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Clinical Study

Assessment of Endothelial Dysfunction: The Role of Symmetrical Dimethylarginine and Proinflammatory Markers in Chronic Kidney Disease and Renal Transplant Recipients

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Objectives. The study was designed to evaluate associations between symmetric dimethylarginine (SDMA), inflammation, and superoxide anion ($O_2^{\bullet-}$) with endothelial function and to determine their potential for screening of endothelial dysfunction in patients with chronic kidney disease (CKD) and renal transplant (RT) recipients. *Materials and Methods*. We included 64 CKD and 52 RT patients. Patients were stratified according to brachial artery flow-mediated dilation (FMD). *Results*. Logistic regression analysis showed that high SDMA and high sensitive C-reactive protein (hs-CRP) were associated with impaired FMD in CKD and RT patients, after adjustment for glomerular filtration rate. The ability of inflammation, SDMA, and $O_2^{\bullet-}$ to detect impaired FMD was investigated by receiving operative characteristic analysis. Hs-CRP (area under the curves (AUC) = 0.754, $P < 0.001$), IL-6 (AUC = 0.699, $P = 0.002$), and SDMA (AUC = 0.689, $P = 0.007$) had the highest ability to detect impaired FMD. SDMA in combination with inflammatory parameters and/or $O_2^{\bullet-}$ had better screening performance than SDMA alone. *Conclusions*. Our results indicate a strong predictable association between hs-CRP, SDMA, and endothelial dysfunction in CKD patients and RT recipients. The individual marker that showed the strongest discriminative ability for endothelial dysfunction is hs-CRP, but its usefulness as a discriminatory marker for efficient diagnosis of endothelial dysfunction should be examined in prospective studies.

1. Introduction

Patients with chronic kidney disease (CKD) are at high risk of developing cardiovascular disease. In addition, renal transplant (RT) recipients are 10 times more likely to suffer from fatal cardiac events compared with the general population [1]. Many risk factors, traditional and nontraditional, are involved in the pathogenesis of atherosclerosis in these patients.

Endothelial dysfunction, oxidative stress, and inflammation are recognised as new risk factors in CKD patients. Nevertheless, interplay between these factors remains largely unexplored.

Impairment of endothelium-dependent vasodilatation has largely been attributed to reduced availability or biological activity of nitric oxide (NO). NO is synthesized from L-arginine in a reaction catalysed by NO synthase (NOS).

Methionine is metabolised to homocysteine by a demethylation pathway. The final result of this pathway is the formation of asymmetric dimethyl-L-arginine (ADMA), an NOS inhibitor. Increased ADMA may limit intracellular L-arginine availability for NO synthesis or inhibit NOS. In

addition, homocysteine mediates superoxide anion ($O_2^{\bullet-}$) production. $O_2^{\bullet-}$ reacts with NO and forms highly reactive peroxynitrite capable of causing tissue damage. Furthermore, $O_2^{\bullet-}$ increases ADMA by affecting the activities of protein arginine N-methyltransferases that synthesize ADMA and dimethylaminohydrolase which metabolises ADMA. Symmetric dimethylarginine (SDMA) is an inactive stereoisomer produced alongside ADMA, and it has recently been described as a risk factor for cardiovascular events. SDMA is probably important as a competitive inhibitor of arginine transport across cell membranes [2, 3]. There is growing evidence that increased plasma ADMA leads to cardiovascular effects in CKD patients [4]. Furthermore, elevated plasma ADMA is associated with increased morbidity, mortality, and the deterioration of graft function in RT recipients [5].

The aetiology of endothelial dysfunction is complicated. It is explained by alter vasoconstrictive/vasodilatatory function, by triggering inflammatory processes (through NO and adhesion molecules such as ICAM-1, VCAM-1, and E-selectin) and by affecting haemostasis (through release of tissue factor, von Willebrand factor, thromboxane A2, and fibrinogen) [6]. It is therefore unlikely that a single biomarker will provide accurate information for endothelial dysfunction occurrence. Accordingly, simultaneous measurement of several biomarkers and formulation of models that have incremental value in endothelial dysfunction screening in comparison to single biomarker analysis would be useful. For this reason, numerous researchers are focussed on finding new markers with high diagnostic accuracy that would provide a powerful clinical tool for endothelial dysfunction screening. We have chosen to centre our study on biomarkers characteristic of nontraditional risk factors: SDMA as an endothelial marker, $O_2^{\bullet-}$ as a marker of oxidative stress, and inflammatory markers such as high sensitive C-reactive protein (hs-CRP), interleukin-6 (IL-6), and serum amyloid A (SAA).

The aim of our study was to determine associations between SDMA, inflammation, and $O_2^{\bullet-}$ with endothelial function and to evaluate their potential for screening of endothelial dysfunction in patients with CKD and RT recipients.

2. Material and Methods

This study included 64 stage 2–5 CKD patients not requiring dialysis and 52 RT recipients at least 6 months after transplantation. All subjects were examined at the Nephrology Clinic, Clinical Centre of Serbia, and provided information regarding medication and medical history. Diabetics and patients with acute inflammatory disease were excluded. Forty patients received a kidney from a related living donor and twelve from a cadaver. These patients had previous transplantation duration of 9.56 ± 5.27 years. Any history of hypertension, ischemic vascular disease including myocardial infarction, angina pectoris, and cerebral stroke, heart failure, and smoking were obtained by interview. All patients with recent myocardial infarction and those with heart failure were excluded. 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 disease received corticosteroids or other immunosuppressive therapy according to standard protocols.

The diameter of the brachial artery was measured for assessment of endothelial function, and blood was drawn for biochemical measurements. Patient data included demographic (sex and age), clinical (duration of renal disease), and biochemical variables. Clinical and immunological observations as well as laboratory parameters and endothelial function were all 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.

2.1. Endothelial Function. This was performed according to the method originally described by Celermajer et al. [7]. Measurements were performed between 1:00 p.m. and 3:00 p.m. All subjects were instructed not to eat for at least 4 hours before the procedure or consume alcohol, or caffeine-containing drinks for at least 24 hours prior to the procedure. Patients lied supine in a quiet temperature-controlled room for 10 minutes before the examination. The diameter of the brachial artery was measured from 2D ultrasound images using a commercially available system (Agilent Image Point HX) and images were recorded on VHS tape. A baseline image was obtained at rest 2 to 15 cm above the antecubital fossa incident with the R-wave on the electrocardiogram. To create a flow stimulus in the brachial artery, a blood pressure cuff was placed on the forearm and inflated to at least 50 mmHg above systolic pressure to occlude arterial inflow for 5 minutes. A second measurement was performed after 60 to 80 seconds after cuff release to endothelial-dependant flow-mediated vasodilatation of the brachial artery (FMD). For each measurement, a minimum of three cardiac cycles was averaged. FMD was calculated as the percentage change in diameter compared with baseline resting diameter.

2.2. Biochemical Measurements. Blood sampling was performed after 12 hours of fasting overnight in order to measure the following parameters: serum SDMA, amino-terminal probrain natriuretic peptide (NT-proBNP), creatinine, urea, uric acid, albumin, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, hs-CRP, IL-6, SAA and plasma fibrinogen, and $O_2^{\bullet-}$.

The serum samples for determination of SDMA, NT-proBNP, and IL-6 were stored at −80°C in aliquots until analysis. Other parameters were analysed on the day of collection. Citrated plasma was stored at −70°C before measurement of fibrinogen. For the measurement of $O_2^{\bullet-}$, plasma from heparinised blood samples was used immediately.

Measurement of serum SDMA was determined using an ELISA (DLD Diagnostica GMBH, Hamburg, Germany).

NT-proBNP was determined with an ELFA (bioMerieux, Vidas, Lyon, France). Creatinine, urea, uric acid, albumin, and lipids were analysed employing 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 level was measured with a highly sensitive colorimetric sandwich ELISA kit (Human IL-6 Quantikine HS ELISA kit; R&D Systems, GmbH, Germany). The rate of nitroblue tetrazolium reduction was used to measure the rate of $O_2^{\bullet-}$ generation, as described by Auclar and Voisin [8] (the intraassay CV was 5.6%, and the interassay CV was 9.5%). The SDMA reference range is 0.3–0.7 μmol/L.

Assessment of renal function was performed via estimated glomerular filtration rate (GFR) using the modified diet in renal disease equation [9]. 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 following formula: weight (kg)/height^2 (m^2).

2.3. Statistical Analyses. Differences in continuous variables between the groups were analysed by Student's *t*-test for normally distributed variables. Variables with non-Gaussian distribution were log-transformed to achieve normality. Adjusted mean levels of SDMA were estimated by analysis of covariance. Group differences for categorical variables were examined by the Chi-square test. The CKD and RT patients were first stratified into tertiles according to inflammatory parameters, SDMA, and $O_2^{\bullet-}$ concentrations. CKD patients with hs-CRP (hs-CRP ≥ 0.89 mg/L), IL-6 (IL ≥ 3.5 pg/mL), SAA (SAA ≥ 2.5 mg/L), SDMA (SDMA ≥ 1.79 μmol/L), and $O_2^{\bullet-}$ ($O_2^{\bullet-}$ ≥ 57.5 μmol/min L) in the upper tertile were defined as having high hs-CRP, IL-6, SAA, SDMA, and $O_2^{\bullet-}$. Similarly, RT patients with hs-CRP (hs-CRP ≥ 2.02 mg/L), IL-6 (IL ≥ 6.1 pg/mL), SAA (SAA ≥ 11.6 mg/L), SDMA (SDMA ≥ 1.30 μmol/L), and $O_2^{\bullet-}$ ($O_2^{\bullet-}$ ≥ 50.1 μmol/min L) in the upper tertile were defined as having high hs-CRP, IL-6, SAA, SDMA, and $O_2^{\bullet-}$. The low risk values for examined parameters (lower than the upper tertile) were coded 0, while the high risk values (higher than the upper tertile) were coded 1. Odds ratios (ORs) were calculated using binary logistic regression analysis to determine whether high levels of inflammatory parameters, SDMA, and $O_2^{\bullet-}$ had any potential for the prediction of impaired FMD. Factors that were significant in univariate analysis were adjusted for GFR concentrations in multivariate logistic regression analysis to determine the adjusted OR. Accuracy of the examined parameters was assessed using receiving operative characteristic (ROC) curve analysis. Parameters with significant accuracy were combined with other parameters, curves for these models were plotted and the area under ROC curves (AUC) was presented as C statistics from the analysis. For internal validation of the statistically significant models, we used the percentile bootstrap method, with 1000 resampling simulations, with the aid of the "boot" package of R.

Data are shown as mean ± standard deviation for normally distributed continuous variables and as relative or 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 (2005, Alpha Omnia, Belgrade, Serbia), MedCalc for Windows version 9.6.3. (Mariakerke, Belgium), and R version 2.15.3. The minimal statistical significance was set at $P < 0.05$.

3. Results

To evaluate the effect of endothelial function on the examined parameters, we stratified CKD and RT patients into FMD groups. Because of the lack of reference ranges for FMD, we defined the cutoff values for impaired and nonimpaired FMD groups [10]. The patients had apparently impaired endothelial function (the first tertile of the FMD values) if the percentage change in diameter compared with baseline resting diameter of the brachial artery was lower than 3.8% in CKD and 2.19% in RT patients. However, if the patients had a higher percentage for FMD (the second and third tertiles of the FMD values), they were considered as individuals with apparently nonimpaired endothelial function.

There were no differences in age and gender between the FMD groups in CKD and RT patients. In CKD patients inflammatory parameters (hs-CRP, IL-6, and SAA) were significantly higher in those with impaired FMD, whereas HDL cholesterol and uric acid were higher in those with nonimpaired FMD (Table 1). Moreover, SDMA was significantly higher in CKD patients with impaired FMD. RT patients with impaired FMD had higher LDL cholesterol and lower albumin.

To explore whether the association between inflammatory parameters, SDMA, lipoproteins, uric acid and albumin with endothelial dysfunction was confounded by other factors, their concentrations in patients with and without impaired FMD belonging to both study groups (CKD and RT groups) were compared after adjusting for GFR. After adjusting for GFR, inflammatory parameters in the CKD patients with impaired FMD were significantly higher than the corresponding values in the CKD patients with nonimpaired FMD ($F = 16.065$, $P < 0.001$ for hs-CRP, $F = 5.624$, $P = 0.026$ for IL-6, and $F = 8.201$, $P = 0.008$ for SAA). However, after adjusting for GFR, SDMA and uric acid were not significantly increased and HDL-cholesterol was not significantly decreased in the patients diagnosed with impaired FMD ($F = 1.33$, $P = 0.262$ for SDMA, $F = 3.58$, $P = 0.069$ for uric acid, and $F = 1.198$, $P = 0.283$ for HDL-cholesterol).

Accordingly, in the RT group, there was a significant effect of impaired FMD on LDL-cholesterol after controlling for the GFR ($F = 4.817$, $P = 0.039$). In contrast, adjusted albumin was not significantly different between RT patients with impaired and nonimpaired FMD ($F = 1.775$, $P = 0.197$).

We used binary logistic regression to determine whether high hs-CRP, IL-6, SAA, SDMA, and $O_2^{\bullet-}$ had any potential

Table 1: Demographic data, lipid status parameters, inflammatory markers and SDMA concentrations in CKD and RT patients with impaired and nonimpaired FMD.

	CKD patients FMD			RT patients FMD		
	Non-impaired ($n = 44$)	Impaired ($n = 20$)	P	Non-impaired ($n = 36$)	Impaired ($n = 16$)	P
Age, years	38.4 ± 12.7	44.2 ± 12.0	0.238	39.5 ± 8.8	39.0 ± 8.6	0.894
Male, %	45.5	40.0	0.773	61.1	62.5	0.946
BMI, kg/m^2	23.63 ± 3.40	23.09 ± 10.44	0.875	25.48 ± 4.30	25.18 ± 3.11	0.856
CKD duration, months*	20.27 (10.59–38.80)	88.80 (25.85–151.74)	0.076	140.44 (103.08–177.81)	145.63 (92.79–198.45)	0.811
Creatinine, μmol/L*	233.94 (189.45–288.88)	412.80 (202.65–622.94)	0.164	165.99 (138.46–198.99)	222.13 (141.40–302.85)	0.202
GFR, mL/min/1.73 m^2	27.98 ± 13.48	19.54 ± 11.78	0.104	40.97 ± 13.86	32.95 ± 13.48	0.182
Albumin, g/L	40.18 ± 5.93	37.40 ± 7.07	0.256	43.47 ± 3.24	40.57 ± 2.70	0.049
Uric acid, μmol/L	475.45 ± 88.20	403.40 ± 79.88	0.035	408.39 ± 71.39	382.38 ± 89.62	0.435
Urea, mmol/L*	15.20 (12.56–17.85)	19.20 (13.44–24.95)	0.157	10.47 (8.36–13.12)	14.02 (9.30–18.74)	0.229
Cholesterol, mmol/L	5.51 ± 1.20	5.25 ± 1.53	0.596	5.63 ± 0.97	6.49 ± 1.19	0.064
LDL-cholesterol, mmol/L	3.38 ± 0.99	3.46 ± 1.36	0.859	3.41 ± 0.64	4.25 ± 1.02	0.019
HDL-cholesterol, mmol/L	1.22 ± 0.22	1.06 ± 0.18	0.045	1.28 ± 0.39	1.30 ± 0.31	0.904
Triglyceride, mmol/L*	1.58 (1.25–2.00)	1.73 (1.16–2.58)	0.653	1.78 (1.47–2.17)	2.05 (1.75–2.40)	0.232
Fibrinogen*, g/L	4.51 (4.02–5.06)	5.38 (4.03–7.21)	0.166	4.58 (3.94–5.31)	4.65 (3.55–6.08)	0.909
hs-CRP*, mg/L	0.45 (0.28–0.73)	5.96 (1.02–34.91)	0.010	1.53 (0.74–3.17)	1.79 (0.87–3.71)	0.772
IL-6*, pg/mL	1.93 (1.44–2.60)	4.58 (3.21–6.53)	<0.001	4.29 (2.96–6.19)	3.97 (2.64–5.97)	0.802
SAA*, mg/L	2.18 (1.92–2.47)	3.78 (2.06–6.97)	0.006	9.86 (5.96–16.37)	8.88 (3.71–21.28)	0.809
NT-proBNP*, ng/L	232.27 (79.43–679.20)	645.65 (245.47–1258.1)	0.104	199.99 (91.83–435.51)	348.34 (183.65–660.69)	0.353
SDMA*, μmol/L	1.34 (1.19–1.51)	1.97 (1.69–2.28)	<0.001	1.25 (1.10–1.42)	1.30 (0.90–1.89)	0.759
$O_2^{\bullet-}$, μmol/min L	49.77 (34.83–71.29)	28.84 (9.86–84.14)	0.154	46.56 (33.73–64.27)	44.67 (35.73–55.85)	0.860

Continuous variables are presented as mean ± standard deviation and compared by Student's t-test, whereas categorical variables are presented as relative frequencies and compared by Chi-square test.

*Values for CKD duration, creatinine, urea, TG, fibrinogen, hs-CRP, IL-6, SAA, NT-proBNP, SDMA, and $O_2^{\bullet-}$ are presented as geometrical mean and 95% confidence intervals (CIs). Logarithmic transformation of the values was performed before the analysis.

for the prediction of impaired FMD. Unadjusted analysis showed that high hs-CRP and SDMA were associated with impaired FMD (Table 2) in CKD and RT patients. SDMA and hs-CRP levels were associated with impaired FMD in multivariate analysis (adjustment for GFR), indicating that high values of both parameters, independently of GFR, could predict impaired FMD (Table 2).

The ability of inflammation, SDMA, and $O_2^{\bullet-}$ to detect impaired FMD was investigated by ROC curve analysis (Table 3). The ROC results indicated that hs-CRP (AUC = 0.754, $P < 0.001$), IL-6 (AUC = 0.699, $P = 0.002$), and SDMA (AUC = 0.689, $P = 0.007$) had the highest ability to detect impaired FMD (Table 3). From examined parameters, the highest sensitivity for impaired FMD was 84.8% for SDMA, and the highest specificity was for hs-CRP (75.8%). A combination of SDMA or $O_2^{\bullet-}$ with hs-CRP did not increase hs-CRP's ability to discriminate impaired FMD from nonimpaired FMD. Nevertheless, the combination of hs-CRP with SDMA and $O_2^{\bullet-}$ slightly increased the discriminative ability of hs-CRP alone (AUC = 0.756, $P = 0.004$). We also investigated the potential benefit of adding SDMA or/and $O_2^{\bullet-}$ to IL-6 in order to better discriminate subjects with impaired FMD from subjects with nonimpaired FMD. The addition of SDMA increased the AUC for IL-6 (AUC = 0.732, $P = 0.001$). On the other hand, the AUC for the combination of SDMA, $O_2^{\bullet-}$, and IL-6 was lower (AUC = 0.724, $P = 0.003$). SDMA in combination with inflammatory parameters and/or $O_2^{\bullet-}$ had better screening performance

Table 2: OR for impaired FMD in CKD and RT patients.

	Unadjusted OR	OR adjusted for GFR
SDMA	5.25 (1.894–14.550) $P = 0.001$	3.342 (1.030–10.847) $P = 0.045$
hs-CRP	4.24 (1.677–10.746) $P = 0.002$	3.738 (1.450–9.638) $P = 0.006$
IL-6	2.10 (0.841–5.262) $P = 0.112$	/
SAA	2.381 (1.000–5.665) $P = 0.051$	/
$O_2^{\bullet-}$	0.844 (0.313–2.280) $P = 0.739$	/

OR: odds ratio; CI: confidence interval. SDMA, hs-CRP, IL-6, SAA, and $O_2^{\bullet-}$: categorical variables.

than SDMA alone. This improvement in AUCs for all models was not higher than the AUC for hs-CRP alone. After internal validation of the models, the mean hs-CRP AUC of the 1000 bootstrap samples was 0.735 (95% CI: 0.628–0.840). The application of the hs-CRP, SDMA, and $O_2^{\bullet-}$ model to the same bootstrap samples yielded a mean AUC of 0.741 (95% CI: 0.616–0.859) (Table 3). Both the mean AUCs showed useful discriminative ability for impaired FMD.

Assuming that no patients with impaired FMD should be missed when screening (i.e., sensitivity of 100%), the hs-CRP, SDMA, and $O_2^{\bullet-}$ model achieved a specificity of 29.2% compared to 24.2% using hs-CRP alone. However, for the low predicted probability (0.10 or less) for impaired FMD, false negative value was 0% for both models.

4. Discussion

Our findings suggest that impaired endothelial function is associated with increased inflammatory activity in CKD patients, as assessed by the measurement of several biochemical markers such as IL-6, hs-CRP and SAA. The current results were expected as IL-6 promotes synthesis of CRP, and SAA in the liver while CRP decreases NOS expression [11, 12].

Only a few reports described associations between IL-6, CRP, and/or SAA and endothelial dysfunction in RT recipients. Cueto-Manzano et al. [13] demonstrated that after renal transplantation, CRP decreases despite a rise in IL-6. Endothelial function is improved after renal transplantation, but not in the early posttransplant period [14]. In the long term, renal transplantation improves endothelial function, but not inflammation. This finding suggests that some other factors influence posttransplant inflammatory activity of individual markers. The results of our study agree with these previous reports because we did not find any differences in inflammatory markers between the FMD groups in RT recipients.

An increase in LDL-cholesterol is common after renal transplantation, due to immunosuppressive therapy and posttransplant renal function. LDL-cholesterol was significantly different when RT patients with and without FMD dysfunction, after adjustment for GFR, were compared [15, 16]. This finding indicates that LDL- cholesterol alters endothelial function which is an early step in the development of cardiovascular disease.

A number of studies have highlighted a connection between ADMA and endothelial dysfunction in nonrenal and renal patients [2, 17–19]. Among patients with chronic renal failure, markers of oxidative excess correlate with endothelial dysfunction. Reactive oxygen species (ROS) decrease NO bioavailability and upregulate adhesion molecules (VCAM-1, ICAM-1, and E-selectin) and chemotactic molecules (macrophage chemoattractant peptide-1) [20]. The expression of these molecules plays an initial role in an inflammatory process and ruinous step of forming foaming cells of atherosclerotic plaque. Probably, there are other factors to contribute to these mechanisms. For instance, in diabetic patients ROS also enhance expression of interleukin-6 leading to a vicious circle of diabetic nephropathy [21]. Also, reduced NO downregulates its function in preventing adhesion and aggregation of other cells such as platelets through cyclic guanosine monophosphate, so that the rolling phenomenon of platelets over von Willebrand factor predominates and the initial signal for thrombosis is ready. All of the factors mentioned above could discriminate consequences, but they cannot start the atherothrombogenesis. Instead of using markers of fire, could we use markers of the first smoke (e.g., ADMA SDMA), primarily in CKD and RT patients?

As the relationship between SDMA and endothelial function in CKD patients and RT recipients remains unclear we assessed whether high SDMA could predict impaired FMD. According to our results, the relationship between SDMA and endothelial dysfunction after adjustment for GFR was statistically significant. ADMA is metabolised mainly by dimethylarginine dimethylaminohydrolase (DDAH). Renal excretion is of less importance. In contrast, excretion via the kidney is the main route for SDMA [22]. Fliser et al. [23] showed that the correlation coefficient for SDMA and GFR was almost identical to that of creatinine and GFR. Such findings suggest that urinary excretion is the main elimination pathway for SDMA in humans. As SDMA accumulates eight times more than ADMA in patients with end stage renal disease, it is reasonable to assume that renal dysfunction has a major influence on SDMA [24]. Even in this case, the high value of SDMA independently of GFR increased the probability for the development of endothelial dysfunction. In addition, SDMA is not a direct inhibitor of NOS unlike ADMA. Nevertheless, the influence of SDMA on the endothelium may be as important as that of ADMA. The fact that we find significant association between SDMA and impaired endothelial dysfunction means that SDMA could be a biomarker with clinical significance. A recent study by Schepers et al. [25] suggested that SDMA, but not ADMA, stimulated monocytic ROS production in CKD thereby exhibiting proinflammatory effects. Interestingly in another study [26], the acute inflammation was characterised by a decrease in ADMA, but not SDMA. A key question is whether SDMA and ADMA have different metabolic pathways and play different pathophysiological roles. Further studies are required to fully evaluate the influence of SDMA on the cardiovascular system in renal patients.

Table 3: The results of ROC analysis for discriminating impaired from non-impaired FMD.

	AUC (95% CI)	Std. error	Sensitivity %	Specificity %	AUC (95% CI)[b]	*P*
SDMA	0.689 (0.549–0.829)	0.071	81.8	58.3	0.686 (0.540–0.820)	0.007
hs-CRP	0.754 (0.602–0.905)	0.054	73.3	75.8	0.735 (0.628–0.840)	<0.001
IL-6	0.699 (0.597–0.802)	0.052	72.3	52.6	0.690 (0.579–0.789)	0.002
SAA	0.605 (0.486–0.725)	0.061	76.7	51.4		0.093
$O_2^{\bullet-}$	0.593 (0.467–0.719)	0.064	69.2	34.5		0.174
Model: hs-CRP and SDMA	0.730 (0.582–0.878)	0.076			0.728 (0.592–0.855)	0.005
Model: hs-CRP and $O_2^{\bullet-}$	0.671 (0.531–0.812)	0.072			0.639 (0.517–0.771)	0.025
Model: hs-CRP, SDMA, and $O_2^{\bullet-}$	0.756 (0.623–0.888)	0.068			0.741 (0.616–0.859)	0.004
Model: IL-6 and SDMA	0.732 (0.614–0.851)	0.061			0.704 (0.593–0.813)	0.001
Model: IL-6 and $O_2^{\bullet-}$	0.628 (0.507–0.749)	0.062				0.070
Model: IL-6, SDMA, and $O_2^{\bullet-}$	0.724 (0.570–0.878)	0.079			0.715 (0.566–0.846)	0.003
Model: SDMA and $O_2^{\bullet-}$	0.737 (0.598–0.876)	0.071			0.655 (0.533–0.778)	0.002

Data in parentheses are 95% confidence intervals (95% CIs). Sensitivity and specificity were calculated for optimal cutoff value. [b]The AUC and 95% confidence intervals of the 1000 bootstrap samples.

Several studies have shown that CRP is a risk factor for cardiovascular events and causes mortality in different cohorts of renal patients [27–30]. In addition, a growing number of studies found a link between inflammation and endothelial function in CKD patients [31–33]. Endothelium synthesized several biochemical markers. Each of them has an important role in the development of inflammation (ICAM-1, VCAM-1, von Willebrand factor, and E-selectin) [34]. Therefore, testing the association between indicators of renal function, inflammation and endothelial dysfunction may provide more evidence that different pathophysiological mechanisms lead to cardiovascular and renal disease. In our study, the individual marker which showed the strongest association with FMD was hs-CRP. Our finding agrees with that of Recio-Mayoral et al. [35], as hs-CRP showed a significant negative correlation with the percentage of FMD in predialysis, dialysis, and kidney transplant recipients. Furthermore, our data provide novel evidence that hs-CRP may predict impaired endothelial function measured by the diameter of the brachial artery in CKD patients.

Our study evaluated the diagnostic accuracy of hs-CRP, IL-6, SAA, SDMA, and $O_2^{\bullet-}$ to detect endothelial dysfunction in CKD patients. In the whole patient group, ROC curves of single markers indicated that only hs-CRP, IL-6 and SDMA had the ability to detect impaired FMD. In addition, the AUC value was higher for hs-CRP than for IL-6, and SDMA. According to our knowledge there is no data about SDMA diagnostic ability, sensitivity and specificity for impaired FMD detection in CKD and RT patients. Schepers et al. [25] examined the discriminative power of SDMA in CKD patients for inflammation. AUC was 0.69 and sensitivity and specificity were 71% and 70%, respectively. In our study SDMA sensitivity for impaired FMD was 81.8%, and it was the best sensitivity compared to other markers. The multimarker strategy that assumes the choice of an adequate set of biomarkers was used to construct ROC curves. All combinations of markers appeared to have comparable AUCs, ranging from 0.724 for IL-6/SDMA/$O_2^{\bullet-}$ to 0.756 for hs-CRP/SDMA/$O_2^{\bullet-}$. None of the models had a significantly better AUC compared with the AUC for hs-CRP alone (0.754). Swets [36] suggests the following guidelines for interpretation of AUCs: 0.5–0.7 rather low accuracy; 0.7–0.9 accuracy useful for some purposes; >0.9 rather high accuracy.

However, the basic intent of the models for predicting impaired FMD is to avoid additional analyses in subjects without impaired FMD. Therefore, a correct classification of subjects with low risk for impaired FMD is important measure of the model performance. According to models in our study, subjects with 0.10 or less predicted probability for impaired FMD had 0% false negative values. This means that no subject with impaired FMD will be classified as a subject without impaired FMD if the values of the parameters in the model are higher than the calculated cutoffs. Similarly for screening tests, the sensitivity is of prime importance and so is the negative predictive value. The level of false-positive values must be kept low enough in order to preserve both specificity and positive predictive value at acceptable levels [37]. In our study, in case of 100% sensitivity, false-positive values (1-specificity) were 70.8% and 75.8% for hs-CRP/SDMA/$O_2^{\bullet-}$ model and hs-CRP alone, respectively. As pointed out by Dodd and Pepe [38], "large monetary costs result from high false-positive rates."

Clearly SAA and $O_2^{\bullet-}$ had poor ability to detect FMD in CKD. This poor performance of $O_2^{\bullet-}$ may be explained by the fact that different oxidative pathways exist in uremia. Which of these pathways have the greatest influence is still unknown. It seems that chlorinated oxidative stress is more important than nitrosative stress [39]. Therefore, other biochemical parameters of oxidative stress should be examined.

Unexpectedly, our study showed that the combined use of chosen biomarkers provides very little, if any, contribution to screening power for endothelial dysfunction comparing with that provided by the sole hs-CRP. It is obvious that different pathophysiological processes provide overlapping information regarding screening potential for endothelial dysfunction. Future investigations should address other biomarkers for each step that leads to endothelial dysfunction, especially biomarkers of endothelial cell activation (vWF, ICAM-1,

VCAM-1, and E-selectin). Practical application of SDMA and other biomarkers demands both basic research and clinical trials. The use of these biomarkers can be recommended in clinical studies for the purpose of better understanding the endothelial dysfunction or to predict it in different population groups.

5. Limitations

The relatively small sample size could influence statistical power (especially when categorical variables were used) for multivariate analysis and there is a possibility of missed associations which could otherwise be detected in a larger study group. In spite of the small number of patients, the study offers an objective comparison of endothelial dysfunction and different biomarkers in CKD patients and RT recipients.

6. Conclusion

The data presented here suggest that a strong predictable association exists between hs-CRP, SDMA, and endothelial dysfunction in CKD patients and RT recipients. Furthermore, the individual marker which showed the strongest discriminative ability for endothelial dysfunction is hs-CRP when compared with IL-6, SDMA, SAA, and $O_2^{\bullet-}$. We did not find any relationship between endothelial dysfunction and $O_2^{\bullet-}$. Further studies incorporating a larger number of patients to evaluate influence of the other markers of oxidative stress and endothelial cell activation on the cardiovascular system in CKD patients and RT recipients are desirable. Also, further studies should consider the cost effectiveness of a screening test with a high number of false positive values.

Conflict of Interests

The authors report no conflict of interests. All authors have read the journal's policy on disclosure of potential conflict of interests.

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