Kidney & Blood Pressure Research

Kidney Blood Press Res 2017;42:244-256
DOI: 10.1159/000477222
Published online: May 16, 2017
Accepted: March 08, 2017

Original Paper

Pre-Interventional Kynurenine Predicts Medium-Term Outcome after Contrast Media Exposure Due to Coronary Angiography

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Key Words
Contrast induced acute kidney injury • Coronary angiography • Major adverse kidney event • Kynurenine • Preinterventional biomarker

Abstract
Background/Aims: Contrast induced acute kidney injury (CI-AKI) remains a serious complication of contrast media enhanced procedures like coronary angiography. There is still a lack of established biomarkers that help to identify patients at high risk for short and long-term complications. The aim of the current study was to evaluate plasma kynurenine as a predictive biomarker for CI-AKI and long-term complications, measured by the combined endpoint "major adverse kidney events" (MAKE) up to 120 days after CM application. ***Methods:*** In this prospective cohort study 245 patients undergoing coronary angiography were analyzed. Blood samples were obtained at baseline, 24h and 48h after contrast media (CM) application to diagnose CI-AKI. Patients were followed for 120 days for adverse clinical events including death, the need for dialysis, and a doubling of plasma creatinine. Occurrence of any of these events was summarized in the combined endpoint MAKE. ***Results:*** Preinterventional plasma kynurenine was not associated with CI-AKI. Patients who later developed MAKE displayed significantly increased preinterventional plasma kynurenine levels ($p<0.0001$). ROC analysis revealed that preinterventional kynurenine is highly predictive for MAKE (AUC=0.838;

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p<0.0001). The optimal cutoff was found at ≥3.5 µmol/L. Using this cutoff, the Kaplan-Meier estimator demonstrated that concentrations of plasma kynurenine ≥3.5 µmol/L were significantly associated with a higher prevalence of MAKE until follow up (p<0.0001). This association remained significant in multivariate Cox regression models adjusted for relevant factors of long-term renal outcome. ***Conclusion:*** Preinterventional plasma kynurenine might serve as a highly predictive biomarker for MAKE up to 120 days after coronary angiography.

Introduction

Over 80 million procedures using iodinated contrast media (CM) are performed annually [1]. Contrast-induced acute kidney injury (CI-AKI) is a prevalent complication of such CM enhanced examinations and procedures [1]. The Contrast Media Safety Committee of the European Society of Urogenital Radiology (ESUR) defines CI-AKI as an increase in plasma creatinine of at least 25 % or 44 µmol/l within 3 days after CM administration in the absence of an alternative etiology [2]. CI-AKI occurs in up to 5% of hospitalized patients with normal renal function prior to CM administration, but the prevalence dramatically increases to 20-30% in high risk patients such as patient with diabetes, congestive heart failure or preexisting renal insufficiency [3, 4]. However, the true prevalence of CI-AKI might be higher, as CI-AKI is often clinically undetected because patients can be asymptomatic [4]. Furthermore, due to technical improvements, many patients undergoing CM interventions are likely to be discharged within 24 hours, complicating an assessment of plasma creatinine concentrations for more than 24 hours after CM exposure [5]. Most patients who experienced CI-AKI usually display a partial or complete recovery of kidney function by the time of hospital discharge [6], but several recent cohort studies have shown that CI-AKI is associated with long-term loss of kidney function, cardiovascular events, and death [6-8]. There is increasing consensus that ultimate clinical outcomes should be moved in the focus of studies investigating contrast media associated nephrotoxicity [9]. The acute endpoint CI-AKI itself, characterized by changes in plasma creatinine or urine excretion over set time intervals, merely indicates transient pathophysiology, which may or may not eventually result in a significant clinical outcome [9]. This is underlined by data, indicating that also subclinical CI-AKI is associated with increased long-term mortality [10, 11]. Until now, there is no established specific biomarker that helps identifying patient subgroups which are at an increased risk for long-term sequelae of CM interventions.

Kynurenine is the first stable metabolite of the least-abundant essential amino acid, L-tryptophan, which plays an important role in protein synthesis and functions as a biochemical precursor for several other important biomolecules [12]. The catabolism of tryptophan to kynurenine is mediated by indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase [12, 13]. The expression of IDO is induced by inflammatory cytokines, in particular by interferon- γ and to a lesser extent by tumor necrosis factor-α [14-16]. Current literature suggests that an activated IDO induces homeostatic mechanisms against excessive immune reactions, promotes immune tolerance and elicits anti-inflammatory effects [14]. IDO is activated in various diseases, leading to decreasing tryptophan and increasing kynurenine blood levels [17]. Moreover, chronic systemic low-grade inflammation enhances IDO activation [14]. Chronic kidney disease (CKD) is accompanied by immune dysregulation and more recent studies have shown that the kynurenine pathway is associated with chronic kidney disease (CKD) [12, 15, 18-20]. It was demonstrated that CKD patients display increased kynurenine plasma levels [19-21]. Furthermore, it was shown in animal and cell culture experiments that kynurenine does not just accumulate in CKD patients due to impaired kidney function, but that the kynurenine pathway might be involved in the pathogenesis of CKD [22, 23]. Given the current evidence, kynurenine might serve as a new biomarker to assess the risk of kidney disease [18-21].

The aim of the current study was to evaluate whether preinterventional plasma kynurenine concentrations can predict short and medium-term outcome in patients with preexisting renal risk factors subjected to CM application due to coronary angiography.

Materials and Methods

Study population and protocol

This prospective cohort study, performed from January 2010 to December 2011, was approved by the authors' institutional review board of the university hospital Charité, Berlin, Germany. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. 245 patients with renal impairment (baseline creatinine > 1.1 mg/dL) or diabetes mellitus (diagnosed before study begin or HbA1c > 46 mmol/mol) undergoing coronary angiography were included. All patients without any present inclusion criteria, patients with terminal kidney disease and patients who did not sign the informative consent were excluded from the study. Additionally, patients who did not or were not able to provide their agreement to participate were excluded. Individual patient risk for CI-AKI was assessed and calculated based on Mehran contrast nephropathy risk score, which calculates an individual patient risk score for CI-AKI based on readily available information [24]. The variables included in this calculation are patient-related characteristics such as an age over 75 years, diabetes mellitus, congestive heart failure, hypotension, anemia, chronic kidney disease and procedure-related characteristics like the use of elective intraaortic baloon pumps or increasing volumes of contrast media [24]. According to the number of present risk factors the total score can be ranked into four groups: low CI-AKI risk (≤ 5), moderate CI-AKI risk (6 - 10), high CI-AKI risk (10-15) and very high CI-AKI risk (≥ 16) [24].

Blood was collected before, 24h, and 48h after CM application, if possible. In 22 patients we were not able to obtain plasma after CM application for a reliable assessment of CI-AKI based on changes in plasma creatinine. Patients were followed for 120 days. Patient treatment was not influenced in any case by this study. All patients received tri-iodonated non-ionic low-osmolar iobitridol (XENETIX® 350, Guerbet GmbH, Sulzbach/Taunus, Germany) in a concentration of 350 mg iodine/mL as contrast medium. Patients at high risk for CI-AKI [2] were subjected to preventive measures, which included hydration before or after CM application, N-acetyl-L-cysteine (ACC), Ca-antagonist, and theophyllin treatment.

Sample treatment and measurement

After collection, EDTA-blood samples were centrifuged for 5 min at 3000 rpm to obtain plasma. Plasma samples were then frozen and stored at −80 °C. For the measurement of creatinine the Jaffé method was used. Glomerular filtration rate was estimated by the modification of diet in renal disease (MDRD) formula. Cystatin C was measured employing an immunonephelometric method using polystyrene particles coated with human cystatin C specific antibodies (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). For the measurement of kynurenine an ELISA assay was used (IDK® Kynurenine ELISA K 7728, Immundiagnostik AG, Bensheim, Germany). The lower detection limit of the assay is 0.12 µmol/L with a limit of quantification of 0.18 µmol/L. Intra-assay coefficients of variation range between 6.2 and 7.6%. Both patients and doctors were blinded to measured kynurenine levels.

Definition of endpoints

CI-AKI was defined as an increase of plasma creatinine by 25% or 0.5 mg/dL from baseline within 48 h. Death was described as the subsumption of death of all causes. The endpoint dialysis was defined as every dialysis in 120 days following CM exposure. The endpoint doubling of plasma creatinine refers to a 100% increase from baseline plasma creatinine levels. The primary composite endpoint of this study was the occurrence of major adverse kidney events (MAKE) until follow up to 120 days after CM administration. In the vast majority of cases (n=233) follow up was organized by interviewing the respective patient, in the remaining cases close relatives or the family physician was questioned. If available, medical reports were obtained. MAKE was defined as one of the following events: death, dialysis or a doubling of plasma creatininie until follow up, as previously described [9, 25].

Statistical analyses

Data were analyzed using SPSS version 20.0 (SPSS, Inc, Chicago, IL, USA). Figures were compiled with Graphpad Prism 5 (GraphPad Software Inc., La Jolla, California, USA) or SPSS version 20.0 (SPSS, Inc, Chicago, IL, USA). All values are given as mean ± standard deviation (SD). Depending on group-specific normal distribution either unpaired t-test or the Mann-Whitney U test was used to compare two groups. For the comparison of categorical variable distribution, the chi-squared test was used. To assess specificity and sensitivity, receiver operating characteristic (ROC) curves were calculated. Representing the best cutoff value, the point of the curve closest to the upper left corner of the coordinate system (sensitivity and specificity equal 1), was calculated and used to transform a continuous parameter into a binary endpoint [26]. To analyze group specific occurrence of events over time, Kaplan-Meier curves were calculated and tested for statistical significant differences using the Log-rank Mantel-Cox test. To adjust for possible confounding, multivariate Cox regression analysis was performed. Probability values <0.05 were considered significant. The authors had full access to the data and take full responsibility for their integrity.

Table 1. Descriptive statistics of the analyzed cohort (continuous data are given as mean ± SD)

Patient characteristics	(n=245)
Age, y	68.7 ± 9.7
Sex, female/male, %	21.6/78.4
Body mass index, kg/m²	28.9 ± 5.5
Ethnicity (Caucasian, Non-Caucasian), %	98.8/1.2
Former smoking, n, %	116/47.3
Current smoking, n,%	40/16.3
Baseline kynurenine, µmol/L	3.18 ± 1.03
Baseline creatinine, mg/dL	1.27 ± 0.48
Baseline cystatin c, mg/L	1.17 ± 0.49
Baseline GFR, ml/min/1.73 m²	64.02 ± 21.63
Diabetes mellitus, n,%	128/52.2
HbA1c, mmol/mol	50 ± 12.0
Renal insufficiency, n, %	220/89.9
Elective/emergency coronary angiography, n, %	232/94.7; 13/5.3
Congestive heart failure, n,%	67/27.3
Coronary artery disease, n,%	184/75.1
Peripheral artery disease, n, %	43/17.6
Arterial occlusive disease, n, %	188/76.7
Hypertension, n,%	218/89.0
Anemia, n,%	67/27.3
Statins, n, %	152/62.0
ARBs, n,%	81/33.1
ACE inhibitors, n, %	141/57.6
Diuretics, n, %	158/64.5
Renal scoring (4 groups low - high risk), %	50.2/29.8/16.3/3.7
Mean renal score, integer score	6.5
CM-volume, mL	115.3 ± 57.0
Hydration before/after CM, n, %	135/55.1
ACC-prophylaxis	118/48.2
Ca antagonist-prophylaxis, n, %	64/26.1
Theophyllin-prophylaxis, n, %	3/1.2
CI-AKI, n, %	19/8.5
Dialysis until follow up, n, %	12/4.9
Death until follow up, n, %	9/3.7
Doubling of creatinine until follow up, n, %	1/0.4
MAKE until follow up, n, %	17/6.9

Results

A total of 245 patients undergoing coronary angiography were statistically analyzed. Table 1 shows descriptive statistics of the cohort. The mean age of the cohort was 68.7 ± 9.7 years, 21.6% (53) of all patients were female, 78.4% (192) were male, 98.8% (242) had a caucasian ethnic background, 1.2% (3) were non-caucasian, 63.7% (156) were former and 16.3% (40) were current smokers. The average body mass index (BMI) was 28.9 ± 5.5 kg/m², mean HbA1c concentration was 50 ± 12 mmol/mol with 52.2% (128) of all patients being diabetics. Of all patients, 89.9% (220) suffered from renal insufficiency. Hypertension was present in 89.0% (218), 75.1% (184) of all patients suffered from coronary artery disease, 17.6% (43) displayed peripheral artery disease, 76.7 (188) presented with artery occlusive disease, 27.3% (67) had congestive heart disease and 27.3% (67) were anemic. The average renal risk score was

6.5, 50.2% (123) displayed a low, 29.8% (73) a moderate, 16.3 (40) a high and 3.7% (9) a very high risk for CI-AKI. Regarding patient medication, 62.0% (152) were treated with statins, 33.1% (81) received angiotensin II receptor blockers (ARBs), 57.6% (141) took angiotensin converting enzyme (ACE) inhibitors, and 64.5% (158) were treated with diuretics. In 94.7% (232) of patients elective coronary angiography was performed, 5.3% (13) required an emergency coronary angiography. Mean administered CM volume was 115.3 ± 57.0 mL. Regarding prophylactic measures during coronary angiography, 55.1% (135) received hydration before or after CM application, 48.2% (118) were treated with N-acetyl-L-cysteine (ACC), 26.1 (64) received Ca-antagonists, and 1.2% (3) received theophyllin. Baseline plasma kynurenine concentrations were 3.18 ± 1.03 µmol/L. The distribution of measured baseline plasma kynurenine concentrations is shown in Fig. 1. Baseline creatinine concentrations were 1.27 ± 0.48 mg/dL and baseline cystatin C concentrations were 1.17 ± 0.49 mg/L. The average GFR was 64.02 ± 21.63ml/min/1.73 m^2. Following CM application, 8.5% (19) developed CI-AKI. Over the course of the study, 4.9% (12) had to undergo dialysis, 3.7% (9) of patients died and 0.4% (1) displayed a doubling of plasma creatinine. Taken together, 6.9% (17) reached the combined endpoint MAKE.

Table 2. Baseline plasma kynurenine (KYN) concentrations (µmol/l) according to clinical events, *) Elevation of plasma creatinine by 25% or 0,5mg/dl from baseline in 48h; †) Death until follow up; ‡) Dialysis until follow up; §) Doubling of plasma creatinine until follow up; ||) Combined endpoint "major adverse kidney events" (MAKE): death or dialysis or doubling of plasma creatinine until follow up

Event		Baseline KYN according to events				
		n	%	mean	SD	p
CI-AKI *)	No	204	91.5	3.18	1.03	0.909
	Yes	19	8.5	3.15	1.08	
Death †)	Alive	236	96.3	3.135	0.990	0.002
	Dead	9	3.7	4.208	1.466	
Dialysis ‡)	No	233	95.1	3.097	0.955	<0.0001
	Yes	12	4.9	4.816	1.118	
Plasma Crea*2 §)	No	234	99.6	3.114	0.951	-
	Yes	1	0.4	6.050	-	
MAKE ||)	No	228	93.1	3.083	0.951	<0.0001
	Yes	17	6.9	4.489	1.190	

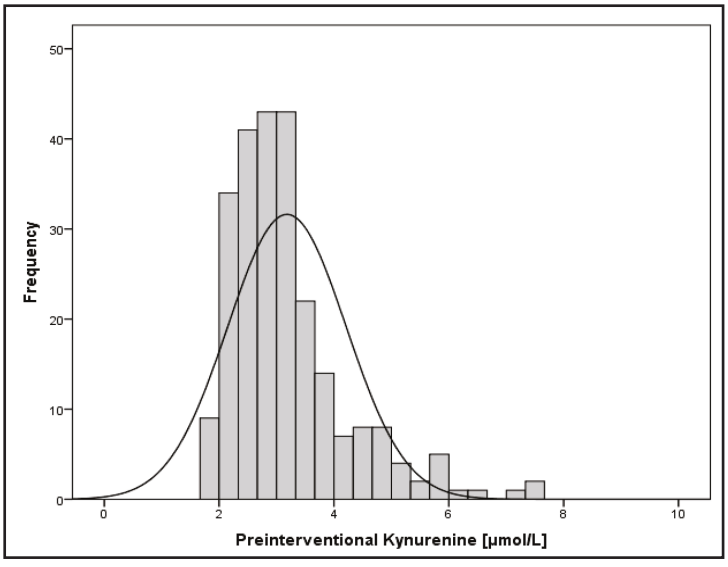

Fig. 1. Distribution of measured baseline kynurenine concentrations

Table 2 shows preinterventional plasma kynurenine concentrations in patient subgroups with or without the occurrence of clinical events. CI-AKI and doubling of plasma creatinine until follow up was not associated with any changes in plasma kynurenine concentrations. However, patients who died, had to receive dialysis, or developed MAKE until follow up, displayed significantly elevated plasma kynurenine levels.

The predictive value of preinterventional kynurenine in regards to the primary endpoint of this study, MAKE, was calculated by the area under the receiver operating characteristic curve (ROC-AUC), which is shown in Fig. 2. Preinterventional kynurenine was very accurate in predicting MAKE (AUC=0.838; 95% C.I.= 0.738-0.939; p=3.3 x 10^{-6}). The opti-

mal cutoff value for kynurenine was ≥ 3.5 μmol/L. Table 3 shows descriptive statistics of the cohort grouped into patients with plasma kynurenine concentrations < 3.5 μmol/L and ≥ 3.5 μmol/L. Significant differences in patients with plasma kynurenine concentrations ≥ 3.5 μmol/L were a higher age (p=0.019), higher baseline concentrations of creatinine and cystatin C (both p<0.0001), a lower GFR (p<0.0001), a higher prevalence for preexisting renal insufficiency (p=0.036), congestive heart failure (p=0.001), and anemia (p=0.003). Patients with higher preinterventional plasma kynurenine were more often taking diuretics (p=0.006), displayed a higher renal risk score (p<0.0001) and an according higher prevalence for high and very high CI-AKI risk (p<0.0001). Correspondingly, higher preinterventional kynurenine was associated with a higher prevalence for hydration before or after CM application (p=0.0001), and more ACC prophylaxis (p<0.0001). Furthermore preinterventional plasma kynurenine concentrations ≥ 3.5 μmol/L were associated with a higher prevalence for dialysis (p<0.0001), death (p=0.004) and the combined endpoint MAKE (p<0.0001).

Fig. 2. ROC curve of baseline plasma kynurenine (KYN) concentrations as test variable and MAKE until follow up as state variable.

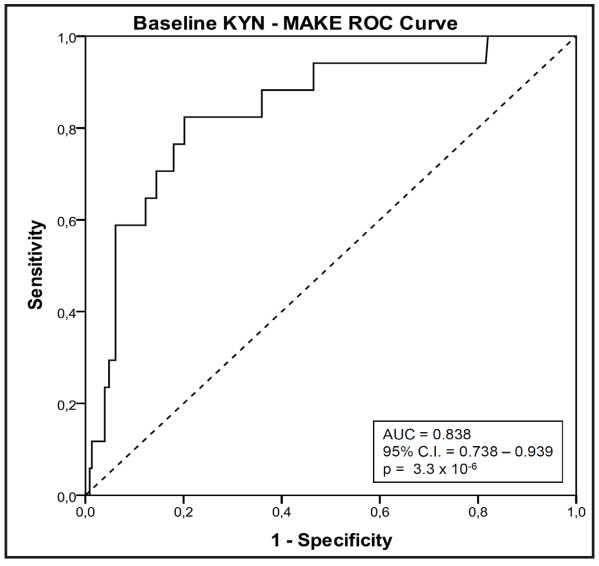

Table 4 displays patient characteristics according to the occurrence of MAKE until follow up. Patients with MAKE until follow up had significantly higher baseline plasma kynurenine, cystatin C and creatinine concentrations (all p<0.0001) and a lower baseline GFR (p<0.0001). Patients who experienced MAKE also had a higher prevalence for congestive heart failure (p=0.003) and anemia (p=0.003) and more often were smokers (p=0.028). MAKE was associated with a higher renal risk score (p=0.0002) and a higher prevalence for high and very high CI-AKI risk (p=0.0005). Furthermore, patients who experienced MAKE more often underwent an emergency coronary angiography (p=0.019).

Employing the Kaplan-Meier estimator, differences in the occurrence of MAKE until the follow up according to plasma kynurenine < 3.5 μmol/L and ≥ 3.5 μmol/L were analyzed. Plasma levels of kynurenine ≥ 3.5 μmol/L were significantly associated with a higher prevalence of MAKE until follow up (Log-rank Mantel-Cox test; chi-squared=31.59; $p=1.9 \times 10^{-8}$; see Fig. 3). Multivariate Cox-regression models using established risk factors for MAKE [27], that were significantly different in the observed cohort stratified into no MAKE or MAKE (Table 3), were calculated to demonstrate an independent association between plasma kynurenine ≥ 3.5 μmol/L and an elevated risk for MAKE (Table 5). Model A used baseline GFR, congestive heart failure, anemia, age, contrast media volume, elective or emergency coronary angiography, and current smoking status as covariates (chi-squared=33.250, p=0.00002) . Adding plasma kynurenine as categorical variable (KYN < 3.5 μmol/L or ≥ 3.5 μmol/L) in model B to the same covariates as used in model A improved the overall strength of the model (chi-squared=48.679, $p=7.33 \times 10^{-8}$) and rendered the association between GFR and MAKE insignificant. In model C plasma kynurenine was applied to the same covariates as used in model A as a continuous variable, resulting in a similar model strength as observed in Model B. (chi-squared=47.171, $p=1.42 \times 10^{-7}$). As there is considerate evidence in literature that CI-AKI also impacts long-term outcome [6-8], model D was furthermore adjusted for the occurrence of CI-AKI. An addition of CI-AKI to the covariates only mildly affected the independent association between plasma kynurenine and MAKE.

Table 3. Descriptive statistics of patients grouped according to baseline kynurenine (KYN) concentrations < 3.5 µmol/L and ≥ 3.5 µmol/L (continuous data are given as mean ± SD)

Patient characteristics	KYN < 3.5 µmol/L (n=183)	KYN ≥ 3.5 µmol/L (n=62)	p-value
Age, y	67.7 ± 9.7	71.5± 9.4	0.019
Sex, female/male, %	20.2/79.8	25.8/74.2	0.356
Body mass index, kg/m²	28.8 ± 5.4	29.3 ± 5.8	0.568
Ethnicity (Caucasian, Non-Caucasian), %	98.9/1.1	98.4/1.6	0.748
Former smoking, n, %	84/45.9	32/51.6	0.436
Current smoking, n,%	32/17.5	8/12.9	0.399
Baseline kynurenine, µmol/L	2.71 ± 0.44	4.58 ± 0.10	<0.0001
Baseline creatinine, mg/dL	1.13 ± 0.27	1.67 ± 0.70	<0.0001
Baseline cystatin c, mg/L	1.01 ± 0.33	1.64 ± 0.57	<0.0001
Baseline GFR, ml/min/1.73 m²	69.77 ± 20.04	47.05 ± 16.75	<0.0001
Diabetes mellitus, n,%	102/55.7	26/41.9	0.060
HbA1c, mmol/mol	51 ± 13.1	46 ± 9.8	0.104
Renal insufficiency, n, %	160/87.4	60/96.8	0.036
Elective/emergency coronary angiography, %	95.6/4.4	91.9/8.1	0.262
Congestive heart failure, n,%	40/21.9	27/43.5	0.001
Coronary artery disease, n,%	140/76.5	44/71.0	0.384
Peripheral artery disease, n, %	29/15.8	14/22.6	0.228
Arterial occlusive disease, n, %	142/77.6	46/74.2	0.584
Hypertension, n,%	159/86.9	59/95.2	0.072
Anemia, n,%	41/22.4	26/41.9	0.003
Statins, n, %	115/62.8	37/59.7	0.657
ARBs, n,%	59/32.2	22/35.5	0.639
ACE inhibitors, n, %	102/55.7	39/62.9	0.324
Diuretics, n, %	109/59.6	49/79.0	0.006
CM-volume, mL	114.7	116.8	0.760
Renal scoring (4 groups low - high risk), %	58/31/9/3	27/27/39/7	<0.0001
Mean renal score, integer score	5.6 ± 4.3	9.1 ± 4.8	<0.0001
Hydration before/after CM, n, %	88/48.1	47/75.8	0.0001
ACC-prophylaxis, n, %	74/40.4	44/71.0	<0.0001
Ca antagonist-prophylaxis, n, %	47/25.7	17/27.4	0.788
Theophyllin-prophylaxis, n, %	1/0.5	2/3.2	0.099
CI-AKI, n, %	14/8.4	5/8.8	0.937
Dialysis until follow up, n, %	1/0.5	11/17.7	<0.0001
Death until follow up, n, %	3/1.6	6/9.7	0.004
Doubling of creatinine until follow up, n, %	0/0	1/1.9	0.067
MAKE until follow up, n, %	3/1.6	14/22.6	<0.0001

Discussion

To the best of our knowledge, this is the first study that investigated kynurenine in the context of renal outcomes after CM application due to coronary angiography. The patient cohort analyzed in this study can be considered representative, featuring similar clinical characteristics and risk factor frequencies as observed in larger studies [5, 24]. Patients who developed MAKE up to 120 days after CM administration displayed significantly elevated preinterventional plasma kynurenine levels. ROC analysis demonstrated that preinterventional plasma kynurenine levels are highly predictive of MAKE, with an optimal cutoff of ≥3.5 µmol/L. Employing the Kaplan-Meier estimator, revealed that patients with plasma kynurenine ≥3.5 µmol/L have a strongly increased risk of MAKE (death, need for dialysis, doubling of plasma creatinine). Multivariate Cox regression models demonstrated

Table 4. Descriptive statistics of patients grouped according to the occurrence of MAKE until follow up (continuous data are given as mean ± SD)

Patient characteristics	No MAKE (n=228)	MAKE (n=17)	p-value
Age, y	68.6 ± 9.6	69.3 ± 11.9	0.969
Sex, female/male, %	78.5/21.5	76.5/23.5	0.844
Body mass index, kg/m²	29.0 ± 5.5	27.4 ± 5.1	0.246
Ethnicity (Caucasian, Non-Caucasian), %	99.1/0.9	94.1/5.9	0.070
Former smoking, n, %	114/50.0	2/11.8	0.002
Current smoking, n,%	34/14.9	6/35.3	0.028
Baseline kynurenine, µmol/L	3.08 ± 0.95	4.49 ± 1.19	<0.0001
Baseline creatinine, mg/dL	1.20 ± 0.35	2.09 ± 1.03	<0.0001
Baseline cystatin c, mg/L	1.12 ± 0.42	1.88 ± 0.76	<0.0001
Baseline GFR, ml/min/1.73 m²	65.57 ± 20.54	43.29 ± 25.59	<0.0001
Diabetes mellitus, n,%	121/53.1	7/41.2	0.344
HbA1c, mmol/mol	50 ± 12.0	44 ± 8.7	0.304
Renal insufficiency, n, %	205/89.9	15/88.2	0.826
Elective/emergency coronary angiography, %	95.6/4.4	82.4/17.6	0.019
Congestive heart failure, n,%	57/25.0	10/58.8	0.003
Coronary artery disease, n,%	173/75.9	11/64.7	0.304
Peripheral artery disease, n, %	41/18.0	2/11.8	0.516
Arterial occlusive disease, n, %	177/77.6	11/64.7	0.224
Hypertension, n,%	201/88.2	17/100.0	0.133
Anemia, n,%	57/25.0	10/58.8	0.003
Statins, n, %	141/61.8	11/64.7	0.814
ARBs, n,%	74/32.5	7/41.2	0.461
ACE inhibitors, n, %	130/57.0	11/64.7	0.536
Diuretics, n, %	144/63.2	14/82.4	0.111
CM-volume, mL	115.7 ± 55.7	108.9 ± 73.6	0.309
Renal Scoring (4 groups low - high risk), %	53/29/15/3	12/41/29/18	0.0005
Mean renal score, integer score	6.2 ± 4.5	10.9 ± 5.2	0.0002
Hydration before/after CM, n, %	125/54.8	10/58.8	0.749
ACC-prophylaxis, n, %	108/47.4	10/58.8	0.362
Ca antagonist-prophylaxis, n, %	59/25.9	5/29.4	0.749
Theophyllin-prophylaxis, n, %	3/1.3	0/0	0.633
CI-AKI, n, %	16/7.7	3/21.4	0.074

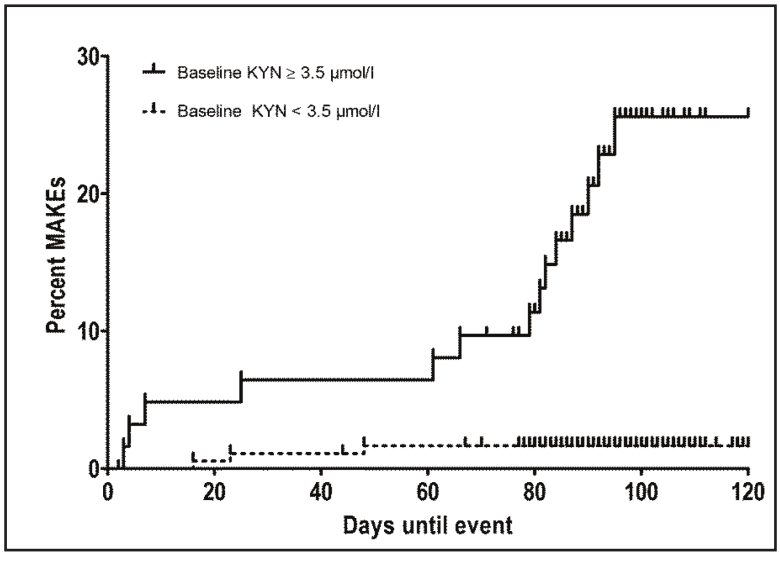

Fig. 3. Kaplan-Meier analysis of MAKE until follow up in patients with baseline kynurenine (KYN) concentrations < 3.5 µmol/L and ≥ 3.5 µmol/L. Log rank (Mantel Cox) test, chi-squared = 31.59; p<0.0001.

Table 5. Multivariate Cox regression analyzing the association between preintervetional serum kynurenine and MAKE, The dependent variable is MAKE up to 120 days after CM intervention. Model A: baseline GFR, congestive heart failure, anemia, age, contrast media volume, elective or emergency coronary angiography (CA), and current smoking status. Model B: same covariates as model A plus plasma kynurenine as categorical variable (KYN < 3.5 µmol/L or ≥ 3.5 µmol/L). Model C: same covariates as model A plus plasma kynurenine as continuous variable. Model D: same covariates as model C with additionally adding the occurrence of CI-AKI to the covariates

Model A	chi-squared=33.250 p=0.00002				95% C.I. for EXP(B)	
	B	S.E.	Sig.	Exp(B)	Lower	Upper
Baseline GFR, ml/min/1.73 m²	-0.050	0.016	0.002	0.951	0.921	0.982
Congestive heart failure (no, yes)	0.944	0.526	0.072	2.571	0.918	7.203
Anemia (no, yes)	0.605	0.515	0.240	1.832	0.667	5.030
Age, years	-0.016	0.028	0.569	0.984	0.931	1.040
Contrast media volume (mL)	-0.002	0.004	0.583	0.998	0.990	1.006
Elective/emergency CA (no, yes)	-0.207	0.825	0.802	0.813	0.162	4.094
Current smoking (no, yes)	1.241	0.617	0.044	3.460	1.032	11.600
Model B	chi-squared=48.679 p=7.33 x 10^{-8}				95% C.I.for EXP(B)	
	B	S.E.	Sig.	Exp(B)	Lower	Upper
Baseline KYN, < 3.5 or ≥ 3.5µmol/L	1.842	0.635	0.004	6.306	1.818	21.875
Baseline GFR, ml/min/1.73 m²	-0.029	0.016	0.077	0.971	0.940	1.003
Congestive heart failure (no, yes)	0.744	0.534	0.163	2.105	0.739	5.992
Anemia (no, yes)	0.558	0.523	0.285	1.748	0.628	4.867
Age, years	-0.016	0.030	0.580	0.984	0.928	1.043
Contrast media volume (mL)	-0.002	0.004	0.604	0.998	0.990	1.006
Elective/emergency CA (no, yes)	-0.139	0.852	0.870	0.870	0.164	4.624
Current smoking (no, yes)	1.320	0.615	0.032	3.745	1.121	12.512
Model C	chi-squared=47.171 p=1.42 x 10^{-7}				95% C.I.for EXP(B)	
	B	S.E.	Sig.	Exp(B)	Lower	Upper
Baseline KYN, µmol/L	0.556	0.196	0.004	1.744	1.189	2.559
Baseline GFR, ml/min/1.73 m²	-0.038	0.016	0.017	0.963	0.933	0.993
Congestive heart failure (no, yes)	0.644	0.546	0.238	1.904	0.653	5.545
Anemia (no, yes)	0.422	0.529	0.426	1.524	0.540	4.301
Age, years	-0.006	0.029	0.841	0.994	0.939	1.053
Contrast media volume (mL)	-0.003	0.004	0.515	0.997	0.989	1.005
Elective/emergency CA (no, yes)	-0.232	0.769	0.763	0.793	0.176	3.581
Current smoking (no, yes)	1.732	0.655	0.008	5.650	1.566	20.378
Model D	chi-squared=51.748 p=5.04 x 10^{-8}				95% C.I.for EXP(B)	
	B	S.E.	Sig.	Exp(B)	Lower	Upper
Baseline KYN, µmol/L	0.594	0.233	0.011	1.811	1.147	2.860
Baseline GFR, ml/min/1.73 m²	-0.044	0.018	0.014	0.957	0.924	0.991
Congestive heart failure (no, yes)	-0.351	0.726	0.629	0.704	0.170	2.920
Anemia (no, yes)	0.844	0.657	0.199	2.325	0.642	8.426
Age, years	-0.029	0.033	0.372	0.971	0.911	1.035
Contrast media volume (mL)	-0.003	0.006	0.575	0.997	0.986	1.008
Elective/emergency CA (no, yes)	0.668	0.950	0.482	1.951	0.303	12.554
Current smoking (no, yes)	1.630	0.797	0.041	5.103	1.071	24.316
CI-AKI (no, yes)	1.375	0.813	0.091	3.957	0.804	19.459

that this association is independent of other important risk factors. Taken together, results of this study show an independent association between baseline plasma kynurenine levels and the occurrence of MAKE up to 120 days after contrast media exposure due to coronary angiography in a population with moderate risk for CI-AKI.

Given the multifaceted contribution of the kynurenine pathway in fundamental biological processes, impacting on inflammation, oxidative stress, and endothelial function,

current literature suggests an involvement of kynurenine in both acute and chronic kidney disease [18, 28]. Previous studies have demonstrated that metabolites of the kynurenine pathway are elevated in CKD animal models as well as in CKD patients [21, 29], and kynurenine metabolites were shown to increase parallely to decreasing renal function [29, 30]. Moreover, studies show that plasma kynurenine is positively associated with plasma markers of chronic inflammation in CKD patients [19, 20]. Results of this study showed that patients with baseline plasma kynurenine levels ≥ 3.5 µmol/L also had a significantly reduced GFR and elevated levels of plasma creatinine and cystatin C, underlining that these patients suffered from a preexisting impaired renal function. However, plasma kynurenine was still significantly associated with MAKE after adjusting for glomerular filtration rate, age, congestive heart failure, anemia, contrast media volume, and CI-AKI — all risk factors evinced in previous larger studies [24]. Also, elective or emergency coronary angiography and smoking — both factors that were significantly increased in MAKE patients — did not strongly affect the independent association between elevated plasma kynurenine and MAKE.

An increased activity of tryptophan-metabolizing enzymes due to inflammatory stimuli is currently accepted as the main mechanism behind elevated kynurenine levels in CKD [12, 18, 20, 31]. Additionally an attenuated renal excretion of kynurenine is suggested by the literature [18, 21]. Until now it is not clear if elevated kynurenine levels are causally involved in the pathogenesis of kidney disease. Interestingly, kynurenine formamidase knock out animals display a significant increase of metabolites of the kynurenine pathway, concomitant to a gradual deterioration of kidney function [22]. Furthermore, it was demonstrated in mesangial cell culture experiments that kynurenine stimulates profibrotic gene expression [23]. The authors concluded that kynurenine metabolites might be associated with the progression of glomerular fibrosis and renal failure [23]. Such observations indicate a possible pathological role of accumulated kynurenine metabolites in the progression of renal failure and might also serve as one possible explanation for the association between elevated plasma kynurenine and an increased risk of MAKE in this study [23]. This notion is to some extent supported by results of a large general population-based longitudinal study performed over the duration of 7 years. It was shown that the baseline kynurenine-to-tryptophane ratio is associated with kidney function decline and CKD incidence years later. However, differently from the current study, no significant association was observed when assessing plasma kynurenine levels solely. Yet, this might have been related to the relatively healthy study population and the different study setting. Alterations of either substrate or metabolite alone could have been too minor to observe an effect, but the impact of this factor was not strengthened by calculating a ratio out of the two oppositely changing components [31].

Results of this study show that, regardless of the underlying mechanism, kynurenine might serve as a preinterventional highly predictive biomarker for MAKE up to 120 days after coronary angiography. However, baseline kynurenine was not associated with CI-AKI. Additionally, there was no significant association between CI-AKI and MAKE, which has already been observed by other studies [10]. These missing associations might be explained by the same underlying reasons. The frequency of CI-AKI in the current study was lower than in other studies, presumably due to under-diagnosis [5]. Blood samples were obtained up to 48h after CM application, but CI-AKI might still have developed after this time threshold [5]. Moreover, diagnostic classifications like ESUR define CI-AKI as an impairment of glomerular function, usually measured by the current gold standard surrogate parameter, plasma creatinine [32, 33]. It is well accepted that the diagnostic properties of plasma creatinine assessment has limitations, as it displays a delayed rise in response to an acute renal failure triggering insult and also lacks specificity due to extrarenal influences on plasma concentrations [5, 9]. Furthermore, current definitions neglect a hallmark of acute renal failure and in particular CI-AKI, which is an impairment of tubular function due to morphological tubular alterations such as tubular necrosis and tubular dilatation [32-36]. There is also emerging observational evidence that subclinical cases (i.e. positive for a novel

biomarker/negative for creatinine) might be at an increased risk for short- and long-term morbidity and mortality [11]. The unexpected missing association between CI-AKI and MAKE, observed in the current study, supports these data. In this context, recent reviews of clinical studies investigating acute renal impairment proposed that employing a renal composite endpoint like MAKE could be used to better define patients with a meaningful poor outcome [37].

Findings of the current study might have relevant clinical implications. Calculating a threshold for kynurenine using the kynurenine ROC curve for MAKE resulted in a cut-off concentration for kynurenine of ≥ 3.5 µmol/L to identify patients at risk for MAKE after coronary angiography. This might be clinically very important information for patient's management, especially as kynurenine can be assessed prior to a CM enhanced procedure. Patients at high risk for MAKE based on kynurenine concentrations before intervention may need a much closer follow-up as compared to patients with low MAKE risk. Preinterventional kynurenine could also be used in decision making in high risk populations with uncertain beneficial effects of a percutaneous intervention, e.g. diabetic patients with multivessel coronary artery disease [38]. Thus, kynurenine could be a tool for personalized patient care management before and after coronary angiography. Future studies should investigate whether or not pharmacological alterations of kynurenine concentrations prior to coronary angiography may alter the MAKE risk, in other words, whether or not kynurenine is just a risk biomarker or a risk factor for MAKE.

Study limitations

The main limitation of the current study is the lack of an independent replication cohort. Thus, findings of the current study have to be replicated in future studies. Another limitation of the current study is that plasma and urine samples were only obtained only until 48h after CM application. As current guidelines define CI-AKI as an increase in plasma creatinine by at least 25 % within 3 days after CM administration, CI-AKI might have been under-diagnosed. Furthermore, the kynurenine pathway could have been analyzed more in-depth, putatively drawing a more detailed picture of the involvement of kynurenine and other tryptophan metabolites in CI-AKI and MAKE.

Conclusion

In patients with moderately impaired renal function, high preinterventional plasma kynurenine levels were associated with a worse medium-term outcome after contrast media enhanced coronary angiography. Importantly, this association was independent of relevant risk factors of medium- and long-term renal morbidity and mortality including estimates of glomerular function. Further investigations are needed to validate the additional benefit of assessing preinterventional plasma kynurenine in predicting medium- and long-term renal outcome after contrast media enhanced procedures. Current evidence suggests that the concomitant measurement of several different renal biomarkers might help stratifying patients into high and low risk groups after CM enhanced interventions.

Abbreviations

CM - contrast media; CI-AKI - contrast induced acute kidney injury; ESUR - European Society of Urogenital Radiology; KYN - kynurenine; MAKE - major adverse kidney events

Disclosure Statement

All authors declared no competing interests.

Acknowledgements

KK is the CEO of Neuroimmun AG, the manufacturer of the kynurenin ELISA Kit used to measure serum kynurenin in the current study (IDK® Kynurenine ELISA K 7728). AK is a research employee of Bayer AG. No industry research grant was received for this project.

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