Comprehensive assessment of cardiovascular-kidney--metabolic (CKM) syndrome: Novel tools for assessment of cardiovascular risk and kidney outcomes in long-term kidney transplant patients

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ABSTRACT

Background: Cardiovascular (CV)-kidney-metabolic (CKM) syndrome, a newly defined entity, offers a framework for assessing CV risk. Emerging evidence suggests that histone deacetylase sir-tuin-1 (SIRT1) and adipokine chemerin hold promise as CKM markers.

Aims: This study aimed to explore the relationship between CKM stage, clinical parameters, and both novel and established markers of CV and renal risk.

Methods: A cohort of 102 patients with long-term, stable kidney transplant (KTx) (>24 months) and median (interquartile range) follow-up of 83 (42–85) months was recruited alongside 32 healthy controls. Patients were classified into modified CKM stages following the American Heart Association guidance. Serum high-sensitivity interleukin-6 (hsIL-6), chemerin, and SIRT1 were measured using commercial immunoassay kits. The incidence of CV events (CVE), CV mortality, and permanent transfer to dialysis therapy were primary endpoints.

Results: CKM stage was associated with higher risk of CVE/CV death (HR 95% CI, 3.79 [1.16–12.42]; $P = 0.03$) and allograft loss (HR 95% CI, 2.05 [1.17–3.57]; $P = 0.01$). Elevated sirtuin-1 was associated with significantly lower risk of CV event/death (HR 95% CI, 0.11 [0.11–0.89]; $P = 0.04$), whereas high chemerin status was tied to dialysis risk (HR 95% CI, 5.77 [1.96–17.00]; $P = 0.001$) alone. In contrast to sirtuin-1, hsIL-6 and chemerin showed incremental changes across more advanced CKM stages, though statistically significant for hsIL-6 alone. In age-adjusted models for CV disease, independent associations with diabetes and total cholesterol were noted.

Conclusions: Classifying patients based on modified CKM stages offers valuable prognostic insights for stable KTx recipients, particularly when assessing reno-cardiovascular risk. The investigated serum markers may serve as supplemental tools for refining risk stratification.

Key words: cardiovascular, chemerin, kidney transplant, prognosis, sirtuin

WHAT'S NEW?

The newly defined cardiovascular-kidney-metabolic (CKM) syndrome offers a framework for evaluating cardiovascular (CV) risk, which is particularly useful in stable kidney transplant (KTx) patients, as CV causes are a leading cause of death and graft loss. This study aimed to explore the relationship between CKM stage, clinical characteristics, and potential markers, like the histone deacetylase sirtuin-1 and adipokine chemerin. We demonstrate that classification into CKM stages allows for both CV and renal risk stratification. Only highly sensitive interleukin-6 differed significantly by CKM class, which implies incremental, low-grade inflammatory burden. High sirtuin-1 concentrations were prognostic for favorable CV and renal outcomes. In contrast, circulating chemerin levels were associated with severity of renal disease. These findings likely indicate intertwined pathophysiological pathways in CKM, which may be indirectly tracked by these candidate marker assessments. This study aligns with the growing emphasis on multiorgan assessment in CV health, reflecting evolving paradigms in clinical practice.

INTRODUCTION

Chronic kidney disease (CKD) continues to pose a significant health burden in developed nations owing to its slow but relentless progression toward end-stage renal disease, which is compounded by enhanced cardiovascular (CV) risk and excess CV mortality [1, 2]. Data from the Global Burden of Disease Study show a concerning upward trajectory in CKD prevalence, more pronounced among males and the elderly. Both CV and metabolic disorders (mainly diabetes, obesity, and hypertension) also emerge as major contributors to excess morbidity [2]. In patients with progressing CKD, kidney transplantation (KTx) represents the final line of therapy. In the post-KTx setting, despite the associated CV risk reduction, enhanced morbidity and mortality remain clinically significant [3]. Firstly, traditional CV risk factors are highly prevalent in CKD populations and commonly persist after KTx. There are also several non-traditional, CKD-specific risk factors, such as low-grade inflammation, altered mineral-bone balance resulting in enhanced vascular calcification), and immunosuppressive medication use (i.e., post-KTx) [3, 4]. Achievement of treatment targets (e.g., blood pressure, lipid levels) also appears to be more difficult, in part, due to concomitant use of immunosuppressive agents [3, 4]. This paints a complex clinical picture of post-KTx management, in which a tailored holistic approach is optimal.

The ongoing research into individualized risk prediction continues with the discovery and validation of novel diagnostic and prognostic tools. For example, the incorporation of serum cystatin and urine albumin-creatinine ratio has led to a marked improvement in serum creatinine-based standards for renal function assessment [1]. Aside from markers tied to estimated glomerular filtration rate (eGFR), recent efforts also focus on deriving functional information from pathophysiologic pathways of renal disease. Panels of inflammatory and immuno-modulatory biomarkers (including cytokines, chemokines, and remodeling factors) that reflect innate and adaptive immune dysregulation have been shown to be significantly associated with eGFR based on data from two population-based studies [5]. For the general population, to address the multiorgan link with CV disease, preliminary guidance for achieving "cardiovascular-kidney-metabolic" (CKM) health was recently proposed by the American Heart Association [6]. This concept indicates a paradigm shift towards a patient-centered (rather than disease-oriented) approach in modern-day medicine, triggered by aging populations and the related burden of multimorbidity.

Chronic inflammation is related to adaptive energy homeostasis. Sirtuin-1 (SIRT1) is a class III NAD+ protein deacetylase localized in nuclei that protects against tissue damage. Its effects are derived from attenuation of inflammation and regulation of cell cycle (i.e., reduced apoptosis) through protein deacetylation, which includes downstream effects on NF-κB and p53 signaling [7, 8]. Experimental evidence shows how treatment with resveratrol, a SIRT1 activator, promotes autophagy and counteracts aging processes in cardiomyocytes [9]. Modulation of carbonyl stress by SIRT1 is also purported to regulate age-related cardiac susceptibility to ischemic injury [10]. While effector immune cells mainly operate on glycolysis, repressive responses (modulated by sirtuins) are fueled by fatty acids [11], which are a predominant energy source in the context of chronic inflammation. SIRT1 has also been identified as a regulator of vascular calcification based on its effects on endothelial vascular smooth muscle and adipose tissue cells [12]. Experimental data indicate that SIRT1 inhibition leads to tubular fibrosis [13], endothelial dysfunction [14], and susceptibility to oxidative stress [15].

Chemerin is a polypeptide adipocytokine involved in coagulation and inflammation cascades [16, 17]. It is mainly secreted by the liver and white adipose tissue, but also by immune cells and endocrine organs [16]. Pooled data from multiple cohorts of CKD patients indicate that higher chemerin concentrations accompany a decline in renal filtration [18]. While enhanced expression has been observed in experimental chemerin-related models of kidney disease, whether local production alone translates to marked shifts in circulating levels remains uncertain [19, 20]. In contrast, evidence accumulates for the importance of chemerin within the vasculature, specifically regarding its regulatory role in arterial blood pressure. While also acting as a potent chemoattract for immune cells, chemerin not only stimulates direct and indirect vasoconstriction but also vascular smooth muscle changes, which has led to the investigation of its role in hypertension [21].

This study was undertaken to incorporate the CKM approach in describing the clinical profile of long-term KTx recipients (KTRs). We aimed to explore the concept of CKM in stable KTRs and evaluate the role of chemerin and sirtuin-1 as new potential markers of prognostic importance related to CV and renal risk. With CKD prevalence on the rise, despite KTx offering an effective therapeutic avenue for CKD patients, persistent CV morbidity and CV mortality highlight the necessity of exploring the use of non-traditional tools.

METHODS

Patients

This longitudinal observational study enrolled 102 adult (≥18 years) long-term KTRs from the University Hospital in Kraków, Poland. Only patients who maintained allograft function for at least 24 months were included (described as "long-term"). Those with a probable history of acute rejection, current active or chronic infection, prior parathyroidectomy, or malignancy were excluded due to potential interference with biochemical assessments at the baseline visit. Clinical data, including demographic, treatment, and comorbidity-related characteristics were collected, based on medical chart review. Most patients received triple immunosuppressive therapy with tacrolimus or cyclosporine. Glomerulonephritis, congenital and reflux nephropathy were the main primary renal causes of KTx.

Patients undergoing ambulatory care visits between September 2016 and October 2017 were screened and queried about interest in participation in the study. Subjects who expressed intent, fulfilled enrollment criteria, and provided written consent were recruited.

A control group of 32 volunteers (16 females, 16 males), without any apparent medical conditions, aged 29 to 74 years (mean 50 years) was recruited through convenience sampling by medical professionals and staff at our center, to serve as reference.

A more detailed description of the study criteria and the control group is presented alongside the flowchart in the Supplementary material (*Figure S1*).

Definitions

Hypertension and dyslipidemia were defined based on prior medical records, while achievement of treatment targets was determined based on office blood pressure measurement at the baseline visit <140/90 mm Hg [22] or total cholesterol <4.0 mmol/l [23], respectively. By convention, fasting blood glucose (FBG) over 99 mg/dl and below 125 mg/dl was treated as impaired fasting glycemia. Metabolic risk factors were defined with body mass index (BMI) cut-off at 25 and 30 kg/m^2 for overweight status/obesity, while information about dyslipidemia or diabetes mellitus (either pre-existing type 1, type 2, or post-transplant) was derived from chart review.

Manifest CV disease (CVD) was defined as the presence of coronary artery disease (CAD) or prior history of myocardial infarction (MI), transient ischemic event/stroke, thromboembolic event (TE; defined as pulmonary embolism or deep venous thrombosis), or heart failure. This reflects a pragmatic definition due to the modest size of this cohort and the relatively low frequency of events. Information about diagnostic or therapeutic invasive procedures was difficult to determine reliably for all patients and was not included within the scope of this definition.

In line with the recent proposal of CKM syndrome definition, a modified concept for assessing global CV risk was developed (see Figure 1):

1. Stages 0 and 1 were defined for individuals with retained graft function (Kidney Disease: Improving Global Outcomes [KDIGO] I or II), in the absence of any overt CV risk disorder (dyslipidemia, diabetes, or manifest CVD). Patients with normal FBG (70–99 mg/dl) and BMI <25 kg/m^2 were classified as Stage 0, while those who were either overweight (25–30 kg/m^2) or had impaired fasting glycemia (100–125 mg/dl) were classified as Stage 1.
2. Stage 4 was defined as the presence of manifest CVD (as defined above) and the concomitant presence of at least one other CV risk factor or disorder (KDIGO ≤IIIA, BMI >25 kg/m^2, diabetes, FBG >7.0 mmol/l, dyslipidemia, total cholesterol >4.0 mmol/l, office BP >140/90 mm Hg).
3. Patients who did not meet the criteria for Stage 0, 1, or 4 were grouped into Stage 2/3, with no further differentiation due to a lack of data for subclinical CV disease.

The nephro package was used to calculate eGFR according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula based on serum creatinine [24], with the kidney disease stage assessed according to the KDIGO guidelines [25].

Additionally, SCORE2 was measured to obtain a quantitative, proxy estimate of the CV risk related to traditional risk factors in the general population [26]. Since this was a supplemental descriptive characteristic due to missing data for high-density lipoprotein, it was calculated with conservative imputation based on the upper reference limit for the general population.

Follow-up

Patient outcomes were tracked by four physicians (AS, MB, KN, KB) who manually examined available hospital records in electronic and paper form. In the first two years, follow-up data were gathered from consecutive visits spaced within a pre-defined time range from 3 to 6 months apart. This process was carried out for the first two years after the baseline study visit. Thereafter, endpoint status was assessed up to December 31, 2023 (censoring date). Outcomes were tracked through medical records and, if possible, telephone contact with the patient, family, and/or

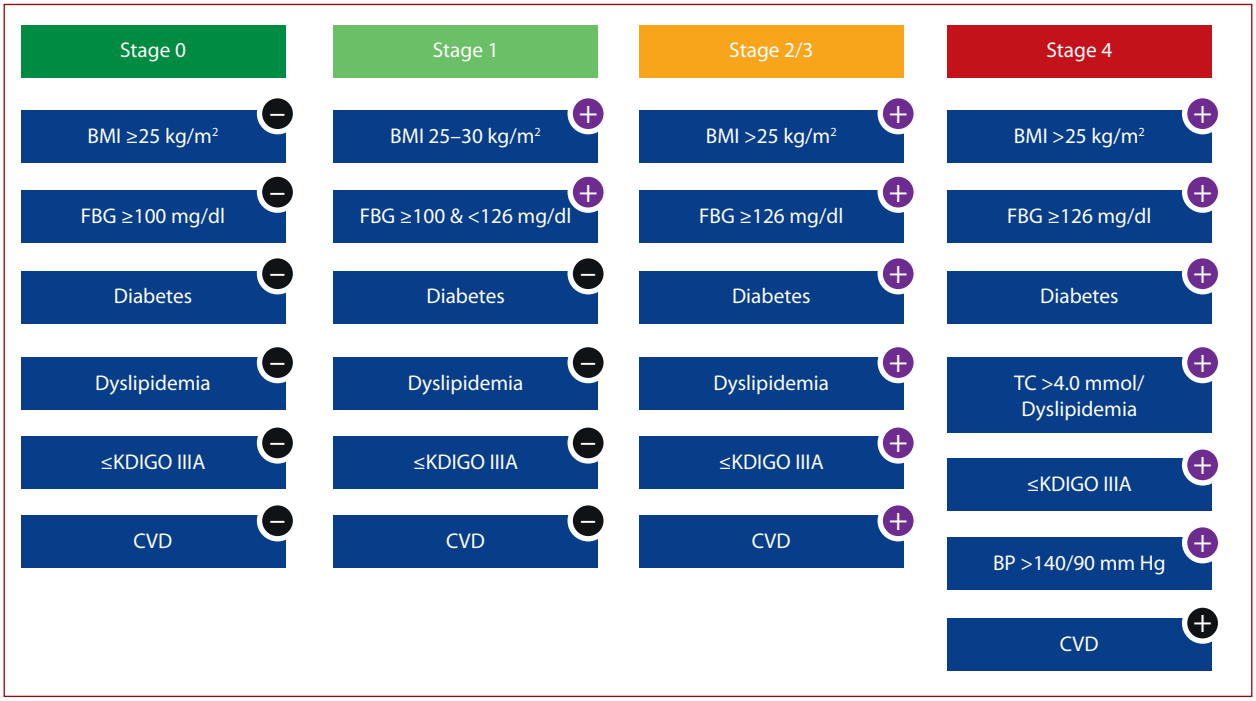

Figure 1. Modified classification system for cardiovascular-kidney-metabolic (CKM) burden in kidney allograft recipients. We stratified patients based on the concomitant presence of risk factors or disorders of CV significance. Black circles with presence (plus) and absence (minus) signs reflect mandatory requirement for classification, while purple circles indicate requirement for at least one disorder or risk factor to be present, aside from the mandatory condition (dark circle). Intermediate cases not fulfilling the definitions listed above were considered nonspecific, but elevated risk (Stage 2/3). The original stage 3, previously termed "Subclinical CVD" in CKM, was not included due to lacking data for subclinical disease assessment (N-terminal pro-B-type natriuretic peptide, troponin levels, computed tomography angiography, etc.) and thus classification uncertainty

Abbreviations: BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; FBG, fasting blood glucose; KDIGO, Kidney Disease: Improving Global Outcomes; TC, total cholesterol

dialysis center. Definitions are as follows: 1) allograft loss — permanent transfer to dialysis therapy, 2) cardiovascular event (CVE) — myocardial infarction, stroke or transient ischemic event, acute limb ischemia or pulmonary embolism, and 3) death from any cause, which was further differentiated into CV, infectious, neoplastic and other (unknown) causes. Due to the study scope, death from non-CV causes was treated as a non-informative censoring event.

Biochemical testing

Blood samples were collected, stored at -70 C, and analyzed using standard biochemistry assays and three automatic analyzers: Hitachi 917 (Hitachi, Japan), Modular P (Roche Diagnostics, Germany), and Sysmex XE 2100 (Sysmex, Japan).

Non-routine assays for serum concentrations of SIRT1, chemerin, and sensitive interleukin-6 (hsIL-6) were analyzed using commercially available immunoenzymatic assays in batches by the experienced study staff. All procedures were performed following the manufacturer's instructions.

Specifically, we tested the serum concentration of sirtuin 1 with a competitive kit (Human NAD-dependent deacetylase sirtuin-1 SIRT1 ELISA Kit, EIAab Science Co. Ltd, Wuhan, China; sensitivity below 32 pg/ml; detection range: 78–5000 pg/ml; intra- and inter-assay precision of <4.3% and <7.2%, respectively). The hsIL-6 concentration was tested using the Quantikine Human Chemerin Immunoassay (R&D Systems, Minneapolis, US; sensitivity: 4.13 pg/ml; reference range: 48–142 pg/ml; intra- and inter-assay precision of <4.5% and <7.9%). The chemerin concentration was tested using the Quantikine HS Human IL-6 Immunoassay (R&D Systems, Minneapolis, MN, US; sensitivity: 0.039 pg/ml; reference range: 0.477–9.96 pg/ml; intra- and inter-assay precision of <7.8% and <9.6%).

Chemerin and sirtuin-1 were stratified into "low" and "high" risk-based pragmatic cut-offs (94 ng/ml and 10 ng/ml, respectively) based on median concentrations in KTRs' serum.

Bioethics statement

The study was performed according to the Declaration of Helsinki and Good Clinical Practice recommendations. Ethical approval was obtained from the bioethics committee of the Jagiellonian University Medical College. All participants provided written informed consent.

Statistical analysis

Statistical analysis was performed in R 4.3.3 (R Core Team, 2024, R Foundation for Statistical Computing, Vienna, Austria). We summarized continuous and nominal variables using means (standard deviations [SD]) and medians (interquartile ranges), or counts and proportions (n, %). A cross-group comparison in Table 1 and Supplementary

Table 1. Baseline demographic and clinical characteristics of patients stratified by cardiovascular risk according to CKM stage

	Stage 0 (n = 11)	Stage 1 (n = 11)	Stage 2/3 (n = 55)	Stage 4 (n = 25)	Total (n = 102)	*P*- value
Age, years	40.00 (24.00–45.50)	52.00 (47.50–54.50)	52.00 (39.00–61.00)	61.00 (56.00–66.00)	53.00 (41.25–61.00)	<0.001
BMI, kg/m^2	22.64 (21.64–24.27)	26.70 (25.64–28.02)	24.86 (23.22–29.95)	28.07 (24.39–30.41)	25.64 (23.18–29.44)	0.01
Male, n (%)	9 (81.8)	6 (54.5)	37 (67.3)	21 (84.0)	73 (71.6)	0.37
KDIGO stage, n (%)						
IV	0 (0.0)	0 (0.0)	5 (9.1)	5 (20.0)	10 (9.8)	<0.001
IIIB	0 (0.0)	0 (0.0)	16 (29.1)	10 (40.0)	26 (25.5)	
IIIA	0 (0.0)	0 (0.0)	16 (29.1)	4 (16.0)	20 (19.6)	
II	8 (72.7)	9 (81.8)	13 (23.6)	6 (24.0)	36 (35.3)	
I	3 (27.3)	2 (18.2)	5 (9.1)	0 (0.0)	10 (9.8)	
Baseline eGFR, ml/ /min/1.73 m^2	80.20 (67.78–88.88)	66.91 (62.47–85.75)	53.82 (40.29–68.20)	40.43 (32.41–59.80)	56.95 (40.41–71.46)	<0.001
Hypertension, n (%)	8 (72.7)	9 (81.8)	51 (92.7)	24 (96.0)	92 (90.2)	0.11
Dyslipidemia, n (%)	0 (0.0)	0 (0.0)	11 (20.0)	5 (20.0)	16 (15.7)	0.17
Diabetes, n (%)	0 (0.0)	0 (0.0)	17 (30.9)	13 (52.0)	30 (29.4)	0.002
Coronary artery disease, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	19 (76.0)	19 (18.6)	<0.001
Prior MI, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	6 (24.0)	6 (5.9)	<0.001
Prior VTE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	4 (16.0)	4 (3.9)	0.01
Prior stroke, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.00
Statin use, n (%)	7 (63.6)	6 (54.5)	23 (41.8)	12 (48.0)	48 (47.1)	0.55
RAAS inhibitor, n (%)	6 (54.5)	2 (18.2)	15 (27.3)	8 (32.0)	31 (30.4)	0.26
Calcium blocker, n (%)	5 (45.5)	6 (54.5)	35 (63.6)	20 (80.0)	66 (64.7)	0.17
β-blocker, n (%)	6 (54.5)	8 (72.7)	34 (61.8)	17 (68.0)	65 (63.7)	0.78
α-blocker, n (%)	2 (18.2)	5 (45.5)	27 (49.1)	13 (52.0)	47 (46.1)	0.32
Total cholesterol, mmol/l	5.30 (4.90–5.55)	4.60 (4.43–5.38)	5.20 (4.60–5.97)	4.30 (3.80– 5.15)	5.10 (4.30– 5.60)	0.03
Fasting glucose, mmol/l	4.84 (4.65–5.31)	5.07 (4.80–5.50)	5.56 (5.01–6.21)	5.63 (5.15–6.42)	5.40 (4.95– 5.99)	0.004
Hemoglobin, g/dl	14.80 (14.00–15.25)	14.10 (12.95–14.70)	14.20 (12.50–14.90)	13.60 (12.90–15.00)	14.15 (12.60–14.90)	0.45
Sirtuin-1, pg/ml	10.85 (8.92– 12.08)	10.47 (7.02–13.93)	10.10 (9.21– 12.41)	9.09 (7.93, 10.79)	10.09 (8.45–12.38)	0.43
Chemerin, ng/ml	80.58 (56.53–103.56)	91.17 (74.00–94.82)	95.72 (83.39– 127.45)	102.40 (85.35–122.60)	94.06 (81.03–121.30)	0.12
hsIL-6, pg/ml	1.76 (1.45–3.84)	3.02 (1.73–4.91)	3.02 (2.12– 5.02)	4.34 (3.22–5.87)	3.20 (2.11–5.07)	0.04
SCORE2	2.00 (2.00–6.00)	5.00 (3.00–6.75)	7.00 (5.00–10.00)	12.00 (7.00–18.00)	7.00 (4.00–10.50)	<0.001
CVE, n (%)	0 (0.0)	0 (0.0)	4 (7.3)	4 (16.0)	8 (7.8)	0.321

Abbreviations: BMI, *body mass index*; CKM, cardiovascular kidney metabolic; CVE, cardiovascular event; eGFR, estimated glomerular filtration rate; hsIL-6 high sensitivity interleukin-6; KDIGO, *Kidney Disease: Improving Global Outcomes*; MI, myocardial infarction; RAAS, *renin–angiotensin–aldosterone* system; SCORE2, Systematic Coronary Risk Estimation *2;* VTE, venous thromboembolism

material, *Figure S2* is provided using more robust tests (Kruskal–Wallis/Wilcox and Fisher's exact test, respectively). Serum marker concentrations are compared between KTx recipients and controls using means and SDs, with P-values based on permutation t-tests (Supplementary material, *Table S1*). Similarly, the comparison across CKM and CKD subgroups is described using means and SDs, with formalized testing using analysis of variance (ANOVA), supplemented with a permutation equivalent (Supplementary material, *Table S1*). For bivariable relationships, Spearman's rho and Pearson's r were utilized to assess monotonic and linear relationships using pairwise observations and are denoted as rho and R, respectively. For log-transformed variables, the minimal offset was utilized to avoid non-finite values. For risk factor analysis, age-adjusted bivariable logistic regression models are reported in Table 2. For all models, calibration plots (observed vs. expected values) and Hosmer–Lemeshow tests were performed to assess goodness of fit. Survival analyses are based on Kaplan–Meier curves supplemented with log-rank tests (Figure 3) and univariable Cox proportional hazard (PH) regression models, which are summarized using hazard ratios (HR) with corresponding 95% confidence intervals (95% CI). The PH assumption was tested for all Cox PH models using Schoenfeld residuals. Multivariable Cox PH models were not considered due to the low event rate.

Table 2. Summary of age-adjusted multivariable logistic regression for the presence of manifest cardiovascular (CV) disease

Feature	Adjusted odds ratios		
	OR	95% CI	*P*-value
Age, years[a]	1.09	1.04–1.15	<0.001
Sex, male	3.96	1.21–16.12	0.03
Diabetes, yes	4.49	1.52–14.16	0.008
Current smoking	0.47	0.02–3.24	0.51
MAP, mm Hg	1.04	0.99–1.09	0.10
BMI, kg/m^2	1.10	0.97–1.24	0.13
Total cholesterol, mmol/l	0.46	0.23–0.84	0.02
hsIL-6, log	2.11	0.87–5.35	0.10
Sirtuin-1, ng/ml	0.87	0.73–1.01	0.08
Chemerin, ng/ml	1.01	0.99–1.03	0.15

[a]Base univariable model

Abbreviations: CI, confidence interval; MAP, mean arterial pressure; OR, odds ratio; other — see Table 1

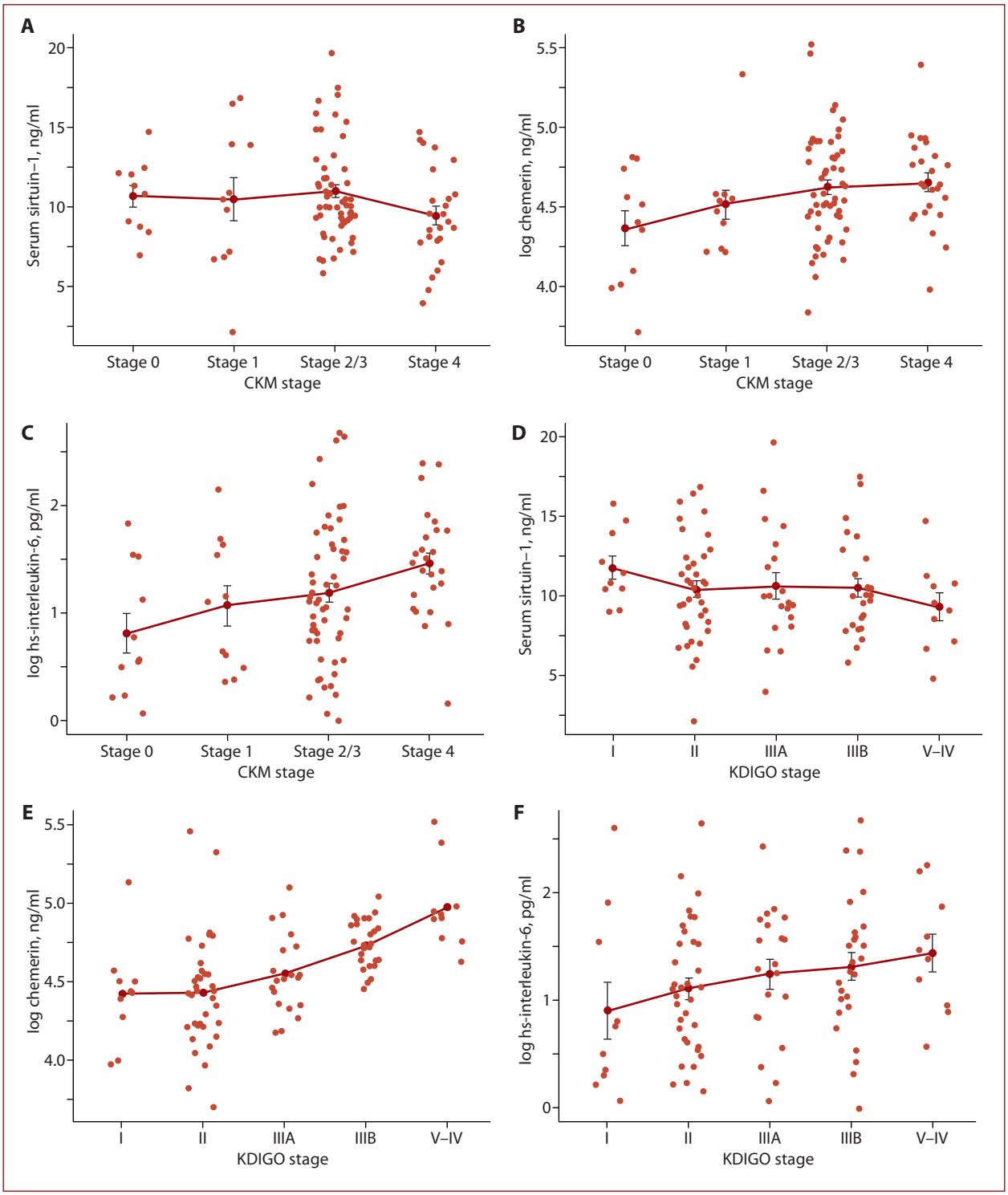

Figure 2. Comparison of mean (standard error) concentrations of serum hsIL-6, chemerin and sirtuin-1 concentrations across CKM and CKD stages

Abbreviations: CKD, chronic kidney disease; CKM, cardiovascular kidney metabolic; hsIL-6, high sensitivity interleukin-6

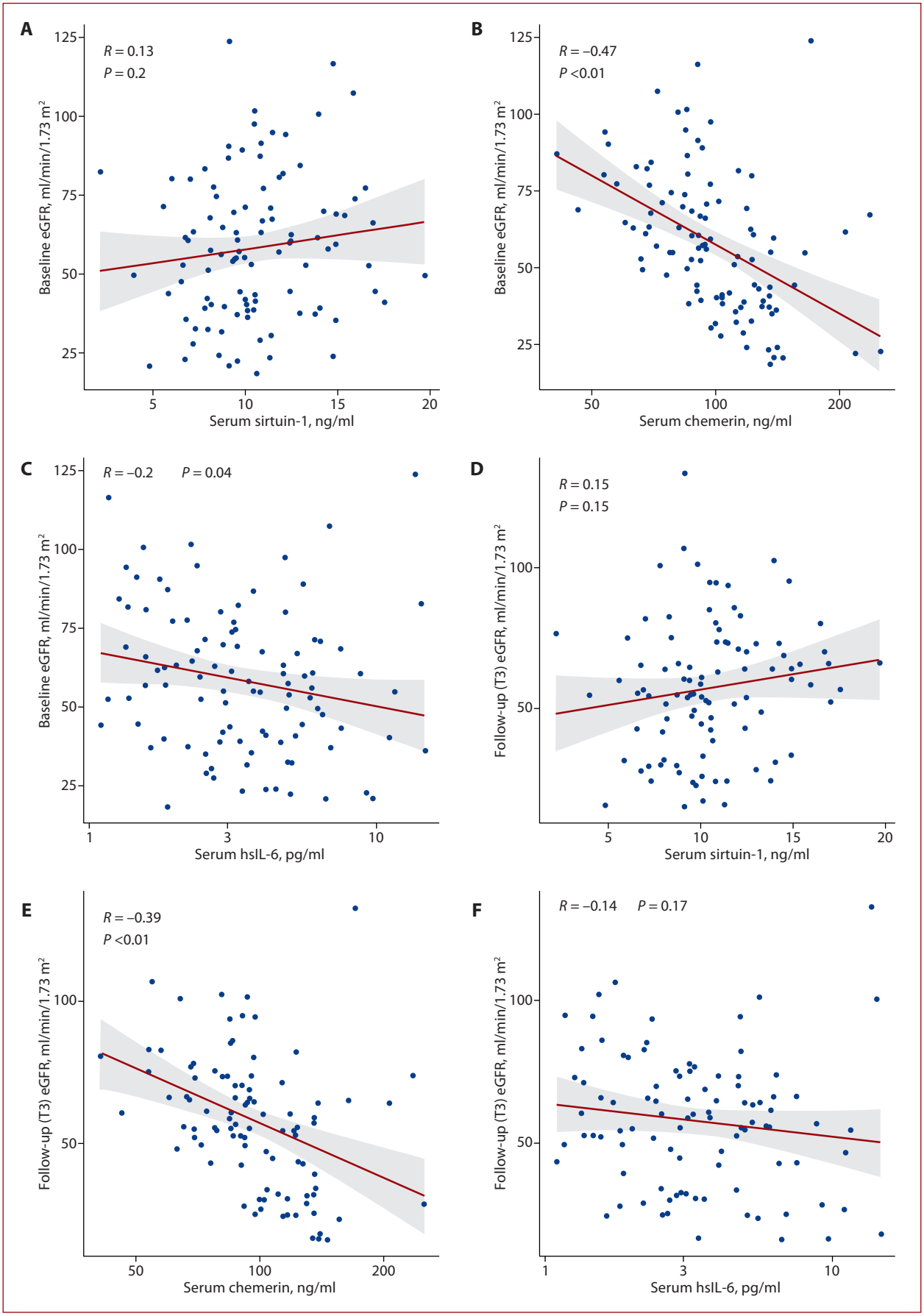

Figure 3. Scatter plot with linear fit illustrating the relationship between baseline eGFR (**A, B, C**) and future eGFR (**D, E, F**) compared according to serum sirtuin-1, chemerin and hsIL-6 concentrations, respectively

Abbreviations: eGFR, estimated glomerular filtration rate; other — see Figure 2

RESULTS

Baseline clinical characteristics of long-term kidney transplant recipients and inter-relationships with sirtuin-1, chemerin, and highly sensitive interleukin 6

Mean (SD) serum sirtuin-1, chemerin, and hsIL-6 concentrations were all significantly higher in KTRs when compared with controls (P <0.001; Supplementary material, *Table S1*).

Baseline clinical characteristics are summarized by the modified CKM staging in Table 1. This cohort represents middle-aged adults, most of whom were males with higher body mass, and can be classified as heightened, but not very high CV risk (Stage 2/3 CKM). Importantly, no patients had renal failure (KDIGO stage V) at baseline, while relatively comparable proportions from each KDIGO stage were recruited. We observed incremental concentrations of hsIL-6 and chemerin (but not sirtuin-1) across CKM stages (Figure 2, Supplementary material, *Table S2*), which was also paralleled by higher SCORE2 values (Table 1). Metabolic disorders, such as diabetic status, higher BMI, or abnormal fasting glucose were more common in higher CKM stages, as can be expected from the classification criteria.

We compared serum concentrations of the studied markers by CKM and KDIGO stage (Figure 2; Supplementary material, *Tables S2–S3*). In line with observations of higher hsIL-6 concentrations with more severe CKM class, we also observed higher hsIL-6 levels associated with the presence of CAD, diabetes, and prior history of MI. In contrast, no significant differences in serum concentrations were observed for chemerin, and only sirtuin-1 levels differed by prior history of cardiac ischemia (Supplementary material, *Figure S2*).

Relationships between clinical features and circulating concentrations of sirtuin-1, chemerin, and highly sensitive interleukin-6

In age-adjusted models predicting manifest cardiovascular disease (Table 2), we observed a significant relationship between diabetic status and total cholesterol. Notably, mean arterial pressure and male sex may be other, potentially relevant predictors.

Of all 3 markers, we only observed a significant positive relationship between incremental serum concentrations of chemerin and more advanced renal impairment (Figure 2E; Supplementary material, *Table S3*), which is consistent with its relationship with renal function over follow-up (Figure 3). Due to the intervals in ambulatory care, the second consecutive visit (T3; 6 to 12 months from baseline visit) was selected as an interim measure for the relationship with future renal function.

Serum sirtuin-1 concentrations showed a weak positive association with total cholesterol (rho = 0.15) and a moderate negative relationship with hsIL-6 (rho = –0.31). Similarly, chemerin levels were positively associated with both hsIL-6 (rho = 0.24) and total cholesterol (rho = 0.23). No monotonic associations (defined as rho >0.10) were observed with age, BMI, and mean arterial pressure for both sirtuin-1 and chemerin.

Relationship between chemerin, sirtuin-1, and interleukin 6 with cardiovascular events, dialysis therapy risk, or death

Over a median follow-up of 83 months (IQR 42–85), we recorded 21 (20.6%) deaths, of which 8 (38.1%) were classified as having a primary CV cause (4 MI, 1 pulmonary embolism, 2 stroke, 1 nonspecific). Among 25 patients requiring permanent transfer to dialysis therapy, 8 individuals died, and 3 of these deaths were CV-related.

Survival free from different types of poor outcomes is visualized in Figure 4. We observed associations between the CKM stage and risk of CVE/CV death (HR 95% CI, 3.788 [1.155–12.42]; P = 0.03), as well as allograft loss (HR 95% CI, 2.048 [1.174–3.573]; P = 0.01). SCORE2 was associated with enhanced risk of CVE/CV death (HR 95% CI, 1.164 [1.014–1.336]; P = 0.03), but not allograft loss (HR 95% CI, 1.039 [0.960–1.123]; P = 0.34).

When stratified by median values, high sirtuin-1 levels were associated with significantly lower risk of CVE/CV death (HR 95% CI, 0.110 [0.113–0.893]; P = 0.04) and allograft loss (HR 95% CI, 0.428 [0.189–0.973]; P = 0.04). In turn, high chemerin status was not linked to CVE/CV risk (HR 95% CI, 0.595 [0.142–2.497]; P = 0.48), in contrast to the permanent dialysis therapy risk (HR 95% CI, 5.77 [1.96–17.00]; P = 0.001).

DISCUSSION

Risk prediction in stable, long-term KTRs remains difficult due to the paucity of existing data, as well as small sample sizes of available studies, their heterogeneity, and temporal instability (i.e., changing immunosuppressive treatment and general management) across cohorts. The response variables are also usually derived from surrogate measures of graft function or review of medical history (e.g., records of hospitalization for MI). Gold-standard diagnostic procedures (e.g., kidney biopsy, coronary angiography) are invasive and conservatively performed due to higher risk [27]. However, following a decline in infection-related deaths in KTRs under modern therapy, CV mortality increased in importance, with CV diseases currently representing the most frequent cause of graft loss [3]. Development of reliable prediction models for poor outcomes in KTRs requires identifying relevant biomarkers and developing risk prediction tools that parallel the main pathophysiological pathways in CKD, but also those involved in enhanced comorbidity. This study was conducted to explore whether using the recent concept of CKM [6] can facilitate CV risk stratification in KTRs and evaluate whether circulating serum SIRT1, chemerin, and hsIL-6 are potential markers due to their link with associated processes.

We observed that the classification of KTRs into modified CKM stages may allow for prediction of both poor

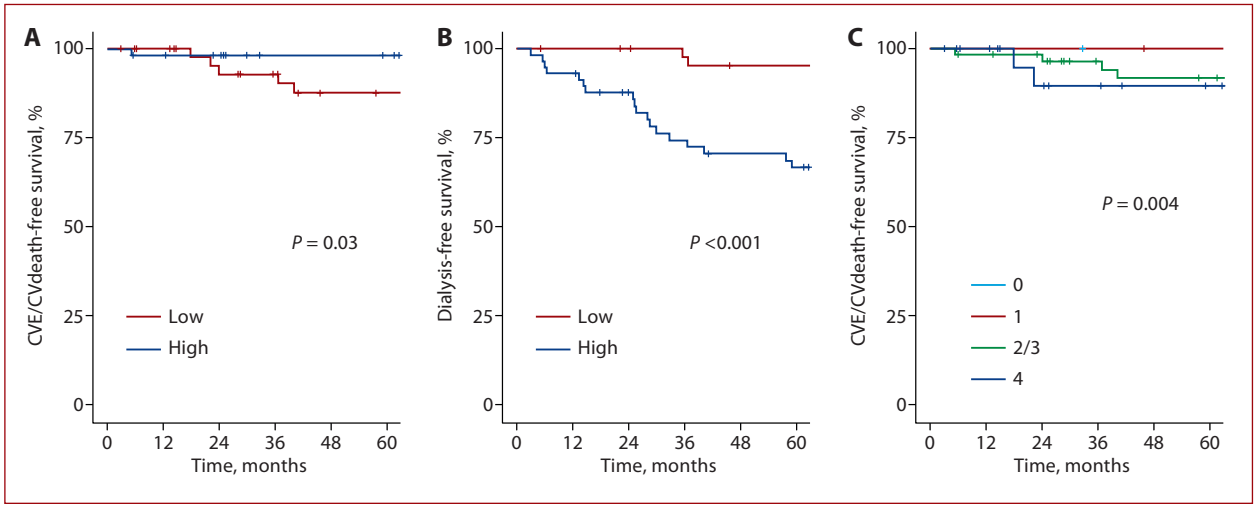

Figure 4. Survival free from poor outcome compared across patients stratified by median concentrations of sirtuin-1 (left) and chemerin (middle), as well as modified CKM class (right), respectively

Abbreviations: see Figure 2

CV and renal outcomes in survival analyses. Due to the incorporation of different clinical characteristics, including comorbidity, CKM staging represents a useful descriptive measure for global CV risk. The underlying theoretical background that ties each of these constituent risk factors together also supports the use of such a tool. Moreover, in post-KTx recipients, traditional and non-traditional CV risk factors often show bidirectional relationships (e.g., hypertension as a cause and result of graft dysfunction) [27, 28].

SIRT1 is considered an anti-aging protein, based on studies in age-associated disorders [29], but also a mediator of immune-metabolic cross-talk in CV diseases [30]. Based on an array of experimental data, SIRT1 is ascribed a protective effect against vascular calcification through regulation of NO bioavailability, cell differentiation, adipose tissue dysfunction, and inflammation [12]. Population-based data indicate that genetic polymorphisms in SIRT1 may affect susceptibility to diabetic kidney disease and atherosclerosis [31, 32]. We observed that higher SIRT1 concentrations were associated with enhanced CV risk. They were also significantly lower in individuals with a prior history of MI, and numerically lower in patients with CAD. By comparison, hsIL-6 levels were significantly higher in patients with both CV disease and diabetes. In studies of patients with type 1 diabetes, sirtuin-1 levels were significantly associated with echo- (posterior and relative wall thickness) and electrocardiographic (QTc) parameters of early cardiac dysfunction [30]; other studies reported an association with ejection fraction [29]. Elevated serum SIRT1 has also been previously associated with worse renal function in both CKD and hemodialysis patients [29, 33], with significantly higher levels when compared with age- and sex-matched controls [33].

There is an array of data demonstrating that chemerin may be associated with CKM driving pathways. In humans, SIRT1 concentrations in serum/plasma are lower in patients with aortic aneurysm [34], stroke [35], and prior history of myocardial infarction [36], but also type 2 diabetic nephropathy [37]. Circulating chemerin is associated with dysfunctional adiposity measures (obesity, lipid alterations) and blood pressure [38]; it is also an independent marker of arterial stiffness, even after adjustment for confounders [39]. In patients with metabolic syndrome, elevated chemerin levels are associated with CAD presence assessed by coronary angiography [40]. Other groups have also demonstrated a relationship with renal function in diabetics [41]. We observed a significant relationship between higher chemerin levels and KDIGO stages in KTRs, which corresponds with pooled observation for the general population [18]. While liver and endocrine tissue appear as the main sources of body chemerin, other origins may be more pronounced under conditions of tissue stress [16]. The role of adipose tissue as a major source of chemerin in CKD remains unclear [42]. Due to the 16 kDA polypeptide size, increased chemerin may result from reduced excretion or increased stimulation of adipokine levels, which is consistent with a decline in concentrations after KTx [18, 43]. Alternatively, excessive activation of inflammatory, endothelial, and oxidative cascades following renal insult may also contribute to higher chemerin levels [18]. In the present study, we observed that serum chemerin concentrations increased with higher CKM (numerical) and CKD stage and also exhibited a prognostic role in assessing the risk of dialysis therapy.

Though it receives much attention, optimal CV screening in the pre- and post-KTx setting has not been defined [44, 45], while the pre-existing baseline risk in the Polish population remains high [46]. This is the first study to evaluate the CKM stage system (though modified), as well as serum SIRT1 and chemerin concentrations in stable long-term KTRs. The major strength is the recruitment of a homogenous population; however, due to the low event

rate, we are unable to account for other, potentially-relevant clinical confounders. It should be noted that the chosen definitions and outcome classification are also prone to attribution bias. We may also be lacking information about non-fatal CV events that occurred outside of our center and affiliated wards. While of great interest, due to sample characteristics and pharmacotherapy variety, we could not reliably evaluate the cardioprotective effects of novel medications, e.g., sodium-glucose cotransporter inhibitors [47]. We also did not assess local expression of tissue markers, which limits the conclusions drawn to inferences based on other reports. Certain molecules that are critical for disease progression on the local level (auto- and paracrine mechanisms), may not translate into marked shifts in systemic concentrations, which precludes their use as serum/plasma biomarkers.

CONCLUSION

This study investigated CV and renal outcomes in long-term KTRs by exploring the predictive value of serum sirtuin-1 and chemerin levels within the context of the CKM staging system. Serum hsIL-6 significantly increased with an advanced CKM stage, in parallel to its positive association with CAD, prior history of MI, and diabetic status. Elevated sirtuin-1 levels were associated with increased risk of CV events/CV death, as well as allograft loss. In contrast, higher chemerin levels were associated with allograft impairment and requirement for permanent dialysis therapy. Alterations in serum SIRT1, chemerin, and hsIL-6 likely reflect the intertwined and multifaceted pathways that drive cardiac, metabolic, and renal disease.

Supplementary material

Supplementary material is available at https://journals.viamedica.pl/polish_heart_journal.

Article information

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