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Serum uric acid is related to cardiovascular events and correlates with N-terminal pro-B-type natriuretic peptide and albuminuria in patients with diabetes mellitus

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Short title: Uric acid predicts cardiovascular events

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Abstract

Background Hyperuricemia is a risk factor for cardiovascular events and renal insufficiency. It correlates to intima-media thickness and microalbuminuria. In this study we evaluated uric acid as an independent marker for cardiac events in patients with diabetes.

Methods In a prospective observational study we recruited 494 patients with diabetes. Patients were then followed for 12.8 months (mean follow-up) and hospitalizations as a result of cardiac events (ischaemic heart disease, arrhythmias, heart failure) were recorded.

Results The median duration of diabetes was 11 ± 10.35 years. Patients were in the mean 60 ± 13 years old and mean HbA_{1c} was 62 ± 13 mmol/mol (7.8 ± 3.3%). At baseline, mean uric acid was 321.2 ± 101.1 μmol/l (range 101.1–743.5 μmol/l), median N-terminal pro-B-type natriuretic peptide was 92 ± 412 pg/ml and median urinary albumin to creatinine ratio was 8 ± 361 mg/g; Uric acid significantly correlated to N-terminal pro-B-type natriuretic peptide ($r = 0.237$, $P < 0.001$) and urinary albumin:creatinine ratio ($r = 0.198$, $P < 0.001$). In a Cox regression model, including age, estimated glomerular filtration rate, gender, systolic blood pressure, smoking and alcohol consumption, uric acid was the best predictor of cardiac events (hazard ratio 1.331, confidence interval 1.095–1.616, $P = 0.04$). However, uric acid lost its prognostic value when the natural logarithm of N-terminal pro-B-type natriuretic peptide was added to the model.

Conclusion Serum uric acid is a predictor of cardiac events and correlates to N-terminal pro-B-type natriuretic peptide and albuminuria, underscoring the importance of uric acid as a cardiovascular risk marker in patients with diabetes.

Keywords diabetes, uric acid

Abbreviations natlog(NT-proBNP), natural logarithm of N-terminal pro-B-type natriuretic peptide

Introduction

Cardiovascular disease is the main cause of morbidity and mortality in patients with diabetes [1]. The relationship between uric acid and cardiovascular disease in patients with diabetes still remains controversial [2,3]. In epidemiological studies, uric acid is an independent predictor of cardiovascular mortality in the general population and in patients with diabetes [4,5]. In patients with Type 2 diabetes, serum uric acid correlates to albuminuria and surrogate markers of atherosclerosis, such as the ankle brachial index or the carotid intima-media thickness [6,7]. Furthermore, it is related to known risk factors such as hypertension, insulin resistance, dyslipidaemia and albuminuria [7]. In fact, renal clearance of uric acid is inversely related to the degree of insulin resistance [8,9]. Most epidemiological evidence concludes that serum uric acid is an independent risk factor for cardiovascular and renal diseases in selected patient cohorts [10].

The mechanisms leading to premature atherosclerosis caused by elevated uric acid are not fully understood. However, there is evidence for a link between uric acid and endothelial dysfunction [11], which is aggravated by increased oxidative stress [12]. In almost all outcome studies, patients were followed for a relatively long time—at least 4 years. To date, no data exist as to whether uric acid remains a reliable marker when used for short-term prognosis.

Among many biomarkers and known risk factors, N-terminal pro-B-type natriuretic peptide (NT-proBNP) is an upcoming marker which allows an exact cardiovascular risk stratification in patients with diabetes [13–15]. Therefore, we wanted to evaluate a possible relationship between NT-proBNP as an established cardiovascular risk marker and uric acid. Furthermore, we calculated the performance of uric acid as a marker for short-term cardiovascular events.

Methods

Design

In this prospective observational study, patients visiting the diabetic outpatient clinics of the Vienna General Hospital and the Hietzing Hospital Vienna between December 2005 and November 2007 were included. All patients gave written informed consent according to the Helsinki II declaration. This

study was approved by the local ethics committees of the Medical University of Vienna and the Hietzing Hospital Vienna. At inclusion, the medical history, including data on the duration of diabetes, presence of any existing cardiovascular and other diseases, was recorded. Furthermore, an electrocardiogram was performed and analysed for the presence of any abnormalities.

Analytical methods

During the patients' regular visits, blood was drawn from an antecubital vein. We analysed plasma glucose, lipid values, liver enzymes, urea and creatinine and uric acid in a certified laboratory. NT-proBNP was determined with the Elecsys Essay by Roche Diagnostics (Vienna, Austria). For evaluating kidney function, we used the four-variable Modification of Diet in Renal Disease (MDRD) formula. Albuminuria was determined as the urinary albumin:creatinine ratio from the first-morning-void urine. Values above 30 mg/g were considered abnormal.

Endpoint

The endpoint was a combination of unplanned hospitalizations because of ischaemic heart disease, chronic heart failure and arrhythmias. Hospitalization data were obtained from the regional hospital data network. Information about hospitalization for cardiac events was obtained from hospital files by a cardiologist who was unaware of the results.

Statistical analysis

Continuous variables are presented as means ± standard deviation; categorical variables are shown as frequencies and percentages. As NT-proBNP is not normally distributed, the Spearman's rank sum correlation coefficient was used for testing the correlation between serum uric acid, NT-proBNP and urinary albumin:creatinine ratio.

For evaluating the influence of uric acid on the cardiovascular endpoint, we calculated four different Cox regression models, with stepwise forward selection to determine the most potent predictors. The *P*-value for entering the stepwise model was set at 0.05 for inclusion and 0.10 for exclusion. Harrell's concordance index (c-index) was used as a measure of the overall performance of the Cox

regression models. The c-index can be interpreted in a similar way to the well-known area under the curve of the receiver operating characteristic curve. Additionally, 500 bootstrap repetitions were performed for the Cox regression models, repeating the variable selection for each sample using the same entering and exclusion rules. This bootstrapping procedure is used as a test against over fitting the Cox regression model because of the limited number of events. It was counted how often a variable was entered into the Cox regression models.

The first model (standard model) was based on systolic blood pressure (mmHg), age (years), gender (0: female; 1: male), smoking status (1: smoker; 0: non-smoker), alcohol status (1: drinker; 0: non-drinker) and estimated glomerular filtration rate (ml min^{-1} 1.73 m^{-2}).

The second model was based on the standard model and serum uric acid (mg/dl).

The third model was based on the standard model and the natural logarithm of NT-proBNP (pg/ml).

The fourth model was based on the standard model, serum uric acid (mg/dl) and NT-proBNP (pg/ml).

All results of the regression model are presented using hazard ratios Exp(B). Hazard ratios were calculated per unit increase.

Proportional hazards assumption was assessed and satisfied for all variables based on time interaction tests.

SPSS 19.0 software (SPSS, Chicago, IL, USA) and GChaos 18.6 statistical software written in C++ by one of the authors (G.S) were used for all statistical analysis.

Results

In this study, we analysed data of 494 consecutive patients (214 women, 280 men) enrolled between April 2005 and November 2007. Patients were 60 ± 13 years old and the mean HbA_{1c} was 62 ± 13 mmol/mol (7.8 ± 3.3%). Hypertension was present in 305 patients (61.9%), mean serum uric acid was 320.6 ± 98.1 μmol/l (range 101.1–743.5 μmol/l). Forty patients (8.1%) were treated with the xanthinoxidase inhibitor, allopurinol. Mean systolic blood pressure was 139 ± 22 mmHg and mean

diastolic blood pressure was 82 ± 12 mmHg. Considering cardiovascular biomarkers, median NT-proBNP was 92 ± 412 pg/ml, 125 patients had an elevated urinary albumin excretion and mean LDL cholesterol was 2.78 ± 0.87 mmol/l (Table 1).

Although this is a highly selected collective, with patients having a long duration of diabetes (11.00 ± 11.8 years), history of ischaemic heart disease was only present in 85 (17.3%) of the patients (Table 2).

During a mean follow-up time of 12.8 months, 31 patients (6.3%) reached the defined endpoint, which was hospitalization because of an unexpected cardiovascular event.

Uric acid significantly correlated to NT-proBNP ($r = 0.237$; $P < 0.01$), urinary albumin:creatinine ratio ($r = 0.189$; $P < 0.01$), systolic blood pressure ($r = 0.162$; $P < 0.01$), diastolic blood pressure ($r = 0.106$; $P < 0.19$) and estimated glomerular filtration rate (eGFR) ($r = –0.355$; $P < 0.001$).

Cox regression models

In the standard model consisting of age, systolic blood pressure, gender, smoking status, alcohol status and eGFR, age (hazard ratio 1.050, confidence interval 1.017–1.084, $P = 0.003$) is the only predictor after stepwise selection. The model has a c-index of 0.654 and age is included in 72.2% of the bootstrap repetitions.

In the second model (standard model and serum uric acid), we found serum uric acid (hazard ratio 1.331, confidence interval 1.095–1.616, $P = 0.004$) and age (hazard ratio 1.045, confidence interval 1.011–1.080, $P = 0.009$) to be significant predictors after stepwise selection. In this model, uric acid is included in 64.6% of the bootstrap analysis, supporting the robustness of the analysis.

The model has a c-index of 0.681, which is higher than for the standard model alone.

In the third model [standard model and natlog(bnp)] we found natlog(bnp) to be the only significant predictors after stepwise selection (hazard ratio 3.059, confidence interval 2.249–4.160, $P < 0.001$).

The model has a c-index of 0.838, which is higher than for the standard model alone. Furthermore, the natural logarithmof NT-proBNP [natlog(NT-proBNP)] is included in 100% of the 500 bootstrap repetitions.

The fourth model [standard model, natlog(bnp), and serum uric acid] is, after stepwise selection, reduced to natlog(bnp), which is again the only significant predictor being included in 100% of the 500 bootstrap repetitions (Table 3).

Discussion

Our results show that uric acid correlates to NT-proBNP and urinary albumin:creatinine ratio, which are currently the best biomarkers for cardiovascular risk stratification in patients with diabetes [14,16]. Among many factors, uric acid is strongly influenced by kidney function. For further evaluating the prognostic value of this marker, we set up four different Cox regression models, which can be summarized in two main findings.

First, uric acid is the best independent predictor of the defined endpoint, thus slightly increasing the c-index from 0.65 to 0.68 when added to traditional factors such as age, kidney function, smoking, alcohol consumption, gender and systolic blood pressure.

Second, uric acid lost its prognostic information when natlog(NT-proBNP) was entered into the model. However, this finding is not surprising considering the fact that NT-proBNP is the most potent predictor for cardiac events in patients with diabetes [13,14], being superior to almost all known biomarkers.

To our mind, the fact that uric acid was the only independent significant predictor of the endpoint when added to a model that included traditional risk factors clearly underscores the prognostic value of this marker being superior to established markers such as age, kidney function, smoking, systolic blood pressure and alcohol consumption.

Generally, our data are in agreement with a recent study by Zoppini and colleagues [17]. In this study (median follow-up of 4.7 years), elevated serum uric acid concentrations independently predicted cardiovascular mortality in patients with Type 2 diabetes [17]. In our study, we could now show that uric acid also performs well in a shorter follow-up period.

As our data underscore the relationship between uric acid and cardiovascular events, we hypothesize that allopurinol could be beneficial in patients with diabetes with elevated uric acid levels. This raises the question whether treatment with allopurinol should be initiated earlier. Generally, allopurinol exerts anti-hypertensive effects and reduces vascular changes [18]. Treatment with allopurinol is able to reverse the uric acid-induced endothelial dysfunction [12]. It has been shown that high doses of allopurinol have a high antioxidative potential, thus improving endothelial dysfunction by reducing oxidative stress [19]. Furthermore, treatment with allopurinol improves myocardial energy metabolism in patients with stable angina pectoris [20].

It is currently unknown whether treatment with allopurinol is able to reduce cardiovascular events in patients with diabetes. Based on our results, we assume that patients with elevated uric acid values might benefit from treatment with allopurinol. Further studies are needed to confirm this hypothesis.

Because only 8.1% of the patients were treated with allopurinol, we did not adjust the regression models for treatment with allopurinol. In our cohort, mean serum uric acid level was 320.6 μmol/l.

In population-based studies, elevated serum uric acid levels increased blood pressure by stimulating the activity of the rennin–angiotensin–aldosterone system [21,22]. Data from the multiple Risk Factor Intervention Trial suggest that elevated uric acid leads to a slow inflammation, which expedites the progression of premature atherosclerosis [10,23]. On a pathophysiological basis, experimental studies demonstrate that uric acid is also associated with inflammatory biomarkers such as C-reactive protein (CRP) and interleukin 6 (IL-6) [24]. However, there are numerous studies providing evidence that uric acid increases oxidative stress and promotes lipid oxidation. In summary, these mechanisms result in increased endothelial dysfunction [12]. In contrast to these results, uric acid has been shown to exert

antioxidative effects, therefore preventing stress-induced cell transformation and cardiac and renal toxicity. In patients with Type 1 diabetes, uric acid is able to restore endothelial function [25].

It is currently not known whether uric acid is a culprit or whether uric acid is a surrogate marker of vascular endothelial dysfunction. We believe that the short follow-up period of 12.8 months underscores the hypothesis that uric acid is an independent marker of cardiovascular events. Although uric acid is related to hypertension, hyperlipidaemia and insulin resistance, none of these markers provided significant prognostic information when used for short-term prognosis.

Although uric acid concentrations and event rates were low, serum uric acid turned out to be a significant predictor of cardiovascular events. In summary, serum uric acid is an independent predictor of cardiac events and correlates to N-terminal pro-B-Type naturetic peptide and urinary albumin:creatinine ratio.

Competing interests

Nothing to declare.

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Table 1 Demographic data

Variable	Primary endpoint not reached	Primary endpoint reached	*P*-value
Duration of diabetes (years) (median)	10 ± 10.33	11 ± 10.74	0.832
HbA_{1c} (mmol/mol)	61 ± 14	66 ± 5	
HbA_{1c} (%)	7.7 ± 3.4	8.23 ± 1.7	0.405
BMI (kg/m^2)	29.0 ± 5.4	29.9 ± 6.3	0.364
LDL cholesterol (mg/dl)	107.92 ± 33.23	100.8 ± 36.5	0.245
Cholesterol (mg/dl)	197 ± 49	186 ± 56	0.248
Triglycerides (mg/dl)	185 ± 351	219 ± 325	0.592
GFR (MDRD)	72.18 ± 18.15	61.90 ± 26.16	0.003
Albumin:creatinine ratio (mg/g) (median)	7.95 ± 331.08	23.15 ± 612.77	< 0.001
Allopurinol treatment	35 patients (7.7%)	5 patients (14.7%)	0.311
Uric acid (mg/dl)	5.3 ± 1.6	6.47 ± 2.2	< 0.001
NT-proBNP (pg/ml) (median)	87 ± 279	486 ± 970	< 0.001

GFR, glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Table 2 Cardiovascular history

Variable	Uric acid ≤ 249.8 μmol/l	Uric acid 249.9–315.2 μmol/l	Uric acid 315.3–380.7 μmol/l	Uric acid ≥ 380.8 μmol/l
Hypertension	57 (46%)	80 (62.0%)	82 (67.2%)	85 (72.0%)
Ischaemic heart disease	8 (6.5%)	20 (15.5%)	25 (20.5%)	32 (27.1%)
Artrial fibrillation	2 (1.6%)	2 (1.6%)	4(3.3%)	7 (5.9%)
Coronary artery bypass graft	2 (1.6%)	6 (4.7%)	12 (9.8%)	11 (9.3%)
Percutaneous coronary intervention	4 (3.2%)	3 (2.3%)	9 (7.4%)	7 (5.9%)

Table 3 Cox regression models

Model	Wald	Significance	Exp(B)	95% lower CI	95% upper CI
Model 1*					
Age	8.854	*P* = 0.003	1.050	1.017	1.084
Model 2†					
Uric acid	8.272	*P* = 0.004	1.331	1.095	1.616
Model 3‡					
natlog(NT-proBNP)	50.799	*P* < 0.001	3.059	2.249	4.160
Model 4§					
natlog(NT-proBNP)	50.799	*P* < 0.001	3.059	2.249	4.160

Variables included in models:

*model 1: age, GFR, gender, systolic blood pressure, smoking, alcohol;

†model 2: age, uric acid, gender, systolic blood pressure, GFR, smoking, alcohol consumption;

‡model 3: age, natlog(NT-proBNP), gender, systolic blood pressure, GFR, smoking, alcohol consumption;

§model 4: age, natlog(NT-proBNP), uric acid, gender, systolic blood pressure, GFR, smoking, alcohol consumption.

GFR, glomerular filtration rate; natlog(NT-proBNP), natural logarithm of N-terminal pro-B-type natriuretic peptide.