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Skin autofluorescence, arterial stiffness and Framingham risk score as predictors of clinical outcome in chronic kidney disease patients: a cohort study

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ABSTRACT

Background. The risk of cardiovascular disease (CVD) is predicted by Framingham's CVD risk scores (FRS) but the high CVD-related mortality in patients with chronic kidney disease (CKD) is only partially explained by traditional CVD risk markers. Therefore, there is a need to explore whether other CVD risk markers may improve risk prediction. Although arterial stiffness measured by augmentation index (AIx) and tissue content of advanced glycation end-products (AGEs) measured by skin autofluorescence (SAF) are two biomarkers that associate with CVD and mortality in CKD, it is not known how they compare with FRS. We evaluated associations between SAF, AIx and FRS, and their associations with CVD and mortality in CKD patients.

Methods. SAF (AGE Reader) and AIx (SphygmoCor; adjusted for 75 heart beats per minute) were measured in 261 clinically stable and extensively phenotyped patients with CKD Stage 5 (median age 56 years, 66% male, 20% diabetes; 130 non-dialysed, 93 patients on peritoneal dialysis and 38 patients on haemodialysis). Multivariate receiver operator characteristics (ROC) curve analysis and multivariate Cox models followed by C-statistics were used to evaluate CVD-related and all-cause mortality risk associated with SAF, AIx and FRS during follow-up for median 25 months with 46 deaths.

Results. In multivariate regression analysis, SAF associated with FRS, haemoglobin, fat body mass index and CVD, and inversely with per cent handgrip strength (HGS). AIx associated with FRS, and inversely with per cent HGS. Associations of SAF and AIx with high-sensitivity C-reactive protein (hsCRP), serum albumin, statin therapy and renal replacement therapy were not statistically significant. In ROC analysis, area under the curve (AUC) for CVD mortality ranged from AUC = 0.72

(AIx and FRS, respectively) to AUC = 0.78 (FRS + AIx), and for all-cause mortality from AUC = 0.70 (AIx) to AUC = 0.79 (FRS + AIx). In multivariate Cox analysis, after adjusting for 1-standard deviation (1-SD) of FRS, 1-SD increase of SAF associated with all-cause mortality and 1-SD increase of AIx associated with CVD mortality and all-cause mortality. After further adjustments for hsCRP, albumin and presence of CVD, AIx (but not SAF) remained independently associated with CVD mortality, hazard ratio (HR) 2.14 [95% confidence interval (95% CI) 1.18–3.89] and all-cause mortality, HR 1.74 (95% CI 1.16–2.60).

Conclusions. In patients with CKD Stage 5, SAF and aortic stiffness associated with mortality, independently of FRS. After adjusting for additional confounders including inflammation, aortic stiffness remained as an independent predictor of outcome. Since the contribution of SAF and aortic stiffness compared with FRS in ROC curve analysis was relatively modest, this underlines the importance of traditional CVD risk factors in CKD.

Keywords: advanced glycation end-products, chronic kidney disease, Framingham risk factors, mortality, vascular stiffness

INTRODUCTION

In the general population, the risk of cardiovascular disease (CVD) predicted by Framingham's CVD risk scores (FRS) is based on traditional risk factors [1] and the prognostic improvement of FRS by using additional biomarkers is in general not great [2]. In contrast, traditional CVD risk markers only partially explain the high prevalence of CVD and the high CVD-related mortality in patients with advanced chronic

kidney disease (CKD), possibly because they are exposed to several uraemia-related CVD risk factors [3, 4]. While some biomarkers, most noticeably plasma interleukin-6 (IL-6), may improve risk prediction, much of the increased CVD risk in CKD can, however, still not be accounted for [5]. Therefore, there is a need to explore whether other CVD risk markers may improve risk prediction in CKD patients.

Advanced glycation end-products (AGEs) accumulate with age and in progeric conditions, such as diabetes mellitus (DM) and CKD [6], and may modify proteins in the body including arterial wall and skin [7, 8]. AGEs cause vascular damage by cross-linking of collagen and elastin, and by interactions with receptors for AGE (RAGE) [6, 9, 10], leading to arterial stiffness [11–13]. Some AGEs are fluorescent and can be measured by autofluorescence that may serve as a representative marker of the total burden of AGEs [7]. The usefulness of skin autofluorescence (SAF) has been validated in healthy subjects and uraemic patients [7, 14, 15]. SAF has been shown to predict progression of microvascular disease [16], cardiovascular events and worse clinical outcomes in DM and CKD patients, including those undergoing dialysis [14, 17–19]. In patients with advanced CKD, the uraemic milieu may contribute to impaired plasticity and other changes leading to premature vascular ageing [20].

Arterial stiffness can be assessed by applanation tonometry and measurements of augmentation index (AIx) [21]. AIx is an independent predictor of all-cause and CVD-related mortality in haemodialysis (HD) patients [22].

The aim of the present study was to explore the association of SAF and AIx with clinical outcome when traditional cardiovascular risk factors represented by FRS are taken into account. For this purpose, we evaluated associations between SAF, AIx and FRS, and their associations—separately and when analysed concomitantly—with presence of CVD and mortality in CKD Stage 5 (CKD5) patients.

MATERIALS AND METHODS

Patients and study design

We investigated SAF and AIx in 261 clinically stable patients with CKD5 including 130 non-dialysed (CKD5-ND) and 131 dialysed (CKD5-D) patients treated by peritoneal dialysis (PD; $n = 93$) or HD ($n = 38$). Patient characteristics are shown in Table 1. Their ages ranged from 19 to 85 years and they were recruited from April 2008 to March 2017. The aetiologies of renal disease were chronic glomerulonephritis ($n = 61$; 23%), hypertension and renovascular disease ($n = 39$; 15%), polycystic kidney disease ($n = 39$; 15%), diabetic nephropathy ($n = 32$; 12%) and others or unknown causes ($n = 90$; 35%). Exclusion criteria were age <18 years, signs of overt clinical infection and unwillingness to participate.

CKD5-ND patients

CKD5-ND patients ($n = 130$) with data on skin AGEs (SAF) and arterial stiffness (AIx) were recruited from an ongoing prospective cohort study on malnutrition, inflammation and atherosclerosis (MIA study) in patients investigated close to the

Table 1. Baseline clinical and biochemical characteristics of 261 CKD5 patients

Demography and clinical characteristics	
Age (years)	56 (29–75)
Males, n (%)	171 (66)
DM, n (%)	53 (20)
CVD[a], n (%)	55 (21)
Current smoker, n (%) ($n = 245$)	23 (9)
SBP (mmHg)	144 (118–175)
Diastolic blood pressure (mmHg)	84 (68–100)
Mean blood pressure[b] (mmHg)	104 (87–123)
Pulse pressure (mmHg)	57 (42–85)
Heart rate (bpm)	73 (60–90)
Nutritional status	
Malnutrition (SGA >1), n (%) ($n = 259$)	92 (36)
BMI (kg/m^2)	24.7 (20.5–30.1)
LBMI (kg/m^2, $n = 221$)	18.1 (14.8–20.8)
FBMI (kg/m^2, $n = 221$)	6.4 (3.4–10.8)
Per cent HGS (%, $n = 258$)	85 (53–113)
Total BMD (g/cm^2, $n = 183$)	1.11 (0.91–1.28)
Biochemicals	
Haemoglobin (g/L)	113 (94–129)
Albumin (g/L)	34 (27–40)
Triglyceride (mmol/L)	1.5 (0.8–2.7)
Total cholesterol (mmol/L)	4.6 (3.2–6.3)
HDL cholesterol (mmol/L)	1.3 (0.8–2.0)
LDL cholesterol (mmol/L)	2.8 (1.6–4.5)
Calcium (mmol/L)	2.3 (2.0–2.5)
Phosphate (mmol/L)	1.7 (1.2–2.5)
Intact-PTH (ng/L, $n = 260$)	260 (87–658)
25OH-Vitamin D (nmol/L, $n = 205$)	35 (14–71)
hsCRP (mg/L)	1.6 (0.2–14.6)
IL-6 (pg/mL, $n = 204$)	3.9 (0.6–13.2)
Medications	
β-Blockers, n (%)	128 (49)
Ca-blocker, n (%)	126 (48)
ACEi/ARB, n (%)	166 (64)
Statins, n (%) ($n = 260$)	107 (41)
Investigated risk markers	
AIx (%)	23 (7.2–35.5)
SAF (AU)	3.2 (2.4–4.3)
Framingham CVD risk score (%)	14.4 (1.9–44.4)

Continuous variables are presented as median (10–90th percentile). Categorical variables are presented as number (n)/percentage (%). LDL, calculated based on Friedewald formula [(total cholesterol) − (HDL cholesterol) – (triglycerides/5)]; intact-PTH, intact parathyroid hormone; ACEi, angiotensin-converting enzyme; ARB, angiotensin 2 receptor blocker.
[a]Defined as clinical history or signs of ischaemic cardiac disease, and/or presence of peripheral vascular disease and/or cerebrovascular disease.
[b]Mean blood pressure, defined as [diastolic pressure + (systolic pressure – diastolic pressure) × 2/3].

initiation of dialysis therapy [23], and an ongoing study on vascular changes in end-stage renal disease (ESRD) patients prior to living donor-renal transplantation (LD-Rtx) [24]. The aetiologies of CKD were chronic glomerulonephritis ($n = 36$), hypertension and renovascular disease ($n = 23$), diabetic nephropathy ($n = 22$) and others or unknown causes ($n = 49$).

CKD5-D patients

Prevalent PD patients ($n = 93$) were recruited from a cross-sectional study with follow-up that aimed at evaluating variation in inflammatory markers in prevalent PD patients, the MIMICK2 study [25], and PD patients (median vintage time 11.3 months) scheduled for LD-Rtx [24]. PD patients were

treated with different combinations of biocompatible glucose-based or amino acid-based, or, for the long dwell, icodextrin-based solutions. The causes of ESRD were chronic glomerulonephritis ($n = 14$), hypertension and renovascular disease ($n = 10$), diabetic nephropathy ($n = 10$) and others or unknown causes ($n = 59$). Prevalent HD patients ($n = 38$) were recruited from the LD-Rtx cohort [24] (median vintage time 12.8 months). HD patients were treated by conventional maintenance HD or haemodiafiltration. The causes of CKD were chronic glomerulonephritis ($n = 11$), hypertension and renovascular disease ($n = 6$) and others or unknown causes ($n = 21$).

AIx measurement

Assessment of arterial stiffness was performed non-invasively by SphygmoCor® System (AtCor Medical, Sydney, Australia), using tonometry-based and cuff-based SphygmoCor Devices. Using tonometry-based SphygmoCor device, the peripheral pulse waveform (PPW) was recorded from the radial artery at the wrist in non-fistula arm using applanation tonometry with a sensor probe. PPW and brachial blood pressure measurements were used to estimate central aortic pressure waveform calculated by the transfer function. Using the cuff-based SphygmoCor Device, brachial artery compression waveforms were obtained by partially inflating a cuff over the brachial artery between the shoulder and the elbow joint. The brachial waveforms were calibrated using cuff-measured brachial systolic and diastolic blood pressures, and then used to generate central aortic pressure waveforms by transfer function. Augmentation pressure (AP) and AIx was derived from this with the technique of pulse wave analysis. The merging of incident and the reflected wave (the inflection point) were identified on the generated central aortic pressure waveform. AP was defined as the maximum systolic pressure minus pressure at the inflection point. AIx was defined as AP divided by pulse pressure and expressed as a percentage. In addition, because AIx is influenced by heart rate, an index normalized for heart rate of 75 beats per minute (bpm) was used in accordance with Wilkinson *et al.* [26]. SphygmoCor adjusts the AIx at an inverse rate of 4.8% for each 10 bpm increment.

SAF measurement

AGEs Autofluorescence® was measured using an Autofluorescence AGE reader™ (DiagnOptics Technologies BV, Groningen, The Netherlands). Patients with tattooed and dark skin were not investigated. The AGE reader illuminates a skin surface of ∼1 cm², guarded against surrounding light, with an excitation light source between 300 and 420 nm. Emission light (fluorescence in the wavelength range between 420 and 600 nm) and reflected excitation light (with a wavelength between 300 and 420 nm) from the skin are measured with a spectrometer [7]. SAF was calculated as the ratio between the emission light and reflected excitation light, multiplied by 100, and expressed in arbitrary units (AU). All measurements were performed at room temperature in a semi-dark environment.

Biochemical assessments

Biochemical analyses of high-sensitivity C-reactive protein (hsCRP) [coefficient of variation (CV) 5%], plasma cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, haemoglobin, creatinine and albumin (CV 3–4%) were performed at the Clinical Chemical Laboratory of Karolinska University Hospital, Stockholm, Sweden. IL-6 (CV 4%) was analysed by immunometric assays on an Immulite 1000 Analyzer (Siemens Healthcare Diagnostics, Los Angeles, CA, USA) using commercial kits. Low-density lipoprotein (LDL) was calculated using the Friedewald formula [27]: [(total cholesterol) − (HDL cholesterol) – (triglycerides/5)]. In CKD5-ND patients, estimated glomerular filtration rate (eGFR) was calculated according to the CKD Epidemiology Collaboration (CKD-EPI) equation [28].

Clinical assessments and body composition

Presence of CVD was defined as a clinical history or signs of ischaemic cardiac disease, and/or presence of peripheral vascular disease and/or cerebrovascular disease. According to the subjective global assessment (SGA) score, patients were classified as well-nourished (SGA = 1) or as having mild (SGA = 2), moderate (SGA = 3) or severe (SGA = 4) signs of malnutrition [29]. For simplicity, the patients were placed in two groups; well-nourished (SGA = 1) and malnourished (SGA ≥2). Handgrip strength (HGS) was evaluated in the non-fistula arm using the Harpenden Dynamometer (Yamar, Jackson, MI, USA) and repeated three times, and the greatest value was recorded and expressed in kilograms. HGS was expressed in per cent of values in healthy individuals, considering the differences between the sexes, when included in the statistical analyses. Blood pressure was presented as mean blood pressure, defined as [diastolic pressure + (systolic pressure – diastolic pressure)/ 3]. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in metres. Lean body mass and fat mass were calculated by anthropometry with measurements of biceps, triceps, subscapular and supra-iliac skinfold thickness using the Durnin and Womersley Caliper Method [30], and by equations proposed by Siri [31]. Lean BMI (LBMI) and fat BMI (FBMI) were calculated according to the method of Kyle *et al.* [32] and expressed as kilograms per metre-squared. Total bone mineral density (BMD) was determined by dual-energy X-ray absorptiometry.

Assessment of Framingham's CVD risk

The FRS was calculated according to sex and age stratified tables with specific scores assigned for systolic blood pressure (SBP), diabetes, anti-hypertensive medication, total cholesterol, HDL cholesterol and smoking status. The FRS provided an estimate of the 10-year risk of developing CVD for each patient [1].

Statistical analyses

Data are expressed as median (10th–90th percentile) or percentage, as appropriate. Statistical significance was set at the level of $P < 0.05$. Comparisons between two groups were assessed with the non-parametric Wilcoxon test for continuous variables and Chi-square test for nominal variables.

Non-parametric Spearman rank correlation analysis was used to determine associations between variables. Multiple linear regression analyses of continuous variables of SAF and AIx were performed and results were shown as standardized β regression coefficients. We selected variables for the multiple linear regression analysis, which showed significant univariate associations. The classifiers of CVD, and predictors of all-cause and CVD mortality, were calculated by the area under the curve (AUC) by the receiver operating characteristic (ROC) curve. Multivariate Cox proportional hazard models were used for all-cause and CVD mortality to obtain hazard ratios for 1-standard deviation (1-SD) increase of SAF, AIx and FRS. Discriminative abilities of the models were estimated as C-statistics for Cox regression models [33]. Statistical analyses were performed using Statistical Software Stata 15.0 (Stata Corporation, College Station, TX, USA) and SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA).

Statement of ethics

The Ethics Committee of the Karolinska Institutet at the Campus Flemingsberg (EPN) Stockholm, Sweden, approved the study protocol and written informed consent was obtained from each patient and the protocol adhered to the statutes of the Declaration of Helsinki.

RESULTS

The clinical and biochemical characteristics of patients are shown in Table 1. Clinical signs or symptoms of CVD were present in 21% of the patients, ranging from 5% in HD patients to 25% among non-dialysed patients investigated prior to dialysis initiation. The median eGFR of non-dialysed patients was 6.1 (4.2–10.0) mL/min/1.73 m^2 while GFR data were lacking in PD and HD patients.

In univariate analysis, SAF significantly associated with AIx (rho = 0.31), FRS (rho = 0.51), hsCRP (rho = 0.31) and FBMI (rho = 0.24, n = 221), and inversely with per cent HGS (rho = −0.35) and BMD (rho = −0.22, n = 183). AIx associated with FRS (rho = 0.41) and hsCRP (rho = 0.25), and inversely with per cent HGS (rho = −0.42), albumin (rho = −0.26), BMD (rho = −0.23; n = 183) and LBMI (rho = −0.20, n = 221).

In multivariate linear regression analysis, 1-SD higher SAF associated with 1-SD higher FRS, haemoglobin and FBMI, presence of CVD and, inversely, with 1-SD higher per cent HGS but not with 1-SD of hsCRP and albumin, statin use or renal replacement therapy (RRT). 1-SD higher AIx associated with 1-SD higher FRS, and inversely with 1-SD higher per cent HGS, but not with CVD, 1-SD of hsCRP, HGS, albumin or FBMI, respectively, nor with statin therapy or RRT modality (Table 2).

ROC curves of SAF, AIx and FRS

Table 3 shows the AUC of the ROC curves of SAF, AIx and FRS as classifiers of CVD and as predictors of CVD-related and all-cause mortality. Classifiers of CVD: AUC for FRS was 0.75 [95% confidence interval (95% CI) 0.69–0.81] and adding SAF (FRS + SAF, AUC = 0.75) or AIx (FRS + AIx, AUC = 0.73) did not increase AUC values. CVD mortality: AUC for FRS was 0.72 (95% CI 0.62–0.83) and AUC increased to 0.75 (95% CI 0.66–0.84) by adding SAF, and to 0.78 (95% CI 0.69–0.87) by adding AIx. All-cause mortality: AUC for FRS was 0.77 (95% CI 0.70–0.84), which increased to 0.79 (95% CI 0.72–0.86) by adding SAF, and to 0.79 (95% CI 0.73–0.86) by adding AIx.

Cox analysis of SAF, AIx and FRS

During follow-up for median 25 months, the CVD mortality rate was 8% (n = 21) and all-cause mortality rate was 18% (n = 46) among the 261 CKD5 patients. Table 4 shows that in multivariate Cox analysis, after adjusting for 1-SD of FRS, 1-SD increase of SAF associated with all-cause mortality and 1-SD increase of AIx associated with CVD mortality and all-cause mortality. After further adjustments for also hsCRP, serum albumin and presence of CVD, 1-SD increase of AIx remained independently associated with all-cause [hazard ration (HR) 1.74; 95% CI 1.16–2.60; P = 0.008] and CVD mortality (HR, 2.14; 95% CI 1.18–3.89; P = 0.01), whereas these associations for 1-SD higher SAF were no longer statistically significant. Discriminative abilities for SAF and AIx, estimated as C-statistics for Cox regression models, showed for SAF versus CVD mortality AUC of 0.79 (95% CI 0.71–0.86) and for all-cause mortality AUC of 0.78 (95% CI 0.71–0.86). Corresponding figures for AIx showed for CVD mortality, AUC of 0.81 (95% CI 0.74–0.88) and for all-cause mortality, AUC of 0.81 (95% CI 0.75–0.87).

DISCUSSION

While risk scores, such as FRS, are invaluable for preventive strategies, a significant gap exists between predicted and actual event rates, and biomarkers may serve as additional tools to stratify further the risk of patients at an individual level [2]. Vascular biomarkers may detect pre-clinical organ damage in different parts of the vascular bed, reflecting ageing processes and adverse effects of specific CVD risk factors, and have been shown to associate with risk burden and prediction of future clinical outcomes [2].

In the present study, we investigated whether two non-invasive CVD risk markers, SAF (reflecting the burden of AGEs) [6, 14–19] and AIx (a marker of vascular stiffness) [16, 34], could independently classify presence of CVD and predict mortality in CKD5 patients when correcting for FRS [1]. While all three markers, FRS, SAF and AIx, associated with increased mortality (and could classify presence of CVD), the additional contribution of SAF and AIx to the calculated risk over and above that provided by FRS was relatively modest.

These results confirm the importance of traditional risk factors for CVD risk prediction in CKD patients [5]. Based on clinical assessment, medical history and routine biochemistry, FRS captures traditional risk factors such as age, sex, diabetes, SBP, anti-hypertensive medication, total cholesterol, HDL cholesterol and smoking status in a systematic way. While some of the factors included in FRS, such as total cholesterol, do not appear to predict increased CVD risk in advanced CKD, rather the opposite [35], our results show that FRS may predict CVD risk in this population. Nevertheless, when adjusting for FRS, both SAF and AIx still associate with increased mortality. After

further adjustments for inflammation and presence of CVD, AIx (but not SAF) remained independently associated with CVD mortality (Table 4).

SAF and AIx were correlated and could—despite their different nature—be causally related as AGEs reflected by SAF may contribute to vascular changes including arterial stiffness reflected by AIx. Arterial stiffness, which is associated with ageing and other CVD risk factors, is caused by various phenomena, i.e. fibrosis, breaks in elastin fibres, calcification and diffusion of macromolecules within the arterial wall; however, AGEs may also play a role. Accumulation of AGEs may lead to

cross linking of proteins in the extracellular matrix, e.g. collagen and elastin, altering their physical properties, which potentially translates into increased arterial stiffness [13, 36, 37]. Experimental studies showed that AGE is capable of calcification of vascular smooth muscle cells through the RAGE/p38 mitogen-activated protein kinase signalling pathway or RAGE/oxidative stress pathway [38–41]. Wang *et al.* [42] demonstrated an association between SAF and vascular calcification in CKD Stages 3–5 patients, and Ueno *et al.* [43] reported association between SAF and arterial stiffening in HD patients. Moreover, Mac-Way *et al.* [44] demonstrated a positive association between SAF and aortic stiffness as determined by carotid–femoral pulse wave velocity (PWV) in dialysis patients, although these associations were lost when adjusted for age [44].

Several studies demonstrate that aortic stiffness estimated by PWV is an independent predictor of CVD and all-cause mortality in hypertensive patients [45], HD patients [46] and in other conditions [2]. Furthermore, PWV was associated with cardiovascular events independent of FRS in hypertensive patients [47]. Arterial stiffening increases PWV and reduces the transit time of pressure wave from the peripheral reflection site towards central arteries [48]. An early return of reflected wave alters the timing of merging with incident wave, which results in an increase of AIx. The AIx is considered as an index of arterial stiffness. AIx predicts CVD morbidity and mortality in non-CKD patients [21, 49, 50] and HD patients [22]. The importance of aortic stiffness is supported by the present study showing that AIx associated with clinical outcome, independent of FRS, and after adjustment for other confounders including inflammation among patients with advanced CKD, the association of AIx with clinical outcome appeared to be stronger than that for SAF.

FRS is widely used in clinical practice [1] and validated in large cohorts of patients with various acute and chronic diseases. In the 261 patients with advanced CKD investigated in the present study, the median (10–90%) FRS was 14.4 (1.9–44.4%) and, during follow-up for median 25 months, the CVD-related mortality rate was 8% (all-cause mortality rate 18%), indicating that this was a high-risk population. The excessive cardiovascular risk in CKD is attributed both to the high prevalence of traditional risk factors in CKD, such as high

Table 2. Multivariate associations, expressed as standardized β-coefficients, of SAF and AIx with other variables

Parameters	1-SD of SAF ($r^2 = 0.32$) β-coefficient (P-value)	1-SD of AIx ($r^2 = 0.18$) β-coefficient (P-value)
1-SD increase of FRS (%)	0.32 (<0.001)	0.15 (0.03)
Presence of CVD (no = 0, yes = 1)	0.16 (0.01)	0.07 (0.32)
1-SD increase of hsCRP (mg/L)	0.11 (0.06)	0.03 (0.70)
1-SD increase of per cent HGS	−0.13 (0.045)	−0.28 (<0.001)
1-SD increase of FBMI (kg/m^2)	0.14 (0.02)	0.09 (0.15)
1-SD increase of albumin (g/L)	−0.06 (0.37)	−0.10 (0.17)
1-SD increase of haemoglobin (g/L)	0.15 (0.02)	0.02 (0.73)
Statin use (no = 0, yes = 1)	−0.005 (0.98)	−0.05 (0.44)
RRT (CKD5-ND versus CKD5-D)	0.10 (0.09)	−0.11 (0.09)

Table 3. Areas under the ROC curve describing the ability of SAF, AIx and FRS to classify presence of CVD and predict CVD mortality and all-cause mortality in 261 patients with CKD5

Model	Prevalence of CVD AUC (95% CI)	CVD death AUC (95% CI)	All-cause death AUC (95% CI)
SAF	0.71 (0.63–0.79)	0.74 (0.63–0.84)	0.75 (0.67–0.83)
AIx	0.64 (0.57–0.72)	0.72 (0.62–0.83)	0.70 (0.62–0.78)
FRS	0.75 (0.69–0.81)	0.72 (0.62–0.83)	0.77 (0.70–0.84)
FRS + SAF	0.75 (0.68–0.81)	0.75 (0.66–0.84)	0.79 (0.72–0.86)
FRS + AIx	0.73 (0.66–0.80)	0.78 (0.69–0.87)	0.79 (0.73–0.86)

CVD, defined as clinical history or signs of ischaemic cardiac disease, and/or presence of peripheral vascular disease and/or cerebrovascular disease. CVD death was defined clinically based on the death certificate.

Table 4. Adjusted hazard ratios of SAF, AIx and FRS for CVD mortality and all-cause mortality in 261 patients with CKD5

	CVD death		All-cause death	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Crude				
1-SD increase of FRS (%)	1.82 (1.31–2.54)	<0.001	1.92 (1.54–2.41)	<0.001
1-SD increase of SAF (AU)	1.52 (1.13–2.03)	0.005	1.56 (1.30–1.89)	<0.001
1-SD increase of AIx (%)	2.56 (1.59–4.12)	<0.001	2.44 (1.74–3.42)	<0.001
Adjusted for 1-SD of FRS				
1-SD increase of SAF (AU)	1.35 (0.91–2.02)	0.14	1.42 (1.10–1.83)	0.007
1-SD increase of AIx (%)	2.52 (1.45–4.36)	0.001	2.29 (1.55–3.38)	<0.001
Adjusted for 1-SD of FRS, hsCRP, albumin and presence of CVD				
1-SD increase of SAF (AU)	1.25 (0.78–2.01)	0.36	1.30 (0.97–1.74)	0.09
1-SD increase of AIx (%)	2.14 (1.18–3.89)	0.01	1.74 (1.16–2.60)	0.008

AIx, adjusted to a heart rate of 75 bpm; CVD, defined as clinical history or signs of ischaemic cardiac disease, and/or presence of peripheral vascular disease and/or cerebrovascular disease. CVD death was defined clinically based on the death certificate.

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age, hypertension and diabetes (whereas dyslipidaemia is linked with better outcomes), and also to non-traditional risk factors linked to CKD and kidney failure, i.e. inflammation, malnutrition, albuminuria, anaemia and alterations of phosphate–calcium metabolism [3]. Thus, FRS does not cover all risk aspects of the atherogenic uraemic milieu. Despite this, various studies have shown that the effect of adding measurements of GFR, serum cystatin-C and proteinuria to FRS lead to only slight improvement of its predictive power [51–53]. In the present study, we found that the AUC of FRS was relatively high and that improvements of AUC values after addition of SAF and AIx were modest. In contrast, Chen et al. [54] showed that addition of albumin, haemoglobin and eGFR may enhance the predictive power of FRS.

The results of the present study should be considered in the light of some limitations and strengths. First, since we employed an observational design no conclusions can be made about causality; furthermore, the true predictive strength of the investigated markers was not ascertained. Secondly, we relied on measurement at one single time point during PD and HD treatment or in conjunction with dialysis initiation in CKD5-ND patients, which may not be an ideal time point for risk prediction as patients are subject to many concomitant disturbances affecting investigated markers as well as their general health status. Thirdly, since SAF measurements have only been validated in subjects with Fitzpatrick skin types 1–4, the results of the present study cannot be applied to patients with other skin types [7]. Fourthly, the patient population was relatively small and since patients were clinically stable, they may not be representative of the typical population of CKD5 patients. Fifthly, we lack data on PWV, such as carotid–femoral PWV, which is considered as gold standard measurement for arterial stiffness, whereas AIx, although it is a predictor of CVD events as well as of all-cause mortality, is less well established as is the relation between the two methods [2]. On the other hand, patients were extensively phenotyped with data on inflammatory and nutritional biomarkers; their CVD risk profiles according to FRS, SAF and AIx varied substantially, which should strengthen the statistical analyses, and none of the patients was lost to follow-up. However, the results need to be confirmed in a larger cohort and after adjusting for additional clinical and biochemical parameters such as coronary artery calcification score, renal failure duration, residual renal function and dialysate glucose exposure, which all are important potential covariates that need to be included in future studies. While this study supports the use of FRS, which is cheap and easy to implement in the clinical setting, and thus the importance of traditional risk factors in CKD, it also highlights the importance of vascular stiffness. Further studies using PWV or other accurate measurements of arterial stiffness are warranted.

In conclusion, this study shows that SAF and AIx after adjusting for FRS were associated with presence of CVD and mortality; however, only AIx independently associated with mortality after adjusting for additional confounders including inflammation. These results suggest that measuring arterial stiffness may help to identify patients at high risk who may benefit from aggressive clinical management. However, it is important to note that the contribution of AIx (and SAF) compared with FRS was relatively modest. Thus, our findings emphasize the importance of considering and manage traditional CVD risk factors also in CKD.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

CONFLICT OF INTEREST STATEMENT

B.L. is employed by Baxter Healthcare Corporation. None of the other authors declare any conflict of interest.

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