weaker associations with MCE (multivariable HR [95% CI], 1.18 [1.03 to 1.35], $P=0.02$ for kynurenine; 0.90 [0.79 to 1.01], $P=0.08$, for tryptophan).

Neopterin and KTR remained significantly associated with risk of MCE even after inclusion of CRP in the model. Adjusted HR were also significant for CRP, though somewhat lower than for the IFN-γ markers (Table 2).

Inflammation Markers and All-Cause Mortality

A total of 9.5% of patients (n=226) died during follow-up. Multivariable HR (95% CI) per SD increment in logarithmically transformed values were 1.40 (1.21 to 1.62), $P<0.001$, for neopterin, 1.23 (1.06 to 1.43), $P=0.006$, for KTR; 1.16 (1.01 to 1.34), $P=0.04$, for kynurenine; and 0.93 (0.81 to 1.06), $P=0.26$, for tryptophan. The corresponding risk estimate for CRP was 1.24 (1.09 to 1.42), $P=0.001$.

Adjustment for CRP moderately attenuated the multivariable associations of neopterin and KTR to all-cause mortality, which, however, remained statistically significant for both markers. Similarly, CRP predicted all-cause mortality after including either of the IFN-γ markers into the multivariable model (Table 2).
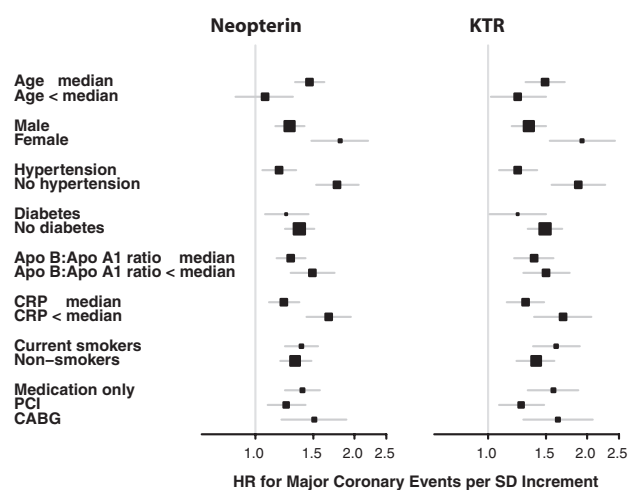
Inflammation Markers and Cardiovascular Mortality

Death from CVD occurred in 5.3% (n=127) during follow-up. Neopterin, KTR and CRP all predicted this secondary end point, with nearly identical multivariable HR (95% CI) per SD increment: neopterin, 1.27 (1.04 to 1.56); KTR, 1.27 (1.05 to 1.54); and CRP, 1.25 (1.05 to 1.49); $P≤0.02$ (Supplemental Table I, available online at http://atvb.ahajournals.org).

Evaluation of Survival Models

The multivariable model without inflammation markers yielded values for the area under the receiver operating characteristics curve of 0.671 and 0.728 for MCE and all-cause mortality, respectively. Inclusion of neopterin increased areas by 0.015 ($P=0.04$; MCE) and 0.018 ($P=0.10$; mortality), whereas KTR provided increments of 0.016 ($P=0.04$; MCE) and 0.019 ($P=0.04$; mortality). CRP did not increase areas for any of these end points ($≤0.006$, $P≥0.42$). Neither biomarker substantially increased area for CVD mortality ($≤0.010$, $P≥0.18$).

For MCE, the addition of neopterin into the multivariable model resulted in a NRI of 6.8% ($P=0.02$). Risk classification was improved as a consequence of 9 (5.3%) of those who subsequently experienced an MCE being up-classified to a higher risk category and 32 (1.5%) of those without MCE being down-classified (Table 3). Corresponding NRIs were 4.6% ($P=0.14$) for KTR and 3.1% ($P=0.22$) for CRP. Risk classifications for all-cause or CVD mortality were not significantly improved by any of the inflammation markers (NRI $≤4.1\%$, $P≥0.37$).

Discussion

Principal Findings

We have identified plasma neopterin and plasma KTR as predictors of adverse prognosis in patients with SAP and angiographically verified obstructive CAD. These biomarkers showed moderate associations with age, creatinine, and hy-

Table 2.   Study Outcomes by Inflammation Markers. Hazard Ratios Are Presented per 1 SD Increase in the Natural Logarithm of the Respective Biomarkers

| | HR (95% CI) | | | | | |
| | Model I, Simple* | | Model II, Multivariable† | | Model III, Multivariable‡§ | |
Marker	Value	P	Value	P	Value	P
MCE						
Neopterin	1.30 (1.18 to 1.43)	<0.001	1.28 (1.10 to 1.48)	0.001	1.23 (1.05 to 1.43)	0.009
KTR	1.34 (1.19 to 1.50)	<0.001	1.28 (1.12 to 1.48)	0.001	1.24 (1.07 to 1.43)	0.003
CRP	1.34 (1.19 to 1.51)	<0.001	1.20 (1.06 to 1.36)	0.005	1.15 (1.01 to 1.31)	0.04
All-cause mortality						
Neopterin	1.34 (1.21 to 1.48)	<0.001	1.40 (1.21 to 1.62)	<0.001	1.34 (1.15 to 1.56)	<0.001
KTR	1.28 (1.13 to 1.45)	<0.001	1.23 (1.06 to 1.43)	0.006	1.18 (1.02 to 1.37)	0.03
CRP	1.41 (1.25 to 1.60)	<0.001	1.24 (1.09 to 1.42)	0.001	1.17 (1.02 to 1.33)	0.03

*Adjustment for age and gender.

†Adjustment for age, gender, body mass index, smoking status, hypertension, diabetes mellitus, LVEF, angiographic extent of coronary artery disease, treatment following baseline coronary angiography (medication only, percutaneous coronary intervention, coronary artery bypass grafting), apoB:apoA1 ratio, creatinine, and statin therapy.

‡As for model II, with additional adjustment for CRP.

§As for model II, with additional adjustment for neopterin. Corresponding risk estimates after multivariable adjustment including KTR were 1.15 (1.01 to 1.31), P=0.03 (MCE) and 1.21 (1.06 to 1.38), P=0.006 (all-cause mortality).

pertension and were weakly related to apoB:apoA1 ratio, CRP, and LVEF. Notably, however, both predicted long-term risk of MCE and mortality even after adjustment for all these factors and several other potential confounders.

Neopterin and KTR in Coronary Heart Disease

Increased plasma neopterin concentrations have been observed in patients with acute coronary syndrome[22] or SAP.[22] Elevated levels were associated with complex morphology of plaques in the carotid[23] and coronary arteries[24] and predicted adverse prognosis in CAD patients.[7–9] Circulating KTR values were shown to be elevated in patients with CAD[12] and correlated with CVD risk factors in presumably healthy populations,[25,26] but they have not previously been related to clinical outcomes. In a large cohort of patients with SAP, we now demonstrate similar risk estimates for KTR and neopterin in the prediction of MCE and mortality. In relation to MCE, these IFN-γ markers seemed to provide more prognostic information than the established inflammation marker CRP.

Possible Mechanisms

IFN-γ is the principal inducer of neopterin and kynurenine formation. Associations of circulating neopterin and KTR levels to adverse outcomes therefore may reflect IFN-γ activity. Among its diverse and complex actions in athero-

Table 3.   Risk Reclassification

| | Model With Neopterin, n (%) | | | |
Model Without Inflammation Markers*	<5% Risk	5% to 15% Risk	>15% Risk	Total
Patients Who Did Not Experience MCE‡				
<5% risk	896 (40.5)	102 (4.6)	0 (0.0)	998 (45.1)
5% to 15% risk	142 (6.4)	885 (40.0)	49 (2.2)	1076 (48.7)
>15% risk	0 (0.0)	41 (1.9)	96 (4.3)	137 (6.2)
Total	1038 (46.9)	1028 (46.5)	145 (6.6)	2211
Patients Who Did Experience MCE				
<5% risk	36 (21.3)	9 (5.3)	0 (0.0)	45 (26.6)
5% to 15% risk	4 (2.4)	72 (42.6)	7 (4.1)	83 (49.1)
>15% risk	0 (0.0)	3 (1.8)	38 (22.5)	41 (24.3)
Total	40 (23.7)	84 (49.7)	45 (26.6)	169‡

*Includes age, gender, body mass index, smoking status, hypertension, diabetes mellitus, LVEF, angiographic extent of artery disease, treatment following baseline coronary angiography (medications only, percutaneous coronary intervention, coronary artery bypass grafting), apoB:apoA1 ratio, creatinine, and statin therapy.

†MCE is a composite of fatal and nonfatal acute myocardial infarction, excluding procedure-related myocardial infarction, including sudden death.

‡Numbers of events differ from those reported in Table 1 because Table 3 is restricted to events occurring during the first 32 months of follow-up.

genesis, this Th1 cytokine enhances collagen degradation and may elicit proapoptotic actions in the lipid-rich core.[5] It is also a potent macrophage activating factor.[27] Plaque disruption generally occurs at sites where the fibrous cap is most heavily infiltrated with activated macrophages.[28] Hence, prevailing evidence suggests a role of IFN-γ in mediating plaque destabilization and acute coronary syndromes.

However, neopterin and kynurenine do not necessarily only serve as passive markers of IFN-γ activity. Neopterin is released in parallel with its partially reduced derivative 7,8-dihydroneopterin.[6] Strong associations have been found between their formation and the production of reactive oxygen species in macrophages.[6] Even though these pteridines may also have antioxidative effects,[29] under most physiological conditions, net effects are likely to be prooxidative[6] and include enhanced modification of low-density lipoprotein cholesterol[29] and promoted apoptosis.[30] Degradation of tryptophan, in contrast, suppresses Th1 cell activity and represents an antiinflammatory pathway.[31] Importantly, a recent experimental study also revealed a novel function of kynurenine in vasculature by identifying this metabolite as a potent vasodilator.[32] Hence, kynurenine formation within atherosclerotic arteries possibly represents a counterregulatory protective mechanism.[14]

Investigations of atherosclerotic lesions demonstrate that neopterin content is elevated[33] and that genes related to the kynurenine pathway are upregulated in coronary plaques,[14] where these processes colocalize with macrophages and are more predominant in culprit lesions from patients with acute coronary syndromes as compared with stable CAD.[33] However, histological examinations of atherectomy specimens have revealed considerable overlap in tissue areas of lesions occupied by inflammatory cells between these 2 categories of patients.[34] In the present study population, elevated levels of neopterin and KTR thus possibly identify subjects with vulnerable lesions despite a clinically stable condition.

Strengths and Limitations
Strengths of our study include its prospective design, the large sample size, and the detailed information about clinical characteristics, including angiographic extent of CAD. Follow-up was ascertained through the use of a patient-administrative and a population-based registry. We cannot exclude the possibility that the report of clinical end points is subjected to some underreporting or other misclassification. However, we do not suspect that such misclassification differs between levels of the inflammation markers, and a bias in this relationship therefore seems unlikely.

Of note, patients with active infectious diseases were not eligible for this study, only a very limited number were diagnosed with cancer (2.0%, n=48), and exclusion of cancer patients had no effects on our results. Thus, these conditions are unlikely to represent confounders for the associations of biomarkers with outcomes.

Limitations of our study include the single measurement of biomarkers, which may have led to underestimation of the true strength of the association by so-called regression dilution bias.[35] Our study cannot provide answers to whether the IFN-γ markers are causally related to outcomes or represent innocent bystanders. However, the prognostic value of a biomarker does not necessarily depend on its pathogenic role in mediating events but rather on its ability to reflect pathways involved in disease progression.[36]

Conclusion
In patients undergoing coronary angiography for SAP, systemic markers of IFN-γ activity, neopterin and KTR, predict long-term prognosis independently of CRP and traditional CVD risk factors. The role of IFN-γ mediated inflammation in atherosclerotic disorders should be explored further, as this may represent a potential target for novel therapeutic strategies in plaque stabilization.[5]

Disclosures
None.

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