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

Short title: CKM and kidney outcomes in stable KTx

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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 stage 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 regarding favorable CV and renal outcome. In contrast, circulating chemerin levels were associated with renal disease severity. These findings likely parallel 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.

ABSTRACT

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

Aims: This study aims 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 American Heart Association guidance. Serum high sensitivity interleukin-6 (hsIL-6), chemerin, and SIRT1 were measured using commercial immunoassay kits. Incidence of CV events (CVE), CV mortality, and permanent transfer to dialysis therapy were primary endpoints.

Results: CKM class was associated with higher risk of CVE/CV death (HR 95% CI, 3.79 [1.16–12.42]; $P = 0.03$), as well as 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]; $P = 0.04$), while in contrast, 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 class offers valuable prognostic insights for stable KTx recipients, particularly when assessing renocardiovascular risk. The investigated serum markers may serve as supplemental tools for refining risk stratification.

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

INTRODUCTION

Chronic kidney disease (CKD) continues to pose a significant health burden in developed nations owing to its slow, but relentless progression towards 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 reveal 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 a final line of therapy. In the post-KTx setting, despite the associated CV risk reduction, enhanced morbidity and mortality still remain clinically significant [3]. Firstly, traditional CV risk factors are highly prevalent in CKD populations and commonly persist after KTx. There are also a number of non-traditional, CKD-specific risk factors, such as low-grade inflammation, altered mineral-bone balance (precipitating 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 discovery and validation of novel diagnostic and prognostic tools. For example, serum cystatin and urine albumin-creatinine ratio incorporation have lead to 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 parallels a paradigm shift towards a patient-centered (rather than disease-oriented) approach in modern day medicine, which follows the aging populations and related burden of multimorbidity.

Chronic inflammation is intertwined with 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, an activator of SIRT1, 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 effects on endothelial, vascular smooth muscle and adipose tissue cells [12]. Experimental data indicate 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 immune cells and endocrine organs [16]. Pooled data from multiple cohorts of CKD patients indicates higher chemerin concentrations parallel 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 for 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 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 explore the concept of CKM in stable KTRs, but also evaluate the role of chemerin and sirtuin-1 as new, candidate markers of prognostic importance related to CV and renal risk. With CKD prevalence on the rise, despite KTx offering a effective therapeutic avenue for CKD patients, persistent CV morbidity and CV mortality highlight the necessity of investigating beyond 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 (otherwise 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 co-morbidity 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 regarding interest in study participation. Subjects who expressed intent, provided consent in written form and fulfilled enrollment criteria 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 from medical professionals and staff at our center, in order to serve as reference.

More detailed descripition of study criteria and the control group is presented alognside a flowchart in 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 at 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 regarding 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 relative infrequence of events. Information regarding diagnostic or therapeutic invasive procedures was difficult to reliably determine for all patients, and was not included within the scope of this definition.

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

1) Stage 0 and 1 was defined for individuals with retained graft function (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 with Stage 0, while those who were either overweight (25–30 kg/m^2) or had impaired fasting glycemia (100–125 mg/dl) were termed as Stage 1.

2) Stage 4 was defined as the presence of manifest CVD (as defined above) and at 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 criteria for Stage 0, 1 or 4 were grouped into Stage 2/3, with not 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 kidney disease stage assessed according to Kidney Disease: Improving Global Outcomes (KDIGO) guidelines [25].

Additionally, SCORE2 was estimated to obtain a quantitative, proxy measure of 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. Within the first two years, follow-up data were gathered from consecutive visits spaced within a pre-defined timerange of 3 to 6 months apart. This process was carried out for the first two years after 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 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 using three automatic analyzers: Hitachi 917 (Hitachi, Japan); Modular P (Roche Diagnostics, Germany); Sysmex XE 2100 (Sysmex, Japan).

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

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, USA; 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 was stratified into "low" and "high" risk based pragmatic cut-offs (94 ng/ml and 10 ng/ml, respectively) based on median concentrations in serum of KTRs.

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

Performed in R 4.3.3 (R Core Team, 2024, R Foundation for Statistical Computing, Vienna, Austria). We summarized continuous and nominal variables using mean (standard deviation [SD]) and median (interquartile range), or count and proportion (n, %). Cross-group comparison in Table 1 and Supplementary material, *Figure S2* is uniformly 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 mean and SD, with P value based on permutation t test (Supplementary material, *Table S1*). Similarly, comparison across CKM and CKD subgroups is described using mean and SD, 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, minimal offset was utilized to availed 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 test (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 Schoenfield residuals. Multivariable Cox PH models were not considered due to low event rate.

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 among KTRs when compared with reference subjects (P <0.001; Supplementary material, *Table S1*).

Baseline clinical characteristics are robustly summarized by modified CKM stage in Table 1. This cohort represents middle-aged adults, most of whom are males, haver higher body-mass and can be classified with 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 with higher CKM stage, 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 with diabetic status and total cholesterol. Of note, mean arterial pressure and male sex may be other, potentially relevant predictors.

Out 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 interval of 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 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 MAP for both sirtuin-1 or chemerin.

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

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

Survival free from different types of poor outcome is visualized in Figure 4. We observed associations between CKM class 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 tied to CVE/CV risk (HR 95% CI, 0.595 [0.142–2.497]; *P* = 0.48), in contrast to 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, with low samples sizes, 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., hospitalization records for MI). Gold-standard diagnostic procedures (e.g., kidney biopsy, coronary angiography) are invasive and conservatively performed due to enhanced risk [27]. However, following a decline in infection-related deaths of 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 among KTRs requires discovery of relevant biomarkers and development of risk prediction tools that parallel the main pathophysiological pathways in CKD, but also those involved in enhanced co-morbidity. This study was conducted to explore whether incorporating the recent concept of CKM [6] can facilitate CV risk stratification in KTRs, but also evaluate whether circulating serum SIRT1, chemerin and hsIL-6 are candidate markers due to their mechanistic link with associated processes.

We observed that classification of KTRs into modified CKM stages may allow for prediction of both poor CV and renal outcome in survival analyses. Due to the conceptual incorporation of different clinical characteristics, including co-morbidity, CKM class 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 as 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 genetic polymorphisms in SIRT1 may affect susceptibility to diabetic kidney disease, but also atherosclerosis [31, 32]. We observed higher SIRT1 concentrations were associated with enhanced CV risk. They were also significantly lower in individuals with prior history of MI, and numerically lower in patients with CAD. By comparison, hsIL-6 levels were significantly enhanced 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], while others reported an association with ejection fraction [29]. Elevated serum SIRT1 has also been previously associated with worse renal function in both CKD and HD patients [29, 33], with significantly higher levels when compared with age- and sex- matched controls [33].

There is an array of data demonstrating chemerin may be intertwined 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], as well as being 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]. Others have also demonstrated a relationship with renal function in diabetics [41]. We observed a significant relationship between higher chemerin and KDIGO stage in KTRs, which falls in line with pooled observation for the general population [18]. While liver and endocrine tissue appears as 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 16kDA polypeptide size, increased chemerin may be derived from reduced excretion or increased stimulation of adipokine levels, which is consistent with 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 serum chemerin concentrations showed an increase with higher CKM (numerical) and CKD stage, but also exhibited a prognostic role for dialysis therapy risk.

Though it receives much attention, optimal CV screening in the pre- and post-KTx setting remains unelucidated [44, 45], while the pre-existing baseline risk of the Polish population remains high [46]. This is the first study to evaluate CKM stage (though modified), as well as serum SIRT1 and chemerin concentrations among stable, long-term KTRs. The major strength is 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, eg., sodium-glucose cotransporter inhibitors [47]. We also did not assess local expression of tissue markers, which limits the conclusions drawn to inference based on other reports. Certain molecules that are critical for disease progression on a local level (auto- and paracrine mechanisms), may not translate into marked shifts in systemic concentrations, and preclude their use as serum/plasma biomarkers.

CONCLUSION

This study investigates 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 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 permanent dialysis therapy requirement. 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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REFERENCES

1. Peralta CA, Shlipak MG, Judd S, et al. Detection of chronic kidney disease with creatinine, cystatin C, and urine albumin-to-creatinine ratio and association with progression to end-stage renal disease and mortality. JAMA. 2011; 305(15): 1545–1552, doi: 10.1001/jama.2011.468, indexed in Pubmed: 21482744.
2. Feng X, Hou N, Chen Z, et al. Secular trends of epidemiologic patterns of chronic kidney disease over three decades: an updated analysis of the Global Burden of Disease Study 2019. BMJ Open. 2023; 13(3): e064540, doi: 10.1136/bmjopen-2022-064540, indexed in Pubmed: 36931681.
3. Małyszko J, Bachorzewska-Gajewska H, Tomaszuk-Kazberuk A, et al. Cardiovascular disease and kidney transplantation-evaluation of potential transplant recipient. Pol Arch Med Wewn. 2014; 124(11): 608–616, indexed in Pubmed: 25311509.
4. Devine PA, Courtney AE, Maxwell AP. Cardiovascular risk in renal transplant recipients. J Nephrol. 2019; 32(3): 389–399, doi: 10.1007/s40620-018-0549-4, indexed in Pubmed: 30406606.
5. Nano J, Schöttker B, Lin JS, et al. Novel biomarkers of inflammation, kidney function and chronic kidney disease in the general population. Nephrol Dial Transplant. 2022; 37(10): 1916–1926, doi: 10.1093/ndt/gfab294, indexed in Pubmed: 34612501.
6. Ndumele CE, Rangaswami J, Chow SL, et al. American Heart Association. Cardiovascular-Kidney-Metabolic health: a presidential advisory from the american heart association. Circulation. 2023; 148(20): 1606–1635, doi: 10.1161/CIR.0000000000001184, indexed in Pubmed: 37807924.
7. Jung YuJ, Lee JE, Lee AeS, et al. SIRT1 overexpression decreases cisplatin-induced acetylation of NF-κB p65 subunit and cytotoxicity in renal proximal tubule cells. Biochem Biophys Res Commun. 2012; 419(2): 206–210, doi: 10.1016/j.bbrc.2012.01.148, indexed in Pubmed: 22330808.

8. Kim DH, Jung YuJ, Lee JE, et al. SIRT1 activation by resveratrol ameliorates cisplatin-induced renal injury through deacetylation of p53. Am J Physiol Renal Physiol. 2011; 301(2): F427–F435, doi: 10.1152/ajprenal.00258.2010, indexed in Pubmed: 21593185.
9. Hosoda R, Nakashima R, Yano M, et al. Resveratrol, a SIRT1 activator, attenuates aging-associated alterations in skeletal muscle and heart in mice. J Pharmacol Sci. 2023; 152(2): 112–122, doi: 10.1016/j.jphs.2023.04.001, indexed in Pubmed: 37169475.
10. Gu C, Xing Y, Jiang Li, et al. Impaired cardiac SIRT1 activity by carbonyl stress contributes to aging-related ischemic intolerance. PLoS One. 2013; 8(9): e74050, doi: 10.1371/journal.pone.0074050, indexed in Pubmed: 24040162.
11. Vachharajani VT, Liu T, Wang X, et al. Sirtuins link inflammation and metabolism. J Immunol Res. 2016; 2016, doi: 10.1155/2016/8167273, indexed in Pubmed: 26904696.
12. Lu CL, Liao MT, Hou YC, et al. Sirtuin-1 and Its Relevance in Vascular Calcification. Int J Mol Sci. 2020; 21(5): 1593, doi: 10.3390/ijms21051593, indexed in Pubmed: 32111067.
13. He W, Wang Y, Zhang MZ, et al. Sirt1 activation protects the mouse renal medulla from oxidative injury. J Clin Invest. 2010; 120(4): 1056–1068, doi: 10.1172/JCI41563, indexed in Pubmed: 20335659.
14. Kida Y, Zullo JA, Goligorsky MS. Endothelial sirtuin 1 inactivation enhances capillary rarefaction and fibrosis following kidney injury through Notch activation. Biochem Biophys Res Commun. 2016; 478(3): 1074–1079, doi: 10.1016/j.bbrc.2016.08.066, indexed in Pubmed: 27524235.
15. Huang XZ, Wen D, Zhang M, et al. Sirt1 activation ameliorates renal fibrosis by inhibiting the TGF-β/Smad3 pathway. J Cell Biochem. 2014; 115(5): 996–1005, doi: 10.1002/jcb.24748, indexed in Pubmed: 24356887.
16. Zhao L, Leung LL, Morser J. Chemerin forms: their generation and activity. Biomedicines. 2022; 10(8): 2018, doi: 10.3390/biomedicines10082018, indexed in Pubmed: 36009565.
17. Mariani F, Roncucci L. Chemerin/chemR23 axis in inflammation onset and resolution. Inflamm Res. 2015; 64(2): 85–95, doi: 10.1007/s00011-014-0792-7, indexed in Pubmed: 25548799.
18. Behnoush AH, Shobeiri P, Bahiraie P, et al. Chemerin levels in chronic kidney disease: a systematic review and meta-analysis. Front Endocrinol (Lausanne). 2023; 14, doi: 10.3389/fendo.2023.1120774, indexed in Pubmed: 36761204.
19. Mocker A, Hilgers KF, Cordasic N, et al. Renal chemerin expression is induced in models of hypertensive nephropathy and glomerulonephritis and correlates with markers of

inflammation and fibrosis. Int J Mol Sci. 2019; 20(24): 6240, doi: 10.3390/ijms20246240, indexed in Pubmed: 31835675.
20. Lin S, Teng J, Li J, et al. Association of chemerin and vascular endothelial growth factor (VEGF) with diabetic nephropathy. Med Sci Monit. 2016; 22: 3209–3214, doi: 10.12659/msm.896781, indexed in Pubmed: 27612613.
21. Ferland DJ, Mullick AE, Watts SW. Chemerin as a driver of hypertension: a consideration. Am J Hypertens. 2020; 33(11): 975–986, doi: 10.1093/ajh/hpaa084, indexed in Pubmed: 32453820.
22. Bekcer GJ, Wheeler DC, Zeeuw DD, et al. Kidney disease: Improving global outcomes (KDIGO) blood pressure work group. KDIGO clinical practice guideline for the management of blood pressure in chronic kidney disease. Kidney International Supplements. 2012; 2(5): 337–414, doi: 10.1038/kisup.2012.46.
23. Mach F, Baigent C, Catapano AL, et al. ESC Scientific Document Group. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2020; 41(1): 111–188, doi: 10.1093/eurheartj/ehz455, indexed in Pubmed: 31504418.
24. Pattaro C, Riegler P, Stifter G, et al. Estimating the glomerular filtration rate in the general population using different equations: effects on classification and association. Nephron Clin Pract. 2013; 123(1-2): 102–111, doi: 10.1159/000351043, indexed in Pubmed: 23797027.
25. Kidney Disease: Improving Global Outcomes (KDIGO) Blood Pressure Work Group. KDIGO 2021 Clinical Practice Guideline for the Management of Blood Pressure in Chronic Kidney Disease. Kidney Int. 2021; 99(3S): S1–S87, doi: 10.1016/j.kint.2020.11.003, indexed in Pubmed: 33637192.
26. SCORE2-Diabetes Working Group and the ESC Cardiovascular Risk Collaboration, SCORE2 working group and ESC Cardiovascular risk collaboration. SCORE2 risk prediction algorithms: new models to estimate 10-year risk of cardiovascular disease in Europe. Eur Heart J. 2021; 42(25): 2439–2454, doi: 10.1093/eurheartj/ehab309, indexed in Pubmed: 34120177.
27. Rangaswami J, Mathew RO, Parasuraman R, et al. Cardiovascular disease in the kidney transplant recipient: epidemiology, diagnosis and management strategies. Nephrol Dial Transplant. 2019; 34(5): 760–773, doi: 10.1093/ndt/gfz053, indexed in Pubmed: 30984976.
28. Aziz F, Jorgenson M, Garg N, et al. New approaches to cardiovascular disease and its management in kidney transplant recipients. Transplantation. 2022; 106(6): 1143–1158, doi: 10.1097/TP.0000000000003990, indexed in Pubmed: 34856598.

29. Zbroch E, Bazyluk A, Malyszko J, et al. The serum concentration of anti-aging proteins, sirtuin1 and αKlotho in patients with end-stage kidney disease on maintenance hemodialysis. Clin Interv Aging. 2020; 15: 387–393, doi: 10.2147/CIA.S236980, indexed in Pubmed: 32214805.
30. Łukawska-Tatarczuk M, Franek E, Czupryniak L, et al. Sirtuin 1, Visfatin and IL-27 Serum Levels of Type 1 Diabetic Females in Relation to Cardiovascular Parameters and Autoimmune Thyroid Disease. Biomolecules. 2021; 11(8): 1110, doi: 10.3390/biom11081110, indexed in Pubmed: 34439776.
31. Yue XG, Yang ZG, Zhang Y, et al. Correlations between SIRT1 gene polymorphisms and diabetic kidney disease. R Soc Open Sci. 2018; 5(6): 171871, doi: 10.1098/rsos.171871, indexed in Pubmed: 30110438.
32. Kedenko L, Lamina C, Kedenko I, et al. Genetic polymorphisms at SIRT1 and FOXO1 are associated with carotid atherosclerosis in the SAPHIR cohort. BMC Med Genet. 2014; 15: 112, doi: 10.1186/s12881-014-0112-7, indexed in Pubmed: 25273948.
33. Bielach-Bazyluk A, Zbroch E, Czajkowska K, et al. Serum sirtuin 1 is independently associated with intact PTH among patients with chronic kidney disease. Clin Interv Aging. 2021; 16: 525–536, doi: 10.2147/CIA.S293665, indexed in Pubmed: 33790547.
34. Krekora J, Matuszewska-Brycht O, Sołek J, et al. Sirtuin 1 and Sirtuin 2 Plasma Concentrations in Patients with Ascending Aortic Dissection and Ascending Aortic Aneurysm. Artery Res. 2023; 29(3): 79–82, doi: 10.1007/s44200-023-00035-3.
35. Esmayel IM, Hussein S, Gohar EA, et al. Plasma levels of sirtuin-1 in patients with cerebrovascular stroke. Neurol Sci. 2021; 42(9): 3843–3850, doi: 10.1007/s10072-021-05074-9, indexed in Pubmed: 33507417.
36. Doulamis IP, Tzani AI, Konstantopoulos PS, et al. A sirtuin 1/MMP2 prognostic index for myocardial infarction in patients with advanced coronary artery disease. Int J Cardiol. 2017; 230: 447–453, doi: 10.1016/j.ijcard.2016.12.086, indexed in Pubmed: 28043667.
37. Shao Y, Ren H, Lv C, et al. Changes of serum Mir-217 and the correlation with the severity in type 2 diabetes patients with different stages of diabetic kidney disease. Endocrine. 2017; 55(1): 130–138, doi: 10.1007/s12020-016-1069-4, indexed in Pubmed: 27522360.
38. Wang D, Yuan GY, Wang XZ, et al. Plasma chemerin level in metabolic syndrome. Genet Mol Res. 2013; 12(4): 5986–5991, doi: 10.4238/2013.November.26.8, indexed in Pubmed: 24338392.

39. Yoo HJ, Choi HY, Yang SJ, et al. Circulating chemerin level is independently correlated with arterial stiffness. J Atheroscler Thromb. 2012; 19(1): 59–68, doi: 10.5551/jat.9647, indexed in Pubmed: 22104178.
40. Dong B, Ji W, Zhang Y. Elevated serum chemerin levels are associated with the presence of coronary artery disease in patients with metabolic syndrome. Intern Med. 2011; 50(10): 1093–1097, doi: 10.2169/internalmedicine.50.5025, indexed in Pubmed: 21576834.
41. Hu W, Feng P. Elevated serum chemerin concentrations are associated with renal dysfunction in type 2 diabetic patients. Diabetes Res Clin Pract. 2011; 91(2): 159–163, doi: 10.1016/j.diabres.2010.11.016, indexed in Pubmed: 21144611.
42. Blaszak J, Szolkiewicz M, Sucajtys-Szulc E, et al. High serum chemerin level in CKD patients is related to kidney function, but not to its adipose tissue overproduction. Ren Fail. 2015; 37(6): 1033–1038, doi: 10.3109/0886022X.2015.1040707, indexed in Pubmed: 25945605.
43. Rutkowski P, Sledzinski T, Zielinska H, et al. Decrease of serum chemerin concentration in patients with end stage renal disease after successful kidney transplantation. Regul Pept. 2012; 173(1-3): 55–59, doi: 10.1016/j.regpep.2011.09.005, indexed in Pubmed: 21971116.
44. Szramowska A, Bielecki M, Grzeszczyk M, et al. High-sensitivity cardiac troponin T in detecting obstructive coronary artery disease in hemodialysis patients listed for kidney transplantation. Pol Heart J. 2024; 82(3): 285–291, doi: 10.33963/v.phj.99498, indexed in Pubmed: 38493456.
45. Bellasi A, Salera D, Ratti C, et al. Cardiovascular screening in chronic kidney disease subjects waiting for transplantation: Unresolved question. Pol Heart J. 2024; 82(4): 474–476, doi: 10.33963/v.phj.100265, indexed in Pubmed: 38716501.
46. Szramowska A, Bielecki M, Grzeszczyk M, et al. High-sensitivity cardiac troponin T in detecting obstructive coronary artery disease in hemodialysis patients listed for kidney transplantation. Pol Heart J. 2024; 82(3): 285–291, doi: 10.33963/v.phj.99498, indexed in Pubmed: 38493456.
47. Stompór T, Winiarska A. Kidneys in heart failure: Impact of flozins. Kardiol Pol. 2023; 81(11): 1071–1080, doi: 10.33963/v.kp.97844, indexed in Pubmed: 37937356.

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	9 (81.8%)	6 (54.5%)	37 (67.3%)	21 (84.0%)	73 (71.6%)	0.37
KDIGO stage						<0.001
IV	0 (0.0%)	0 (0.0%)	5 (9.1%)	5 (20.0%)	10 (9.8%)	
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	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
Beta blocker, n (%)	6 (54.5%)	8 (72.7%)	34 (61.8%)	17 (68.0%)	65 (63.7%)	0.78
Alpha 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, mg/dl	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/l	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	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

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

	Adjusted odds ratios		
Feature	**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

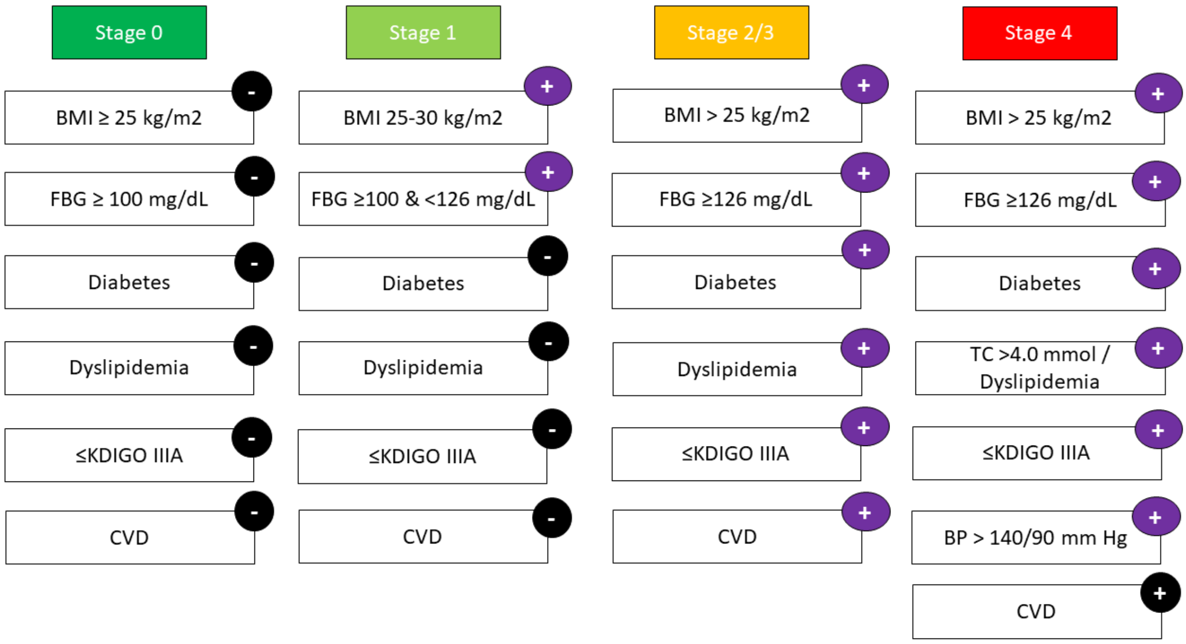

Figure 1. Modified classification system for cardiovascular-kidney-metabolic (CKM) burden in kidney allograft recipients. We stratified patients based on the concomitant presence of risk factors or disorders of CV significance. Black circles with presence (plus) and absence (minus) signs reflect mandatory 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

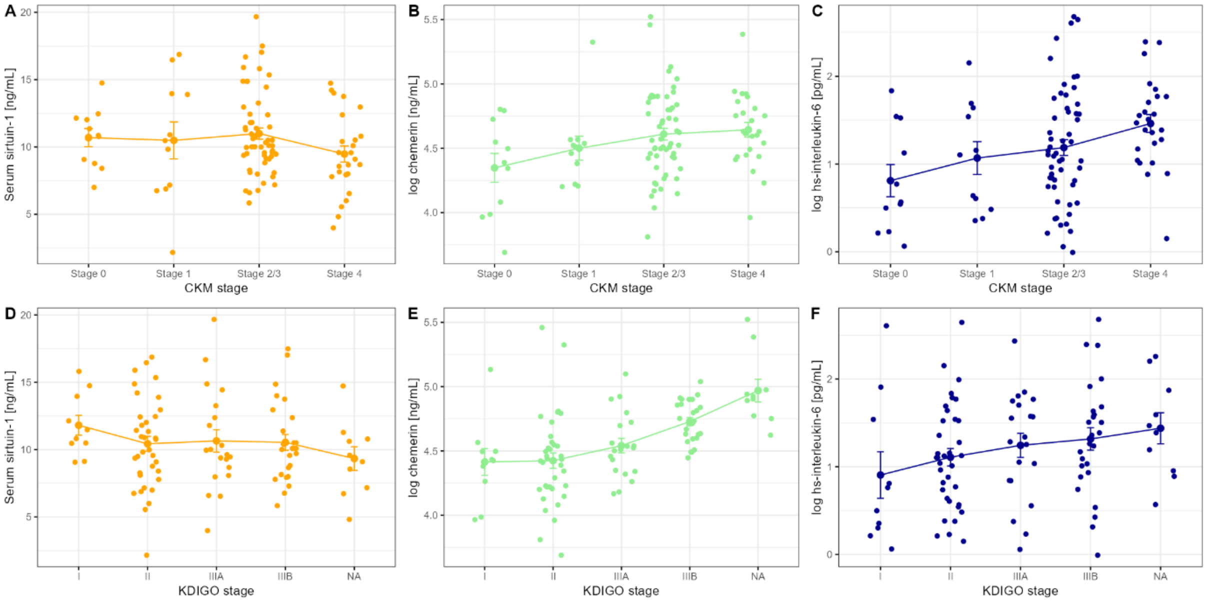

Figure 2. Comparison of mean (standard error) concentrations of serum hsIL-6, chemerin and sirtuin-1 concentrations across CKM and CKD stages

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

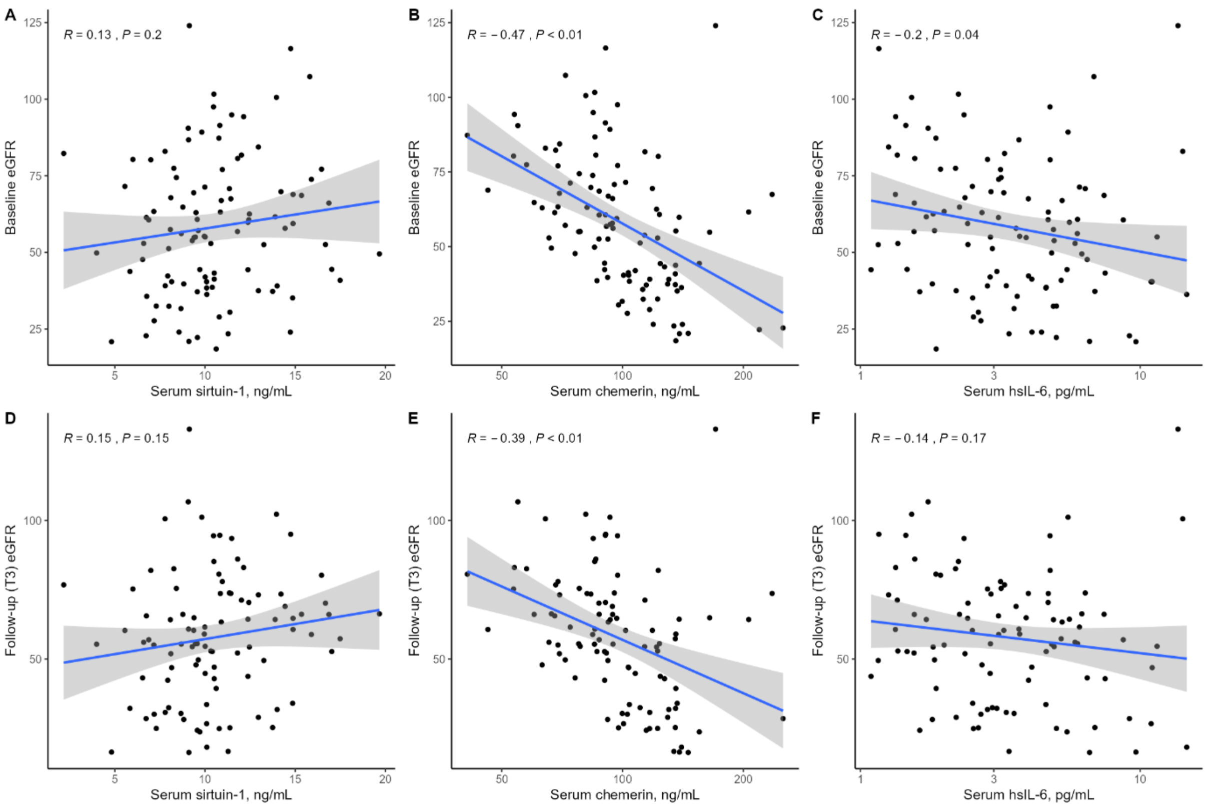

Figure 3. Scatter plot with linear fit illustrating the relationship between baseline eGFR (**A, B, C**) and future eGFR (**D, E, F**) compared according to serum sirtuin-1, chemerin and hsIL-6 concentrations, respectively

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

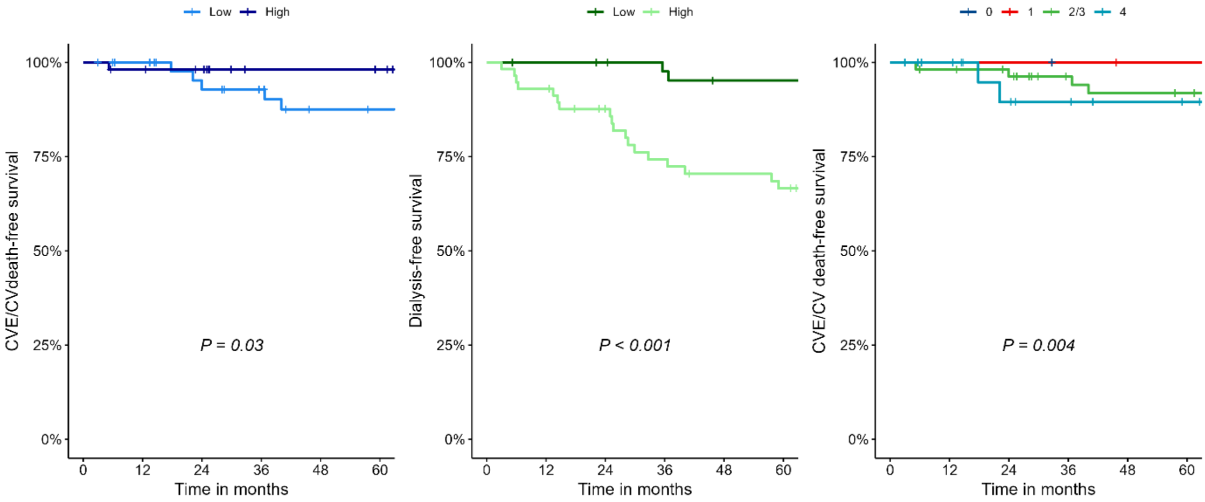

Figure 4. Survival free from poor outcome compared across patients stratified by median concentrations of sirtuin-1 (left) and chemerin (middle), as well as modified CKM class (right), respectively

Abbreviations: see Figure 2