CIRCULATING S100A12 (EN-RAGE), SOLUBLE RAGE, AND MORTALITY IN CHRONIC KIDNEY DISEASE STAGE 5 PATIENTS

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Introduction

Chronic kidney disease (CKD) patients have an increased risk of cardiovascular disease (CVD)-mortality that associate with biomarkers of inflammation, oxidative stress and endothelial function. Recently, the advanced glycation end-products (AGE) - receptor of AGE (RAGE) system, including circulating RAGE-ligand S100A12, also known as EN-RAGE and soluble RAGE (sRAGE), have gained increased attention. RAGE functions as a multi-ligand pattern recognition receptor mediating pro-inflammatory signals following binding to circulating AGEs, S100A12 and other circulating peptide and protein ligands. Up-regulation of RAGE is linked to inflammation, obesity, insulin resistance, diabetes, CKD and CVD.

S100A12 is overexpressed on the cell surface of macrophages, lymphocytes and endothelium at sites of local inflammation where it acts as co-facilitator/initiator of the AGE-RAGE mediated inflammatory response. Circulating S100A12 correlates with inflammatory markers and may reflect an individual's disease activity.

Circulating sRAGE shed from the cellular membrane acts as a decoy receptor that binds to AGEs and other circulating RAGE-ligands, thereby alleviating intracellular RAGE signaling and the pro-inflammatory effects of these ligands. Low rather than high plasma sRAGE associate with inflammation, and it has been suggested that sRAGE is a potentially protective factor for atherosclerosis.

In chronic pro-inflammatory conditions such as atherosclerosis, plasma S100A12 is up-regulated and plasma sRAGE down-regulated, and these alterations associate with increased risk of CVD, both in diabetic and in non-diabetic patients. Moreover, in a study not focusing on CKD, plasma S100A12 and sRAGE were inversely correlated.

The roles of sRAGE and S100A12 as biomarkers in CKD are not fully understood. Higher S100A12 and lower sRAGE levels have been reported to associate with inflammation, CVD and mortality in CKD patients; however, this is not a consistent finding. Whereas in prevalent hemodialysis (HD) patients, plasma concentrations of both S100A12 and sRAGE were elevated compared to healthy individuals, only S100A12 but not sRAGE, associated with CVD-related mortality. Furthermore, there is a scarcity of studies on S100A12 and sRAGE in CKD stage 5 patients initiating dialysis treatment.

In the current study, we evaluated the mortality predictive role of S100A12 and sRAGE in CKD stage 5 patients starting on dialysis therapy. In addition, for comparative analyses, we measured S100A12 and sRAGE also in CKD 5 patients who had been treated by dialysis for a median of 12 months, patients with CKD stages 3-4, and community-dwelling control subjects.

Materials and Methods

Patients and Study Design

The current study is based on ***post hoc*** analyses of data from an ongoing prospective cohort study of CKD stage 5 patients. At the time of the baseline investigation, patients were on the edge of starting, or had just started, on dialysis treatment at the Karolinska University Hospital Huddinge, Stockholm, Sweden. Exclusion criteria were age below 18 years, HIV or hepatitis B/C, signs of acute infection, unwillingness to participate and, in the current study, lack of sufficient blood sample volume for measurements of plasma S100A12 and sRAGE concentrations.

The current study comprised 200 CKD stage 5 patients (62% men) with a median age of 56 (range of 25th to 75th percentile, 46 to 64) years and a median glomerular filtration rate (GFR) of 6.2 (range of 25th to 75th percentile, 5.0 to 8.0) ml/min per 1.73 m^2 calculated as the mean of renal urea and creatinine clearances from 24-hour urine collection. Fifty-eight patients had already started dialysis treatment with HD (71% of patients) or peritoneal dialysis (PD; 29%) at the time of blood sampling and these patients had, prior to the investigation, been treated by dialysis for a median time period of 9 (range of 2 to 47) days. As the S100A12 and sRAGE levels among the 58 patients who had already started dialysis treatment did not differ significantly from the levels in the 142 patients who had not yet started on dialysis (data not shown), we included all 200 patients in the current study.

Comorbidities were assessed based on medical records and included diabetes mellitus (DM) in 67 (34%) patients, and CVD, defined as cardiac, cerebrovascular (including stroke) or peripheral vascular disease, in 78 (39%) patients. The patients received medications such as phosphate binders and other drugs as indicated clinically; 192 (96%) patients were on antihypertensive medications. Survival was recorded from the day of examination and for a follow-up time of up to five years. As the rate of renal transplantation was high, survival analysis was censored for transplantation.

Table 1. Characteristics and laboratory variables in 200 CKD stage 5 patients as well as in 50 control subjects, 56 CKD stages 3-4 patients, and 58 HD patients and 78 PD patients who had been on dialysis for one year.

	Controls (n=50)	CKD 3-4 (n=56)	CKD 5 (n=200)	HD (n=58)	PD (n=78)
Age (year)	63 (58-70)	62 (47-72)	56 (46-64)	59 (43-65)	54 (47-62)
Males (%)	31 (62%)	51 (88%)	123 (62%)	39 (67%)	45 (58%)
GFR (ml/min/1.73m^2)	82 (76-91)	25 (20-36)	6.2 (5.0-8.0)	NA	NA
DM (%)	0 (0%)	3 (5%)	67 (34%)	22 (38%)	19 (24%)
CVD (%)	0 (0%)	2 (4%)	78 (39%)	23 (40%)	22 (29%)
BMI (kg/m^2)	25.4±3.9	26.5±4.0	24.9±4.4	25.7±4.7	24.2±4.1)
SGA>1 (%)	2 (4%)	2 (4%)	46 (23%)	16 (28%)	14 (18%)
S-albumin (g/l)	39.0±2.8	36.9±3.5	33.5±5.0	39.0±4.7	33.7±5.0
S-creatinine (µmol/l)	79±15	292±136	761±248	NA	NA
HbA1c (%)[a]	4.7 (4.5-5.0)	5.0 (4.6-5.7)	4.4 (4.0-4.6)	4.4 (3.9-4.7)	4.8 (4.4-5.4)
Chol (mmol/l)	5.2±0.8	5.2±1.2	4.5±1.3	5.3±1.7	5.8±1.8
hsCRP (mg/l)	1.2 (0.6-2.6)	2.9 (1.2-4.9)	4.3 (1.4-11.2)	3.5 (1.5-12.0)	4.5 (1.3-11.0)
S100A12 (ng/ml)	6.7(4.6-10.0)	**26.9(21.0-38.9)**	**33.2(21.0-68.5)**	**29.5(22.4-48.6)**	**34.7(21.2-53.9)**
sRAGE (ng/ml)	1.3 (0.8-1.7)	**2.1 (1.4-2.5)**	**3.1 (2.3-4.4)**	**2.7 (2.1-4.1)**	**2.9 (2.1-3.7)**
S100A12/sRAGE ratio	5.2 (4.0-8.4)	**12.8 (9.0-23.6)**	**11.8 (6.4-22.4)**	**10.9 (7.8-19.9)**	**12.1 (6.2-24.6)**

Data are expressed as mean ± SD or median (interquartile range, IQR), or percentage.
GFR, glomerular filtration rate; DM, diabetes mellitus; CVD, cardiovascular disease; BMI, body mass index; SGA>1, subjective global assessment score (indicating protein-energy wasting); HbA1c, Hemoglobin A1c, Chol, total cholesterol, hsCRP, high-sensitivity C-reactive protein.
[a]HbA1c was assessed in non-diabetic subjects comprising 120 CKD stage 5 patients, 31 HD patients, 51 PD patients, 48 CKD stage 3-4 patients, and 50 controls.

Table 2. Multivariate logistic models examining predictors of cardiovascular disease in 200 CKD stage 5 patients. According to analysis by the receiver operating characteristics (ROC) curve, the cut off value of S100A12 as predictor of all-cause mortality was 40.2 ng/ml. In a multivariate logistic regression analysis for predicting CVD, S100A12 levels above 40.2 ng/ml associated with a positive, but non-significant trend toward increased risk of CVD (1.8 [0.95-3.48]; p=0.07) after adjusting for age and sex.

	OR (95% CI)	P-value
Intercept	--	0.001
Age less than 55 years, ref	4.64 (2.31-9.32)	0.001
Sex, female ref	2.88 (1.45-5.71)	0.002
S100A12, >40.2 ng/ml, ref	1.80 (0.95-3.48)	0.07

Pseudo r^2= 0.14, OR, odds ratio; CI, confidence interval

Table 3. Multivariate Cox regression models examining the hazard ratio (HR) for all-cause mortality risk of 1-SD higher plasma concentration of S100A12 and sRAGE, respectively, in 200 CKD stage 5 patients.

	S100A12 HR (95% CI)	P-value	sRAGE HR (95% CI)	P-value
Crude	1.34 (1.06-1.69)	0.01	1.19 (0.74-1.92)	0.47
Model 1: Adjusted for age, sex	1.36 (1.08-1.71)	0.008	1.22 (0.78-1.92)	0.38
Model 2: Adjusted for age, sex, DM and CVD	1.36 (1.06-1.74)	0.015	1.21 (0.77-1.90)	0.41
Model 3: Adjusted for age, sex, DM, CVD, SGA, s-albumin and hsCRP	**1.32 (1.01-1.73)**	**0.044**	1.23 (0.76-2.01)	0.40

HR, hazard ratio; CI, confidence interval; DM, diabetes mellitus; CVD, cardiovascular disease; SGA, subjective global assessment; hsCRP, high-sensitivity C-reactive protein.

In addition, for comparison purposes, S100A12 and sRAGE were measured also in 58 prevalent HD (39 men, median age of 59 [43 to 65] years) with preceding median dialysis duration (dialysis vintage) of 12 months) and in 78 prevalent PD patients (54 men, median age of 54 [47 to 62] years with median dialysis vintage of 12 months; 56 CKD stage 3-4 patients (51 men, median age of 62 [47 to 72] years, median GFR of 25 [20 to 36] ml/min per 1.73 m^2) and 50 control subjects (31 men, median age of 63 [58 to 70] years, median GFR of 82 [76 to 91] ml/min per 1.73 m^2) from a population-based cohort of controls randomly selected by Statistics Sweden among individuals in the Stockholm region; these individuals underwent similar measurements as the CKD 5 patients.
The local ethics committee of Karolinska Institutet at Karolinska University Hospital Huddinge approved the study protocol, and informed consent was obtained from each individual.

Results

Figure 1. Plasma S100A12 (**A)** and sRAGE (**B**) levels in control subjects (n=50), CKD stage 3-4 patients (n=56), CKD stage 5 patients starting on dialysis (n=200), and in prevalent PD (n=78) and HD (n=58) patients who had been on dialysis for one year. As compared to controls, S100A12 and sRAGE concentrations were significantly higher in CKD patients (CKD 3-4, CKD 5, PD and HD groups; all P<0.05). The concentration of sRAGE was significantly higher in CKD 5 than in CKD 3-4 patients (P<0.05).

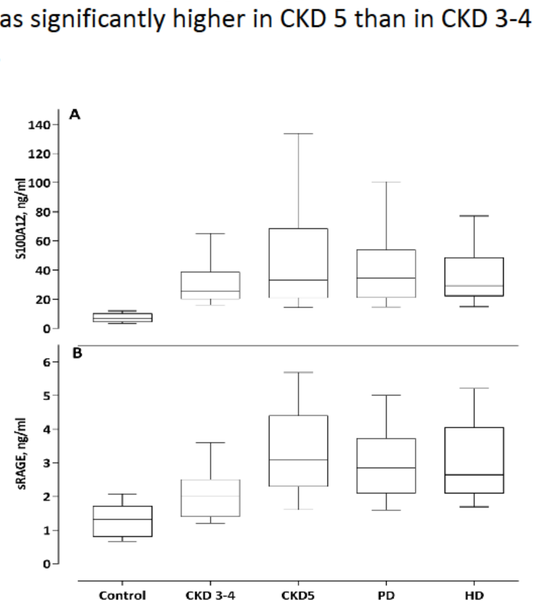


Figure 2. Plasma S100A12 levels among 200 CKD stage 5 patients were higher in those with diabetes mellitus (DM; panel A) or cardiovascular disease (CVD; panel B). Differences in S100A12 levels between non-DM (n=133) and DM (n=67) groups and between non-CVD (n=122) and CVD (n=78) groups were sta[illegible]est).

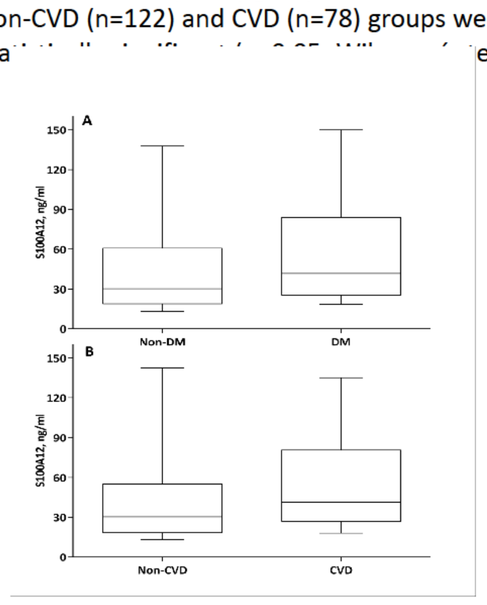
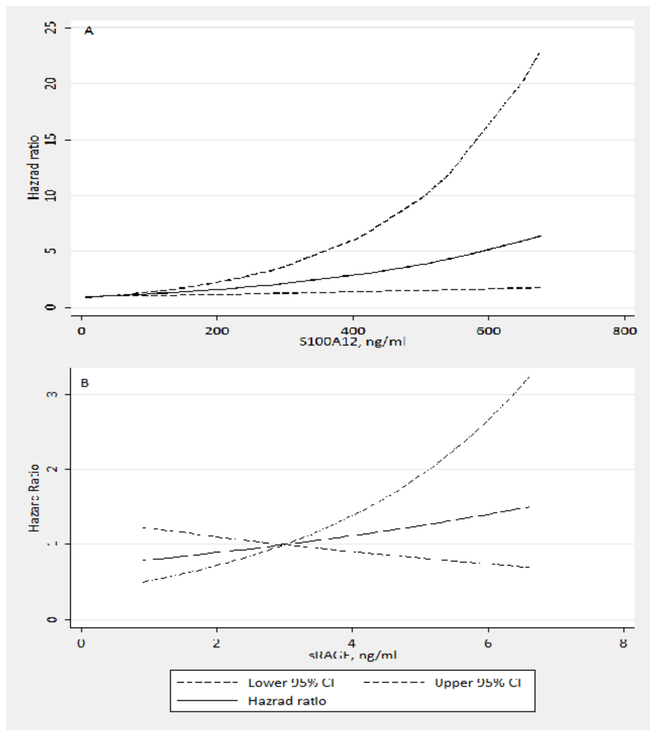


Figure 3. Spline curves of hazard ratios for all-cause mortality in relation to plasma S100A12 levels (**panel A**) and plasma sRAGE levels (**panel B**) among 200 CKD stage 5 patients initiating maintenance dialysis therapy.



Conclusion

The circulating levels of both sRAGE and S100A12 are elevated in CKD stage 5 patients as compared with those in control subjects. Whereas sRAGE was not associated with clinical outcome, a 1-SD increase of S100A12 was a predictor of increased all-cause mortality, independent of other biomarkers. These results suggest that S100A12 may be a useful biomarker that could be of value to identify CKD 5 patients at high mortality risk while sRAGE seems to be a more unspecific marker.

Conflicts of interest

Bengt Lindholm is affiliated with Baxter Healthcare. None of the other authors have any conflicts of interest to declare.

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H) Clinical Nephrology, CKD. H1) Lab methods, progression & risk factors for CKD 1-5, nutrition in CKD, renal diseases (except GNs and cystic diseases).
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