International Journal of Cardiology 168 (2013) 1435–1440

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Neopterin and kynurenine–tryptophan ratio as predictors of coronary events in older adults, the Hordaland Health Study ☆

Gerhard Sulo [a,*,1], Stein E. Vollset [a,b,1], Ottar Nygård [c,d,1], Øivind Midttun [e,1], Per Magne Ueland [f,g,1], Simone J.P.M. Eussen [a,f,1], Eva R. Pedersen [c,1], Grethe S. Tell [a,1]

[a] Department of Public Health and Primary Health Care, University of Bergen, Bergen, Norway
[b] Norwegian Institute of Public Health, Bergen, Norway
[c] Section for Cardiology, Institute of Medicine, University of Bergen, Bergen, Norway
[d] Department of Heart Disease, Haukeland University Hospital, Bergen, Norway
[e] Bevital AS, Bergen, Norway
[f] Section for Pharmacology, Institute of Medicine, University of Bergen, Bergen, Norway
[g] Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway

ARTICLE INFO

Article history:
Received 31 May 2012
Received in revised form 12 November 2012
Accepted 18 December 2012
Available online 18 January 2013

Keywords:
Coronary artery disease
Epidemiology
Immune system
Neopterin
Kynurenine
Tryptophan ratio

ABSTRACT

Background: Immune system activation is involved in atherosclerosis. Neopterin production and tryptophan catabolism through the kynurenine pathway, measured by the kynurenine–tryptophan ratio (KTR), are induced by interferon gamma, thus both are considered markers of cell mediated immune activation. This study prospectively investigated their predictive value on acute coronary events among Norwegian community-dwelling older adults without previous coronary heart disease.
Methods: 1112 men and 1631 women, 71–74 years old were examined during 1997–99 as part of the Hordaland Health Study. They were followed until an acute coronary event (defined as unstable angina, non-fatal or fatal acute myocardial infarction or sudden death) or December 31, 2006. Kaplan–Meier hazard curves were constructed for quartiles of plasma neopterin and KTR. Cox proportional hazards models adjusted for sex, body mass index, smoking, hypertension, renal function and cholesterol were used to examine the relation between neopterin and KTR quartiles and the study endpoint.
Results: Median (interquartile range) values were 8.6 (7.2–10.4) nmol/L for neopterin and 25.8 (25.3–31.1) nmol/μmol for KTR. During the follow up, 265 participants had at least one acute coronary event. Increased baseline levels of plasma neopterin and KTR were associated with continuous increased risk of developing the study endpoint (P-values for trend <0.001 and 0.019, respectively). Adjusted hazard ratios comparing the fourth quartile to the first were 1.65 (95% CI; 1.11–2.47; $P=0.013$) for neopterin and 1.57 (95% CI 1.03–2.39; $P=0.036$) for KTR.
Conclusion: Plasma neopterin and KTR levels predict acute coronary events in older adults without previous coronary heart disease.

1. Introduction

Atherosclerosis is a chronic inflammatory disease. Inflammation contributes to both initiation of atheroma and its thrombotic complications [1] leading to 'acute coronary syndrome' (ACS). The cell mediated immune system is involved in the development of atherosclerotic lesions. T lymphocytes and monocyte-derived macrophages have been found in atherosclerotic plaques [2]. Activated T cells produce several cytokines including interferon gamma (IFN-γ) which plays complex roles in different stages of atherogenesis [3]. Both neopterin production and tryptophan catabolism via the kynurenine pathway are stimulated by IFN-γ.

Neopterin is a marker of immune cell activation, released by activated macrophages upon stimulation with IFN-γ [4]. Higher levels of plasma neopterin are found in ACS patients compared to patients with stable coronary heart disease and to healthy individuals [5]. Longitudinal studies have found an association between high neopterin levels and increased risk for adverse coronary events in patients with stable angina pectoris (SAP) [6] and recurrent events among those with an ACS [7]. In newly diagnosed diabetics, a positive association was found between levels of neopterin and subsequent death from ischemic heart disease [8].

Tryptophan is an essential amino acid in humans. Almost 90% of tryptophan is catabolized through the kynurenine pathway [9]. Tryptophan

☆ Gerhard Sulo is funded by a 'Nasjonalforeningen for folkehelsen' Fellowship. This work was also funded in part by the non-profit 'Foundation to Promote Research into Functional Vitamin B12 Deficiency'.

* Corresponding author at: Department of Public Health and Primary Health Care, Kalfarveien 31, PB 7804, N-5020 Bergen, Norway. Tel.: +47 55586100; fax: +47 55586130.
E-mail address: gerhard.sulo@isf.uib.no (G. Sulo).

[1] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

0167-5273/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ijcard.2012.12.090

2, 3-dioxygenase (TDO) and indoleamine 2, 3-dioxygenase (IDO) initiate this pathway and kynurenine is its first stable metabolite. IDO is found in many tissues of the human body, including antigen presenting cells and is up regulated in response to IFN-γ [10]. Up regulated IDO activity increases the conversion of tryptophan to kynurenine, thus resulting in higher levels of the kynurenine–tryptophan ratio (KTR). The KTR is considered a marker of cell mediated immune activation [11]. Increased KTR levels are seen in many infectious, neurodegenerative and neoplastic disorders [12]. Higher KTR levels have also been found in patients with established coronary heart disease compared to healthy controls [13], and a prospective study in patients with SAP found that higher KTR at baseline predicted adverse prognosis [14].

Little is known about the association between plasma neopterin and KTR with coronary events in people without coronary heart disease. Thus, the purpose of this study was to prospectively investigate this association in a population of community-dwelling elderly, free of previously known coronary heart disease.

2. Methods

2.1. Study population

The Hordaland Health Study (HUSK) was conducted during 1997–1999 as collaboration between the National Health Screening Service, the University of Bergen and local health services. Detailed information about participants and procedures are given previously [15]. The source population for the current study included 1868 men and 2470 women born 1925–27. Participation rates were 79% for men and 76% for women.

Data collection involved a personal invitation sent by mail along with a questionnaire including questions on various health behaviors and personal and family history of diseases. For people who agreed to participate and filled out the first questionnaire, a brief medical examination was scheduled. Baseline measurements included height, weight, waist and hip circumferences and blood pressure. A non-fasting blood sample was drawn from each participant and time since last meal was recorded. The study protocol was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. All participants gave written consent before being enrolled in the study.

Participants with missing data on plasma neopterin and/or KTR (n = 52) were excluded. Other exclusion criteria applied were; previous hospitalizations for coronary heart disease or coronary revascularization procedures (n = 422), other atherosclerotic-related conditions such as stroke (n = 94), peripheral artery disease (n = 51) and congestive heart failure (n = 60). Thus, this analysis included 2743 participants; 1112 men and 1631 women.

2.2. Blood samples and biochemical analyses

Serum cholesterol and triglycerides were measured at baseline by enzymatic methods using reagents from Boehringer Mannheim, FRG (Roche, Basel, Switzerland). Plasma creatinine was measured colorimetrically using the alkaline picrate method with reagents from Roche (Basle, Switzerland). Plasma concentrations of neopterin, tryptophan and kynurenine were analyzed by liquid chromatography/tandem mass spectrometry [16] at Bevital A/S (Bergen, Norway).

2.3. Baseline variables

Detailed information regarding baseline variables is given in the Supplementary materials online-methods. Briefly, weight and height were measured and body mass index (BMI) was calculated. Information on education levels, physical activity and smoking habits were derived from the self-reported questionnaires. Hypertension, diabetes and hypercholesterolemia were defined using information from measurements of blood pressure, glucose levels and cholesterol in combination with the information on medication use given by the participants. Renal function was defined using creatinine levels to calculate estimated glomerular filtration rates (eGFR).

2.4. Follow up time and the study endpoint

Baseline examinations took place between April 1998 and June 1999. Participants were followed through December 31, 2006. Median (interquartile range) follow up time measured by the reversed Kaplan–Meier estimator [17] was 7.95 (7.72–8.23) years. The study endpoint was a composite of unstable angina pectoris (UAP (ICD-10 code, I20)), non-fatal or fatal acute myocardial infarction (AMI (ICD-10 code, I21)) or sudden death (ICD-10 codes, R96 or R98). Only the first event was used for the analysis. Information on discharge diagnosis was retrieved from the Regional Register of Cardiovascular Diseases which includes all hospitalizations due to cardiovascular disease or diabetes from all hospitals in Western Norway [18]. Information on deaths was obtained from the national Cause of Death Registry. Linkages between baseline variables and the endpoint were made possible through the personal identification number, unique to each Norwegian resident.

2.5. Statistical analysis

Continuous variables are presented as mean (SD) or median (interquartile range) if data distribution was skewed. Independent sample t-tests and one-way ANOVA were used for comparisons of normally distributed variables and Mann–Whitney and Kruskal–Wallis tests are used for comparisons of variables not normally distributed. Categorical variables were compared using the chi square test. Associations between continuous variables were presented using Spearman's rank correlation coefficients.

In the survival analysis, neopterin and KTR were treated as categorical variables with four categories based on sex specific quartile cut off points. Kaplan–Meier cumulative hazard curves were constructed for neopterin and KTR quartiles and the corresponding P values of log-rank tests for linear trend are given.

To examine the relationship of neopterin and KTR quartiles with the study endpoint, we performed Cox proportional hazards modelling. Two models were constructed separately for neopterin and KTR. The first model was adjusted for gender; the second model was further adjusted for BMI categories, smoking, hypertension, renal function, and cholesterol quartiles. Model 2 was also applied after exclusion of participants who were taking glucocorticoids and cytostatics (known as immunosuppressive medications) or statins. This model is referred to as 'model 2-restricted'. Hazard ratios (HR) and corresponding 95% confidence intervals (CI) are given for each of the quartiles 2–4 compared to the first. In addition, analyses for trend were performed for neopterin and KTR quartiles treated as continuous variables. HR, 95% CI and corresponding P values are presented for one quartile increment in neopterin and KTR levels.

Table 1
Baseline characteristics of the study participants, the Hordaland Health Study.

Characteristics of participants	All participants n = 2743	Men n = 1112	Women n = 1631	P[a]
Age, y, mean (range)	72 (71–74)	72.4 (71–74)	72.4 (71–74)	0.36
Education, %				<0.001
Less than high school	72.1	63.1	78.7	
High school	10.6	12.6	9.2	
College or university	17.2	24.3	12.1	
BMI (kg/m^2), %				0.01
<25	40.9	39.9	41.5	
25–30	45.0	51	40.8	
≥30	14.1	9.1	17.7	
Smoking, %				<0.001
Never smokers	47.0	24.4	62.6	
Former smokers	37.0	57.8	22.7	
Current smokers	16.0	17.8	14.7	
Physical activity, %				<0.001
None/light	59.4	46.5	68.8	
Moderate/vigorous	40.6	53.5	31.2	
Diabetes, %				0.004
No diabetes	92.4	90.2	94.0	
IFG or IGT	3.4	4.9	2.3	
Diabetes	4.2	4.9	3.7	
Hypertension, %	71.5	71.9	71.3	0.76
Hypercholesterolemia, %	50.3	33.4	61.8	<0.001
eGFR<60 (mL/min/1.73 m^2), %	34.3	18.3	45.2	<0.001
Antihypertensive medications[b], %	27.2	25.6	28.3	0.14
Hypoglycemic medications[c], %	3.3	3.5	3.2	0.67
Statins, %	7.3	4.8	9.1	<0.001
Immunosuppressive medications[d], %	7.8	8.7	7.1	0.13
Neopterine (nmol/L)	8.6 (7.2–10.4)	8.5 (7.2–10.3)	8.6 (7.3–10.5)	0.21
KTR (nmol/μmol)	25.8 (21.3–31.1)	25.4 (21.2–31.1)	26.1 (21.4–31.1)	0.46

During the follow up period, 265 study outcome events occurred; 148 in men and 117 in women.
Neopterin and KTR levels are expressed as median (interquartile range).
IFG, impaired fasting glucose; IGT, impaired glucose tolerance.
eGFR, estimated glomerular filtration rate; KTR, kynurenine–tryptophan ratio.
[a] P values from the independent sample t-test, Mann–Whitney test or chi-square test comparing men to women.
[b] At least one class of antihypertensive medications.
[c] Oral hypoglycemic agents or insulin.
[d] Glucocorticoid or cytostatic medications.

The dose-response relationship between neopterin and KTR and risk of the study endpoint were also visualized by generalized additive regression model (GAM) plots. Neopterin and KTR values were modelled using a smoothing spline fit in an adjusted Cox proportional hazard model which included the same variables as the model 2.

Two-sided tests with a 0.05 significance level were used. Analyses were performed with SPSS 18 and STATA 11. GAMs were constructed with 'survival' package in R (version 2.10 for Windows).

3. Results

Baseline characteristics of the study participants are shown in Table 1. Levels of neopterin and KTR were similar in men and women. Compared to women, a higher proportion of men were current smokers (P = 0.04), physically active (P<0.001) and classified as having impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) (P = 0.001). A higher proportion of women than men had never smoked (P<0.001), had less education (P<0.001), were obese (P<0.001), and had impaired renal function (P<0.001) and hypercholesterolemia (P<0.001). The use of statins was higher in women compared to men (P<0.001), while there were no significant sex differences in the use of other classes of reported medications.

Neopterin and KTR median (interquartile range) values across levels of other baseline variables are given in Table 2. Hypertensive participants and those with impaired renal function had significantly higher levels of neopterin compared to participants with normal blood pressure values and renal function. KTR levels were significantly higher in the lowest compared to the highest education category, in overweight and obese participants compared to those with normal BMI and in former smokers compared to current smokers. Similar to neopterin, a higher level of KTR was found in hypertensive participants and in those with impaired renal function.

We also observed a strong correlation of neopterin with KTR (r = 0.51; P<0.001).

Table 2
Neopterin and KTR median (interquartile range) values across levels of baseline variables, the Hordaland Health Study.

Baseline variables	Neopterin (nmol/L)	P*	KTR (nmol/μmol)	P*
Education level		0.13		0.009
Less than high school	8.6 (7.3–10.6)		26.1 (21.7–31.5)	
High school	8.4 (7.2–10.3)		25.3 (20.6–30.6)	
College or university	8.4 (7.1–10.3)		24.6 (20.7–30.3)	
Body mass index, (kg/m^2)		0.35		<0.001
<25	8.6 (7.2–10.5)		24.6 (20.4–30.0)	
25–30	8.5 (7.2–10.2)		26.3 (21.9–31.1)	
≥30	8.7 (7.3–10.7)		28.0 (23.0–34.0)	
Smoking		0.15		0.004
Never smokers	8.5 (7.2–10.3)		25.6 (21.1–30.6)	
Former smokers	8.7 (7.3–10.5)		26.4 (21.9–32.1)	
Current smokers	8.4 (7.1–10.3)		25.1 (20.9–31.4)	
Physical activity		0.32		0.54
None/light	8.7 (7.2–10.5)		25.8 (21.3–31.1)	
Moderate/vigorous	8.4 (7.2–10.1)		25.5 (21.2–30.7)	
Diabetes		0.2		0.17
No diabetes	8.6 (7.3–10.4)		25.9 (21.3–31.1)	
IFG or IGT	8.4 (7.2–10.9)		24.7 (21.3–28.8)	
Diabetes	7.8 (6.8–10.2)		26.5 (22.7–32.9)	
Hypertension		0.02		<0.001
Yes	8.6 (7.3–10.6)		26.1 (21.7–31.5)	
No	8.4 (7.1–10.2)		24.8 (20.7–30.3)	
eGFR<60 (mL/min/1.73 m^2)		<0.001		<0.001
Yes	9.6 (7.9–11.7)		29.0 (23.7–35.4)	
No	8.1 (6.9–9.7)		24.5 (20.4–29.1)	

IFG, impaired fasting glucose; IGT, impaired glucose tolerance.
eGFR, estimated glomerular filtration rate; KTR, kynurenine–tryptophan ratio.
* P values from Mann–Whitney or Kruskal–Wallis test.

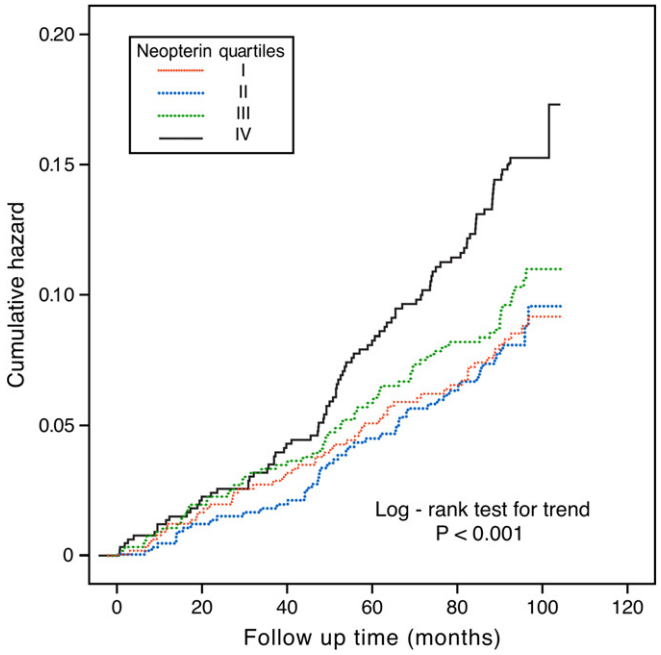

Fig. 1. Kaplan–Meier cumulative hazard curves for acute coronary events according to neopterin quartiles, the Hordaland Health Study.

3.1. Follow up time and study endpoint events

During the follow up time, 265 participants; 148 men and 117 women suffered one of the study endpoints. Table 1 in Supplementary material online-tables shows the distribution of the study outcome and its components across neopterin and KTR quartiles. Kaplan–Meier cumulative hazard curves show that quartile increments of neopterin (Fig. 1) and KTR (Fig. 2) are associated with increased risk of acute coronary events (P for trend<0.001 and 0.019, respectively).

The adjusted generalized additive Cox regression models visualize a positive dose response relationship between plasma neopterin levels and risk of the study endpoint (Fig. 3).

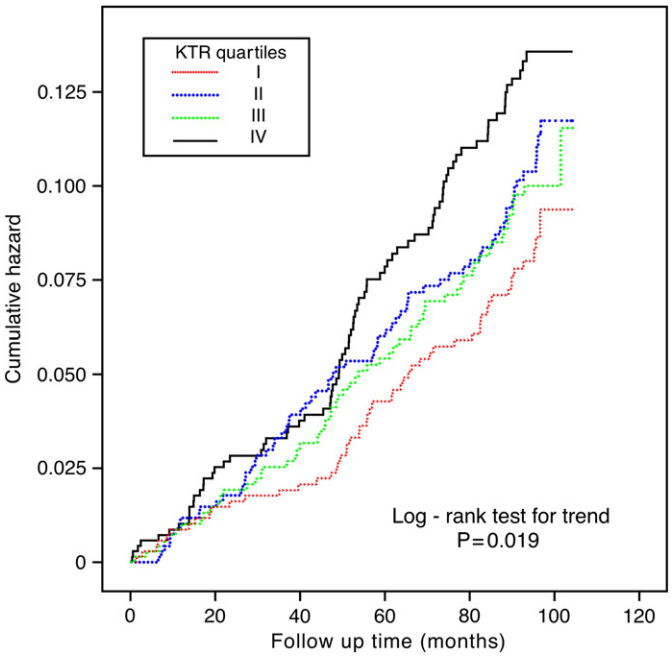

Fig. 2. Kaplan–Meir cumulative hazard curves for acute coronary events according to KTR quartiles, the Hordaland Health Study.

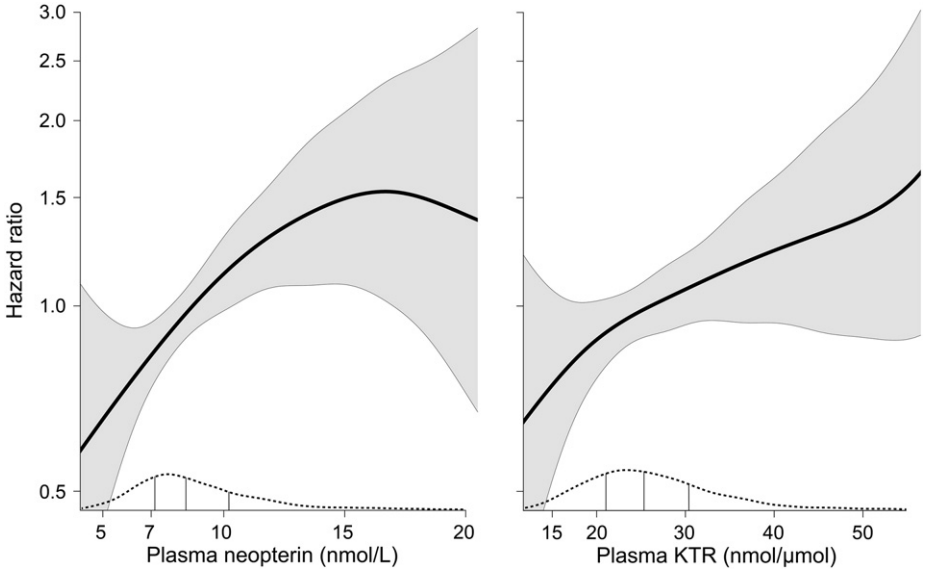

Fig. 3. Dose-response relationships between plasma neopterin or KTR levels (1–99% of all participants) and hazard ratios (HRs) of acute coronary events obtained from generalized additive Cox regression. Models are adjusted for gender, BMI categories, smoking status, hypertension, impaired renal function (eGFR<60 mL/min/1.73 m^2) and cholesterol (in quartiles). The solid lines show HRs and the shaded areas show 95% CIs. Density plots show the distribution of biomarkers, and vertical lines denote the 25th, 50th, and 75th percentiles.

Results from the adjusted Cox models are shown in Table 3. The fourth quartile of neopterin was associated with an increased risk compared to the first in the analysis including all participants (HR, 1.63; 95% CI 1.15–2.33; P = 0.007) and in analysis restricted to participants not taking immunosuppressive medications or statins (HR, 1.65; 95% CI 1.11–2.46; P = 0.013). For KTR (Table 3), we observed a borderline significant increased risk of acute coronary events when comparing the fourth quartile to the first (HR, 1.44; 95% CI 0.99–2.08; P = 0.054). This association became significant (HR, 1.57; 95% CI 1.03–2.39; P = 0.036) in the analysis restricted to participants not taking immunosuppressive medications or statins.

In sex specific analyses, we observed that the fourth quartile was associated with increased risk for the study endpoint compared to the first in men for both neopterin and KTR. In women, no significant associations were observed (Tables 2 and 3 respectively in Supplementary material online-tables).

Table 3
Hazard ratios (HR) for acute coronary events according to neopterin and KTR quartiles, the Hordaland Health Study.

Variables	Model 1 HR (95% CI) (n = 2743)	P[a]	Model 2 HR (95% CI) (n = 2743)	P[a]	Model 2-restricted HR (95% CI) (n = 2339)	P[a]
Neopterin (nmol/L)						
Quartile 1 (<7.2)	1.00 reference		1.00 reference		1.00 reference	
Quartile 2 (7.2–8.6)	1.01 (0.67–1.82)		1.00 (0.68–1.46)		0.80 (0.51–1.25)	
Quartile 3 (8.6–10.4)	1.24 (0.86–1.77)		1.23 (0.85–1.77)		1.18 (0.78–1.78)	
Quartile 4 (>10.4)	1.81 (1.30–2.53)		1.63 (1.15–2.33)		1.65 (1.11–2.46)	
Per quartile increment[b]	1.24 (1.11–1.38)	<0.001	1.19 (1.06–1.34)	0.003	1.23 (1.08–1.40)	0.002
KTR (nmol/μmol)						
Quartile 1 (<21.3)	1.00 reference		1.00 reference		1.0 reference	
Quartile 2 (21.3–25.8)	1.27 (0.89–1.82)		1.25 (0.87–1.80)		1.24 (0.82–1.90)	
Quartile 3 (25.8–31.1)	1.21 (0.84–1.75)		1.25 (0.86–1.82)		1.22 (0.79–1.88)	
Quartile 4 (>31)	1.60 (1.14–2.26)		1.44 (0.99–2.08)		1.57 (1.03–2.39)	
Per quartile increment[b]	1.15 (1.03–1.28)	0.012	1.11 (0.99–1.25)	0.069	1.14 (1.02–1.31)	0.047
Gender (male)	2.03 (1.59–2.59)	<0.001	2.01 (1.53–2.66)	<0.001	1.90 (1.39–2.61)	<0.001
BMI (kg/m^2)				0.49		0.2
<25			1.00 reference		1.00 reference	
25–30			1.18 (0.89–1.55)		1.32 (0.97–1.79)	
≥30			1.15 (0.77–1.71)		1.09 (0.68–1.73)	
Smoking				<0.001		<0.001
Never smokers			1.00 reference		1.00 reference	
Former smokers			1.11 (0.82–1.50)		1.30 (0.91–1.85)	
Current smokers			2.16 (1.55–3.02)		2.77 (1.90–4.03)	
Hypertension			1.53 (1.13–2.08)	0.006	1.80 (1.26–2.56)	0.001
eGFR<60 (mL/min/1.73 m^2)			1.14 (0.86–1.51)	0.36	1.28 (0.94–1.75)	0.12
Cholesterol (mg/dL)				0.25		0.6
Quartile 1			1.00 reference		1.00 reference	
Quartile 2			0.85 (0.61–1.20)		1.03 (0.68–1.53)	
Quartile 3			0.69 (0.48–0.99)		0.80 (0.52–1.22)	
Quartile 4			0.88 (0.63–1.23)		1.02 (0.69–1.51)	

Model 1, adjusted for gender; model 2, adjusted for gender, BMI, smoking, hypertension, renal function (eGFR) and cholesterol.
Model 2-restricted, the same adjustments made to model 2, but applied to participants not taking immunosuppressive medications or statins.
[a] P for trend.
[b] HR (95% CI) for one quartile increment in neopterin and KTR respectively.

4. Discussion

In this study among community-dwelling elderly without pre-existing coronary heart disease we observed that higher levels of neopterin and KTR were associated with an increased risk of subsequent acute coronary events. The associations were slightly stronger after excluding participants taking statins or immunosuppressive medications.

Several prospective studies have investigated the association between neopterin and adverse coronary events or mortality in hospitalized patients with coronary artery disease. In SAP patients, higher baseline levels of plasma neopterin were associated with an increased risk of adverse coronary events [6,14,19]. Similarly, in patients hospitalized for an ACS event, higher levels of plasma neopterin at admission increased the risk for recurrence or death during follow up [7,20,21]. A positive association between neopterin levels and adverse coronary events or death has also been reported from studies including patients with both SAP and ACS [22], or diabetes [8]. In treated hypertensive patients [23], higher neopterin levels have been associated with greater risk of future adverse coronary events. These studies provide evidence that neopterin predicts future coronary events with similar effect estimates in SAP and ACS patients. Another study found that neopterin levels predicted a rapid progression of plaques in patients with established coronary heart disease [24].

The estimates in our study are similar in direction with studies conducted among patients with underlying coronary heart disease [6–8,14,20,22]. Differences in effect size may be attributable to different categorization levels used for the exposure variable and different population characteristics. Our findings support previous knowledge on the predictive role of neopterin on future coronary events in patients with pre-existing coronary disease [25], and expand it to older adults without previous coronary heart disease.

The relationship between levels of KTR and subsequent coronary events has not previously been extensively studied. A cross-sectional study reported higher levels of KTR in patients with established coronary disease compared to healthy subjects [13]. In a population of older adults, baseline levels of KTR were positive predictors of all-cause mortality in a 4-year follow up period [26]. We have previously reported that one standard deviation increase of logarithmically transformed KTR was associated with a relative risk of 1.28 for major coronary events in SAP patients [14]. In the present study, we observed an association between baseline KTR and subsequent risk of acute coronary events in participants not taking immunosuppressive medications or statins, thus replicating previous findings among coronary heart disease patients, now in a population of adults without underlying coronary heart disease.

In line with previous publications [14,27], we found a strong correlation between KTR and neopterin, suggesting that increasing KTR is related to tryptophan catabolism through the IDO route due to immune system activation. Nevertheless, additional contribution from the TDO route cannot be totally excluded and may partially explain the weaker predictive value of KTR for coronary events compared to neopterin.

Studies of atherosclerotic plaque composition have identified more areas rich of macrophages in plaques from ACS patients compared to plaques from patients with SAP [28]. Plaque rupture is considered to be the main cause of ACS, and activated macrophages contribute to plaque instability through the release of many effector molecules [29]. Neopterin, one of the many products of activated macrophages, appears to amplify the effects of reactive oxygen species, promoting oxidative stress, [30] which in turn plays a role in mediating apoptosis on T cells [31]. Thus, apart from reflecting an active status of the immune system, neopterin may also modulate the immune response.

There is evidence that tryptophan catabolism through the kynurenine pathway modulates the immune system response during inflammation [32]. Activation of the immune system and the release of IFN-γ up regulate IDO activity in antigen presenting cells, leading to increased tryptophan catabolism. This process has anti-proliferative qualities [33] by depleting tryptophan in the microenvironment and subsequently arresting the proliferation of T cells [34]. In addition, several metabolites of the tryptophan catabolism induce apoptosis of T cells [35]. Enhanced IDO activity, expressed by increased KTR levels might represent a protective mechanism against accelerated inflammation.

To our knowledge, this is the first study prospectively assessing the relationship between plasma neopterin and KTR levels with ACS events in a population of older adults without coronary heart disease. Our study includes a large number of participants and the follow up time was 8 years. The personal identification number for each participant and good records on hospitalization and causes of death, allowed us to follow the study population without loss to follow up. The composite endpoint of non-fatal and fatal ACS events reflects the pathophysiological changes taking place during development and complication of the atherosclerotic plaque.

Participants' comorbidities and the use of different medications were also considered when dealing with the main associations under investigation in our study. Because statins [36] and immunosuppressive medications [37] are reported to suppress both formation of neopterin and tryptophan catabolism induced by IFN-γ, we repeated the multivariable analyses after excluding participants who were taking these medications. The magnitude of effects observed were similar to those from analyses in all participants, and in the case of KTR, the associations became stronger and statistically significant.

In the sex specific analyses, we observed similar effect estimates in men and women although the number of events among women was fewer and were not statistically significant.

Our study has also several limitations. Participants were community dwelling adults born in 1925–27. Due to the small differences in age, we did not include it as a possible confounder in the final analysis. Further work is needed to validate our results in a broader age range. Plasma levels of neopterin and KTR were measured only once at the beginning of the study, and therefore changes over time in their levels were not possible to be taken into consideration while conducting the analyses.

5. Conclusions

Increased levels of neopterin and KTR indicate ongoing inflammation with subsequent immune system activation. This may contribute to a faster progression of atherosclerotic plaques and render them more susceptible to rupture, causing an ACS event. Our study shows that neopterin and KTR may predict future coronary events years ahead of the acute episode, among community-dwelling older adults without prior coronary heart disease.

Acknowledgments

We thank Marit Krokeide, Anne-Kirstin Thoresen and Gry Kvalheim for their technical assistance.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2012.12.090.

References

[1] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135–43.
[2] Hansson GK, Jonasson L. The discovery of cellular immunity in the atherosclerotic plaque. Arterioscler Thromb Vasc Biol 2009;29:1714–7.
[3] Harvey EJ, Ramji DP. Interferon-gamma and atherosclerosis: pro- or anti-atherogenic? Cardiovasc Res 2005;67:11–20.

[4] Huber C, Batchelor JR, Fuchs D, et al. Immune response-associated production of neopterin. Release from macrophages primarily under control of interferon-gamma. J Exp Med 1984;160:310–6.
[5] Schumacher M, Halwachs G, Tatzber F, et al. Increased neopterin in patients with chronic and acute coronary syndromes. J Am Coll Cardiol 1997;30:703–7.
[6] 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;26:457–63.
[7] 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;115:3071–8.
[8] Vengen IT, Dale AC, Wiseth R, Midthjell K, Videm V. Neopterin predicts the risk for fatal ischemic heart disease in type 2 diabetes mellitus: long-term follow-up of the HUNT 1 study. Atherosclerosis 2009;207:239–44.
[9] Richard DM, Dawes MA, Mathias CW, Acheson A, Hill-Kapturczak N, Dougherty DM. L-Tryptophan: basic metabolic functions, behavioral research and therapeutic indications. Int J Tryptophan Res 2009;2:45–60.
[10] Taylor MW, Feng GS. Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J 1991;5:2516–22.
[11] Schrocksnadel K, Wirleitner B, Winkler C, Fuchs D. Monitoring tryptophan metabolism in chronic immune activation. Clin Chim Acta 2006;364:82–90.
[12] Chen Y, Guillemin GJ. Kynurenine pathway metabolites in humans: disease and healthy states. Int J Tryptophan Res 2009;2:1–19.
[13] Wirleitner B, Rudzite V, Neurauter G, et al. Immune activation and degradation of tryptophan in coronary heart disease. Eur J Clin Invest 2003;33:550–4.
[14] Pedersen ER, Midttun O, Ueland PM, et al. Systemic markers of interferon-gamma-mediated immune activation and long-term prognosis in patients with stable coronary artery disease. Arterioscler Thromb Vasc Biol 2011;31:698–704.
[15] Vikse BE, Vollset SE, Tell GS, Refsum H, Iversen BM. Distribution and determinants of serum creatinine in the general population: the Hordaland Health Study. Scand J Clin Lab Invest 2004;64:709–22.
[16] Midttun O, 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;23:1371–9.
[17] Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials 1996;17:343–6.
[18] Oyen N, Nygard O, Igland J, et al. Hospital admission rates for cardiovascular diseases in Western Norway, 1992–2001. Tidsskr Nor Laegeforen 2008;128:17–23.
[19] Garcia-Moll X, Cole D, Zouridakis E, Kaski JC. Increased serum neopterin: a marker of coronary artery disease activity in women. Heart 2000;83:346–50.
[20] Kaski JC, Consuegra-Sanchez L, Fernandez-Berges DJ, et al. 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;201:176–83.
[21] 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;92:1201–3.
[22] 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;55:1135–46.
[23] Avanzas P, Arroyo-Espliguero R, Cosin-Sales J, Quiles J, Zouridakis E, Kaski JC. Prognostic value of neopterin levels in treated patients with hypertension and chest pain but without obstructive coronary artery disease. Am J Cardiol 2004;93:627–9.
[24] Zouridakis E, Avanzas P, Arroyo-Espliguero R, Fredericks S, Kaski JC. Markers of inflammation and rapid coronary artery disease progression in patients with stable angina pectoris. Circulation 2004;110:1747–53.
[25] Avanzas P, Arroyo-Espliguero R, Kaski JC. Neopterin and cardiovascular disease: growing evidence for a role in patient risk stratification. Clin Chem 2009;55:1056–7.
[26] Pertovaara M, Raitala A, Lehtimaki T, et al. Indoleamine 2,3-dioxygenase activity in nonagenarians is markedly increased and predicts mortality. Mech Ageing Dev 2006;127:497–9.
[27] Frick B, Schroecksnadel K, Neurauter G, Leblhuber F, Fuchs D. Increasing production of homocysteine and neopterin and degradation of tryptophan with older age. Clin Biochem 2004;37:684–7.
[28] Moreno PR, Falk E, Palacios IF, Newell JB, Fuster V, Fallon JT. Macrophage infiltration in acute coronary syndromes. Implications for plaque rupture. Circulation 1994;90:775–8.
[29] Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352:1685–95.
[30] Hoffmann G, Wirleitner B, Fuchs D. Potential role of immune system activation-associated production of neopterin derivatives in humans. Inflamm Res 2003;52:313–21.
[31] Fuchs D, Gruber A, Uberall F, Wachter H. Oxidative stress and apoptosis. Immunol Today 1994;15:496.
[32] Mandi Y, Vecsei L. The kynurenine system and immunoregulation. J Neural Transm 2012;119:197–209.
[33] Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL. Inhibition of T cell proliferation by macrophage tryptophan catabolism. J Exp Med 1999;189:1363–72.
[34] Mellor AL, Munn DH. Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation? Immunol Today 1999;20:469–73.
[35] Fallarino F, Grohmann U, Vacca C, et al. T cell apoptosis by tryptophan catabolism. Cell Death Differ 2002;9:1069–77.
[36] Neurauter G, Wirleitner B, Laich A, Schennach H, Weiss G, Fuchs D. Atorvastatin suppresses interferon-gamma-induced neopterin formation and tryptophan degradation in human peripheral blood mononuclear cells and in monocytic cell lines. Clin Exp Immunol 2003;131:264–7.
[37] Schroecksnadel S, Sucher R, Kurz K, Fuchs D, Brandacher G. Influence of immunosuppressive agents on tryptophan degradation and neopterin production in human peripheral blood mononuclear cells. Transpl Immunol 2011;25:119–23.