ORIGINAL ARTICLE

Interplay of nitric oxide metabolites and markers of endothelial injury, inflammation, and vascular disease in the spectrum of advanced chronic kidney disease

Krzysztof Batko[1], Marcin Krzanowski[1], Agata Pietrzycka[2], Mariusz Gajda[3], Paulina Dumnicka[4], Danuta Fedak[5], Paulina Gołasa[1], Karolina Woziwodzka[1], Piotr Jaśkowski[1], Władysław Sułowicz[1], Marek Kuźniewski[1], Jan A. Litwin[3], Katarzyna Krzanowska[1]

1 Chair and Department of Nephrology, Jagiellonian University Medical College, Kraków, Poland
2 Department of Pharmacobiology, Jagiellonian University Medical College, Kraków, Poland
3 Chair and Department of Histology, Jagiellonian University Medical College, Kraków, Poland
4 Department of Medical Diagnostics, Jagiellonian University Medical College, Kraków, Poland
5 Department of Clinical Biochemistry, Jagiellonian University Medical College, Kraków, Poland

KEY WORDS
atherosclerosis, biomarkers, calcification, endothelial injury, nitric oxide

ABSTRACT

BACKGROUND Chronic kidney disease is linked to cardiovascular morbidity; therefore, relevant biomarkers are widely investigated.

AIMS We aimed to assess the relationship between nitric oxide (as measured by its metabolites, NOx), a key endothelial molecule, with markers of endothelial dysfunction, inflammation, antioxidant status, and mineral disorders as well as histologically assessed vascular calcification in uremic and hemodialysis patients with chronic kidney disease.

METHODS Plasma and serum samples were obtained from 62 patients with renal failure. NOx was assessed by the Griess method, while the other biomarkers were measured by the immunoenzymatic assay. Morphological analysis of arterial calcification was performed in a blinded, semiquantitative manner. Common carotid intima-media thickness and atherosclerotic plaques were assessed by ultrasonography.

RESULTS In the simple analysis, NOx levels correlated positively with the parameters of renal function, mineral metabolism, endothelial injury, and inflammation. NOx predicted carotid intima-media thickness in simple ($P = 0.014$) and multiple analysis ($P = 0.036$) adjusted for the Framingham risk score, C-reactive protein, serum creatinine, and parathormone. The occurrence of atherosclerotic plaques in the common carotid artery was correlated with higher NOx concentrations ($P = 0.021$).

CONCLUSIONS In chronic renal failure, NOx is associated with surrogate markers of atherosclerosis, even after adjustment for traditional cardiovascular risk factors, inflammation, and renal function, but not with the presence or grade of medial arterial calcification. Endothelial injury, inflammation, and mineral metabolism markers are associated with NOx levels, though a causal link requires further study.

Correspondence to: Katarzyna Krzanowska, MD, PhD, Chair and Department of Nephrology, Jagiellonian University Medical College, ul. Kopernika 15c, 31-501 Kraków, Poland, phone: +48 12 424 78 00, email: kasiajanda@op.pl
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INTRODUCTION Cardiovascular disease is the primary cause of morbidity, mortality, and years of life lost in Poland.[1] Chronic kidney disease (CKD) is frequently complicated with cardiovascular morbidity, and in itself is an independent risk factor for cardiovascular disease.[2] Endothelial dysfunction caused by imbalance of vasoactive molecules, oxidative stress, and

WHAT'S NEW?

Chronic kidney disease, particularly its advanced stages, is connected with severe vascular impairment and the associated deleterious sequelae. Both atherosclerosis and medial arterial calcification are processes that have been linked to clinical outcomes in renal failure populations. Recently, cardiovascular risk models have been developed; however, the idea of a biomarker panel dedicated for chronic kidney disease has only been partially explored. The present study provides an overview of new and established biomarkers of processes involved in vascular disease (ie, inflammation, mineral and bone disorder, endothelial injury), which is accompanied by ultrasonography assessments and semiquantitative morphological investigations on artery samples. This study of the relationships between these markers and/or mediators provides insight into the unique interplay occurring in chronic kidney disease, which is shown in the uremic and hemodialysis populations. Our data may serve as a benchmark for future studies that could aim to establish a cardiovascular risk model tailored to chronic renal failure.

inflammation[3,4] is one of the initial events and an indicator of vascular pathology in cardiovascular complications of CKD. Impaired nitric oxide (NO) synthesis is a hallmark of vascular disease[5]; however, NO is highly reactive and has to be assessed indirectly through its relatively stable biologic metabolites (NOx), nitrite and nitrate, by a modified Griess assay.[6] Nitric oxide homeostasis plays a crucial role in the pathology of the cardiovascular system.[7] The offending processes in vascular injury are complex in CKD, therefore, establishing the interplay between disease-driving pathways (through a purported relationship between respective biomarkers) is important to develop a risk model for this population.

We examined the relationship between NOx and surrogate parameters of vascular disease as well as biochemical indicators of endothelial impairment, inflammation, antioxidant status, and mineral bone disease in uremic and hemodialysis patients with end-stage renal disease.

METHODS **Patients** The study population included consecutive patients with advanced CKD from a convenience sample at our university center. Sixty-two patients fulfilled the predefined inclusion criteria, namely, stage 5 CKD and planned first-time arteriovenous fistula (AVF) procedures. There were 35 men and 27 women with a mean (SD) age 63 (16) years. Clinical, imaging, biochemical, and morphological data were gathered in a cross-sectional fashion, and analyzed for 20 predialysis and 42 hemodialysis patients. Our reference population (for NOx measurements) included 44 healthy subjects (24 men and 20 women) with a mean (SD) age of 61 (10) years, mean (SD) body mass index of 27.2 (5.6) kg/m^2, and mean (SD) creatinine level of 86 (16) µmol/l. This study is part of a concluded research initiative, and follows the design of our previous publications.[8] We also estimated that the inclusion of 60 patients allows for 90% power to detect a correlation of moderate strength (rho = 0.4) at the significance level of 0.05. All patients provided written and informed consent prior to their recruitment. The Bioethics Committee of the Jagiellonian University approved the study.

Imaging Ultrasonography was performed using the Acuson 128 XP/10 system (Acuson Corp., Mountain View, California, United States). An experienced, blinded operator examined common carotid artery intima-media thickness (CCA-IMT) bilaterally, using a linear 5/7 MHz probe in B mode. Assessment at 2 fixed (predefined) locations, respectively, 0.5 cm and 2 cm below CCA bifurcation on each side was performed during diastole. Data are reported as arithmetic means for both arteries in each patient. CCA-IMT measurements were taken outside the locations of possible atherosclerotic plaques. The atherosclerotic carotid plaque was defined as an echoic focal structure protruding into the lumen or focal wall thickening which is at least 50% greater than that of the surrounding vessel and is clearly different from the surroundings. No qualitative and quantitative analysis of atherosclerotic plaques was used.

Biochemistry On the morning preceding the surgical procedure, patients underwent a complete medical examination. Shortly afterwards, following an overnight fast, venous blood was drawn from all patients and samples of plasma and serum were collected.

Specimens were kept at –70°C until analysis performed within 3 months. Plasma for biochemical analysis of oxidative activity was stored under recommended operating procedures; protected from light exposure, positioned on ice, centrifuged in a 2-hour time frame, aliquoted, and kept at –30°C for up to 1 month. Antioxidant capacity of plasma was assessed by a previously described method based on scavenging of 2,2-diphenyl-1-picrylhydrazy (DPPH).[9] NOx were determined using the Griess assay, as reported previously[6]: a reaction in which sulfanilamide and N (1-naphthyl)-ethylenediamine dihydrochloride in acidic medium (phosphoric acid) in combination with nitrates gives the azo dye, with maximum absorption at 540 nm. Spectrophotometric measurements were performed using a microplate reader, the Polar Star Omega (BMG Labtech, Ortenberg, Germany).

In all patients, selected biochemical parameters were measured, including creatinine, intact parathyroid hormone (iPTH), total calcium (Ca) and phosphate (Pi), high-sensitivity C-reactive protein (hs-CRP), interleukin 6 (IL-6), PTX3, soluble tumor necrosis factor receptor 2 (sTNFR2), fibroblast growth factor 23

(FGF-23), osteopontin, osteoprotegerin, osteocalcin, DPPH, NOx, transforming growth factor β1 (TGF-β1), and thrombomodulin (TM).

Plasma/serum samples were assessed using an enzyme-linked immunosorbent assay microplates and an ELX808 automatic reader (BIO-TEK Instruments Inc., Winooski, Vermont, United States). The following commercial kits were applied: IL-6, pentraxin 3, sTNFR2, TGF-β1, TM (R&D Systems, Minneapolis, Minnesota, United States); osteoprotegerin (BioVendor, Brno, Czech Republic); osteopontin (R&D Systems, Minneapolis, Minnesota, United States); osteocalcin (Metra/Quidel, San Diego, California, United States) and FGF-23 (Immunotopics Int., San Clemente, California, United States).

Routine biochemical tests were carried out using automatic biochemical analyzers: Hitachi 917 (Hitachi, Japan) and Modular P (Roche Diagnostics, Mannheim, Germany). hs-CRP was assessed nephelometrically (Nephelometer BN II, Siemens Healthcare Diagnostics, Munich, Germany).

Histology The methodology adopted was described elsewhere.[8] Reproducibility of calcification assessment and superior sensitivity of alizarin red over other histological staining methods are discussed therein. In 36 patients (22 on hemodialysis and 14 predialysis; 16 with NO levels below or equal to the median and 20 with NO levels above the median), radial artery samples were collected during AVF surgery. The samples were fixed in formalin, washed, frozen, and cut into cross-sections. The localization and extent of calcifications were assessed by 2 independent blinded observers. Microscopic imaging was performed using the Olympus DP-71 digital CCD camera coupled to Olympus BX-50 microscope (Olympus, Tokyo, Japan).

Statistical analysis Numbers of patients (percentage of the group) are reported for categories and mean (SD) or median (interquartile range) for continuous variables, in accordance with sample distribution (assessed with the Shapiro–Wilk test). Contingency tables were analyzed using the χ^2 test. Comparisons between the groups were done with the *t* test or the Mann–Whitney test. Simple correlations were analyzed using the Pearson correlation coefficient, after log transformation of right-skewed variables. Multiple linear regression model was calculated using the prespecified predictors associated with cardiovascular risk, that is, the Framingham risk score, serum CRP, iPTH, and creatinine concentrations. A *P* value of less than 0.05 was considered significant. The Statistica 10 software (StatSoft, Tulsa, Oklahoma, United States) was used for computations.

RESULTS There were no differences in demographic and cardiovascular risk factors, including smoking status and an established index of cardiovascular risk, in the recruited population of uremic (predialysis) patients and those already on hemodialysis (TABLE 1).[10] In patients with NOx levels above the median, female sex was more common and renal function (serum creatinine) was more impaired than in those with NOx levels below or equal to the median (TABLE 1). This relationship between NOx and kidney function was further examined as a continuous variable: log-transformed NOx concentrations correlated positively with log(creatinine) ($R = 0.32$; $P = 0.012$).

When considering all patients, the median (interquartile range [IQR]) NOx concentration was 5.40 (4.52–6.99) μmol/l. The median (IQR)

TABLE 1 Demographic and clinical characteristics in patients predialysis (uremia) and on hemodialysis with respect to nitric oxide

Variable	Hemodialysis (n = 42)	Uremia (n = 20)	*P* value	NOx ≤median (n = 31)	NOx >median (n = 31)	*P* value
Age, y, mean (SD)	61 (18)	67 (12)	0.2	64 (18)	62 (14)	0.4
Male sex, n (%)	24 (57)	11 (55)	0.9	22 (71)	13 (42)	0.02
Hemodialysis, n (%)	–	–	–	20 (65)	22 (71)	0.6
Dialysis therapy duration, mo, median (IQR)	20 (4–48)	–	–	13 (4–32)	30 (6–64)	0.2
Diabetes, n (%)	13 (31)	8 (40)	0.5	12 (39)	9 (29)	0.3
Dyslipidemia, n (%)	19 (45)	9 (45)	0.9	11 (35)	17 (55)	0.2
Current smoking, n (%)	11 (26)	6 (30)	0.8	11 (35)	6 (19)	0.1
Hypertension, n (%)	34 (81)	19 (95)	0.1	25 (83)	28 (87)	0.6
BMI, kg/m^2, mean (SD)	24.6 (3.9)	26.4 (4.6)	0.06	24.5 (5)	24.8 (3.2)	0.8
Framingham risk score, %, median (IQR)	11 (6–22)	13 (8–21)	0.3	14 (6–22)	9 (7–20)	0.2
Serum creatinine, μmol/l, median (IQR)	470 (383–634)	315 (234–405)	<0.001	385 (302–488)	454 (361–687)	0.02

Abbreviations: BMI, body mass index; IQR, interquartile range; NOx, nitric oxide (as indirectly measured through its metabolites)

NOx did not differ between hemodialysis and predialysis patients (5.62 µmol/l [4.53–7.25] vs 5.10 µmol/l [4.48–6.20], respectively; $P = 0.3$). A comparison to an internal healthy reference population revealed that NOx concentrations in our study were low (reference population, 27.73 µmol/l [19.31–76.39]). This is in line with a reference benchmark from the literature and the original publication from which we adopted our method for the Griess reaction.[6,11]

When comparing uremic and hemodialysis patients, we observed that FGF-23, Ca × Pi product, and osteopontin were higher in the latter group. This might suggest a more adverse mineral-bone imbalance, as hemodialysis patients showed a greater degree of renal impairment. In patients with higher NOx concentrations, similarly, Ca × Pi product and FGF-23 were substantially elevated (TABLE 2). When examining continuous variables, log-transformed NOx concentrations correlated positively with log(iPTH) ($R = 0.38$; $P = 0.005$), log(Pi) ($R = 0.31$; $P = 0.017$), log(Ca × Pi) ($R = 0.38$; $P = 0.003$), and log(FGF-23) ($R = 0.40$; $P = 0.003$).

The markers of inflammation, but not of antioxidant capacity, were higher among hemodialysis patients (TABLE 3) as compared to those predialysis. No association of NOx levels above and below or equal to the median with parameters of inflammation and antioxidant capacity was observed (TABLE 3). Log-transformed NOx concentrations correlated positively with log(sTNFR2) ($R = 0.28$; $P = 0.035$), suggesting a relationship between systemic inflammation and NOx changes.

Serum TM was significantly higher in patients undergoing hemodialysis and among patients with higher NOx levels (TABLE 4). Log-transformed NOx concentrations correlated positively with log(TM) ($R = 0.27$; $P = 0.045$).

We further assessed the relationship between NOx and vascular disease surrogate markers. In 39 patients who underwent CCA ultrasound, a positive correlation was found between log(NOx) and CCA-IMT ($R = 0.39$; $P = 0.014$ in a simple analysis) (FIGURE 1A) and it remained significant after adjustment for the Framingham risk score, parameters of renal function,

TABLE 2 Markers of mineral-bone imbalance in patients predialysis and on hemodialysis and their respective relationship to nitric oxide levels

Marker	Hemodialysis (n = 42)	Uremia (n = 20)	*P* value	NOx ≤median (n = 31)	NOx >median (n = 31)	*P* value
iPTH, pg/ml	278 (164–560)	294 (175–512)	0.9	279 (164–386)	289 (180–705)	0.4
Ca, mmol/l	2.21 (0.17)	2.23 (0.26)	0.6	2.17 (0.16)	2.26 (0.24)	0.1
Pi, mmol/l	1.60 (1.26–2.05)	1.37 (1.18–1.44)	0.06	1.35 (1.17–1.69)	1.56 (1.35–1.96)	0.05
Ca × Pi, $mmol^2/l^2$	3.60 (2.75–4.24)	2.94 (2.61–3.03)	0.04	2.88 (2.43–3.59)	3.59 (2.93–4.14)	0.001
FGF-23, RU/ml	2275 (754–10 558)	465 (280–1120)	0.002	706 (346–2234)	2276 (977–8208)	0.01
Osteopontin, ng/ml	355 (189–588)	213 (159–352)	0.03	282 (179–522)	341 (222–587)	0.2
Osteoprotegerin, pmol/l	8.13 (6.16–10.61)	6.59 (5.12–9.23)	0.1	7.13 (5.61–9.44)	8.37 (5.94–11.49)	0.2
Osteocalcin, ng/ml	51.1 (36.0–84.5)	38.6 (31.4–54.1)	0.09	40.0 (31.4–76.6)	52.7 (37.7–78.8)	0.2

Data are presented as median (interquartile range).

Abbreviations: Ca, calcium; FGF-23, fibroblast growth factor 23; iPTH, intact parathyroid hormone; Pi, phosphate; others, see TABLE 1

TABLE 3 Markers of inflammation and antioxidant capacity in predialysis and hemodialysis patients and their respective relationship to nitric oxide

Biomarker	Hemodialysis (n = 42)	Uremia (n = 20)	*P* value	NOx ≤median (n = 31)	NOx >median (n = 31)	*P* value
hs-CRP, mg/dl	9.73 (4.59–22.1)	3.56 (1.59–8.47)	0.02	7.95 (4.59–20.5)	4.80 (1.59–19.80)	0.3
IL-6, pg/ml	5.58 (2.81–9.25)	3.6 (2.21–5.17)	0.08	5.02 (2.63–9.36)	4.11 (2.24–6.16)	0.4
sTNFR2, µg/ml	16.2 (13.7–20)	11.6 (9.2–15.7)	0.003	14.7 (11–17.9)	15.6 (11.6–20.6)	0.2
PTX, ng/ml	1.85 (1.09–2.69)	0.8 (0.54–1.48)	0.01	1.41 (0.74–2.33)	1.56 (0.89–2.79)	0.7
DPPH scavenging, %	38.9 (33.7–43.4)	35.7 (33.2–45.2)	0.5	37.0 (32.8–43.5)	38.9 (34.7–43)	0.4
NOx, µmol/l	5.62 (4.53–7.25)	5.1 (4.48–6.2)	0.3	4.53 (4.09–4.88)	6.86 (6.20–8.26)	–

Data are presented as median (interquartile range).

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; hs-CRP, high-sensitive C-reactive protein; IL-6, interleukin 6; PTX, pentraxin; sTNFR2, soluble tumor necrosis factor receptor 2; others, see TABLE 1

TABLE 4 Parameters of vascular remodeling / disease in predialysis and hemodialysis patients and their respective relationship to NOx

Parameter	HD (n = 42)	Uremia (n = 20)	*P* value	NOx ≤ median (n = 31)	NOx> median (n = 31)	*P* value
TGF-β1, µg/ml, median (IQR)	5.01 (4.02–6.45)	6.19 (4.05–8.64)	0.3	5 (4.02–8)	5.57 (4.10–6.56)	0.9
Thrombomodulin, ng/ml, median (IQR)	18.9 (14.9–24.7)	14.6 (13.5–17.5)	0.007	15.0 (13.4–17.7)	17.6 (14.9–24)	0.02
CCA-IMT[a], mm, mean (SD)	0.97 (0.15)	0.96 (0.14)	0.9	0.93 (0.15)	0.99 (0.14)	0.2
Atherosclerotic plaque in CCA, n (%)[a]	11 (48)	5 (31)	0.3	5 (25)	11 (55)	0.04
Radial artery calcifications[b], n (%)	14 (64)	8 (57)	0.7	10 (62)	12 (60)	0.8

a Data available for 39 patients (23 on hemodialysis and 16 predialysis; 20 with nitric oxide levels equal to or lower than the median and 19 with above the median)

b Data available for 36 patients (22 on hemodialysis and 14 predialysis; 16 with nitric oxide levels equal to or lower than the median and 20 with above the median)

Abbreviations: CCA-IMT, common carotid artery-intima media thickness; TGF-β1, transforming growth factor β1; others, see TABLE 1

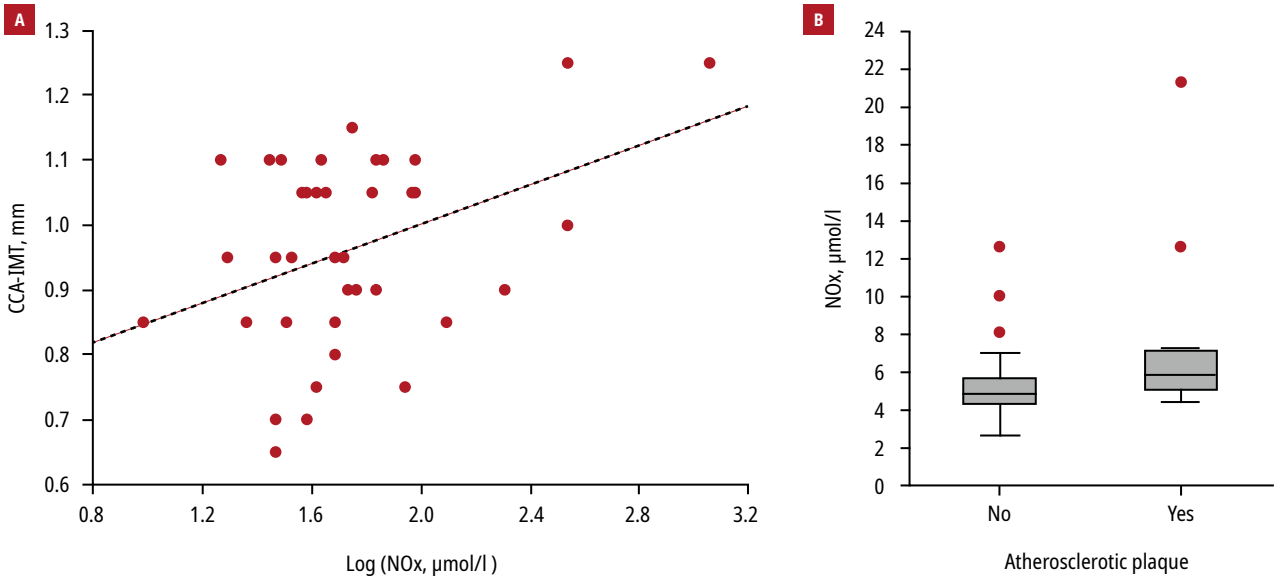

FIGURE 1 Association between nitric oxide concentrations and ultrasound findings: common carotid artery-intima media thickness (**A**) and presence of atherosclerotic plaques (**B**)

Abbreviations: see TABLES 1 and 4

TABLE 5 Multiple linear regression to predict values of the common carotid artery-intima media thickness

Independent variables	β (SE)	*P* value
Log(NOx)	0.34 (0.16)	0.046
Log(Framingham risk score)	0.62 (0.17)	0.002
Log(hs-CRP)	–0.18 (0.16)	0.3
Log(creatinine)	–0.01 (0.19)	0.9
Log(iPTH)	–0.05 (0.17)	0.8

Abbreviations: see TABLES 1–4

inflammation, and secondary hyperparathyroidism (TABLE 5). The prevalence of atherosclerotic CCA plaques was 2-fold higher in patients with NOx levels above the median (TABLE 4). This is further supported by the observation that patients with atherosclerotic plaques had significantly higher median (IQR) NOx concentrations 5.84 µmol/l (5.05–7.16) as compared with 4.88 µmol/l (4.35–5.67) (P = 0.021) (FIGURE 1B). Radial artery samples obtained during AVF formation were assessed in 36 patients, 14 samples (38%) did not reveal any apparent signs of calcification. Among the remaining samples, calcifications ranged from a few small dispersed concretions (grade 1) to heavy mineral deposits occupying large areas of the vascular wall (grade 4). Irrespective of their advancement, calcifications were observed primarily in the tunica media. However, no significant associations were observed between NOx concentrations and the presence or grade of radial artery calcifications (TABLE 4, FIGURE 2).

DISCUSSION Our main findings include a surprising positive relationship between NOx and surrogates of atherosclerosis, adjusted

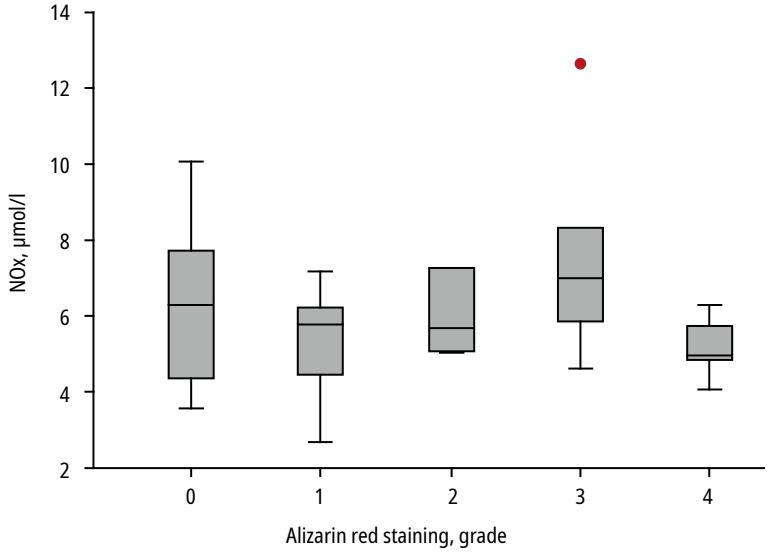

FIGURE 2 Nitric oxide concentrations associated with grades of radial artery calcification. Data are shown as median, interquartile range (box), nonoutlier range (whiskers), and outlier (dot).

for potential confounding parameters of renal function,[12] inflammation,[13] the Framingham cardiovascular risk score,[14] and secondary hyperparathyroidism[15] in chronic renal failure. Our data may enable an adequate assessment of NOx, as a candidate for longitudinal evaluation in advanced renal disease, where it may serve as an indicator of progressing atherosclerosis. Patients with higher NOx levels showed a greater degree of renal impairment and elevated markers of endothelial injury and phosphate imbalance. This could imply a relationship with medial arterial calcification, which, although prevalent and associated with hard outcomes in CKD,[16] can occur without a traditional cardiovascular risk profile. However, NOx levels were not correlated with incidence nor grade of medial arterial calcification, indicating that these processes may be intercorrelated, rather than directly linked. Plasma antioxidant markers likewise did not differ in patients with uremia and on hemodialysis and were not affected by NOx levels.

Atherosclerosis is the underlying setting for future cardiovascular events, interplaying with inflammatory pathways and matrix alterations, both of which have become targets of interest.[17,18] Molecular methods (eg, miRNA profiling) hold future promise for diagnostics in cardiovascular conditions, though their clinical applications still remain to be fully elucidated.[19] To date, CCA-IMT and plaque presence, surrogate markers of atherosclerosis, have been established as a useful diagnostic measure to evaluate cardiovascular risk.[20,21] Meta-analyses have shown that although CCA-IMT assessments predict vascular events, considerable heterogeneity may be observed, partially owing to carotid segment definition and measurement protocols.[22] As such, the mode of ultrasound assessment should be kept in mind when comparing across studies. Similarly to our findings, a previous cross-sectional study of patients on peritoneal dialysis reported that NOx correlated positively with plaques and CCA-IMT.[15] However, results obtained in studies on peritoneal dialysis are not directly comparable with those concerning hemodialysis, with dialysis modality itself exerting an impact.[23] The relationship between NOx and CCA-IMT in hemodialysis is not clear. Ocak et al[24] recently assessed serum NOx levels in patients on hemodialysis and with uremia, reporting higher levels in both groups as compared with healthy controls. In the former, a significant positive relationship with hs-CRP levels was observed, which substantiated a hypothesis of an endothelial protective mechanism in response to inflammation. Diabetes and metabolic syndrome have also been associated with higher serum NOx levels,[25] though, in the present study, we did not observe a direct relationship, possibly because of insufficient patient sample. In patients with diabetes, high NOx levels have been attributed to putative, induced nitric oxide synthase (NOS) isoform production perpetrated by inflammation, oxidative stress and hypoxia.[25,26] These processes are also integral to CKD, where uremic toxins, inflammation, and oxidative stress are the perpetrators of endothelial injury.[4] Progressing CKD and hemodialysis are associated with an increase in the levels of inflammatory cytokines, including TNF,[27] which might partly explain the association between NOx and thrombomodulin, a marker of endothelial injury and dysfunction,[28] which is released in TNF-stimulated inflammatory settings,[29] as well as between NOx and sTNFR2, which is an independent marker of mortality and cardiovascular risk in CKD.[30] Our findings highlight the endothelium as the critical setting in CKD, and point to the complexity of processes involved in its impairment.

Previous studies in patients with coronary artery disease and healthy controls have shown that a lower NOx concentration and an increased level of asymmetric dimethyl arginine, an endogenous NOS inhibitor, occur in vascular disease.[31] Passauer et al[32] studied patients undergoing hemodialysis using an invasive technique, reporting impaired NOS and dilatory vascular response, while the baseline nitric oxide generation was increased. Some conflicting data reported so far may be due to heterogenous populations and different approaches to nitric oxide measurements. We hypothesize that the severe progression of atherosclerosis in the milieu of CKD corresponds with an endogenous failing response of an increase in NO, which does not alter the overall deficiency of NO characteristic of CKD,[33] as suggested by the low (overall) NOx levels observed in our patients.

Limitations Our study was designed in an exploratory manner, and the cross-sectional nature precludes any statements over causality, which could be inferred from statistical analyses. Secondly, it should be noted that a comparison of plasma and serum levels of markers may not reflect the relevant biological processes occurring in a local milieu, which also extends to associations with morphological and imaging findings. The array of biomarkers investigated has been well established in the literature; however, our data extend these findings to the spectrum of advanced CKD. While several of the molecules of pathogenic significance to CKD are well recognized, their interaction is not fully known. Associated pathophysiological pathways in which they are involved can be interconnected or occur in parallel throughout the course of progressive nephropathy. The assessment of a highly reactive molecule has also limitations, and each method holds some inherent drawbacks.[34] NOx levels can be measured in plasma and serum by the Griess assay, a simple and rapid method reported to be reproducible.[35,36] It is widely used as shown in the literature, though without certain methodological considerations it may be inaccurate; interference from components of bodily fluids (eg, amino acids, proteins, ascorbate), and rapid oxidation of nitrite to nitrate by, for example, oxyhemoglobin are factors to account for.[34] Our adopted method[6] involves deproteinization[35] and use of nitrate reductase to recover nitrite. This method is also in line with prior studies,[25] which have attempted to circumvent the influence of diet on NOx levels through investigating fasting blood samples, as it has been demonstrated that an elevation in plasma NOx concentrations following dietary intake returns to baseline following 12 hours.[37] Our approach holds a narrow scope in being limited to assessments of NOx and DPPH scavenging, as we did not assay other components involved in nitrosative stress and / or redox status.

Conclusions We provide data on a large array of biomarkers implicated in renal and cardiovascular pathophysiology and report their relationship in uremia and hemodialysis. Our findings indicate that NOx remains a positive predictor of CCA-IMT, a surrogate atherosclerosis marker, even after adjustment for traditional and non-traditional confounders in a multiple linear regression analysis. No evident relationship with the presence or grade of medial artery calcification, assessed in a semiquantitative manner, was observed. However, the assessment of a highly reactive molecule through its biological metabolites holds several limitations. Considering NOx as a potential biomarker in CKD requires validation in homogenous populations to account for its relationship with comorbidity status.

ARTICLE INFORMATION

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CONTRIBUTION STATEMENT KK and KB conceived the study, were the major participants in its design, coordination, interpretation of the results and statistical analysis, and prepared draft manuscript. MG carried out histological examinations. PD performed statistical analysis. AP, MKr, DF, PG, KW, and PJ participated in the design of the study, interpretation of the results and statistical analysis. JAL participated in data analysis and in preparation of the final manuscript version. MKu and WS participated in study design and coordination. All authors were involved in data collection, draft manuscript modifications, and approved the final version of the manuscript.

CONFLICT OF INTEREST None declared.

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REFERENCES

1 Kruger PC, Guzik TJ, Eikelboom JW. How can the results of the COMPASS trial benefit patients with coronary or peripheral artery disease in Poland. Kardiol Pol. 2019; 77: 661-669.

2 Hill NR, Fatoba ST, Oke JL, et al. Global prevalence of chronic kidney disease - a systematic review and meta-analysis. PLoS One. 2016; 11: e0158765.

3 Ghebre YT, Yakubov E, Wong WT, et al. Vascular aging: implications for cardiovascular disease and therapy. Transl Med (Sunnyvale). 2016; 6: 183.

4 Malyszko J. Mechanism of endothelial dysfunction in chronic kidney disease. Clin Chim Acta. 2010; 411: 1412-1420.

5 Barbato JE, Tzeng E. Nitric oxide and arterial disease. J Vasc Surg. 2004; 40: 187-193.

6 Guevara I, Iwanejko J, Dembińska-Kieć A, et al. Determination of nitrite/nitrate in human biological material by the simple Griess reaction. Clin Chim Acta. 1998; 274: 177-188.

7 Heidrich FM, Jercke MC, Ritzkat A, et al. The endothelial nitric oxide synthase cofactor tetrahydrobiopterin shields the remote myocardium from apoptosis after experimental myocardial infarction in vivo. Kardiol Pol. 2017; 75: 1339-1350.

8 Janda K, Krzanowski M, Gajda M, et al. Impaired fasting glucose and diabetes as predictors for radial artery calcification in end stage renal disease patients. Int J Endocrinol. 2013; 2013: 969038.

9 Janaszewska A, Bartosz G. Assay of total antioxidant capacity: comparison of four methods as applied to human blood plasma. Scand J Clin Lab Invest. 2002; 62: 231-236.

10 National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002; 106: 3143-3421.

11 Ghasemi A, Zahediasl S, Azizi F. Reference values for serum nitric oxide metabolites in an adult population. Clin Biochem. 2010; 43: 89-94.

12 Ekart R, Hojs R, Bevc S, Balon BP. Asymptomatic atherosclerosis and hypertension in nondiabetic patients with chronic kidney disease. Artif Organs. 2008; 32: 220-225.

13 Yilmaz MI, Sonmez A, Saglam M, et al. A longitudinal study of inflammation, CKD-mineral bone disorder, and carotid atherosclerosis after renal transplantation. Clin J Am Soc Nephrol. 2015; 10: 471-479.

14 Janda K, Krzanowski M, Gajda M, et al. Cardiovascular risk in chronic kidney disease patients: intima-media thickness predicts the incidence and severity of histologically assessed medial calcification in radial arteries. BMC Nephrol. 2015; 16: 78.

15 Rebić D. Serum nitric oxide level and carotid arteries atherosclerosis in peritoneal dialysis patients. Folia Medica Facultatis Medicinae Universitatis Saraeviensis. 2014; 49: 93-98.

16 London GM, Guérin AP, Marchais SJ, et al. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. Nephrol Dial Transplant. 2003; 18: 1731-1740.

17 Wang Q, Zhang J, Li Y, et al. Green tea polyphenol epigallocatechin-3-gallate increases atherosclerotic plaque stability in apolipoprotein E-deficient mice fed a high-fat diet. Kardiol Pol. 2018; 76: 1263-1270.

18 Batko K, Krzanowski M, Gajda M, et al. Proteoglycan/glycosaminoglycan and collagen content in the arterial wall of patients with end-stage renal disease - new indicators for vascular disease. Pol Arch Intern Med. 2019; 129: 781-789.

19 Badacz R, Przewłocki T, Gacoń J, et al. Circulating miRNA levels differ with respect to carotid plaque characteristics and symptom occurrence in patients with carotid artery stenosis and provide information on future cardiovascular events. Postepy Kardiol Interwencyjnej. 2018; 14: 75-84.

20 Gacoń J, Przewłocki T, Podolec J, et al. The role of serial carotid intima-media thickness assessment as surrogate marker of atherosclerosis control in patients with recent myocardial infarction. Postepy Kardiol Interwencyjnej. 2019; 15: 74-80.

21 Kim JK, Song YR, Kim MG, et al. Clinical significance of subclinical carotid atherosclerosis and its relationship with echocardiographic parameters in non-diabetic chronic kidney disease patients. BMC Cardiovasc Disord. 2013; 13: 96.

22 Lorenz MW, Markus HS, Bots ML, et al. Prediction of clinical cardiovascular events with carotid intima-media thickness. Circulation. 2007; 115: 459-467.

23 Borràs M, Cambray S, Crespo-Masip M, et al. Peritoneal dialysis is an independent factor associated to lower intima media thickness in dialysis patients free from previous cardiovascular disease. Front Physiol. 2018; 9: 1743.

24 Ocak N, Dirican M, Ersoy A, et al. Adiponectin, leptin, nitric oxide, and C-reactive protein levels in kidney transplant recipients: comparison with the hemodialysis and chronic renal failure. Ren Fail. 2016; 38: 1639-1646.

25 Zahedi Asl S, Ghasemi A, Azizi F. Serum nitric oxide metabolites in subjects with metabolic syndrome. Clin Biochem. 2008; 41: 1342-1347.

26 Assmann TS, Brondani LA, Bouças AP, et al. Nitric oxide levels in patients with diabetes mellitus: a systematic review and meta-analysis. Nitric Oxide. 2016; 61: 1-9.

27 Tbahriti HF, Meknassi D, Moussaoui R, et al. Inflammatory status in chronic renal failure: The role of homocysteinemia and pro-inflammatory cytokines. World J Nephrol. 2013; 2: 31-37.

28 Drożdż D, Łątka M, Drożdż T, et al. Thrombomodulin as a new marker of endothelial dysfunction in chronic kidney disease in children. Oxid Med Cell Longev. 2018; 2018: 1619293.

29 Boehme MW, Deng Y, Raeth U, et al. Release of thrombomodulin from endothelial cells by concerted action of TNF-alpha and neutrophils: in vivo and in vitro studies. Immunology. 1996; 87: 134-140.

30 Neirynck N, Glorieux G, Schepers E, et al. Soluble tumor necrosis factor receptor 1 and 2 predict outcomes in advanced chronic kidney disease: a prospective cohort study. PLoS One. 2015; 10: e0122073.

31 Ilhan N, Seckin D, Ilhan N, Ozbay Y. Abnormal asymmetric dimethylarginine/nitric oxide balance in patients with documented coronary artery disease: relation to renal function and homocysteine. J Thromb Thrombolysis. 2007; 23: 205-211.

32 Passauer J, Büssemaker E, Range U, et al. Evidence in vivo showing increase of baseline nitric oxide generation and impairment of endothelium-dependent vasodilation in normotensive patients on chronic hemodialysis. J Am Soc Nephrol. 2000; 11: 1726-1734.

33 Baylis C. Nitric oxide deficiency in chronic kidney disease. Am J Physiol Renal Physiol. 2008; 294: F1-F9.

34 Hunter RA, Storm WL, Coneski PN, Schoenfisch MH. Inaccuracies of nitric oxide measurement methods in biological media. Anal Chem. 2013; 85: 1957-1963.

35 Moshage H, Kok B, Huizenga JR, Jansen PL. Nitrite and nitrate determinations in plasma: a critical evaluation. Clin Chem. 1995; 41: 892-896.

36 Titheradge MA. The enzymatic measurement of nitrate and nitrite. Methods Mol Biol. 1998; 100: 83-91.

37 Node K, Kitakaze M, Yoshikawa H, et al. Reduced plasma concentrations of nitrogen oxide in individuals with essential hypertension. Hypertension. 1997; 30: 405-408.