Are Levels of NT-proBNP and SDMA Useful to Determine Diastolic Dysfunction in Chronic Kidney Disease and Renal Transplant Patients?

Lidija Memon,¹ Vesna Spasojevic-Kalimanovska,² Natasa Bogavac Stanojevic,^{2*} Jelena Kotur-Stevuljevic,² Sanja Simic-Ogrizovic,³ Vojislav Giga,⁴ Violeta Dopsaj,² Zorana Jelic-Ivanovic,² and Slavica Spasic²

¹Clinical Chemistry Laboratory, Clinical Centre Bezanijska Kosa, Belgrade, Serbia

²Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

³Nephrology Clinic, Clinical Centre of Serbia, Belgrade, Serbia

⁴Cardiology Clinic, Clinical Centre of Serbia, Belgrade, Serbia

Background: The aim of the study was to determine the clinical usefulness of N-terminal pro-B-type natriuretic peptide (NT-proBNP) and symmetric dimethylarginine (SDMA) for detection of renal and left ventricular (LV) diastolic dysfunction in chronic kidney disease (CKD) patients and renal transplant (RT) recipients. **Methods:** We included 98 CKD and 44 RT patients. We assessed LV function using pulsed-wave Doppler ultrasound. Diastolic dysfunction was defined when the E:A ratio was <1. **Results:** Independent predictors of NT-proBNP levels were age, creatinine, and albumin in CKD patients and age and urea in RT patients. Determinants of SDMA in CKD patients were glomerular filtration rate (GFR) and

NT-proBNP and creatinine in RT patients. In RT patients with diastolic dysfunction, NT-proBNP and SDMA were significantly higher than in patients without diastolic dysfunction ($F = 7.478$, $P < 0.011$; $F = 2.631$, $P < 0.017$). After adjustment for GFR, the differences were not seen. In CKD patients adjusted NT-proBNP and SDMA values for GFR were not significantly higher in patients with diastolic dysfunction than in patients without diastolic dysfunction. **Conclusions:** NT-proBNP is useful for detection of LV diastolic dysfunction in RT recipients. When evaluating both NT-proBNP and SDMA it is necessary to consider GFR as a confounding factor. *J. Clin. Lab. Anal.* 27:461–470, 2013. © 2013 Wiley Periodicals, Inc.

Key words: chronic kidney disease; renal transplant recipients; N-terminal pro B-type natriuretic peptide; symmetric dimethylarginine; diastolic dysfunction

INTRODUCTION

It is well known that cardiovascular dysfunction is the major cause of death in patients with chronic kidney disease (CKD). Kidney replacement is the ultimate step of end-stage renal disease. Unfortunately, transplanted kidney function still remains lower than that of a healthy one. Renal transplant (RT) recipients are more than ten times likely to die from cardiac dysfunction and 50 times more likely to suffer from nonfatal cardiovascular events on an annual basis compared to the general population (1). The relationship between cardiovascular disease (CVD) and CKD is complex. Many markers for cardiac dysfunction are also predictors of CKD progression. Prime examples are natriuretic peptides (2).

The natriuretic peptides (ANP and BNP) are structurally similar but genetically distinct cardiac hormones playing important role in cardiovascular homeostasis.

Grant sponsor: Ministry of Education, Science and Technological Development, Republic of Serbia; Grant number: 175035; Grant sponsor: European Cooperation in Science and Technology (COST) BM0904 Action.

*Correspondence to: Natasa Bogavac-Stanojevic, Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, P.O. Box 146, 11000 Belgrade, Serbia. E-mail: naca@pharmacy.bg.ac.rs

Received 5 September 2012; Accepted 30 April 2013

DOI 10.1002/jcla.21628

Published online in Wiley Online Library (wileyonlinelibrary.com).

As hormones, the natriuretic peptides initiate natriuresis, vasodilatation, and inhibition of the renin–angiotensin–aldosterone system. Also, they exert antimitogenic effects on endothelial, smooth muscle, and myocardial cells (3). ANP is generally described as an atrial hormone while BNP is described as a ventricular hormone. BNP is synthesized by cardiomyocytes as a prohormone, which then splits into two fragments: the inactive one (N-terminal pro-B-type natriuretic peptide (NT-proBNP)) and the active hormone (BNP). The released hormone then acts by binding to its specific receptors in blood vessels or kidneys.

The mechanisms responsible for production and release of BNP and its related peptides in the physiological and pathophysiological states are complex. They include mechanical, chemical, neurohormonal, and immunological factors. The main physiological stimulus for BNP release is ventricular muscle stretch that occurs in hemodynamic stress condition. Based on the recent reports, there is a large number of powerful stimulators of synthesis and/or secretion of BNP and other natriuretic peptides. Some of them are glucocorticoids, growth factors, other hormones, and cytokines (IL-1, IL-6, TNF- α). Understanding the relationship of heart endocrine and contractile function and other regulatory mechanisms is crucial for interpretation of natriuretic peptide levels, especially BNP and NT-proBNP (4).

When compared to BNP, NT-proBNP has some respective advantages for detection such as its longer half-life in circulation, higher blood levels, and less degradation in vivo and in vitro (5). Nowadays, measurement of NT-proBNP is a useful diagnostic and prognostic marker of congestive heart failure and left ventricular (LV) systolic dysfunction (6). Diastolic dysfunction is a sign of LV hypertrophy, which is common in renal disease patients. Conventionally, diastolic dysfunction has been assessed on the basis of the echocardiography. However, in a recent population-based study, it has been suggested that BNP might be a suboptimal marker to detect diastolic dysfunction (7). Unfortunately, there is a lack of published data regarding the relationship between NT-proBNP concentrations and diastolic dysfunction particularly in renal patients.

Symmetric dimethylarginine (SDMA) is an inactive stereoisomer produced alongside asymmetric dimethyl-L-arginine (ADMA) and has recently been described as a risk factor for cardiovascular events. ADMA directly inhibits endogenous nitric oxide synthase, leading to endothelial dysfunction, while SDMA is probably important as a competitive inhibitor for arginine transport across cell membranes (8,9). Vallance et al. were the first to report increased plasma levels of ADMA and SDMA in patients with end-stage renal disease (10). However, it has been hypothesized that SDMA accumulation in renal failure is associated with hypertension and CVD (11). In a study

on renal failure patients, Fleck and colleagues concluded that not only ADMA but also SDMA were probably responsible for hypertension, possibly by competition for reabsorption between SDMA and arginine in the kidney (12). It is clear that hypertension, atherosclerosis, fluid overload, and anemia in patients with CKD provoke structural abnormalities on the heart, which may lead to diastolic and systolic dysfunction (13). Due to the lack of clarity concerning the pathophysiological interactions between heart and kidneys, and the considerable relevance of this issue, we hypothesized that increased levels of NT-proBNP and SDMA may be markers of diastolic dysfunction and heart failure in patients with kidney dysfunction.

In this current study, we have measured NT-proBNP and SDMA in patients with CKD and in RT recipients. The aims of the study were to determine (1) the confounding effects of renal function parameters and classical risk factors for atherosclerosis on SDMA and NT-proBNP concentrations and (2) the relationship between SDMA and NT-proBNP and diastolic dysfunction.

PATIENTS AND METHODS

Patients

This study included 98 stage 2–5 CKD patients not requiring dialysis and 44 RT recipients (at least 6 months after transplantation). The subjects were examined at the Nephrology Clinic, Clinical Centre of Serbia and provided information regarding medication and medical history. Diabetics and patients with acute inflammation disease were excluded.

Thirty-four patients received a kidney from a related living donor and ten from a cadaver. These patients had a previous transplantation duration of 9.56 ± 5.27 years. Any history of hypertension, ischemic vascular disease (myocardial infarction, angina pectoris, and cerebral stroke), and current smoking habits were obtained by interview.

The origin of kidney diseases within the examined patients were chronic glomerulonephritis ($n = 28$ in RT and $n = 33$ in CKD patients), chronic pyelonephritis and congenital urinary tract anomalies ($n = 5$ in RT and $n = 8$ in CKD patients), nephrosclerosis ($n = 2$ in RT and $n = 28$ in CKD patients), polycystic kidney disease ($n = 2$ in RT and $n = 12$ in CKD patients), other ($n = 0$ in RT and $n = 17$ in CKD patients), and unknown ($n = 7$ in RT and $n = 0$ in CKD patients). The immunosuppressive protocol in renal RT patients consisted of calcineurin inhibitors (cyclosporine or tacrolimus), mycophenolate acids, or azathioprine and prednisolone. CKD patients with some type of glomerulonephritis as the origin of the disease received corticosteroids or other immunosuppressive therapy according to standard protocols. An echocardiogram was

obtained to assess diastolic function and blood was drawn for biochemical measurements. The patients data included demographic (sex and age), clinical (duration of renal disease), and biochemical variables. Clinical observations, as well as laboratory parameters and the echocardiogram were performed on the same day. All patients gave informed consent prior to their enrolment in the study, which was planned according to the ethical guidelines following the Declaration of Helsinki. The institutional review committee approved our study protocol thereby following local biomedical research regulations.

Echocardiography

LV dimensions were measured from 2D-guided M-mode echocardiograms of the left ventricle at the papillary muscle level using the parasternal short-axis view and the same M-mode recording of 2D measurements of LV dimensions were used for the calculation of the ejection fraction (LVEF) using Teicholz method in the absence of wall motion abnormalities. In patients with preexistent wall motion abnormalities LVEF was calculated using bi-plane Simpson method. Preserved systolic function was defined as LVEF > 50%. The thickness of the ventricular posterior wall and that of the ventricular septum were measured from the same M-mode echocardiogram. The size of the left atrium was determined from the parasternal long-axis view at end systole (14). Pulsed-wave (PW) Doppler was performed in the apical four-chamber view to obtain mitral inflow velocities to assess LV filling and to calculate the E/A ratio (15).

Biochemical Measurements

A fasting blood sample was drawn from each patient in order to measure the following parameters: serum concentrations of SDMA, NT-proBNP, creatinine, urea, uric acid, albumin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, homocysteine (tHcy), high-sensitive C-reactive protein (hsCRP), interleukin-6 (IL-6), serum amyloid-A (SAA), and plasma fibrinogen. The serum samples for determination of SDMA, NT-proBNP, IL-6, and tHcy were stored at -80°C in aliquots until analysis. Other parameters were analyzed on the day of collection. Citrated plasma was stored at -70°C before measurement of fibrinogen.

Measurement of serum SDMA was determined using an ELISA (DLD Diagnostica GMBH, Hamburg, Germany). NT-proBNP was determined using an ELFA (bioMerieux, Vidas, Lyon, France). The assay principle combines a one-step immunoassay sandwich method with a final fluorescent detection (ELFA). This method is based on two polyclonal antibodies. The analytical characteristics were calculated by manufacturer using the protocol based on the recommendations of the CLSI EP5-A2 doc-

ument. Functional sensitivity at interassay coefficient of variation of 20% was less than 50 ng/l. Interlot reproducibility was 5.4% at 116.9 ng/l and 3.5% at 1,066.6 ng/l. The method was compared with the ELECSYS Roshe method (coefficient of correlation: 0.99).

Creatinine, urea, uric acid, albumin, and lipids were analyzed by routine methods (Olympus System Reagents using an Olympus analyzer AU 2700, Hamburg, Germany). SAA and hs-CRP were measured using immunonephelometric assays (Dade-Behring, BN II, Marburg, Germany). Serum IL-6 levels were measured with highly sensitive colorimetric sandwich ELISA kits (Human IL-6 Quantikine HS ELISA kit; R&D Systems, GmbH, Germany). Serum tHcy was measured by high-performance liquid chromatography after reduction of the disulfide bonds by dithiothreitol (normal range: 10–15 $\mu\text{mol/l}$). The SDMA reference range is (0.3–0.7 $\mu\text{mol/l}$). For patients <75 years old, optimal NT-proBNP cut-off value for ruling out heart failure is 125 ng/l. Assessment of renal function was performed via estimated glomerular filtration rate (GFR) using the Modified Diet in Renal Disease equation (16). Arterial hypertension was diagnosed when the systolic blood pressure was ≥ 140 mmHg and/or diastolic pressure was ≥ 90 mmHg, or if antihypertensive treatment was prescribed. Body mass index (BMI) was calculated according to the formula: weight (kg)/height² (m²).

Statistical Analyses

Differences in continuous variables between the groups were analyzed by the Student's *t*-test for normally distributed variables. Values for HD duration, GFR, creatinine, TG, hsCRP, IL-6, SAA, NT-proBNP, and SDMA were log transformed to achieve normality. Adjusted mean levels of NT-proBNP and SDMA were estimated by analysis of covariance (ANCOVA). Group differences for categorical variables were examined by the chi-square test. Univariate associations were evaluated by Pearson's correlation analysis. Factors that were significant in univariate analysis were entered into multivariate linear regression analysis. We also used the variance inflation factor (VIF) function to determine whether any of the covariates had a VIF of >10 (a VIF of >10 is comparable to a tolerance of >0.1). According to this analysis, we investigated the determinants of NT-proBNP and SDMA levels using a forward stepwise paradigm. For each model we calculated β coefficients and standard error (SE) of the predictor variables. Data are shown as mean $\pm$ standard deviation for normally distributed continuous variables and as absolute frequencies for categorical variables. Log-transformed variables were expressed as geometrical mean and 95% confidence interval (CI) for mean. All calculations were performed using MS Excel, EduStat 2.01

TABLE 1. Group Differences Between CKD and RT Patients

	CKD patients <i>n</i> = 98	RT patients <i>n</i> = 44	<i>P</i>
Age (years)	44.9 ± 16.95	41.4 ± 10.69	0.214
Gender, male (%)	49.0	63.6	0.105
BMI (kg/m ²)	23.60 ± 4.74	24.98 ± 3.59	0.105
HTA (%)	66.3	45.5	0.038
HD duration (months) ^a	27.95 (20.50–38.00)	117.98 (92.60–150.30)	0.001
E/A ratio	1.13 ± 0.21	1.08 ± 0.29	0.391
LVEF (%)	58.57 ± 6.35	61.87 ± 6.52	0.279
GFR (ml/min/1.73m ²)	25.23 (21.09–30.20)	37.37 (32.433–43.05)	0.002
Creatinine (μmol/l) ^a	236.86 (204.7–274.0)	168.66 (148.8–191.2)	0.001
Albumin (g/l)	39.85 ± 5.52	42.53 ± 3.20	0.005
Uric acid (μmol/l)	448.40 ± 108.9	400.14 ± 85.78	0.012
Urea (mmol/l)	17.40 ± 8.45	12.10 ± 5.94	<0.001
TC (mmol/l)	5.66 ± 1.25	5.87 ± 1.09	0.350
LDL-C (mmol/l)	3.63 ± 1.14	3.70 ± 0.953	0.731
HDL-C (mmol/l)	1.19 ± 0.36	1.30 ± 0.34	0.119
TG (mmol/l) ^a	1.81 (1.63–2.10)	1.88 (1.63–2.16)	0.753
tHcy (μmol/l)	22.82 ± 6.38	23.66 ± 12.12	0.758
Fibrinogen (g/l)	4.81 ± 0.85	4.80 ± 1.56	0.980
hs-CRP ^a (mg/l)	0.82 (0.53–1.28)	1.43 (0.96–2.13)	0.070
IL-6 ^a (pg/ml)	2.52 (2.09–3.05)	4.24 (3.44–5.23)	<0.001
SAA ^a (mg/l)	3.37 (2.68–4.23)	9.15 (6.74–12.41)	<0.001
NT-proBNP ^a (ng/l)	423.67 (260.78–688.32)	274.79 (177.36–425.75)	0.262
SDMA ^a (μmol/l)	2.07 (1.78–2.41)	1.20 (1.07–1.28)	<0.001

Continuous variables are presented as mean ± standard deviation and compared by the Student *t*-test, whereas categorical variables are presented as relative frequencies and compared by the chi-square test.

^aValues for HD duration, GFR, creatinine, TG, hs-CRP, IL-6, SAA, NT-proBNP, and SDMA are presented as geometrical mean and 95% confidence intervals (CIs). Logarithmic transformation of the values was performed before the analysis.

(2005, Alpha Omnia, Belgrade, Serbia), and MedCalc for Windows version 9.6.3. (Mariakerke, Belgium). The minimal statistical significance was set at *P* < 0.05.

RESULTS

The baseline characteristics of both study groups are shown in Table 1. There were no differences in age or sex between the groups. Duration of renal disease in the RT group [117.98 (92.60–150.30) months] was longer than in the CKD group [27.95 (20.50–38.00) months]. Renal function of RT patients was significantly higher than that in CKD patients. When inflammatory parameters were assessed, only IL-6 and SAA were higher in RT patients. There were no significant differences in serum lipid parameters between the two study groups. We found a significantly higher concentration of SDMA in CKD patients. The NT-proBNP concentration was also higher in CKD patients but did not reach statistical significance. RT patients had better diastolic function expressed as E/A ratio and better LVEF than CKD patients, but without statistical significance.

Distribution of NT-proBNP values in CKD, RT patients, and control subjects, 45 males and 43 females (results from unpublished study) matched by ages and gender

are presented in Figure 1. Since healthy individuals and patients were younger than 75 years, all values in control group were lower than recommended cut-off value for heart failure. Also, 35% CKD patients and 33% RT patients had NT-proBNP values lower than cut-off value of 125 pg/ml (ng/l).

Table 2 shows the results of correlation analysis between NT-proBNP and SDMA and other examined parameters in joint CKD and RT patients. Of all the renal function parameters, GFR (*r* = −0.596 for NT-proBNP and *r* = −0.742 for SDMA) and creatinine (*r* = 0.693 for NT-proBNP and *r* = 0.731 for SDMA) indicated the strongest correlations, although the correlation was stronger for urea than for albumin with both parameters. Both NT-proBNP and SDMA were positively associated with age but they correlated oppositely with HDL-C and E/A ratio. NT-proBNP positively correlated with inflammatory parameters (IL-6, hsCRP, fibrinogen) and SDMA. In contrast, SDMA did not significantly associate with inflammatory parameters but instead positively correlated with tHcy.

Multiple linear regression analysis was performed to identify the determinants of serum NT-proBNP and SDMA concentration. Significant factors in univariate analysis were entered into forward stepwise linear

Fig. 1. Distribution of NT-proBNP values in CKD and RT patients and control subjects matched by ages and gender. Dotted line is cut-off value for NT-proBNP (125 ng/l). Box plots are presented as median value, 25th–75th percentile values for NT-proBNP.

regression analysis. The final model only included those variables that emerged as statistically significant. In CKD patients, independent predictors of higher NT-proBNP were older age ($\beta = 0.030$; $SE = 0.004$; $P < 0.001$; $VIF = 1.094$), higher creatinine ($\beta = 0.003$, $SE = 0.0001$, $P < 0.001$; $VIF = 1.160$), and lower albumin ($\beta = -0.039$, $SE = 0.009$, $P = 0.015$; $VIF = 1.236$). GFR ($\beta = -0.237$, $SE = 0.001$, $P < 0.001$) and NT-proBNP ($\beta = 0.053$, $SE = 0.012$, $P = 0.001$) entered into the final regression equation that described determinants of SDMA (VIF for both parameters was 2.316). The same analysis showed that age ($\beta = 0.016$, $SE = 0.007$, $P = 0.038$) and urea ($\beta = 0.064$, $SE = 0.011$, $P < 0.001$) were independent determinant factors affecting NT-proBNP in RT patients (VIF for both parameters was 1.008). In contrast, when the dependent variable was SDMA, only creatinine was an independent predictor ($\beta = 0.572$, $SE = 0.093$, $P < 0.001$) after including age, GFR, albumin, urea, HDL-C, NT-proBNP, E/A ratio, and tHcy in RT patients.

We performed an analysis of the relationship between changes in renal function, lipid, lipoprotein, inflammatory parameters as well as both NT-proBNP and SDMA and the cardiac structural and functional abnormalities. A small number of CKD patients (three patients) were excluded because they fulfilled the criteria for diagnosis of systolic dysfunction. Therefore, we evaluated changes of the examined parameters according to LV diastolic dysfunction (defined as an E:A ratio < 1).

CKD patients with diastolic dysfunction were older, had higher serum LDL-C, hs-CRP, and NT-proBNP than patients without diastolic dysfunction (Table 3). We detected higher SDMA in patients with diastolic dysfunction than in those without diastolic dysfunction, although the difference failed to reach significance. RT patients with diastolic dysfunction showed significantly increased creatinine, urea, and SAA in comparison with patients without diastolic dysfunction. However, GFR in those patients was significantly lower. In RT patients diagnosed with diastolic dysfunction, SDMA and NT-proBNP were significantly higher than in patients diagnosed without diastolic dysfunction.

To explore whether the association between SDMA and NT-proBNP and diastolic dysfunction were confounded by factors of renal function, SDMA and NT-proBNP in patients with and without diastolic dysfunction belonging to both study groups (CKD and RT groups) were compared after adjusting for GFR and duration of renal disease. After adjusting for duration of disease serum NT-proBNP in the RT patients with diastolic dysfunction were significantly higher than in the RT patients without diastolic dysfunction ($F = 7.478$, $P < 0.011$). However, after adjusting for GFR, NT-proBNP was not significantly increased in the patients diagnosed with diastolic dysfunction ($F = 2.631$, $P = 0.117$). NT-proBNP in patients with diastolic dysfunction was decreased by 36% (from 489.69 to 313.44 ng/l)

TABLE 2. Correlations Between NT-proBNP, SDMA and Demographic Markers of Renal Function, Serum Lipid, and Inflammatory Parameters

	NT-proBNP		SDMA	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Age (years)	0.294	0.001	0.388	<0.001
BMI (kg/m ²)	−0.200	0.058	−0.179	0.082
HD duration (months) ^a	0.171	0.118	−0.018	0.867
E/A ratio	−0.335	0.012	−0.282	0.027
LVEF (%)	−0.192	0.405	−0.172	0.469
GFR (ml/min/1.73m ²) ^a	−0.596	<0.001	−0.742	<0.001
Creatinine (μmol/l) ^a	0.693	<0.001	0.731	<0.001
Albumin (g/l)	−0.358	<0.001	−0.301	<0.001
Uric acid (μmol/l)	0.052	0.579	0.041	0.657
Urea (mmol/l)	0.584	<0.001	0.475	<0.001
TC (mmol/l)	−0.051	0.579	−0.136	0.136
LDL-C (mmol/l)	0.085	0.435	−0.069	0.509
HDL-C (mmol/l)	−0.434	<0.001	−0.370	<0.001
TG (mmol/l) ^a	0.014	0.884	−0.007	0.423
tHcy (μmol/l)	0.318	0.052	0.327	0.023
Fibrinogen (g/l)	0.379	<0.001	0.215	0.051
hs-CRP ^a (mg/l)	0.416	<0.001	0.082	0.452
IL-6 ^a (pg/ml)	0.234	0.042	0.061	0.576
SAA ^a (mg/l)	0.028	0.808	0.036	0.739
SDMA ^a (μmol/l)	0.499	<0.001	—	—

^aLogarithmic transformation of HD duration, GFR, creatinine, TG, hsCRP, IL-6, SAA, NT-proBNP, and SDMA values was performed before the analysis.

after adjusting for renal function. Similarly, in RT patients there was a significant effect of diastolic function on SDMA after controlling for the duration of disease ($F = 6.958$, $P = 0.014$), whereas GFR-adjusted SDMA did not show significantly different between RT patients with and without diastolic dysfunction ($F = 0.162$, $P = 0.690$). In RT patients with diastolic dysfunction SDMA decreased by 11% (from 1.39 to 1.24 μmol/l) after adjusting for GFR.

We also performed the analysis in CKD patients. The association between NT-proBNP, SDMA, and diastolic function was weaker in this group. Adjusted NT-proBNP and SDMA were not significantly higher in patients with diastolic dysfunction than in patients without diastolic dysfunction.

DISCUSSION

The current study has assessed the association between NT-proBNP and SDMA and impaired renal function in CKD and RT patients as well as the usefulness of such parameters as markers of diastolic dysfunction.

Measurement of BNP and NT-proBNP can identify patients at high risk of structural and functional cardiac abnormalities at different stages of CKD (17). We chose the determination of NT-proBNP as a more sensitive biomarker of heart damage in CKD and RT patients. In our study, CKD patients have higher NT-proBNP

than RT patients, but without statistical significance (Table 1). In addition, we found that NT-proBNP was still higher than the cut-off value to rule out heart failure (cut-off value ≤ 125 ng/l). Our results agree with those of Roberts et al. (18) who measured NT-proBNP in CKD patients, patients on dialysis, and RT patients (median levels: 559 ng/l, 3,909 ng/l, and 371 ng/l, respectively). Differences across groups were statistically significant. Within the groups, patients with a history of CVD had significantly higher NT-proBNP than those with no history of CVD, with the exception of RT patients. There are two possible explanations for this observation. First, renal transplantation had not completely restored the renal function in these patients. Second, although the RT patients had better kidney function than CKD patients, their heart function remain damaged due to long-term kidney disease. In our study, in accordance with other published data, NT-proBNP increased in parallel with decreasing renal function estimated as GFR ($r = -0.596$, $P < 0.001$). Mark et al. (19) also found significant negative correlation between BNP and GFR ($r = -0.40$, $P < 0.001$) in patients with renal disease not requiring hemodialysis and patients with renal allografts.

In multiple linear regression analysis independent predictors of higher NT-proBNP in the CKD group were older age, higher creatinine, and lower albumin. Mark et al. (19) found that the major determinants of log-transformed BNP were GFR, albumin, and age in joint

TABLE 3. Demographic Data, Lipid Status Parameters, Inflammatory Markers, NT-proBNP, and SDMA Concentrations in CKD and RT Patients With Different Diastolic Functions

	CKD patients Diastolic dysfunction			RT patients Diastolic dysfunction		
	Without (<i>n</i> = 75)	With (<i>n</i> = 20)	<i>P</i>	Without (<i>n</i> = 23)	With (<i>n</i> = 21)	<i>P</i>
Age (years)	34.7 ± 11.7	50.25 ± 10.09	0.002	34.6 ± 6.84	47.0 ± 8.49	<0.001
BMI (kg/m ²)	23.77 ± 4.17	20.63 ± 10.07	0.192	25.34 ± 3.87	25.31 ± 4.19	0.985
HD duration (months) ^a	17.57 (11.48–29.26)	41.28 (15.31–111.17)	0.108	94.32 (60.67–146.55)	156.8 (109.65–222.84)	0.072
GFR (ml/min/1.73m ²) ^a	24.24 (19.50–30.13)	23.97 (12.71–45.29)	0.963	45.09 (38.46–52.85)	28.49 (21.87–37.09)	0.004
Creatinine (μmol/l) ^a	274.26 (208.93–299.72)	217.69 (133.97–353.99)	0.498	146.07 (17.35–167.49)	200.01 (166.34–264.85)	0.008
Albumin (g/l)	40.67 ± 4.52	42.25 ± 2.76	0.355	43.38 ± 3.26	41.14 ± 2.91	0.059
Uric acid (μmol/l)	472.57 ± 109.03	452.25 ± 81.19	0.629	385.06 ± 89.65	416.33 ± 60.35	0.263
Urea (mmol/l)	15.81 ± 5.88	14.00 ± 7.82	0.481	9.54 ± 3.38	15.67 ± 7.33	0.004
TC (mmol/l)	5.41 ± 0.97	5.74 ± 1.17	0.425	5.68 ± 1.08	5.89 ± 1.17	0.613
LDL-C (mmol/l)	3.17 ± 0.74	3.96 ± 0.98	0.036	3.49 ± 0.88	3.72 ± 1.13	0.538
HDL-C (mmol/l)	1.19 ± 0.32	1.32 ± 0.26	0.286	1.26 ± 0.32	1.23 ± 0.31	0.763
TG (mmol/l) ^a	1.89 (1.57–2.27)	1.46 (0.87–2.46)	0.227	1.94 (1.47–2.54)	1.97 (1.62–2.40)	0.919
tHcy (μmol/l)	26.46 ± 8.39	18.73 ± 0.69	0.043	19.35 ± 4.92	24.25 ± 16.65	0.408
Fibrinogen (g/l)	4.28 ± 0.70	4.93 ± 1.04	0.073	4.51 ± 1.08	5.29 ± 2.14	0.206
hs-CRP ^a (mg/l)	0.37 (0.20–0.71)	1.98 (0.83–4.73)	0.009	1.20 (0.69–2.09)	2.08 (0.90–4.79)	0.236
IL-6 ^a (pg/ml)	2.21 (1.61–3.04)	1.75 (1.14–2.69)	0.393	3.44 (2.46–4.80)	5.54 (3.78–8.13)	0.052
SAA ^a (mg/l)	2.14 (1.65–2.78)	2.99 (1.64–5.47)	0.215	7.22 (5.22–9.99)	15.31 (7.88–29.72)	0.027
NT-proBNP ^a (ng/l)	78.43 (34.39–178.85)	277.05 (96.82–792.6)	0.047	121.76 (69.87–212.17)	489.69 (235.29–1019.2)	0.003
SDMA ^a (μmol/l)	1.34 (1.26–1.62)	1.40 (1.04–1.89)	0.708	1.07 (0.94–1.22)	1.39 (1.19–1.62)	0.009

Continuous variables are presented as mean ± standard deviation and compared by the Student *t*-test, whereas categorical variables are presented as relative frequencies and compared by the chi-square test.

^aValues for HD duration, GFR, creatinine, TG, hs-CRP, IL-6, SAA, NT-proBNP, and SDMA are presented as geometrical mean and 95% confidence intervals (CIs). Logarithmic transformation of the values was performed before the analysis.

E/A > 1 without diastolic dysfunction; E/A < 1 with diastolic dysfunction.

CKD and RT patients. Vickery et al. (20) reported that estimated GFR had an independent effect on plasma BNP ($P = 0.0028$) and, to a greater extent, plasma NT-proBNP ($P < 0.0001$) in patients with CKD. Relatively minor renal abnormalities such as a slightly reduced GFR or microalbuminuria even within the normal range may be associated with increased risk of cardiovascular events (21). The Prevention of Renal and Vascular End Stage Renal Disease (PREVEND) Study demonstrated that a twofold increase in urine albumin was associated with a 29% increase in relative risk for CKD mortality (22).

In the present study, we found significant correlations between NT-proBNP and inflammatory biomarkers: IL-6, hs-CRP, and fibrinogen (Table 2). The causes of this correlation could be related to a multitude of different factors. It had been known that proinflammatory cytokines promote the body's response to inflammation. In addition, IL-6 stimulates the liver to produce acute-phase reactants such as CRP and fibrinogen. The IL-6 is a particularly interesting molecule, since it has effects on volume overload, renal function, retention of uremic solutes, and chronic heart failure (23). Tanaka and colleagues reported that IL-6 significantly increases expression of ANP and BNP mRNA levels in cultured rat ventricular myocytes. This finding suggests that IL-6 might regulate levels of circu-

latory natriuretic peptides through IL-6 receptors in the myocyte (24).

Therefore, the cause of correlation between NT-proBNP and inflammatory biomarkers in our patients may reflect influence of IL-6 on cardiac and renal function.

The current challenge is to ascertain the mechanism underlying the relationship between NT-proBNP and renal function. NT-proBNP clearance in humans is not well understood, but small amounts of intact NT-proBNP are recoverable in urine, suggesting that its blood level depends on renal clearance. Elevated levels of NT-proBNP in our patients may simply reflect decreased clearance and it is in accordance with findings of Spanaus et al. (25) that increased NT-proBNP predisposes to increased risk of accelerated progression of CKD to end-stage renal disease. However, the widespread of NT-proBNP concentrations among patients in the PRIDE study suggests that reduced clearance is only one mechanism of the elevation of NT-proBNP levels in renal patients (26). Because NT-proBNP clearance occurs only in the kidney, increased NT-proBNP in blood depends on both renal and cardiac functions (27).

Recent reports have identified ADMA and SDMA to be associated with early renal impairment in CVD patients

(28). We examined whether SDMA was related to other variables important for kidney disease and cardiovascular risk. Our study revealed that elevated serum SDMA was present in both examined groups with regard to reference range. SDMA was significantly higher in CKD than in RT patients (Table 1). Fleck et al. (29) found that plasma ADMA and SDMA were significantly elevated in patients with chronic renal failure. The increase was more pronounced for SDMA (mean value 2.05 $\mu\text{mol/l}$). After kidney transplantation, SDMA returned to its baseline value (mean value 1.15 $\mu\text{mol/l}$) but that of ADMA remained raised. Fleck's results are similar to ours.

Interestingly, SDMA did not significantly correlate with inflammatory parameters such as hsCRP and IL-6 in our CKD group. In a study by Bode-Böger et al. (30), SDMA did not significantly correlate with CRP, but it did with IL-6. A recent study showed that SDMA had a proinflammatory effect via stimulation of reactive oxygen species (ROS) production in monocytes, which could be a trigger of vascular damage (31). This proinflammatory effect together with the indirect effects of SDMA on NO synthesis, and the relationship between SDMA and renal function may be mechanisms through which SDMA and CVD are linked. Determining the exact relationship between SDMA and inflammation in CKD is a goal for further investigative studies.

Using multiple regression analysis we demonstrated that SDMA, after adjustment for the examined parameters was significantly associated with GFR and NT-proBNP in CKD patients and creatinine in RT patients, which agrees with the study of Codognotto et al. (32). Fliser et al. (33) showed that SDMA highly correlated with both GFR and serum creatinine in 227 patients with nondiabetic kidney disease and mild to moderate renal failure. Meinitzer et al. (34) showed that in linear regression analyses including common cardiovascular risk factors as covariates, SDMA and ADMA were significantly associated with NT-proBNP in 3,229 patients who underwent coronary angiography and that the regression coefficients were higher for SDMA than for ADMA.

In RT patients, age and urea were independent predictors of NT-proBNP and creatinine was an independent predictor of SDMA. Fliser et al. (33) found that the correlation coefficient for SDMA and GFR was almost identical to that of serum creatinine and GFR. Such findings suggest that urinary excretion is the main elimination pathway for SDMA. The results of our study agree with previous reports and we confirmed that SDMA was a sensitive marker of renal dysfunction.

Our aim was also to investigate the usefulness of NT-proBNP and SDMA as biomarkers of diastolic dysfunction as the utility of biomarkers has been more extensively studied for detection of systolic than diastolic heart function. Lubien et al. (35) found that, in the absence of

LV systolic dysfunction, plasma BNP levels were significantly higher in patients with LV diastolic dysfunction (as assessed by echocardiography) compared with subjects without LV diastolic dysfunction.

In our study, the association between the plasma level of NT-proBNP as well as SDMA and diastolic dysfunction appeared to be greater among RT patients than among CKD patients. However, RT patients with diastolic dysfunction had higher IL-6 and SAA as markers of inflammation. This could be a reason for accelerating atherosclerosis and its clinical complications in such patients despite better kidney function. Numerous studies have already reported an independent association between cardiovascular outcomes and different markers of inflammation in patients with CKD.

Likewise, RT patients with diastolic dysfunction had higher levels of NT-proBNP and SDMA than patients without diastolic dysfunction. This could be due to either heart failure or a consequence of long-term renal disease. We adjusted NT-proBNP and SDMA for duration of renal disease to show that an increase in both parameters in subjects with diastolic dysfunction was not a consequence of duration of renal disease. According to the results of ANCOVA analysis, both parameters were significantly higher in patients with diastolic dysfunction, regardless of the duration of renal disease. Meanwhile, after adjustment of NT-proBNP and SDMA for GFR both parameters were not significantly different between the two examined groups. Natriuretic peptide and SDMA were dependent on GFR in patients with diastolic dysfunction. These observations provide a mechanistic link between heart-kidney interactions. Mark et al. (19) found that GFR was a more important determinant of serum BNP than ventricular function in CKD and RT patients.

This study highlights the importance of renal dysfunction in determining serum natriuretic peptide levels. Reduced GFR had the greatest influence on elevated peptide concentrations. Although elevated peptide concentrations are associated with worse prognosis, there is agreement in the literature that current decision thresholds for BNP without adjustment for kidney function should not be applied (19). Because BNP is a good indicator of congestive heart failure, one would assume that NT-proBNP would be as well. The data presented here support this assumption (17). Moreover, we showed an association of NT-proBNP levels with inflammatory markers in these patients. However, we are aware that present study has an important limitation that must be addressed. Because of our rather small cohorts, further study will be needed in a larger number of patients to confirm these findings and to explore the associated mechanisms. This study should serve as a basis for further clinical investigation, in order to evaluate a potential role for examined biomarkers

in clinical decision making and management of patients with both renal and cardiac disease.

CONCLUSION

Our results indicate that CKD influences NT-proBNP and SDMA. In addition, our findings indicate that NT-proBNP represents a biochemical marker providing positive evidence of the presence of diastolic dysfunction with an absence of systolic dysfunction in CKD and RT patients. We also confirmed that NT-proBNP and SDMA are sensitive markers of renal function decline. Clinical trials aimed to determine such markers, especially SDMA, are certainly required. We believe that future studies will shed light on the observed associations and their possible consequences on CVD risk in this category of patients.

ACKNOWLEDGMENTS

The work was financially supported by the Ministry of Science and Technological Development, Republic of Serbia (Project No. 145036) and COST ACTION BM0904.

REFERENCES

- Liefeldt L, Budde K. Risk factors for cardiovascular disease in renal transplant recipients and strategies to minimize risk. *Transplant Int* 2010;23:1191–1204.
- Kronenberg F. Emerging risk factors and markers of chronic kidney disease progression. *Nat Rev Nephrol* 2009;5:677–689.
- Suzuki T, Yamazaki T, Yazaki Y. The role of the natriuretic peptides in the cardiovascular system. *Cardiovasc Res* 2001;51:489–494.
- Clerico A, Recchia FA, Passino C, Emdin M. Cardiac endocrine function is an essential component of the homeostatic regulation network: Physiological and clinical implications. *Am J Physiol Heart Circ Physiol* 2006;290:H17–H29.
- Clerico A, Emdin M. Diagnostic accuracy and prognostic relevance of the measurement of cardiac natriuretic peptides: A review. *Clin Chem* 2004;50:33–50.
- MCCullough PA, Duc P, Omland T, McCord J, Nowak RM, Hollander JE. B-type natriuretic peptide and renal function in the diagnosis of heart failure: An analysis from the Breathing Not Properly Multinational Study. *Am J Kidney Dis* 2003;41:571–579.
- Redfield MM, Rodeheffer RJ, Jacobsen SJ, Mahoney DW, Bailey KR, Burnett JC. Plasma brain natriuretic peptide to detect preclinical ventricular systolic or diastolic dysfunction. A community-based study detect preclinical ventricular systolic or diastolic dysfunction. A community-based study. *Circulation* 2004;109:3176–3181.
- Leiper J, Vallance P. Biological significance of endogenous methylarginines that inhibit nitric oxide synthases. *Cardiovasc Res* 1999;43:542–548.
- Kendrick J, Chonchol MB. Non-traditional risk factors for cardiovascular disease in patients with chronic kidney disease. *Nat Clin Pract Nephrol* 2008;4:672–681.
- Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 1992;339:572–575.
- Kielstein JT, Böger RH, Bode-Böger SM, et al. Asymmetric dimethylarginine plasma concentrations differ in patients with ESRD: Relationship to treatment method and atherosclerotic disease. *J Am Soc Nephrol* 1999;10:594–600.
- Fleck C, Janz A, Schweitzer F, Karge E, Schwertfeger M, Stein G. Serum concentrations of asymmetric (ADMA) and symmetric (SDMA) dimethylarginine in renal failure patients. *Kidney Int* 2001;59(Suppl 78):S14–S18.
- Sarnak MJ, Levey AS, Schoolwerth AC, et al. Kidney disease as a risk factor for development of cardiovascular disease. A statement from the American Heart Association Councils on kidney in cardiovascular disease, high blood pressure research, clinical cardiology, and epidemiology and prevention. *Circulation* 2003;108:2154–2169.
- Lang RM, Bierig M, Devereaux RB, et al. Chamber Quantification Writing Group, American Society of Echocardiography's Guidelines and Standards Committee, European Association of Echocardiography. Recommendations for chamber quantification: A report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr* 2005;18:1440–1463.
- Appleton CP, Jensen JL, Hatle LK, Oh JK. Doppler evaluation of left and right ventricular diastolic function: A technical guide for obtaining optimal flow velocity recordings. *J Am Soc Echocardiogr* 1997;10:271–291.
- Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation: Modification of diet in renal disease study group. *Ann Intern Med* 1999;130:461–470.
- Luchner A, Hengstenberg C, Lowel H, Riegger GAJ, Schunkert H, Holmer S. Effect of compensated renal dysfunction on approved heart failure markers: Direct comparison of brain natriuretic peptide (BNP) and N-terminal pro-BNP. *Hypertension* 2005;46:118–123.
- Roberts MA, Srivastava PM, Macmillan N, et al. B-type natriuretic peptides strongly predict mortality in patients who are treated with long-term dialysis. *Clin J Am Soc Nephrol* 2008;3:1057–1065.
- Mark PB, Stewart GA, Gansevoort RT, et al. Diagnostic potential of circulating natriuretic peptides in chronic kidney disease. *Nephrol Dial Transplant* 2006;21:402–410.
- Vickery S, Price CP, Ian John R, et al. B-type natriuretic peptide (BNP) and amino-terminal proBNP in patients with CKD: Relationship to renal function and left ventricular hypertrophy. *Am J Kidney Dis* 2005;46(4):610–620.
- Schiffrin EL, Lipman ML, Mann JFE. Chronic kidney disease: Effects on the cardiovascular system. *Circulation* 2007;116:85–97.
- Hillege HL, Fidler V, Diercks GFH, et al. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation* 2002;106:1777–1782.
- Stenvinkel P, Ketteler M, Johnson RJ, et al. IL-10, IL-6, and TNF- α : central factors in the altered cytokine network of uremia—the good, the bad, and the ugly. *Kidney Int* 2005;67:1216–1233.
- Tanaka T, Kanda T, Takanashi T, Saegusa S, Moriya J, Kurabayashi M. Interleukin-6-induced reciprocal expression of SERCA and natriuretic peptides mRNA in cultured rat ventricular myocytes. *J Int Med Res* 2004;32:57–61.
- Spanaus KS, Kronenberg F, Ritz E, et al. B-type natriuretic peptide concentrations predict the progression of nondiabetic chronic kidney disease: The Mild-to-Moderate Kidney Disease Study. *Clin Chem* 2007;53(7):1264–1272.
- Anwaruddin S, Lloyd-Jones DM, Baggish A, et al. Renal function, congestive heart failure and amino-terminal pro-brain natriuretic

- peptide measurement: Results from the proBNP investigation of dyspnea in the emergency department (PRIDE) study. *J Am Coll Cardiol* 2006;47:91–97.
27. Srisawasdi P, Vanavan S, Charoenpanichkit C, Kroll MH. The effect of renal dysfunction on BNP, NT-proBNP and their ratio. *Am J Clin Pathol* 2010;133:14–23.
 28. Wang J, Sim AS, Wang XL, et al. Relations between markers of renal function, coronary risk factors and the occurrence and severity of coronary artery disease. *Atherosclerosis* 2008;197:853–859.
 29. Fleck C, Janz A, Schweitzer F, et al. Serum concentrations of asymmetric (ADMA) and symmetric (SDMA) dimethylarginine in renal failure patients. *Kidney Int* 2001;59(Suppl 78):S14–S18.
 30. Bode-Böger SM, Scalera F, Kielstein JT, et al. Symmetrical dimethylarginine: A new combined parameter for renal function and extent of coronary artery disease. *J Am Soc Nephrol* 2006;17:1128–1134.
 31. Schepers E, Glorieux G, Dhondt A, Leybaert L, Vanholder R. Role of symmetric dimethylarginine in vascular damage by increasing ROS via store-operated calcium influx in monocytes. *Nephrol Dial Transplant* 2009;24:1429–1435.
 32. Codognotto M, Piccoli A, Rubini C, et al. Determinants of circulating asymmetric and symmetric dimethylarginines in patients evaluated for acute dyspnea. *Clin Chem Lab Med* 2011;49(2):237–242.
 33. Fliser D, Kronenberg F, Kielstein JT, et al. Asymmetric dimethylarginine and progression of chronic kidney disease: The Mild to Moderate Kidney Disease Study. *J Am Soc Nephrol* 2005;16:2456–2461.
 34. Meinitzer A, Kielstein JT, Pilz S, et al. Symmetrical and asymmetrical dimethylarginine as predictor for mortality in patients referred for coronary angiography: The Ludwigshafen risk and cardiovascular health study. *Clin Chem* 2011;57(1):112–121.
 35. Lubien E, DeMaria A, Krishnaswamy P, et al. Utility of B-natriuretic peptide in detecting diastolic dysfunction: Comparison with Doppler velocity recordings. *Circulation* 2002;105:595–601.