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Urinary excretion of kynurenine and tryptophan, cardiovascular events, and mortality after elective coronary angiography

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Aims Kynurenine is a potent endothelium-derived vasodilator. Its synthesis from tryptophan is stimulated by interferon γ and may represent an important compensatory pathway for the regulation of vascular function in inflammatory conditions. We assessed associations of urine kynurenine to tryptophan ratio (KTR) levels to incident major coronary events (MCEs), acute myocardial infarction (AMI), and ischaemic stroke and mortality in patients with suspected stable coronary artery disease (CAD).

Methods and results A total of 3224 patients (mean age 62 years, 69% men) underwent urine and blood sampling prior to elective coronary angiography and were subsequently followed up for median 55 months. A total of 8.4% experienced an MCE, 7.8% suffered an AMI, and 7.6% died. In age- and gender-adjusted analyses, the hazard ratios [HRs; 95% confidence intervals (CI)] of MCE, AMI, and all-cause mortality were 1.43 (1.29–1.59), 1.44 (1.29–1.59), and 1.38 (1.23–1.54) per standard deviation increment of the (log-transformed) urinary KTR, respectively. These estimates were only minimally attenuated after adjustment for potential confounders. The addition of the urine KTR to a model of conventional risk factors significantly improved goodness of fit, discrimination, and risk classification for these clinical endpoints. No association was seen between the urine KTR and the risk of incident ischaemic stroke.

Conclusion A novel urinary inflammation marker, KTR, is strongly associated with adverse prognosis in patients with suspected stable CAD. Underlying pathomechanisms should be further elucidated.

Keywords Coronary artery disease • Risk prediction • Urinary biomarker • Kynurenine • Tryptophan • Inflammation

Introduction

Coronary artery disease (CAD) and other manifestations of atherosclerosis are recognized as chronic inflammatory diseases in which activated macrophages and T lymphocytes are centrally involved.[1] The helper T cell (Th1) cytokine interferon γ (IFN-γ) is highly expressed within atherosclerotic arteries[2] and stimulates the catabolism of the amino acid tryptophan into kynurenine by inducing the rate-limiting enzyme, indoleamine 2,3-dioxygenase (IDO).[3] Tryptophan catabolism has immunosuppressive effects on Th1 lymphocytes.[4] This metabolic pathway may also have important regulatory roles in atherogenesis and vascular function. Kynurenine was recently identified as a potent endothelium-derived vasodilator, mediating its effects independently of nitric oxide (NO).[5]

Since IDO is not expressed unless stimulated by IFN-γ,[5] increased degradation of tryptophan is typically seen in states of activated cellular (Th1) immune responses.[6] Lowered endogenous tryptophan and kynurenine levels, however, occur when dietary intake of tryptophan is restricted. Consequently, the kynurenine:tryptophan ratio (KTR) provides a more reliable measure of IDO activation than the

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absolute concentrations of kynurenine.[6] We have previously demonstrated that elevated plasma KTR levels are associated with increased risk of major coronary events (MCEs) and mortality in patients with stable CAD, participating in the Bergen Coronary Angiography Cohort (BECAC).[7]

Despite practical advantages of spot urine testing in a clinical setting, urinary biomarkers have not been extensively studied for prognostication of CAD patients. Atherosclerotic and inflammatory kidney lesions are common in such patients[8] and may result in renal induction of the IDO enzyme.[5] In the present study, we therefore explored the associations of urine KTR levels to long-term prognosis among participants in the BECAC.

Methods

Study population

The Bergen Coronary Angiography Cohort includes 3314 consecutively recruited patients who were referred to elective coronary angiography due to symptoms suggestive of stable angina pectoris in the period of January 2000 to April 2004 at the Department of Heart Disease, Haukeland University Hospital (Bergen, Norway), constituting ~99% of the patients undergoing such a procedure at our hospital during the given time period. Most patients had undergone an exercise test prior to the coronary angiography, there were no exclusion criteria and patients were recruited subsequently, unless they did not approve enrolment.

A total of 3224 of the participants delivered spot urine samples at baseline and were thus eligible for the present study. The study complied with the Declaration of Helsinki and was approved by the regional Committee for Medical and Health Research Ethics and the Norwegian Data Inspectorate. All patients provided written informed consent.

Baseline data

Patients completed a self-administered questionnaire that provided information about medical history, risk factors, and medications. These data were checked against medical records and subsequently entered into a computerized database by trained study personnel. Hypertension and diabetes mellitus were classified by pre-existing diagnosis, and diabetes mellitus includes both type 1 and 2. Smokers include current smokers and those reporting having quit within the last 4 weeks. Standardized measurements of systolic (SBP) and diastolic blood pressure (DBP) were performed by study nurses at a clinical examination before coronary angiography. Left ventricular ejection fraction (LVEF; %) was determined by ventriculography or echocardiography.

Coronary angiography

Coronary angiograms were performed by invasive cardiologists. Angiographically verified significant CAD was defined by the presence of any lesion with ≥50% diameter stenosis in the main coronary arteries, i.e. left ascending artery, circumflex artery, or right coronary artery (RCA), including their major side branches. The extent was scored 0–3 according to the number of main vessels with significant stenosis. The presence of left main-stem artery stenosis was classified as double-vessel disease if no RCA stenosis was present or as triple-vessel disease if RCA was stenotic or hypoplastic.

Follow-up and clinical endpoints

Patients were followed from the time of first angiography in 2000–2004 and throughout the year 2006. Information on clinical events was collected from the Cause of Death Registry at Statistics Norway and from the Western Norway Cardiovascular Registry. The latter contains all cardiovascular disease (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 endpoints were MCEs, acute myocardial infarction (AMI), stroke, and total mortality. Major coronary event included fatal and non-fatal AMI, 'sudden cardiac death', and 'sudden death' [International Statistical Classification of Disease Tenth Revision (ICD-10) codes I46 and R96, respectively]. Acute myocardial infarction was classified according to the diagnostic criteria of the revised definition published in 2000.[9] Ischaemic stroke was classified according to the definition by the American College of Cardiology Committee in 2001.[10] Events occurring within 24 h after percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) were considered as procedure-related and were not included. Cardiovascular disease and non-CVD mortality were analysed as secondary endpoints. Cardiovascular disease mortality included causes of death coded I00–I99 or R96 according to the ICD-10 system. All events were adjudicated by at least two experienced clinicians who had no information on baseline biochemical characteristics.

Biochemical analyses

Plasma and serum samples were collected before coronary angiography and were immediately frozen at −80° C, until later analysed at Bevital AS (www.bevital.no) by laboratory personnel who were blinded to the clinical outcomes. The patients were not instructed to be fasting and blood samples were drawn between 8 and 12 h. Urine specimens had been collected by the patients at home, on the day of angiography, and were frozen at the same time and temperature.

Urine concentrations of kynurenine and tryptophan were analysed by gas chromatography tandem mass spectrometry, whereas levels of these metabolites in plasma were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS).[11] Urine and plasma creatinine were determined by including it and its deuterated internal standard (d3-creatinine) in an established LC-MS/MS assay[12] using the ion pairs 114/44.2 and 117/47.2, respectively. For the urinary metabolites, the lower limits of detections were 0.1 μmol/L (kynurenine), 0.5 μmol/L (tryptophan), and 10 μmol/L (creatinine). Coefficients of variation were as follows: urine kynurenine (5.0%), urine tryptophan (5.0%), urine creatinine (7.2%), and plasma creatinine (4.0%). Fractional kidney excretion (FE) for tryptophan and kynurenine was calculated by the formula

$$FE_s = \frac{[S]_{urine} \times [creatinine]_{plasma}}{[creatinine]_{urine} \times [S]_{plasma}},$$

where S denotes the analyte of interest.

We applied the Chronic Kidney Disease Epidemiology Collaboration equation to estimate the glomerular filtration rate (GFR).[13] Urine albumin was analysed using the Dade Behring BN2 nephelometer analyser and serum C-reactive protein (CRP) by an ultra-sensitive immunoassay, using the Behring nephelometer II system N Latex CRP mono (both Behring Diagnostics, Marburg, Germany). Serum levels of apolipoprotein A1 and apolipoprotein B were measured on the Hitachi 917 and 912 systems, respectively (Roche Diagnostics, GmbH, Mannheim, Germany). Glycated haemoglobin (HbA1c) was determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.[14]

Statistical analysis

Variables were reported as counts (percentages) and means (SD) when appropriate. Right skewed variables were logarithmically transformed before being used in parametrical analyses, but in tables we present untransformed medians and corresponding interquartile ranges (IQRs).

Differences in baseline characteristics according to quartiles of the urine KTR were explored using linear regression for continuous variables and logistic regression for categorical variables. Associations between continuous variables were assessed with the Spearman rank correlation adjusted for age and gender, and all correlations with urinary markers were performed with values normalized to urinary creatinine.

Hazard ratios (HRs) were calculated using the Cox regression and are reported per (log-transformed) SD increment and for quartile 4 vs. quartile 1 of the urine KTR. The multivariable model included age, gender, body mass index (BMI), smoking status, hypertension, diabetes mellitus, LVEF, angiographic extent of CAD (0–3), apolipoprotein A1, apolipoprotein B, plasma KTR, serum CRP, estimated GFR (eGFR), urine albumin:creatinine ratio, treatment following baseline coronary angiography (medication only, PCI, CABG), use of statins, angiotensin converting enzyme inhibitors, dual antiplatelet therapy, and loop diuretics.

Interactions were tested by adding product terms to the model. We performed log–log plots and plots of Schoenfeld residuals to ensure that assumptions of proportional hazards were not violated.[15] Dose–response relationships between urine KTR levels and risk of clinical events were visualized by generalized additive regression plots.[16] In these plots, urine KTR values (log-transformed) were modelled with a 4 degrees of freedom smoothing spline fit in multivariable Cox proportional hazard models. All gender- and age-adjusted analyses were performed for the total study population. However, 299 patients were excluded from the multivariable models due to missing data on either urine microalbumin:creatinine ratio, eGFR, CRP, BMI, or plasma KTR.

Overall model fit was compared using the Akaike's information criterion.[17] Discrimination, or the ability of risk prediction models to distinguish those who experience an event from those who do not, was evaluated by calculating areas under receiver operating characteristics curves in logistic regression models including the same variables as the Cox models. By determining net reclassification improvement (NRI), we assessed the extent to which the urine KTR assigned study participants into more correct levels of risk.[18] Since there are no established categories of risk in patients with known CVD, we obtained the continuous, or category-free, NRI (NRI $>$ 0).[19] Discrimination and reclassification analyses were computed using a follow-up time of 32 months, which approximately was the shortest individual follow-up time.

A subgroup of BECAC participants ($n = 1674$) was also included in the Western Norway B Vitamin Intervention Trial (WENBIT)[20] and was followed-up with clinical controls 1 and 3 years after inclusion. Altogether, 1294 of the patients provided urine samples at all three study visits and 196 patients at two visits. These repeated measurements were applied for evaluating test–retest reliability of metabolites. Coefficients of reliability were estimated from linear mixed models as the proportion of variance between persons divided by the total variance.[21]

Reported probability values are two-tailed and were considered significant when <0.05. We used the statistical packages R[22] (version 2.11 for Windows, Vienna, Austria) and PASW (version 18 for Windows).

Results

Patient characteristics and urine kynurenine to tryptophan ratio at baseline

For the 3224 patients, the mean (SD) age at inclusion was 61.9 (10.6) years and 69.2% ($n = 2232$) were men. Altogether, 28.8% ($n = 930$) of patients were diagnosed with triple-vessel disease, single- or double-vessel disease was present in 42.6% ($n = 1374$), and 28.5% ($n = 920$) did not have significant CAD. The median (IQR) values of kynurenine and tryptophan in urine (both presented as relative to urine creatinine) were 195 (125–303) nmol/mmol and 5.00 (3.72–6.66) μmol/mmol, respectively. The median (IQR) urine KTR was 38.2 (28.5–53.8) nmol/μmol. The median (IQR) fractional kidney clearance was 0.87 (0.55–1.33) for kynurenine and 0.53 (0.38–0.72) for tryptophan.

A total of 14.2% ($n = 457$) of participants reported to be fasting at the time of blood sampling. The median levels of plasma kynurenine and plasma tryptophan were lower in fasting patients (169 vs. 173 nmol/L and 69 vs. 73 μmol/L, respectively; $P \leq 0.002$). For the plasma KTR, urine kynurenine, urine tryptophan, and urine KTR, there were no significant differences in levels according to fasting status ($P \geq 0.46$).

Baseline characteristics of the study population are given in *Table 1*. Compared with patients in the first quartile of the urine KTR, those in the fourth quartile were older, more likely to have extensive CAD or diabetes mellitus, were less likely to smoke, and a higher number of patients used angiotensin-converting enzyme inhibitors and loop diuretics. The proportions receiving PCI or CABG following coronary angiography were similar across quartiles of the urine KTR.

In age- and gender-adjusted analyses, urine levels of kynurenine and tryptophan were strongly correlated ($r = 0.78$). The urine KTR was also strongly associated with urine kynurenine ($r = 0.70$), moderately correlated with plasma KTR ($r = 0.37$) and neopterin ($r = 0.29$), and weakly positively related to serum CRP ($r = 0.16$), urine albumin:creatinine ratio ($r = 0.11$; P for all <0.001), and plasma tryptophan ($r = 0.04$; $P = 0.04$). Negative correlations were observed to eGFR ($r = -0.18$; $P < 0.001$), apolipoprotein A1 ($r = -0.12$; $P < 0.001$), apolipoprotein B ($r = -0.05$; $P = 0.004$), and LVEF ($r = -0.04$; $P = 0.04$). Patients with elevated urine KTR levels at baseline were more likely to be treated for hypertension (*Table 1*). However, urine KTR levels were only weakly correlated with SBP ($r = 0.04$; $P = 0.03$) and not correlated with DBP ($r = 0.003$; $P = 0.85$) *per se*.

Supplementary material online, *Tables S1* and *S2* give patient characteristics across quartiles of the tryptophan:creatinine ratio and the kynurenine:creatinine ratio, respectively; the latter showing associations essentially similar to those of the urine KTR.

Urine kynurenine to tryptophan ratio levels and risk of clinical events

During a median (IQR) follow-up of 55 (43–69) months, 8.4% ($n = 270$) of patients experienced an MCE. Urine KTR levels were strongly associated with this endpoint (*Figure 1*) and provided consistent risk estimates across a range of baseline characteristics (*Figure 2*, P for interactions ≥ 0.07). Per SD increment of (log-transformed) urine KTR levels, the age- and gender-adjusted HR [95% confidence intervals (CI)] was 1.43 (1.29–1.59). This estimate was only weakly attenuated after extensive adjustment for confounding risk factors [HR (95% CI), 1.31 (1.15–1.49)]. Moreover, in the multivariable Cox model, there was no statistically significant association between MCE and eGFR [HR (95% CI), 1.01 (1.00–1.02)], urine albumin:creatinine ratio [HR (95% CI), 1.00 (0.99–1.01)], CRP [HR (95% CI), 1.09 (0.91–1.29)], or plasma KTR [HR (95% CI), 1.00 (0.86–1.15)].

Table I Baseline characteristics according to quartiles of the urine kynurenine:tryptophan ratio ($n = 3224$)

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
Urine KTR (nmol/μmol)	23.7 (20.3–26.2)	32.9 (30.8–35.3)	44.4 (40.8–48.4)	70.1 (60.4–90.0)	
Urine creatinine (mmol/L)	13.0 (9.25–18.3)	11.6 (7.64–16.6)	11.2 (7.13–15.4)	10.2 (6.97–10.2)	<0.001
Urine kynurenine:creatinine (nmol/mmol)	107 (80–149)	168 (125–222)	230 (170–304)	371 (261–529)	<0.001
Urine tryptophan:creatinine (μmol/mmol)	4.74 (3.56–6.35)	5.14 (3.83–6.69)	5.14 (3.81–6.75)	4.98 (3.66–6.80)	0.57
Urine albumin:creatinine (mg/mmol)	0.49 (0.36–0.83)	0.50 (0.37–0.90)	0.56 (0.39–1.03)	0.73 (0.44–2.06)	<0.001
Age (years)	57.3 (9.8)	60.4 (10.0)	63.2 (10.0)	66.8 (10.2)	<0.001
Gender [male; *n* (%)]	586 (72.7)	532 (66.0)	559 (69.4)	555 (68.9)	0.26
Systolic blood pressure (mm Hg)	137 (20)	141 (20)	142 (21)	143 (22)	<0.001
Diastolic blood pressure (mm Hg)	81 (10)	81 (10)	81 (11)	81 (10)	0.84
Body mass index (kg/m^2)	26.5 (3.7)	26.8 (4.0)	26.6 (3.9)	26.9 (4.6)	0.18
LVEF (%)	65 (11)	65 (10)	63 (12)	64 (11)	0.01
Fasting at blood sampling [*n* (%)]	115 (14.3)	120 (14.9)	116 (14.4)	106 (13.2)	0.48
Apolipoprotein A1 (g/L)	1.36 (0.26)	1.37 (0.26)	1.36 (0.26)	1.32 (0.27)	0.001
Apolipoprotein B (g/L)	0.95 (0.26)	0.94 (0.25)	0.92 (0.24)	0.89 (0.23)	<0.001
Estimated GFR (mL/min/1.73 m^2)	84 (71–98)	83 (68–97)	76 (63–93)	68 (53–89)	<0.001
Serum CRP (mg/L)	1.52 (0.80–2.90)	1.74 (0.87–3.31)	1.76 (0.83–3.73)	2.37 (1.12–5.20)	<0.001
Plasma neopterin (nmol/L)	7.1 (6.0, 8.6)	7.6 (6.4, 9.4)	8.4 (6.9, 10.4)	10.1 (7.9, 13.3)	<0.001
Plasma KTR (nmol/μmol)	20.6 (17.0–24.0)	22.9 (19.2–31.6)	24.5 (20.3–29.5)	28.8 (23.8–35.7)	<0.001
HbA1c (%)	6.0 (1.4)	6.0 (1.3)	6.2 (1.4)	6.2 (1.5)	0.01
Cardiovascular history and risk factors [*n* (%)]					
Prior acute myocardial infarction	277 (34.4)	308 (38.2)	323 (40.1)	337 (41.8)	0.002
Prior PCI	134 (16.6)	144 (17.9)	160 (19.9)	167 (20.7)	0.02
Prior CABG	78 (9.7)	69 (8.6)	90 (11.2)	103 (12.8)	0.01
Hypertension	314 (39.0)	368 (45.7)	378 (46.9)	467 (57.9)	<0.001
Diabetes mellitus	29 (3.6)	63 (7.8)	101 (12.5)	187 (23.2)	<0.001
Current smoking	261 (32.4)	221 (27.4)	182 (22.6)	163 (20.3)	<0.001
Angiographic evidence of CAD [*n* (%)]					
No significant CAD	270 (33.5)	244 (30.3)	238 (29.5)	168 (20.8)	<0.001
Single-vessel disease	169 (21.0)	187 (23.2)	159 (19.7)	176 (21.8)	0.86
Double-vessel disease	182 (22.6)	169 (21.0)	160 (19.9)	172 (21.3)	0.45
Triple-vessel disease	185 (23.0)	206 (25.6)	249 (30.9)	290 (36.0)	<0.001
Treatment following baseline coronary angiography [*n* (%)]					
No or medications only	422 (52.4)	390 (48.4)	401 (49.8)	368 (45.7)	0.02
PCI	233 (28.9)	229 (28.4)	222 (27.5)	265 (32.9)	0.12
CABG	151 (18.7)	187 (23.2)	183 (22.7)	173 (21.5)	0.24
Medication prior to baseline visit [*n* (%)]					
Aspirin	651 (80.8)	653 (81.0)	644 (80.8)	621 (77.2)	0.04
Statins	566 (70.2)	558 (69.2)	547 (67.9)	573 (71.1)	0.94
Beta blockers	590 (73.2)	594 (73.7)	565 (70.2)	588 (73.1)	0.32
ACEIs	133 (16.5)	156 (19.4)	151 (18.8)	210 (26.1)	<0.001
Loop diuretics	58 (7.2)	65 (8.1)	70 (8.7)	140 (17.4)	<0.001
Dual antiplatelet therapy	12 (1.5)	20 (2.5)	21 (2.6)	24 (3.0)	0.06
Medication at discharge from baseline visit [*n* (%)]					
Aspirin	626 (77.7)	645 (80.0)	659 (81.8)	656 (81.4)	0.04
Statins	615 (76.3)	626 (77.7)	633 (78.5)	637 (79.0)	0.17
Beta blockers	560 (69.5)	577 (71.6)	560 (69.5)	589 (73.1)	0.26
ACEIs	134 (16.6)	160 (19.9)	158 (19.6)	216 (26.8)	<0.001
Loop diuretics	61 (7.6)	71 (8.8)	74 (9.2)	145 (18.0)	<0.001
Dual antiplatelet therapy	102 (12.7)	103 (12.8)	96 (11.9)	107 (13.3)	0.85

Continued

Table 1 *Continued*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
Clinical endpoints during follow-up [*n* (%)]					
Major coronary events	27 (3.3)	61 (7.6)	68 (8.4)	114 (14.1)	<0.001
AMI	27 (3.3)	57 (7.1)	61 (7.6)	106 (13.2)	<0.001
Ischaemic Stroke	21 (2.6)	17 (2.1)	19 (2.4)	39 (4.8)	0.10
All-cause Mortality	27 (3.3)	48 (6.0)	63 (7.8)	106 (13.2)	<0.001
CVD Mortality	9 (1.1)	22 (2.7)	36 (4.5)	65 (8.1)	<0.001
Non-CVD Mortality	18 (2.2)	26 (3.2)	27 (3.3)	41 (5.1)	0.003

Values are given as means (SD), medians (IQR) or counts (percentages). ACEI, angiotensin-converting enzyme inhibitor; AMI, acute myocardial infarction; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CRP, C-reactive protein; CVD, cardiovascular disease; GFR, glomerular filtration rate; KTR, kynurenine:tryptophan ratio; LVEF, left ventricular ejection fraction; PCI, percutaneous coronary intervention.

Acute myocardial infarction occurred in 7.8% (251) patients. Per SD increment in log urine KTR, the HR (95% CI) was 1.44 (1.29–1.59) in the age- and gender-adjusted and 1.32 (1.15–1.51) in the multivariable Cox regression model.

A total of 7.6% of patients ($n = 244$) died during follow-up, and deaths from CVD occurred in 4.1% ($n = 132$). Per SD increment of log urine KTR, multivariable adjusted HR (95% CI) were 1.23 (1.07–1.42) and 1.31 (1.09–1.56) for all-cause and CVD mortality, respectively. The urine KTR was not related to the risk of non-cardiovascular death or incident ischaemic stroke (*Table 2*). Hazard ratios for clinical endpoints across all quartiles of the urine KTR are given in Supplementary material online, *Table S3*. Including only subjects with significant coronary artery stenoses at angiography yielded similar estimates (Supplementary material online, *Table S4*), although the association between urine KTR and CVD mortality was significantly attenuated (*P* for interaction = 0.002).

For individual components of the urine KTR, no associations were observed between urine tryptophan levels and risk of clinical events, except for an inverse relationship with the risk of stroke in multivariable analyses (Supplementary material online, *Tables S5* and *S6*). Urine kynurenine was a significant predictor of MCE, all-cause mortality, and CVD mortality. However, risk estimates were weaker than for the urine KTR, particularly in multivariable analyses. No relationship was seen between urine kynurenine and stroke or non-CVD mortality (Supplementary material online, *Tables S7* and *S8*).

About 50% of the participants were also included in the WENBIT and randomized to treatments with folic acid, vitamin B6, or placebo. Notably, urine KTR outcome associations were not modified by such interventions (*P* for interactions ≥ 0.34).

Goodness of fit, discrimination, and risk reclassification

Addition of the urine KTR to the multivariable model significantly improved goodness of fit and increased the discriminatory power and reclassification for MCE, AMI, and all-cause and CVD mortality, but not for ischaemic stroke and non-CVD mortality (*Table 3*). Similar, albeit somewhat weaker results were seen when exclusively investigating patients with significant coronary stenoses at angiography (Supplementary material online, *Table S9*).

Coefficients of reliability

The test–retest stability of the urine KTR was evaluated applying longitudinal measurements of excretion in patients with samples donated at least once during follow-up ($n = 1490$). We found a coefficient of reliability of 0.74 for the urine KTR, which showed small variations according to age or gender (0.69–0.75), and compared favourably with that of the urine albumin:creatinine ratio (0.70), plasma KTR (0.67), and serum CRP (0.51).

Discussion

Major findings

In a large cohort of patients referred for suspected stable CAD, levels of the urine KTR at baseline showed a strong dose–response relationship with incident MCE, AMI, and all-cause and CVD mortality. This urinary biomarker showed moderate positive associations with age, hypertension, and diabetes mellitus and was weakly negatively related to eGFR and smoking. However, extensive multivariable adjustment hardly attenuated the risk estimates, suggesting that its association with adverse prognosis was not mediated through classical CVD risk factors. We did not observe any relationship between urine KTR and non-CVD mortality or incident ischaemic stroke, the latter possibly because of a low number of events.

Goodness of fit, discrimination, and risk classification were all improved for MCE, AMI, and all-cause and CVD mortality by adding the urine KTR to a model of conventional risk indicators. Moreover, for individual patients, urine KTR levels were relatively stable over time, which is a prerequisite for a potential clinical application of a biomarker.

Kynurenine pathway of tryptophan metabolism in coronary heart disease

Levels of the KTR in plasma were associated with CVD risk factors in presumably healthy populations[23,24] and were elevated in patients with CAD.[25] Moreover, IDO and other genes related to the kynurenine pathway are shown to be up-regulated in atherosclerotic plaques.[26] We previously found that the plasma KTR predicted MCE and mortality in patients with stable CAD included in the

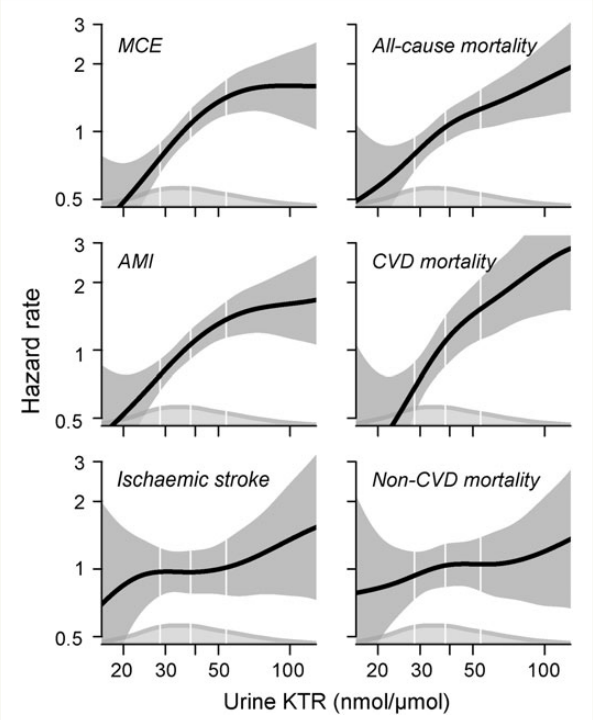

Figure 1 Dose–response relations between the (log-transformed) urine kynurenine:tryptophan ratio and risk of MCE, AMI, ischaemic stroke, all-cause, cardiovascular disease, and non-cardiovascular disease mortality, obtained by generalized additive regression. Models are adjusted for age, gender, smoking status, hypertension, diabetes mellitus, left ventricular ejection fraction (%), angiographic extent of coronary artery disease (1–3), treatment following baseline coronary angiography (medication only, percutaneous coronary intervention, coronary artery bypass grafting), apolipoprotein A1, apolipoprotein B, plasma kynurenine:tryptophan ratio, C-reactive protein, estimated glomerular filtration rate, urine albumine:creatinine ratio, and the use of statins, angiotensin-converting enzyme inhibitors, dual antiplatelet therapy, and loop diuretics. The solid lines show hazard ratios and the shaded areas 95% confidence intervals. The distribution of the urine kynurenine:-tryptophan ratio concentrations is shown at the bottom of the panels, and vertical lines denote 25th, 50th, and 75th percentiles. The plots are trimmed at the 2.5 and 97.5 percentiles. AMI, acute myocardial infarction; CVD, cardiovascular disease; MCE, major coronary event.

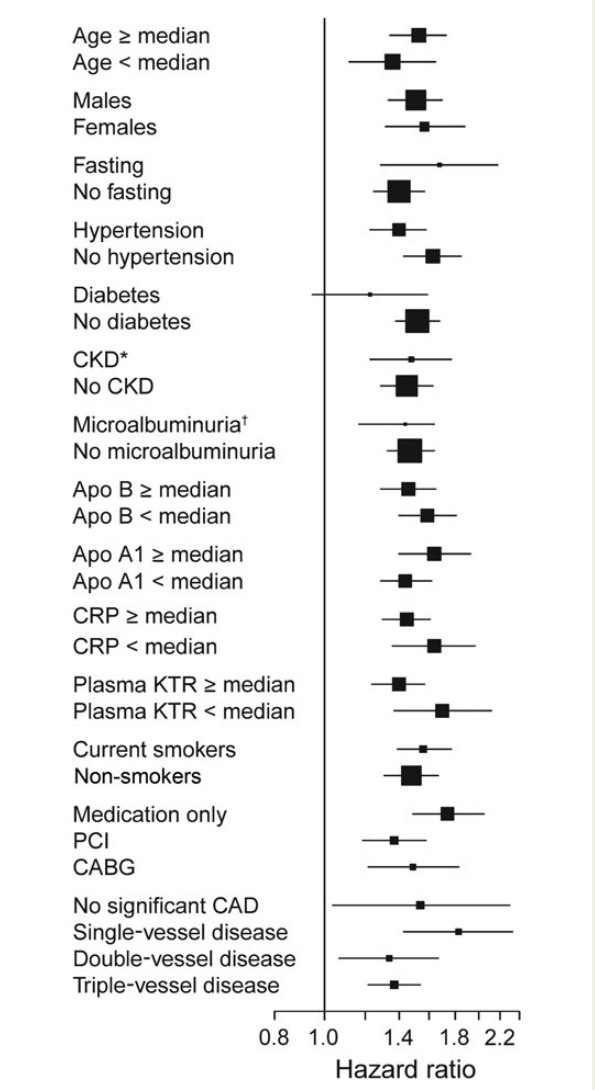

Figure 2 Unadjusted hazards ratios for major coronary events across a range of baseline characteristics. Hazard ratios, represented by squares, are reported per standard deviation (for the total study population) increment of the (log-transformed) urine kynurenine:tryptophan ratio levels. Areas are proportional to the number of patients in each group. Horizontal lines indicate 95% confidence intervals. *Estimated glomerular filtration rate <60 mL/min/1.73 m^2. †Urine albumin:creatinine ratio ≥ 3.0 mg/mmol. Apo, apolipoprotein; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CRP, C-reactive protein; CKD, chronic kidney disease; KTR, kynurenine:tryptophan ratio; PCI, percutaneous coronary intervention.

BECAC study,[7] and we now demonstrate that urine KTR levels are even more strongly associated with adverse prognosis in this cohort.

Urinary biomarkers in coronary heart disease

Microalbuminuria is an established CVD risk marker both in diabetic patients and in the general population,[27] but except for albumin, urinary biomarkers have not been extensively studied in atherosclerotic disorders. Levels of several inflammation markers were elevated in the urine of patients with diabetes mellitus[28] and a cross-sectional study indicated a value of urinary proteomics for the assessment of CAD severity.[29] However, associations of urinary inflammation markers to long-term prognosis in CAD patients have not been evaluated in large-scale epidemiological surveys.

Possible mechanisms

The conversion of tryptophan into kynurenine is induced by IDO.[6] This enzyme can be expressed in fibroblasts, macrophages, and

Table 2 Study endpoints by urine kynurenine:tryptophan ratio levels

Endpoint	Hazard ratio (95% confidence interval)							
	Model I, simple[a]				Model II, multivariable[b]			
	Per SD increment	*P*-value	Q4 vs. Q1	*P*-value	Per SD increment	*P*-value	Q4 vs. Q1	*P*-value
Major coronary events	1.43 (1.29–1.59)	<0.001	3.63 (2.35–5.62)	<0.001	1.31 (1.15–1.49)	<0.001	2.93 (1.80–4.76)	<0.001
Acute myocardial infarction	1.44 (1.29–1.59)	<0.001	3.47 (2.23–5.38)	<0.001	1.32 (1.15–1.51)	<0.001	2.74 (1.67–4.48)	<0.001
Ischaemic Stroke	1.12 (0.91–1.37)	0.30	0.96 (0.54–1.70)	0.88	1.21 (0.97–1.52)	0.09	1.11 (0.57–2.19)	0.76
All-cause mortality	1.38 (1.23–1.54)	<0.001	2.54 (1.63–3.96)	<0.001	1.23 (1.07–1.42)	0.005	2.32 (1.34–4.04)	0.003
CVD mortality	1.53 (1.33–1.75)	<0.001	4.60 (2.24–9.44)	<0.001	1.31 (1.09–1.56)	0.004	4.00 (1.60–9.96)	0.003
Non-CVD mortality	1.20 (1.00–1.44)	0.05	1.50 (0.84–2.70)	0.17	1.10 (0.88–1.39)	0.40	1.53 (0.74–3.16)	0.25

CVD, cardiovascular disease.
[a]Adjusted for age and gender.
[b]Adjusted for age, gender, hypertension, diabetes mellitus, smoking status, left ventricular ejection fraction, angiographic extent of CAD, treatment following baseline coronary angiography (medications only, percutaneous coronary intervention, coronary artery bypass grafting), apolipoprotein A1, apolipoprotein B, estimated glomerular filtration rate, body mass index, C-reactive protein, plasma kynurenine:tryptophan ratio, urine albumin:creatinine ratio, use of statins, angiotensin-converting enzyme inhibitors, dual antiplatelet therapy, and loop diuretics.

dendritic cells throughout the human body.[30] However, vascular endothelial cells appear to be the primary site for IDO expression in systemic inflammatory conditions.[31] Experimental data have revealed immunosuppressive roles of IDO activation.[4,6] Tryptophan depletion in the microenvironment has been shown to cause starvation and stress of immune cells and subsequently reduced cell function. Moreover, cytotoxic effects on Th1 lymphocytes have been revealed for kynurenine and several of its downstream metabolites.[4,32] Recently, it was demonstrated that feeding atherosclerosis prone mice with such a metabolite (3-hydroxyanthranilic acid), led to both inhibited immune responses, a more favourable lipid profile, less uptake of oxidized LDL particles in macrophages and reduced atherosclerosic lesions.[33]

Besides its effects on immune responses, kynurenine was recently identified as a potent endothelium-derived vasodilator.[5] Endothelial dysfunction is an inevitable component of the atherosclerotic process and is characterized by impaired NO-mediated vasodilatation of arteries.[1] IDO-induced conversion of tryptophan therefore may represent an important back-up system in conditions of reduced NO activity. As a haeme enzyme, IDO can be inhibited by NO.[34] Conversely, metabolites of the kynurenine pathway has been shown to inhibit NO synthase.[35] Thus, balanced activation of these two vasodilator systems may potentially be pivotal for maintenance of vascular function in inflammatory conditions.

Urinary albumin excretion is frequently elevated in patients with impaired endothelial function and is considered not only to reflect kidney injury, but a more generalized vascular damage.[36] Notably, neither the urine albumin:creatinine ratio nor eGFR predicted MCEs in the multivariable Cox model. Moreover, risk estimates were similar across strata of these renal indices, suggesting that the associations between urine KTR and adverse outcomes were not solely mediated by renal dysfunction.

Tryptophan and kynurenine are both freely filtered in the glomeruli.[37] According to the present findings, tryptophan is effectively reabsorbed in the proximal tubuli, and only a minor fraction is excreted in the urine. Reabsorption of kynurenine, in contrast, becomes saturated, leading to higher excreted fractions at increasing circulating concentrations.[37] Since adjustment for the plasma KTR and serum CRP only minimally weakened the associations of the urine KTR to adverse outcomes, however, it is unlikely that elevated kynurenine excretion solely mirrors the low-grade systemic inflammation that accompanies CAD.

Atherogenesis is a generalized process and frequently affects multiple organs. In CVD patients, glomerular, tubular, and renal microvascular inflammatory changes are common without overt ischaemic nephropathy.[8] Moreover, histological examinations have revealed close similarities between glomerulosclerosis and atherosclerotic lesions, suggestive of a common pathogenesis.[38] Upon stimulation by IFN-γ, kynurenine can be synthesized locally in the kidneys.[5] Elevated urine KTR, therefore, may reflect renal induction of the IDO enzyme, possibly mediating counter-regulatory vascular protective effects. Due to the observational nature of our work, however, we cannot provide definite answers to whether it represents an epiphenomenon or a causative pathway in atherosclerosis and its clinical manifestations.

Strengths and limitations

The present work is the first to evaluate a urinary marker of endothelial function and inflammation for prognostication of CAD patients. Strengths of the study include its prospective design, the large sample size and detailed information about clinical characteristics. Repeated measurements allowed us to calculate intra-individual variability of biomarkers. Follow-up was ascertained through the use of a patient-administrative and a population-based registry. We cannot exclude the possibility that recordings of clinical endpoints are subjected to some underreporting or other misclassification, but we do not suspect that misclassification differs according to biomarker levels. Urine samples were stored at room temperature for some hours prior to freezing. This is unlikely to have introduced a bias, however, since levels of tryptophan and kynurenine in urine are reported to be stable for at least 48 h under such conditions.[39]

Table 3 Model fit, discrimination, and reclassification indices

	Model* without the urine KTR	Model* with the addition of the urine KTR	*P*-value
Major coronary events			
AIC[a]	3571.330	3558.699	<0.001
ROC-AUC[b] (95% CI)	0.775 (0.738–0.813)	0.786 (0.751–0.822)	0.03
NRI continuous (95% CI)		0.307 (0.146–0.468)	<0.001
Acute myocardial infarction			
AIC[a]	3312.107	3300.015	<0.001
ROC-AUC[b] (95% CI)	0.762 (0.722–0.802)	0.774 (0.736–0.812)	0.03
NRI continuous (95% CI)		0.272 (0.104–0.440)	0.002
Ischaemic stroke			
AIC[a]	1333.054	1332.570	0.12
ROC-AUC[b] (95% CI)	0.819 (0.768–0.870)	0.823 (0.774–0.873)	0.35
NRI continuous (95% CI)		0.139 (−0.141–0.418)	0.33
All-cause mortality			
AIC[a]	2968.879	2963.422	0.001
ROC-AUC[b] (95% CI)	0.795 (0.750–0.840)	0.813 (0.771–0.856)	0.02
NRI continuous (95% CI)		0.296 (0.093–0.498)	0.004
CVD mortality			
AIC[a]	1504.338	1498.664	0.01
ROC-AUC[b] (95% CI)	0.847 (0.800–0.894)	0.867 (0.825–0.910)	0.01
NRI continuous (95% CI)		0.326 (0.064–0.588)	0.01
Non-CVD mortality			
AIC[a]	1464.424	1465.706	0.40
ROC-AUC[b] (95% CI)	0.772 (0.698–0.846)	0.774 (0.698–0.850)	0.77
NRI continuous (95% CI)		0.083 (−0.229–0.395)	0.60

AIC, Akaikes information criteria; CVD, cardiovascular disease; KTR, kynurenine:tryptophan ratio; NRI, net reclassification improvement; ROC-AUC, area under receiver operator characteristics curve.
*Adjusted for age, gender, hypertension, diabetes mellitus, smoking status, left ventricular ejection fraction, angiographic extent of coronary artery disease, treatment following baseline coronary angiography (medications only, percutaneous coronary intervention, coronary artery bypass grafting), apolipoprotein A1, apolipoprotein B, estimated glomerular filtration rate, body mass index, C-reactive protein, plasma KTR, urine albumin:creatinine ratio, use of statins, angiotensin-converting enzyme inhibitors, dual antiplatelet therapy, and loop diuretics.
[a]Lower values indicate better models.
[b]Higher values indicate better models.

Conclusion

In patients referred to coronary angiography for suspected stable CAD, the urine KTR is a strong predictor of MCE, AMI, and mortality, with a minor incremental prognostic value over and above that obtained by classical risk factors. Its striking dose–response relationship and specificity to acute atherosclerotic events prompt further investigations into the role of tryptophan catabolism and renal inflammation in atherogenesis and plaque rupture.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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