Research Article

Plasma Kynurenine Predicts Severity and Complications of Heart Failure and Associates with Established Biochemical and Clinical Markers of Disease

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Keywords

Heart failure · Biomarker · Mortality · Exercise · Tryptophan

Abstract

Background: Kynurenine, a metabolite of the L-tryptophan pathway, plays a pivotal role in neuro-inflammation, cancer immunology, and cardiovascular inflammation, and has been shown to predict cardiovascular events. **Objectives:** It was our objective to increase the body of data regarding the value of kynurenine as a biomarker in chronic heart failure (CHF). **Methods:** We investigated the predictive value of plasma kynurenine in a CHF cohort (CHF, $n = 114$); in a second cohort of defibrillator carriers with CHF (AICD, $n = 156$), we determined clinical and biochemical determinants of the marker which was measured by enzyme immunoassay. **Results:** In the CHF cohort, both kynurenine and NT-proBNP increased with NYHA class. Univariate binary logistic regression showed kynurenine to predict death within a 6-month follow-up (OR 1.43, 95% CI 1.03–2.00, $p = 0.033$) whereas NT-proBNP did not contribute significantly. Kynurenine, like NT-proBNP, was able to discriminate at a 30% threshold of left ventricular ejection fraction (LVEF; AUC-ROC, both 0.74). Kynurenine correlated inversely with LVEF ($\rho = -0.394$), glomerular filtration fraction (GFR; $\rho = -0.615$), and peak VO_2 ($\rho = -0.626$). Moreover, there was a strong correlation of kynurenine with NT-proBNP ($\rho = 0.615$). In the AICD cohort, multiple linear regression analysis demonstrated highly significant associations of kynurenine with GFR, hsCRP, and tryptophan, as well as a significant impact

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of age. **Conclusions:** This work speaks in favor of kynurenine being a new and valuable biomarker of CHF, with particular attention placed on its ability to predict mortality and reflect exercise capacity.

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Introduction

Kynurenine (Kyn) is a metabolite of L-tryptophan produced by the enzymes indoleamine 2,3-dioxygenase (IDO-1 and -2) [1] and tryptophan 2,3-dioxygenase [2]. The key enzyme, IDO-1, is prevalently activated by lipopolysaccharide, tumor necrosis factor- α , interleukin-1, and interferon- γ [1, 3, 4]. Apart from exerting effects on neuro-regulation [5] and cancer immunology [6], Kyn and its metabolites have a role in vascular inflammation [7], endothelium-dependent vasodilation [8], and oxidative stress [9]. Kyn or the Kyn/tryptophan ratio have recently been suggested to predict cardiovascular prognosis, particularly in coronary artery disease (CAD) [10, 11] and after cardiac arrest [12].

Data on chronic heart failure (CHF) and Kyn, however, are scarce. We have recently co-authored a report demonstrating plasma Kyn to be significantly elevated in patients with stable CHF, enrolled in the SICA-HF study, as compared to control individuals [13]. That report also showed inverse associations of circulating Kyn with measures of functional capacity, such as handgrip strength, peak oxygen consumption, and 6-min walk distance.

In the present work, we aimed at gathering more details on Kyn's performance as a CHF biomarker. In our first cohort of CHF patients, we investigated Kyn's ability to predict death in CHF as well as its association with parameters of CHF severity, and we compared its performance with that of the established CHF biomarker, NT-proBNP. In a second CHF cohort of AICD carriers, we evaluated several clinical and biochemical parameters as determinants of plasma Kyn using multiple linear regression analysis. Overall, the findings corroborate the upcoming notion of Kyn predicting CHF severity and complications as well as reflecting exercise capacity. Kyn furthermore associates with markers of inflammation and renal function.

Materials and Methods

Patients

All patients in the cohorts mentioned below provided informed written consent to have data from their medical records used in research. All data were fully anonymized before being accessed by researchers.

CHF Cohort

This study was approved by the Ethics Committee of the Justus Liebig University of Giessen (Kerckhoff Clinic Bad Nauheim; BioData Bank 144/11) and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The study enrolled 114 patients (mean age 71 ± 12 years, 72% male) with varying degrees of CHF, either heart failure with preserved or with reduced ejection fraction: NYHA I, 6%; NYHA II, 60%; NYHA III, 31%, and NYHA IV, 3%. Peripheral venous blood was drawn at baseline for the determination of the different biomarkers (see below), and routine laboratory and clinical parameters were recorded.

Owing to logistical limitations, time-to-event data were not available in this cohort. The patients were followed up for 6 months.

Table 1. Clinical and biochemical characteristics in both study cohorts

	CHF cohort (n = 114)	AICD cohort (n = 156)
Deaths	16 (14)	nd
Female sex	32 (28)	27 (17)
Age, years	71±12	69±11
NYHA I	6 (6)	–
NYHA II	60 (60)	–
NYHA III	31 (31)	–
NYHA IV	3 (3)	–
T2D	18 (16)	42 (27)
CAD	50 (44)	111 (71)
LVEF (%)	38±15	36±12
LVEDD, mm	59±9	58±9
GFR (MDRD), mL/min/1.73m ²	66±22	59±19
NT-proBNP, pg/mL	900 (313–2756)	nd
Kyn, µmol/L	2.7 (2.1–3.5)	3.4 (2.7–4.3)
Tryptophan, µmol/L	64±8	70±17
hsCRP, mg/L	nd	2.4 (0.9–5.3)
CML-calprotectin, ng/mL	nd	116 (18–362)
Carbonylated proteins, U/mL	nd	173 (119–228)
Zonulin, ng/mL	16.5 (13.1–22.4)	10.2 (8.6–11.9)
Plasma sodium, mmol/L	140±4	nd

Continuous variables are given as the median (IQR) if not normally distributed or as the mean ± SD, categorical variables are presented as n (%). NYHA, symptomatic severity of heart failure according to the New York Heart Association; T2D, type-2 diabetes; CAD, coronary artery disease; LVEF, left ventricular ejection fraction (echo); LVEDD, left ventricular end-diastolic diameter (echo); Kyn, kynurenine; hsCRP, high-sensitivity C-reactive protein; CML-calprotectin, carboxy-methyl-lysine calprotectin; nd, not determined.

AICD Cohort

This study was approved by the Ethics Committee of the Charité University Medicine Berlin (approval No. EA1/004/13). The study enrolled 156 patients (mean age 68 ± 11 years, 17% female) from an out-patient department carrying AICD for primary or secondary prevention of sudden cardiac death. All of them suffered from CHF (33 [20%] from heart failure with preserved ejection fraction), 71% from CAD, and 27% from type-2 diabetes. These patients routinely presented for device control every 6 months. Peripheral venous blood was drawn for the determination of several laboratory parameters (see below), and the routine laboratory and clinical parameters were recorded. In this cohort, neither current NT-proBNP levels nor NYHA classification were available owing to logistic limitations. The following clinical and routine biochemical parameters were available: creatinine, severity of CAD (1-, 2-, or 3-vessel disease), and left ventricular ejection fraction (LVEF).

Determination of Kyn and Other Parameters

EDTA plasma was stored at –70 °C for subsequent determinations. Plasma Kyn was measured using a competitive enzyme immunoassay (No. K7728, Immundiagnostik AG, Bensheim, Germany). Nitrotyrosine, zonulin, high-sensitivity C-reactive protein (hsCRP), carbonylated proteins, myeloperoxidase, tryptophan, carboxy-methyl-lysine calprotectin,

Fig. 1. NT-proBNP and Kyn in the CHF cohort. Circulating NT-proBNP (upper plot) and Kyn (lower plot) in dependence of NYHA functional class, grouped into NYHA I–II versus NYHA III–IV (NYHA I, $n = 7$; NYHA II, $n = 69$; NYHA III, $n = 35$; NYHA IV, $n = 3$). Group comparison was done using the Mann-Whitney U test. Data are shown as box plots: boxes indicate the interquartile range, the inner line being the median; whiskers indicate 1.5-fold of the interquartile range; values above 1.5 times (outlier) and 3 times the interquartile range (extreme values) are depicted as circles and stars, respectively.

and oxidized LDL were determined using test kits from Immundiagnostik according to the manufacturer's instructions. The NT-proBNP ELISA was from Roche.

Statistical Analysis

Data are given as the median (interquartile range) unless stated otherwise. First, normality of distribution was tested using the Kolmogorov-Smirnov test. In the CHF cohort, discriminatory power was assessed using ROC analysis; combined ROC curves were produced by multivariate binary logistic regression (with stepwise backward regression). The DeLong test was used to compare individual ROC curves. To obtain a final model for death prediction, we included the significant univariate candidates into a multivariate binary logistic regression analysis.

In the AICD cohort, laboratory and clinical parameters showing a significant univariate correlation with Kyn according to Spearman's rho (ρ) were selected. Those parameters were then included in a multiple linear regression analysis (generalized linear model). Categorical

Fig. 2. **A** NT-proBNP and Kyn as death predictors in the CHF cohort. Circulating NT-proBNP (left) and Kyn (right) in survivors ($n = 98$) versus non-survivors ($n = 16$). Data are shown as box plots: boxes indicate the interquartile range, the inner line being the median; whiskers indicate 1.5-fold of the interquartile range; values above 1.5 times (outlier) and 3 times the interquartile range (extreme values) are depicted as circles and stars, respectively. **B** ROC analysis for death in the CHF cohort. ROC curves for death prediction by Kyn (solid line, AUC 0.70) versus NT-proBNP (dotted line, AUC 0.63). **C** ROC analysis for low LVEF in the CHF cohort. ROC curves for prediction of LVEF $<30\%$ by Kyn (blue line), NT-proBNP (green line), and a combination thereof (yellow line). The combined ROC curve was constructed by multivariate binary logistic regression: Kyn, OR 1.57 (95% CI 1.05–2.35); NT-proBNP, OR 1.029 (95% CI 1.003–1.055).

variables were included in the model if the Mann-Whitney U test indicated significant differences of Kyn between categories.

In general, differences between groups were analyzed using the non-parametric Mann-Whitney U test. $p < 0.05$ was regarded as significant and $p < 0.005$ as highly significant. All analyses were carried out with IBM SPSS Statistics 23.

Table 2. Death prediction in the CHF cohort – logistic regression analysis

	Coefficient B	OR	95% CI	p value
<i>Univariate analysis</i>				
Kyn	0.36	1.43	1.03–2.00	0.033
Height	–0.08	0.93	0.87–0.99	0.026
LVEF	–0.05	0.95	0.90–0.997	0.037
LVEDD	0.06	1.07	1.001–1.14	0.047
Plasma sodium	–0.22	0.80	0.69–0.93	0.005
CAD	1.22	3.38	1.09–10.5	0.035
NT-proBNP	0.001	1.001	0.99–1.01	0.90
<i>Multivariate analysis – model 1</i>				
Kyn	0.40	1.49	1.05–2.10	0.025
Height	–0.08	0.92	0.86–0.99	0.021
<i>Multivariate analysis – model 2</i>				
Kyn	0.32	1.37	0.95–2.00	0.09
Height	–0.09	0.91	0.85–0.98	0.015
CAD	1.20	3.30	0.96–11.3	0.06
<i>Multivariate analysis – final model 3</i>				
Height	–0.08	0.93	0.86–0.99	0.048
LVEDD	0.08	1.08	1.004–1.16	0.039
Sodium	–0.21	0.81	0.69–0.95	0.011

The following parameters were also tested in univariate analysis, but found to be non-significant: age, sex, weight, body mass index, heart rate at rest and during peak exercise, systolic and diastolic blood pressure, right ventricular end-diastolic diameter, left ventricular end-systolic diameter, history of myocardial infarction, NYHA class; presence of: sinus rhythm, pacemaker or defibrillator, diabetes, hypertension, atrial fibrillation, hyperuricemia; plasma/serum parameters: creatinine, urea, hemoglobin, potassium, C-reactive protein, nitrotyrosine, zonulin, tryptophan, oxidized LDL, glomerular filtration rate (MDRD formula and cystatin C-based formula). For multivariate analyses, the following models were constructed: Model 1: kyn, height. Model 2: kyn, height, CAD. Model 3: kyn, height, CAD, LVEF, LVEDD, sodium; for this model, only the final significant contributors after backward regression (Wald procedure) are shown. CI, confidence interval; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; Kyn, kynurenine. Odds ratios are given per unit increase for continuous variables.

Results

Table 1 summarizes the clinical and biochemical characteristics of the patients in the CHF and AICD cohorts.

CHF Cohort

Baseline Kyn Increases with NYHA Class and Is Predictive of Death

Figure 1 shows the significant increase of both NT-proBNP and Kyn with NYHA functional class. As there were only 3 patients in NYHA IV, we decided to group patients into NYHA I–II versus NYHA III–IV. Moreover, both Kyn and NT-proBNP were significantly higher in non-survivors as compared with survivors (Fig. 2A).

Table 2 shows the results of the logistic regression analysis with death as the dependent variable. We identified the following significant univariate predictors: plasma Kyn, LVEF,

Fig. 3. Correlations of Kyn in the CHF cohort. Kyn correlates negatively with peak VO_2 (A), GFR (MDRD; B), as well as LVEF (C), and positively with NT-proBNP (D).

left ventricular end-diastolic diameter (LVEDD), height, plasma sodium, and presence of CAD. It is noteworthy that NT-proBNP was not predictive upon univariate analysis. Correspondingly, the area under the ROC curve for Kyn (0.70) tended to be higher than that for NT-proBNP (0.63; $p = 0.26$; Fig. 2B). When we combined Kyn and NT-proBNP by multivariate binary logistic regression, only Kyn contributed significantly (OR 1.75, 95% CI 1.13–2.70); thus, the combined ROC analysis did not result in any improvement (not shown). Regarding the multivariate prediction model shown in Table 2, we chose the following model building: as significant univariate correlations of Kyn with LVEDD ($r = 0.326$, $p = 0.001$), LVEF ($r = -0.394$, $p < 0.001$), and sodium ($r = -0.212$, $p = 0.031$), but not with height ($r = -0.023$, $p = 0.809$) were found, we constructed Models 1 (Kyn, height) and 2 (Kyn, height, CAD) as preliminary, stepwise multivariate models to exclude any possible collin-

Table 3. Results of multiple linear regression: determinants of Kyn in the AICD cohort

Parameter	Regression coefficient B	Standard error	Significance
GFR (MDRD)	–0.005	0.0015	$p < 0.001$
hsCRP	0.091	0.0211	$p < 0.001$
Tryptophan	0.007	0.0014	$p < 0.001$
Age	0.005	0.0023	$p = 0.046$
Zonulin	–0.193	0.1092	$p = 0.077$
LVEF	–0.003	0.0022	$p = 0.140$
CML-calprotectin	–0.017	0.0114	$p = 0.133$

Only determinants with $p < 0.20$ are shown. Kyn, kynurenine; hsCRP, high-sensitivity CRP; CML-calprotectin, carboxy-methyl-lysine calprotectin; LVEF, left ventricular ejection fraction.

earity issues. The final Model 3 incorporates all univariate candidates whether or not they correlate with the covariable Kyn. Kyn was still a significant multivariate predictor in Model 1 (Table 2), close to significance in Model 2, but not part of the final model which contained sodium, LVEDD, and height.

Kyn Predicts Severely Compromised Left Ventricular Function

We compared the ability of Kyn or NT-proBNP to discriminate at a threshold of LVEF < 30% (Fig. 2C). Both single ROC curves had an AUC of 0.74; a combined ROC analysis did not result in improved discrimination which may be attributable to the high degree of correlation between Kyn and NT-proBNP (see below).

Kyn Associates with Established Markers of CHF Severity

Figure 3 summarizes the different univariate associations of Kyn with markers of disease severity: Kyn correlated inversely with peak VO_2 (Fig. 3A; determined in 18 of 114 patients in whom exercise testing by spiroergometry had been performed), glomerular filtration fraction (GFR) according to the MDRD formula (Fig. 3B), and LVEF (Fig. 3C). Moreover, there was a strong correlation of Kyn with NT-proBNP (Fig. 3D).

AICD Cohort

Kyn Associates with Metabolic Inflammation, Kidney Function, and Tryptophan

The following parameters showed significant univariate associations with Kyn and were therefore included in the multiple linear regression analysis: zonulin ($q = -0.265$), carbonylated proteins ($q = 0.295$), creatinine ($q = 0.478$), hsCRP ($q = 0.322$), tryptophan ($q = 0.342$), carboxy-methyl-lysine calprotectin ($q = -0.112$), LVEF ($q = -0.215$), GFR according to MDRD ($q = -0.521$), age ($q = 0.345$), and severity of CAD (1-, 2-, or 3-vessel disease; $q = 0.177$). In addition, patients with type-2 diabetes ($n = 42$; Table 1) had significantly higher Kyn levels ($3.70 \mu\text{mol/L}$, range 3.12–4.55) than non-diabetic patients ($3.30 \mu\text{mol/L}$, range 2.60–4.22). Table 3 summarizes the results of the multiple linear regression analysis which demonstrated highly significant associations of Kyn with GFR, hsCRP, and tryptophan, as well as a significant correlation with age.

Discussion

This clinical investigation was undertaken in order to substantiate the role of Kyn as a biomarker in CHF. To this end, we gathered data in 2 independent CHF cohorts and arrived at the following main conclusions.

Kyn increased with severity of CHF; for predicting death from any cause it performed better than the established CHF biomarker, NT-proBNP. Kyn exhibited highly significant inverse correlations with peak VO_2 , LVEF, and GFR, and it correlated closely with NT-proBNP. In an attempt to identify clinical and biochemical key determinants of Kyn, we found that GFR and hsCRP as well as tryptophan plasma levels had the most significant impact.

The general finding of plasma levels of Kyn being elevated in patients with CHF was first reported by Konishi et al. [13]. The authors found circulating Kyn increased in patients with HFrEF (mean $\pm$ SD 3.5 ± 1.5 $\mu\text{mol/L}$) and HFpEF (3.4 ± 1.3 $\mu\text{mol/L}$) as compared with control individuals (2.4 ± 1.1 $\mu\text{mol/L}$), with most of their CHF patients being in NYHA class II. Our current investigation extends this report by adding data on the course of Kyn levels with NYHA class. Whereas plasma levels in our NYHA I and NYHA II/III patients are comparable to those measured by Konishi et al. [13] in controls and CHF, respectively, circulating Kyn rose as high as 6–8 $\mu\text{mol/L}$ in our patients in NYHA IV. As already mentioned in the Introduction, the key stimulus to Kyn increase is the well-established upregulation of inflammatory cytokines in CHF [14].

In our logistic regression analysis, Kyn proved to be the only parameter among a number of CHF (NT-proBNP), inflammatory (CRP, zonulin), and oxidative stress markers (nitrotyrosine, oxidized LDL) that showed predictive value for death (Table 2). Kyn was no longer significant in the final multivariate model comprising sodium, height, and LVEDD, which, however, may be attributable to the relatively small event numbers in this pilot study. While low sodium and high LVEDD are established risk predictors in CHF, the appearance of height seems counterintuitive at first sight. Nevertheless, low height has repeatedly been demonstrated to represent an independent risk factor, e.g., for all-cause mortality and hospitalization in heart failure patients with cardiac resynchronisation therapy [15], for early mortality after transplantation [16], as well as for major adverse coronary events in a general population [17].

To date, there is only one additional publication regarding death prediction in CHF by Kyn [18]. The authors determined that out of 42 biologically relevant serum amine-based metabolites, only N-methyl-L-histidine, symmetric dimethylarginine, and L-Kyn predicted ventricular arrhythmia-induced AICD shocks and all-cause mortality in two independent cohorts of AICD carriers with CHF, PROSE-ICD, and GRADE. There is circumstantial evidence in support of this work from findings in rodents demonstrating protection against pancreatitis-related multiple organ failure and death by genetic deficiency or selective pharmacological inhibition of Kyn monooxygenase, an enzyme converting Kyn into 3-hydroxyKyn [19]. Along the same lines, in 245 patients suffering from out-of-hospital cardiac arrest enrolled in the FINNRESUSCI Study [12], metabolites of the Kyn pathway (Kyn, kynurenic acid, 3-hydroxyanthranilic acid) were significantly increased in patients dead versus alive both at the ICU and after 12 months. Multivariable logistic regression showed that all 3 Kyn metabolites impacted significantly on ICU mortality, whereas high kynurenic acid and 3-hydroxyanthranilic acid were revealed as significant predictors of a 12-month poor outcome.

In our previous work [13], we reported on the inverse Kyn-spiroergometric peak VO_2 association in CHF patients, which remained significant after adjustment for age, sex, BMI, and hemoglobin. This inverse correlation could be confirmed here, albeit in a much smaller subcohort (Fig. 3). Exercise-induced upregulation of Kyn aminotransferases via PGC-1 α contributes to local Kyn degradation in skeletal muscle [20], which may account for the

observed relation between plasma Kyn and exercise performance. Besides, this exercise-related mechanism was shown to protect against depression since kynurenic acid, the local Kyn metabolite produced by skeletal Kyn aminotransferases, cannot permeate the blood-brain barrier [20]. Another fact that may contribute to the described constellation between Kyn and exercise capacity is tryptophan depletion by IDO upregulation [21]: enhanced tryptophan degradation was shown to inhibit the proliferation of bone marrow cells, which may result in anemia. This holds particularly true of end-stage renal or cardiac disease [22, 23] in which low circulating tryptophan levels were found.

In our AICD cohort, we established highly significant associations of Kyn with GFR, hsCRP, and tryptophan, as well as a significant increase with age (Table 3). While the increase with tryptophan obviously reflects the common metabolic pathway [1], the inverse relation to renal function is certainly attributable to chronic immune upregulation/dysregulation in renal patients. Thus, plasma IDO levels and activity were reported to be increased with the severity of renal disease [24, 25]. IDO levels, in turn, correlated well with T cell depletion in renal disease [25], which established a link to immunosuppression. Vice versa, we have recently demonstrated baseline Kyn prior to coronary angiography to reflect the propensity to develop contrast-induced nephropathy [26], which renders Kyn an appropriate risk marker for cardiorenal syndrome.

Eventually, despite significant univariate (inverse) correlations of Kyn with LVEF in both cohorts, LVEF was no longer a significant determinant of Kyn in multiple regression analysis (Table 3). This fact – together with hsCRP appearing among the major determinants in that analysis – most likely points to the fact that Kyn is primarily an inflammation-driven, not a remodeling-driven, parameter.

As to the limitations of our study, we should mention that both study cohorts were of modest size with low absolute numbers of events (deaths) and a rather short follow-up period in the CHF cohort, which necessitates larger studies to exclude chance findings. Also, we had no time-to-event data owing to logistical limitations which rendered Cox analysis impossible.

Conclusion

This work reinforces the concept of Kyn being a new and valuable biomarker of CHF, with particular attention placed on its ability to predict mortality and reflect exercise capacity.

Acknowledgements

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Statement of Ethics

All patients in the cohorts described provided informed written consent to have data from their medical records used in research. All data were fully anonymized before being accessed by researchers. This study was approved by the Ethics Committee of the Charité University Medicine Berlin (approval No. EA1/004/13) and by the Ethics Committee of the Justus Liebig University of Giessen (Kerckhoff Clinic Bad Nauheim; BioData Bank 144/11) and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Disclosure Statement

The authors declare the following conflicts of interest. T.B.D. is an employee of Immunodiagnostik. F.P.A. holds the majority of shares in Immunodiagnostik. K.-H.K. is CEO and owner of Neuroimmun GmbH, the manufacturer of the kynurenine kit which is distributed by Immunodiagnostik. K.-H.K. has applied or owns patents on “immunoassays for biogenic amines” (PCT/EP2011/069936, EP20110797208, EP2612147A1, WO2012065912A1). V.M. is an employee of the Kerckhoff Clinic GmbH, Bad Nauheim.

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Author Contributions

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