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IL 6 but not TNF is linked to coronary artery calcification in patients with chronic kidney disease

Joanna Kamińska[a,1], Marek Stopiński[a,1], Krzysztof Mucha[b,c], Anna Jędrzejczak[b], Marek Gołębiowski[d], Monika A. Niewczas[e,f], Leszek Pączek[b,c], Bartosz Foroncewicz[b,*]

[a] Department of Internal Diseases and Dialysis Unit, West Hospital of Saint John Paul II, Daleka 11, 05-825 Grodzisk Mazowiecki, Poland
[b] Department of Immunology, Transplantology and Internal Diseases, Medical University of Warsaw, Nowogrodzka 59, 02-006 Warszawa, Poland
[c] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5A, 02-106 Warszawa, Poland
[d] Department of Clinical Radiology, Medical University of Warsaw, Chałubińskiego 5, 02-004 Warszawa, Poland
[e] Research Division, Joslin Diabetes Center, 1 Joslin Pl, Boston, MA 02215, USA
[f] Department of Medicine, Harvard Medical School, 25 Shattuck St, Boston, MA 02115, USA

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ABSTRACT

Introduction: Patients with chronic kidney disease (CKD) have a high risk of death mainly due to cardiovascular diseases (CVD). Early risk identification may allow interventions and prevention of fatal events.
Objectives: The study aim was to assess the usefulness of selected CVD biomarkers as predictors of 5-year mortality in patients with different CKD stages.
Patients and Methods: Study included 57 CKD patients: 38 in stage 5 (ESRD), 19 in stage 3 and 4 (CKD3-4), and 19 healthy controls. Blood samples were obtained once to measure fetuin A, adiponectin, leptin, tumor necrosis factor (TNF), interleukin-6 (IL-6), metalloproteinase-9 (MMP9), intracellular-1 (ICAM1) and vascular-1 (VCAM1) adhesion molecules (ELISA or Luminex platform). Computed tomography was performed to assess the calcium score (CS). Patients were prospectively followed for 5 years to evaluate their all-cause mortality.
Results: Serum VCAM1, TNF and IL-6 were significantly higher in more advanced CKD stages. VCAM1 correlated significantly with ICAM1, TNF and IL-6. TNF and IL-6 were also significantly correlated with each other. No significant changes were detected for other markers. IL-6 correlated significantly with CS, age, renal function and CRP. Elevated CS and IL-6 increased over 3 times the 5-year all-cause and cardiovascular mortality risks in patients with CKD or ESRD at baseline.
Conclusions: IL-6 and CS were significantly associated with 5-year risk of all-cause mortality in CKD patients. Our study suggests an involvement of chronic inflammation linked to coronary artery calcification that is likely to contribute to the cardiovascular mortality in patients with impaired renal function.

1. Introduction

Chronic kidney disease (CKD) is a major public health concern affecting 10–13% of the general population [1]. Patients with CKD have a high risk of death mainly due to cardiovascular diseases (CVD), including stroke, peripheral vascular disease, coronary artery disease (CAD), congestive heart failure or sudden cardiac death. An estimated 35% of patients with CKD have a history of myocardial infarction or angina [2]. Accordingly, the left ventricular hypertrophy (LVH) prevalence increases at each CKD stage, reaching approximately 75% at the time of dialysis initiation [2,3]. Therefore, it is not surprising that cardiovascular mortality in patients with advanced CKD stages, particularly end stage renal disease (ESRD), is high [4,5]. The high prevalence and incidence of CVD and related mortality may result from numerous CKD-associated factors such as systolic hypertension, LVH, hyperlipidemia and chronic inflammation. Moreover, decreased renal clearance leads to accumulation of potentially harmful substances such as uremic toxins [6].

* Corresponding author at: Klinika Immunologii, Transplantologii i Chorób Wewnętrznych, Warszawski Uniwersytet Medyczny, Nowogrodzka 59, 02-006 Warszawa, Poland.
E-mail addresses: joaniszka@wp.pl (J. Kamińska), interna@szpitalzachodni.pl (M. Stopiński), kjmucha@gmail.com (K. Mucha), anamarie.jedrzejczak@gmail.com (A. Jędrzejczak), marek.golebiowski@wum.edu.pl (M. Gołębiowski), Monika.Niewczas@joslin.harvard.edu (M.A. Niewczas), leszek.paczek@gmail.com (L. Pączek), bartosz.foroncewicz@wum.edu.pl (B. Foroncewicz).
[1] Both authors equally contributed to this study

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Early identification of CKD patients at risk of CVD may allow for early therapeutic interventions and prevention of fatal events. Therefore, biomarkers of CVD are extensively sought. The calcium score (CS) has been used for estimating stages of vascular calcification since 1996. Its results obtained in computed tomography (CT) have 85% sensitivity and 75–85% specificity for stratifying the risk of CAD even in patients without clinical symptoms [7]. There are also protein markers available, including N-terminal pro-brain natriuretic peptide (NTproBNP) or cardiac troponins T and I (TnT and TnI) [8,9]. These markers are useful in clinical practice to monitor disease progression, however, their value as predictive biomarkers in subjects at risk is limited, if any. Therefore, novel biomarkers that could predict CVD development before the clinical disease onset are urgently needed. The list of candidates is long and includes markers of chronic inflammation and endothelial dysfunction [10,11]. For example, C-reactive protein (CRP), interleukin 6 (IL-6) and tumor necrosis factor (TNF) have been associated with CVD and mortality in CKD patients [12,13]. Adhesion molecules: intracellular 1 (ICAM1) and vascular 1 (VCAM1) are increased in ESRD and get upregulated by chronic inflammation, and this upregulation is involved in endothelial dysfunction [14,15]. IL-6, CRP and ICAM1 were reported to be significantly associated with mortality in dialysis patients, whereas TNF and VCAM-1 failed to predict mortality [14]. Recent studies implicated leptin as being associated with endothelial dysfunction and the underlying mechanisms of injury were identified [16].

Many studies aimed to determine a predictive role of these factors in CKD patients, but various study designs and non-homogenous populations preclude definite conclusions. The aim of this study was to assess the usefulness of selected CVD biomarkers as predictors of 5-year mortality in patients with different CKD stages.

2. Patients and methods

2.1. Patients and study design

The study was approved by the Bioethics Committee and all patients signed informed consent to participate in the study. The study population consisted of 76 patients divided into 3 subgroups depending on renal function: ESRD group (n = 38), CKD3-4 group (n = 19) and healthy controls (n = 19). Their characteristics are presented in Table 1. Blood samples were obtained at baseline to perform the measurements, CT was performed to assess the CS and then patients were prospectively followed for 5 years in order to evaluate their all-cause mortality. The date and cause of death was determined based on the patients' hospital records. The person who collected the mortality data was blinded to the results of laboratory tests and CT.

2.2. Methods

Blood was collected after fasting. In ESRD patients it was obtained before hemodialysis. Serum samples were centrifuged for 10 min, at 1800g and +4 °C and subsequently stored at-70 °C until the analysis. Serum samples were stored in small aliquots (Sartsted tubes, Numbrecht, Germany) to ensure a small and consistent number of freeze-thaw cycles for all the immunoassays performed. The classical markers of CVD: troponin T, CK-MB, NTproBNP, CPK, total cholesterol, LDL, HDL TG, uremic acid) and of inflammation: CRP or high-sensitivity CRP (hsCRP) in patients with CRP below 5 mg/l, erythrocyte sedimentation rate and procalcitonin were evaluated. Anti-nuclear and anti-neutrophil cytoplasmic antibodies, thyroid hormones and prostate-specific antigen in males were measured. Glomerular filtration rate (eGFR) was estimated with the MDRD equation.

2.2.1. Inflammatory biomarkers

All inflammatory biomarkers were run by antibody-based quantitative methods in baseline serum specimens subjected only to a single freeze-thaw cycle. IL-6 was assayed with high-sensitive ELISA (HS600B, R&D Systems, Minneapolis, MN, assay sensitivity: 0.05 pg/ml), fetuin was measured with ELISA in samples diluted 10,000 times (43-FETHU-E01, ALPCO Diagnostics, Salem NH). The remaining biomarkers were measured on the Luminex-based platform. This is a multiplex particle-enhanced, sandwich type immunoassay with laser-based detection system based on flow cytometry. We used Human Serum Adipokine Panel B to assay TNF and leptin in undiluted samples (HADK2-61 K-B, sensitivity to detect TNF: 0.14 pg/ml, sensitivity to detect leptin: 85.4 pg/ml, respectively) and Human Cardiovascular Disease Panel 1 to assay ICAM1, VCAM1 and MMP9 in 100 times diluted samples (HCVD1-67AK). Millipore, Billerica, MN was the manufacturer of both assays. Although it is possible to multiplex IL-6 on the Luminex platform, our in-house data and other independent reports strongly suggest a superior performance of high sensitive ELISA assay in comparison with Luminex-based methods. Inter-assay coefficient of variation was below 18% for all assays. Samples were balanced by caseness for the measurements. Manufacturer's protocols were followed. Values of

Table 1
Baseline clinical characteristics stratified by 3 study groups.

Clinical characteristics	Control group n = 19	CKD3-4 group n = 19	ESRD group n = 38	p value
Mean age (SD), years	61.89 (8.75)	64.84 (15.15)	59.87 (15.57)	0.45
Male n (%)	10 (53)	9 (47)	21 (55)	0.87
Prevalent				
Diabetes n (%)	2 (11)	7 (37)	12 (32)	0.14
Hypertension n (%)	5 (26)	16 (84)	22 (59)	**0.001**
CVD n (%)	2 (11)	7 (37)	13 (34)	0.12
Biochemical tests, mean (SD)				
eGFR, ml/min/ 1,73 m^2	91.28 (17.34)	28.59 (11.18)	6.70 (1.94)	by design
CRP, mg/l	1.66 (1.23)	7.51 (11.68)	12.48 (31.84)	< **0,001**
hs-CRP, mg/l	1.73 (1.19)	2.89 (3.13)		0.15
ESR, mm/h		39.10 (23.85)	45.95 (27.38)	0.55
PCT, ng/ml		0.12 (0.11)	1.13 (2.84)	< **0,001**
HbA1c, g/dl		5.72 (1.30)	5.53 (1.39)	0.63
Urea, mg/dl		93.26 (31.49)	136.66 (37.50)	by design
Uric acid, mg/dl		7.23 (1.64)	6.22 (1.30)	**0.02**
pH		7.33 (0.11)	7.37 (0.02)	**0.007**
Total protein, g/d		7.45 (0.58)	6.84 (0.63)	< **0,001**
Albumin, g/dl		4.09 (0.33)	3.99 (0.78)	0.61
CK, U/l		116.26 (91.81)	92.65 (42.79)	0.19
CK MB, U/l		3.23 (4.45)	2.79 (1.72)	0.59
TnT, ng/ml		0.01 (0.03)	0.04 (0.05)	**0.02**
NTproBNP, pg/ml		1267.74 (1891.58)	14369.02 (13169.56)	< **0,001**
TC, mg/dl		214.58 (62.08)	170.08 (49.12)	**0.005**
HDL, mg/dl		45.11 (10.19)	44.53 (14.12)	0.87
LDL, mg/dl		135.52 (52.93)	91.57 (41.75)	**0.001**
TG, mg/dl		170.42 (76.10)	192.97 (233.85)	0.69

Abbreviations: CK – creatine kinase, CKD - chronic kidney disease, CK-MB – heart isoenzyme of creatine kinase, CRP - C-reactive protein, CVD - cardiovascular disease, ESR – erythrocyte sedimentation rate, ESRD - End Stage Renal Disease, eGFR – estimated glomerular filtration rate, HbA1c – glycated hemoglobin A1c, HDL – high density lipoprotein, hs-CRP – high sensitivity C-reactive protein, LDL – low density lipoprotein, NTproBNP - N-terminal pro-brain natriuretic peptide, PCT – procalcitonin, TC – total cholesterol, TG – triglycerides, TnT – troponin T. Conversion factors to SI units are as follows: for CRP and hs-CRP (μg/l) – 1000; for HbA1c (g/l) – 0,1; for NTproBNP (pmol/l) – 0,118; for TC, HDL and LDL (mmol/l) – 0,0259; for TG (mmol/l) – 0,0113, for TnT (μg/l) – 1,0; total protein and albumin (g/l) – 10; for urea (mmol/l) – 0,357; uric acid (μmol/l) – 59,48.
Bold = statistically significant.

Table 2
Circulating baseline inflammatory biomarkers expressed as a median (25th and 75th percentile) in the controls, chronic kidney disease at stage 3–4 and end stage renal disease groups, respectively.

Biomkarker	Control group n = 19	CKD3-4 group n = 19	ESRD group n = 38	p value CKD3-4 vs Control	p value CKD3-4 vs ESRD
Adhesion molecules					
ICAM1, ng/ml	125 (109, 140)	193 (146, 258)	119 (103, 171)	**0.001**	0.82
VCAM1, ng/ml	1138 (983, 1239)	1345 (1117, 1653)	1492 (1174, 1811)	**0.008**	**< 0,001**
Inflammatory markers					
TNF, pg/ml	6.3 (5.1, 8.1)	13 (10, 19)	20 (15, 25)	**< 0,001**	**< 0,001**
IL-6, pg/ml	1.2 (1.1, 2.6)	3.8 (3.0, 5.3)	5.8 (3.0, 9.5)	**< 0,001**	**< 0,001**
Leptin, ng/ml	9.0 (3.9, 13.1)	19 (4, 33)	11 (2.6, 68)	0.16	0.28
Fetuin, µg/ml	740 (617, 906)	652 (517, 891)	570 (485, 651)	0.83	0.35
MMP9, ng/ml	326 (236, 476)	390 (317, 584)	469 (355, 585)	0.32	0.17

Abbreviations: IL6 – interleukin 6, ICAM1 – intercellular adhesion molecule 1, MMP9 – metalloproteinase 9, TNF – tumor necrosis factor, VCAM1 – vascular cell adhesion molecule 1, others - see Table 1.
Bold = statistically significant.

optical density (ELISA) or median fluorescence intensity (Luminex) were fitted using 5-parametric logistic standard curve [17].

2.2.2. Biochemical tests

Routine biochemical tests were carried out using automatic biochemical analyzers: Cobas Integra 400 plus (Roche Diagnostics, Mannheim, Germany) and Elecsys 2010 Roche. The concentration of hs-CRP was measured with use of Roche Diagnostics test; the protein electrophoresis - with Beckman, Apprasise Paragon; the NTproBNP - with Elecsys 2010 Roche; and blood morphology - with Sysmex SF3000 and Sysmex K4500.

2.2.3. Calcium score

CS was performed using 64-row CT scanner (Aquilion 64, Toshiba Medical Systems, Japan). Imaging protocol included a non-contrast enhanced, ECG-gated CT scan with the following parameters: number of scans 40–52 (dependent on the size of the heart and its position in the chest), slice thickness 3 mm, tube voltage 120 kV, tube current 300 mA. Rotation time was adopted to the heart rate. Quantitative CS was performed on dedicated Workstation (Vitrea 2 software V3.9, Vital Images Inc., USA) according to the algorithm described by Agatston et al. [18]. Lesions were identified by density level of at least 130 HU and were colour-marked by the software. Calcifications located within left or right coronary arteries and their branches were pointed by an experienced radiologist resulting in a total area of the lesions measured for all coronaries. Additionally, each lesion was scored between 1 and 4 depending on their density. CS was calculated as a sum of products of each lesion area and its density index [18].

2.2.4. Statistical analysis

Descriptive characteristics were provided as mean and standard deviations, median (25th, 75th percentile) or proportions as applicable. Departures from normality were examined (skewness and kurtosis) and logarithmic transformations were performed as applicable. Cross-sectional comparisons of biomarkers among the three groups were performed with the regression analysis for unbalanced design with a biomarker considered as a dependent variable with its values transformed to base 10 logarithms (SAS command: proc glm). Correlations were examined based on Spearman non-parametric correlation coefficients. Cox proportional-hazards models tested associations of relevant biomarkers with the prospective outcome expressed as hazard ratios per one tertile change of the monotonic marker distribution with corresponding 95% confidence intervals (one degree of freedom). Ties in the failure time were handled with the exact conditional probabilities. Stepwise selection model was used with the criteria: significance level for entry: 0.25 and for stay: 0.1). Such clinical covariates as age, gender, prevalence of diabetes and hypertension, NTproBNP, total protein and albumin together with CS and IL-6 were considered in building of the final model for the all-cause mortality. Cox models evaluating risks of cardiovascular mortalities considered age, gender, NTproBNP, total protein and albumin together with CS and IL-6. We were not able to adjust the latter models for the prevalence of diabetes and hypertension. The analysis was conducted in the testing framework of a two-sided $\alpha = 0.05$. Analyses were performed in SAS v.9.4 (Carry, NC).

3. Results

3.1. Clinical characteristics

The three study groups did not differ in regard to age or gender (Table 1). All clinical and laboratory indices related to kidney function significantly differed between the groups by study design. Subjects with more advanced stages of CKD were more frequently hypertensive. There was a tendency for higher prevalence of diabetes and prevalent CVD, but these associations did not reach statistical significance. Traditional markers of CVD such as total cholesterol and LDL were significantly lower, whereas NTproBNP and TnT were significantly higher in ESRD than in CKD3-4 group. Noticeably, classical inflammation indices, such as procalcitonin and CRP were also significantly higher in ESRD group. The difference in CRP between healthy controls and CKD3-4 group was significant only when standard method of measurement was used. However, it was within normal range in both groups. Therefore, a high sensitivity method was applied, and no significant difference could be observed.

3.2. Inflammatory biomarkers, calcium score and baseline CKD status

We found that concentrations of VCAM1, TNF and IL-6 increase significantly with the progression of renal disease (Table 2). Serum level of ICAM1 was significantly higher in the CKD3-4 group than in the ESRD group and in healthy controls. Moreover, VCAM1 concentration correlated significantly with ICAM1, TNF and IL-6 (table 3). TNF and IL-6 were also significantly correlated. No statistically significant changes could be detected for leptin, fetuin nor MMP9. Interestingly, among the studied inflammatory biomarkers only IL-6 correlated significantly with the calcium score (Table 3). Furthermore, IL-6 was

Table 3
Spearman correlation matrix among the biomarkers. Correlation coefficients are presented (* p value < 0.05).

Biomarker	IL6	TNF	ICAM1	VCAM1	CS
IL6	1.00	0.31 *	0.12	0.34 *	0.53 *
TNF		1.00	0.19	0.47 *	0.10
ICAM1			1.00	0.32 *	0.06
VCAM1				1.00	0.17
CS					1.00

Abbreviations: CS – calcium score, others - see Table 2.

Table 4
Spearman correlation matrix among the biomarkers and clinical characteristics. Correlation coefficients are presented (* p value < 0.05).

	Age	eGFR	urea	pH	CRP	PCT	hSCRP	TP	Alb	CK-MB	TnT	NTproBNP
Biomarker												
IL6	0.33 *	−0.42 *	0.23	0.08	0.66 *	0.04	0,46 *	0.14	−0,53 *	0.13	0.17	0.24
TNF	0.01	−0.65 *	0.11	0,42 *	0.24 *	0.50 *	0.13	−0,41 *	−0.17	0.15	0.39 *	0.42 *
ICAM1	0.06	0.09	−0.46 *	−0.18	0.11	−0.11	0,39 *	0.06	−0.11	−0.22	−0.23	−0.29 *
VCAM1	0.26 *	−0.42 *	0.10	0.05	0.28 *	0.06	0.29	−0.21	−0.03	0,26 *	0.21	0.18
CS	0.62 *	−0.02	0.15	0.02	0.19	0.03	−0.16	−0.05	−0.21	0,31 *	0.19	0.27

Abbreviations: Alb – albumin, others - see Tables 1 and 2.

correlated to age, eGFR, CRP and albumin (Table 4). None of the studied biomarkers correlated to uric acid or cholesterol concentration.

Importantly, calcium score values did not differ between the CKD3-4 and ESRD groups (median and interquartile ranges for CKD3-4 group: 338 (67, 563); ESRD group: 375 (38, 1144) UNITS, respectively. Nevertheless, subjects with ESRD displayed more heterogeneity in comparison with the CKD3-4 group (data not shown). Of note, CS correlated with age, IL-6 and CK-MB only. Other correlations between clinical characteristics and circulating biomarkers are presented in Table 4.

3.3. Inflammatory biomarkers, calcium score, all-cause and cardiovascular mortality

Prospective outcome ascertainment was available for 53 study subjects from the CKD3-4 or ESRD groups. During the 5-year follow-up 16 study subjects died (13 subjects in the ESRD group and 3 subjects in the CKD group, respectively) due to the all-cause mortality. Cardiovascular mortality contributed to 11 out of 13 deaths in the ESRD group and to 2 out of 3 deaths in the CKD group, respectively. In Cox proportional hazards models, CS and IL-6 were associated with the outcome in the crude analysis. They remained significant further in the model that considered all the biomarkers. CS remained the only biomarker significantly associated with all-cause mortality in the model adjusted for clinical covariates (Table 5). The results remained comparable in the Cox analysis evaluating cardiovascular mortality (Supplemental Table 1).

Stratification of the absolute risk of death, based on the CS thresholds is presented in Fig. 1. The cut-off value of 300U increased the death hazard ratio, however the number of patients is too small and confidence intervals are too broad to conclude about the threshold.

4. Discussion

To our best knowledge, our study is the very first to evaluate indices of calcification together with inflammatory biomarkers in the context of CKD and cardiovascular disease. The chief finding of this study is that IL-6 is significantly correlated with the calcium score and that both of them are significantly associated with an increased 5-year risk of all-cause and cardiovascular mortalities in CKD patients.

It was previously reported that IL-6 is a strong independent predictor of clinical outcomes in patients with CKD [19,20] and is involved in high cardiovascular risk in the general population [21]. In ESRD patients, plasma IL-6 levels have been shown to predict death better than IL1, TNF and CRP concentrations [22]. It was proposed that one of the reasons for the strong IL-6 performance is the premature ageing phenotype in patients with CKD, most probably mediated by uremic toxins driving vascular smooth muscle cell damage and phenotypic changes that promote vascular calcification [23]. Along with the IL-6 polymorphisms reported to be important genetic factors for atherosclerosis development [24] these observations support the IL-6 involvement in vascular damage and may explain our finding of IL-6 and CS correlation.

Despite multiple studies on CS in the general population, data for the incidence, prevalence, and prognosis of CS in CKD are limited, particularly in non-dialysis patients [7]. Agatson CS varies between patients with different CKD stages: non-dialysis patients with CKD have an increased CS compared with controls, but lower than that observed in patients with ESRD [25,26]. Moreover, data from studies of CS specific to patients with CKD suggest that many patients have an elevated CS, but it may not be associated with significant CAD [7]. According to the American Heart Association, the CS > 100 Agatson units is associated with high risk of coronary incident [27]. In our study, moving the cut-off value up to 300U increased the death hazard ratio from 4.3 to 9.8. Based on this observation and on the previous studies, we consider CKD patients specific subpopulation requiring higher SC thresholds. Patients on hemodialysis would probably require the highest CS cut-off value to predict the cardiovascular risk. In the previous studies, patients with CS > 400 U identified an increased risk of death independent of demographic data, risk factors, and comorbid

Table 5
Cox proportional hazard models of incident all-cause mortality in subjects with chronic kidney disease at stages 3–4 or end stage renal disease at baseline.

Marker	Crude	Partially adjusted	Final model
	HR (95%CI)	HR (95%CI)	HR (95%CI)
Imaging marker			
Calcium score	4.65 (2.14, 10.10)	3.11 (1.18, 8.17)	4.31 (1.72, 10.81)
Adhesion molecules			
ICAM1	1.14 (0.63, 2.08)		
VCAM1	1.45 (0.83, 2.52)		
Inflammatory markers			
TNF	1.30 (0.72, 2.35)		
IL6	2.60 (1.43, 4.71)	3.26 (1.28, 8.32)	
Leptin	1.04 (0.61, 1.78)		
Fetuin	0.77 (0.43, 1.38)		
MMP9	0.76 (0.44, 1.33)		

Legend: Effect of the respective biomarker is shown per tertile change. A partially adjusted model includes markers significant in the univariable analysis. All models are controlled for the baseline renal status (ESRD vs CKD3-4). For details about the final model, please refer to the methods section.
Abbreviations: see Tables 2 and 3.

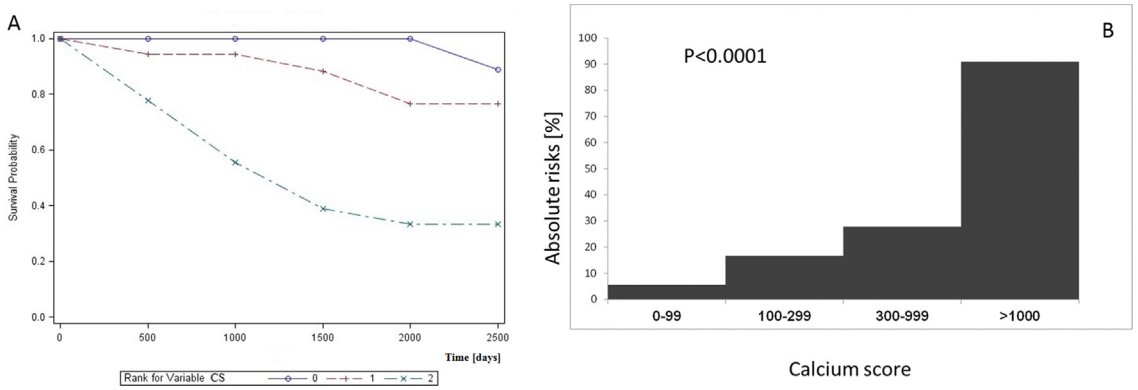

Fig. 1. Absolute risks of death stratified by clinical thresholds of calcium score at baseline. (A) Kaplan-Meier curve of the 5-year survival in the model stratified by tertiles of calcium score distributions at baseline: rank 0 - the lowest, rank 1 – medium, and rank 2 – the highest calcium score; (B) Absolute risks of death stratified by four thresholds of calcium score at baseline. Number of subjects at risk for calcium score thresholds: < 100 (n = 18); 100–299 (n = 6), 300–999 (n = 18), > 1000 (n = 11), respectively.

conditions [28,29]. Validation studies are necessary to stratify CS based CAD risks in CKD patients at different disease stages.

Thus, we confirmed the link of IL-6 to atherosclerosis manifested by an increased CS in patients with different degrees of renal impairment. TNF was found to be correlated with IL-6 and increased with the progression of CKD, too. However, it did not predict mortality in our patients (HR 1,3). It was previously reported that TNF-driven microinflammation is directly involved in the pathogenesis and progression of CKD. The TNF pathway activates cellular damage and apoptosis, recruits inflammatory cells, and causes tubulointerstitial changes [30,31].

Over the last decade, multiple studies have attempted to identify better risk prediction tools specific to the CKD population [23]. Most small- to medium-sized polypeptides circulate in elevated concentrations in CKD and appear to rise contemporaneously with a drop in GFR [32,33]. Their elevated levels may reflect not only increased production, but also a prolonged half-life caused by impaired renal clearance. Accordingly, we found that concentrations of IL-6 and TNF but also of the VCAM1 and ICAM1 increase significantly with progression of renal disease.

Endothelial dysfunction plays a critical role in the development of atherosclerosis and vascular lesions, which may be a shared common pathogenic pathway for CKD and CVD [34,35]. However, the interrelationship of multiple dysfunctions of endothelium with CKD is not well studied [36]. Cell adhesion molecules, such as ICAM1 and VCAM1 trigger leukocyte homing, adhesion, and migration into the subendothelial space. These processes are essential for atherogenesis [37]. Elevated circulating levels of ICAM1 and VCAM1 were found in patients on hemodialysis in some studies but not in others, whereas serum VCAM1 levels were significantly increased in non-dialysis CKD patients [36]. In our study, VCAM1 concentration was significantly increased in CKD3-4 patients in comparison to healthy controls, and significantly higher in ESRD than in the CKD3-4 group. Moreover, VCAM1 correlated significantly with IL-6 and TNF indicating association of inflammation and endothelial dysfunction.

Our study has certain limitations. First, the relatively small number of patients included may account for insufficient power to detect important differences. However, our study also offers the advantage of comparing patients with different stages of CKD, in contrast to most of the previous studies that focused either on dialysis, or non-dialysis CKD patients [23,29,35,36]. Second, this study relies on measurement of biomarkers at a single time point. An intra-individual IL-6 variability was reported even in healthy adults [38]. It is likely that uremic patients who are exposed to persistent low-level inflammation or patients on dialysis encounter more pronounced intra- and inter-individual variations. Third, the follow-up time can be considered both an advantage and a limitation of our study. Five-year follow-up is long enough to determine clinical outcomes with high accuracy. On the other hand, studies using single measurements of inflammatory biomarkers lasted from 2 to 10 years and some authors postulated that single measurement should only be considered for short-term outcomes [20]. In fact, from a clinical point of view, it would be unreasonable to assume that a single CRP measurement would predict mortality risk within 5 or 10 years. But other, less variable factors such as CS may be used for long-term prognosis. Obviously, repeated measurements give a better chance to yield higher sensitivity. It was recently reported that persistently elevated IL-6 levels predict CVD prognosis better than a single baseline measurement of this cytokine [20].

5. Conclusions

In conclusion, a number of inflammatory markers such as: ICAM1, VCAM1, TNF, leptin are associated with increasing renal function impairment in our cross-sectional evaluation. Our study failed to detect differences in leptin, fetuin and MMP9, but we found IL-6 and CS to be significantly associated with 5-year risk of all-cause and cardiovascular mortality in CKD and ESRD patients. Therefore, their predictive power should be validated in further studies. Our study suggests an important involvement of chronic inflammation linked to coronary artery calcification that is likely to contribute to the cardiovascular mortality in patients with impaired renal function.

6. Contribution statement

JK and MS conceived the idea for the study. BF and LP contributed to the design of the research. All authors were involved in data collection. MN, JK and MS analyzed the data. BF coordinated the project. All authors edited and approved the final version of the manuscript.

Conflict of interest

There is no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.04.002.

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