Plasma S100A12 and soluble receptor of advanced glycation end product levels and mortality in chronic kidney disease Stage 5 patients

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ABSTRACT

Background. Alterations in the advanced glycation end-products (AGE)—receptor of AGE (RAGE) system are linked to several chronic diseases, which may result from vascular damage. A high circulating level of the pro-inflammatory RAGE-ligand S100A12, also known as EN-RAGE, is thought to promote while a high level of soluble RAGE (sRAGE) is thought to protect against development of atherosclerotic cardiovascular disease (CVD). We evaluated circulating S100A12 and sRAGE in relation to clinical characteristics, nutritional status, inflammation and mortality risk in chronic kidney disease (CKD) Stage 5 patients starting on dialysis.

Methods. Plasma S100A12 and sRAGE, biomarkers of inflammation and nutritional status, and comorbidities were investigated in 200 CKD Stage 5 patients [median age of 56 years, 62% men and median glomerular filtration rate (GFR) of 6.2 mL/min/1.73 m²] in conjunction with initiation of dialysis therapy. Associations between mortality risk and S100A12 or sRAGE were assessed after a median follow-up period of 23 months. In addition, for comparative analyses, S100A12 and sRAGE levels were assessed also in 58 haemodialysis and 78 peritoneal dialysis patients after 1 year of dialysis, 56 CKD Stages 3–4 patients and 50 community-based control subjects.

Results. The median level of S100A12 was 4-fold higher, median sRAGE 2.4 higher and median ratio S100A12/sRAGE 2.27 times higher in CKD 5 patients than in controls. Similar alterations were observed in CKD 3–4 patients; however, CKD 5 patients had a higher median level of sRAGE than the CKD 3–4 patients. In the CKD 5 patients, S100A12 levels were higher in those with diabetes or CVD than in those without these comorbidities. Furthermore, S100A12 correlated with high-sensitivity C-reactive protein (hsCRP) levels (ρ = 0.53; P < 0.001) and a 1-SD higher level of S100A12 associated with increased all-cause mortality risk (hazard ratio 1.32, 95% confidence interval 1.01–1.73) after adjustment for age, sex, comorbidity, nutritional status and inflammation (hsCRP). In the CKD 5 patients, sRAGE correlated negatively with GFR (ρ = −0.26; P < 0.01) but sRAGE did not associate with hsCRP, comorbidities or mortality.

Conclusions. Plasma concentrations of sRAGE, S100A12 and the ratio S100A12/sRAGE, are markedly elevated in CKD 5 patients starting on dialysis as well as in CKD 3–4 patients and prevalent dialysis patients suggesting that these alterations are typical for patients with moderate or severe CKD. In CKD 5 patients, an increased concentration of S100A12 are associated with inflammation, comorbidities and increased mortality risk whereas no such associations were observed for sRAGE. These results suggest that while high plasma S100A12 is an independent predictor of increased mortality risk, sRAGE does not seem to be a valid risk marker in this patient population.

Keywords: cardiovascular disease, end-stage renal disease, inflammation, mortality, RAGE

INTRODUCTION

Chronic kidney disease (CKD) patients have an increased risk of cardiovascular disease (CVD) mortality [1] that are associated with biomarkers of inflammation, oxidative stress and endothelial function [2–5]. Recently, biomarkers linked to 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 attracted increasing attention [6]. RAGE functions as a multi-ligand pattern recognition receptor mediating pro-inflammatory signals following binding to circulating AGES, S100A12 and other
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 [11]. Circulating S100A12 correlates with inflammatory markers and may reflect an individual’s disease activity [12–14].

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 signalling and the pro-inflammatory effects of these ligands [15, 16]. Low rather than high plasma sRAGE is associated with inflammation, and it has been suggested that sRAGE is a potentially protective factor for atherosclerosis [17].

In chronic pro-inflammatory conditions such as atherosclerosis, plasma S100A12 is up-regulated and plasma sRAGE down-regulated, and these alterations are associated with increased risk of CVD, both in diabetic and in non-diabetic patients [18]. Moreover, in a study not focussing on CKD, plasma S100A12 and sRAGE were inversely correlated [18].

The roles of sRAGE and S100A12 as biomarkers in CKD are not fully understood. Higher S100A12 and lower sRAGE levels have been reported to be associated with inflammation, CVD and mortality in CKD patients; however, this is not a consistent finding [11, 19–28]. Whereas in prevalent haemodialysis (HD) patients, plasma concentrations of both S100A12 and sRAGE were elevated compared with healthy individuals, only S100A12 but not sRAGE, associated with CVD-related mortality [25]. 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 5 patients [29]. At the time of the baseline investigation, patients were on the verge 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.

The current study comprised 200 CKD 5 patients (62% men) with median age of 56 (range of 25–75th percentile, 46–64) years and median glomerular filtration rate (GFR) of 6.2 (5.0–8.0) mL/min/1.73 m² calculated as the mean of renal plasma S100A12 and sRAGE were inversely correlated [18]. Moreover, in a study not focussing on CKD, patients with CKD Stages 3–4, and community-dwelling control subjects.

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S100A12/EN-RAGE ELISA kit (Cyclax Co., Ltd., Nagano, Japan) were measured using commercially available ELISA kits according to the instructions of the manufacturers. High Mobility Group Box Protein-1 (HMGB1) was measured by western blot according to [33], in a limited number of the investigated CKD 3–4 patients (n = 46), CKD 5 patients (n = 38) and controls (n = 37). Circulating levels of albumin, creatinine, haemoglobin A1c (HbA1c), total cholesterol (Chol) and high-sensitivity C-reactive protein (hsCRP) were analysed using certified methods at the Department of Clinical Chemistry, Karolinska University Hospital Huddinge.

Statistical analyses

Values are expressed as mean ± SD or median (range of 25–75th 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 and differences among the three groups were analysed using the non-parametric Kruskal-Wallis test. Spearman rank correlation analysis was used to determine associations between S100A12 and sRAGE, whereas logistic regression analyses with selected parameters. Receiver operating characteristics (ROC) analysis was performed to determine the cut-off value of S100A12 as predictor of all-cause mortality. Multivariate regression analyses were used to assess independent predictors of S100A12 and sRAGE, whereas logistic regression analyses were used to assess determinants of existing CVD. Survival analyses were made with the Cox proportional hazard model. The multivariate Cox regression analyses are presented as hazard ratios (HR) and 95% confidence intervals (CI). The proportionality assumptions were checked through visual inspection of the log of the incidence rates. Restricted cubic spline graphs were used to graphically evaluate systematic relationships between S100A12 and sRAGE and mortality. All statistical analyses were performed using statistical software SAS version 9.4 (SAS Campus Drive, Cary, NC, USA).

RESULTS

S100A12 and sRAGE levels in controls and CKD patients

Clinical and laboratory characteristics of the 200 CKD 5 patients as well as data for control subjects (n = 50), CKD Stage 3–4 patients (n = 56) and HD (n = 58) and PD (n = 78) patients who had been treated by dialysis for 1 year are summarised in Table 1. CKD 5 patients were younger, had higher burden of comorbidities including DM, CVD and PEW (SGA > 1), lower serum albumin, higher hsCRP, higher serum creatinine and lower GFR as compared with CKD Stage 3–4 patients.

The median level of S100A12 was 4-fold higher, median sRAGE 2.4 higher and median ratio 100A12/sRAGE 2.27 times higher in CKD 5 patients than in controls and similar alterations were observed in CKD 3–4 patients; however, CKD 5 patients had a higher median level of sRAGE than the CKD 3–4 patients (Table 1). S100A12 and sRAGE levels, and the ratio S100A12/sRAGE, were similarly elevated also patients who had been treated by dialysis for 1 year with no significant differences between PD and HD patients or in comparison with CKD 5 patients starting on dialysis (Figure 1).

Univariate and multivariate correlations with S100A12 and sRAGE levels in CKD 5 patients

S100a12. In CKD 5 patients, S100A12 levels correlated with presence of DM (ρ = 0.18; P < 0.01) and CVD (ρ = 0.19; P < 0.01), serum creatinine (ρ = 0.20; P < 0.01) and hsCRP (ρ = 0.53; P < 0.001) levels, and correlated negatively with serum albumin (ρ = −0.29; P < 0.001). In the CKD 5 patients, the median S100A12 level was higher in patients with DM [41.6 (25.5–84.0) versus 30.2 (18.9–60.8) ng/mL, P < 0.01] or with CVD [41.2 (26.9–80.6) versus 30.3 (18.4–55.1) ng/mL, P < 0.01] than in patients without DM and CVD respectively. In contrast, for sRAGE, no such differences were found between patients with DM 3.0(1.7–5.6) and without DM 3.1

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 1 year

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 50)</th>
<th>CKD 3–4 (n = 56)</th>
<th>CKD 5 (n = 200)</th>
<th>HD (n = 58)</th>
<th>PD (n = 78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63 (58–70)</td>
<td>62 (47–72)</td>
<td>56 (46–64)</td>
<td>59 (43–65)</td>
<td>54 (47–62)</td>
</tr>
<tr>
<td>Males (%)</td>
<td>31 (62%)</td>
<td>51 (88%)</td>
<td>123 (62%)</td>
<td>39 (67%)</td>
<td>45 (58%)</td>
</tr>
<tr>
<td>GFR (mL/min/1.73 m²)</td>
<td>82 (76–91)</td>
<td>25 (20–36)</td>
<td>62 (5.0–8.0)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>DM (%)</td>
<td>0 (0%)</td>
<td>3 (5%)</td>
<td>67 (34%)</td>
<td>22 (38%)</td>
<td>19 (24%)</td>
</tr>
<tr>
<td>CVD (%)</td>
<td>0 (0%)</td>
<td>2 (4%)</td>
<td>78 (39%)</td>
<td>23 (40%)</td>
<td>22 (29%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4 ± 3.9</td>
<td>26.5 ± 4.0</td>
<td>24.9 ± 4.4</td>
<td>25.7 ± 4.7</td>
<td>24.2 ± 4.1</td>
</tr>
<tr>
<td>SGA &gt; 1 (%)</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
<td>46 (23%)</td>
<td>16 (28%)</td>
<td>14 (18%)</td>
</tr>
<tr>
<td>S-albumin (g/L)</td>
<td>39.0 ± 2.8</td>
<td>36.9 ± 3.5</td>
<td>33.5 ± 5.0</td>
<td>39.0 ± 4.7</td>
<td>33.7 ± 5.0</td>
</tr>
<tr>
<td>S-creatinine (μmol/L)</td>
<td>79 ± 15</td>
<td>292 ± 136</td>
<td>761 ± 248</td>
<td>719 ± 213</td>
<td>711 ± 200</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.7 (4.5–5.0)</td>
<td>5.0 (4.6–5.7)</td>
<td>4.4 (4.0–4.6)</td>
<td>4.4 (3.9–4.7)</td>
<td>4.8 (4.4–5.4)</td>
</tr>
<tr>
<td>Chol (mmol/L)</td>
<td>5.2 ± 0.8</td>
<td>5.2 ± 1.2</td>
<td>4.5 ± 1.3</td>
<td>5.3 ± 1.7</td>
<td>5.8 ± 1.8</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>1.2 (0.6–2.6)</td>
<td>2.9 (1.2–4.9)</td>
<td>4.3 (1.4–11.2)</td>
<td>3.5 (1.5–12.0)</td>
<td>4.5 (1.3–11.0)</td>
</tr>
<tr>
<td>S100A12 (ng/mL)</td>
<td>6.7 (4.6–10.0)</td>
<td>26.9 (21.0–38.9)</td>
<td>33.2 (21.0–68.5)</td>
<td>29.5 (22.4–48.6)</td>
<td>34.7 (21.2–53.9)</td>
</tr>
<tr>
<td>sRAGE (ng/mL)</td>
<td>1.3 (0.8–1.7)</td>
<td>2.1 (1.4–2.5)</td>
<td>3.1 (2.3–4.4)</td>
<td>2.7 (2.1–4.1)</td>
<td>2.9 (2.1–3.7)</td>
</tr>
<tr>
<td>S100A12/sRAGE ratio</td>
<td>5.2 (4.0–8.4)</td>
<td>12.8 (9.0–23.6)</td>
<td>11.8 (6.4–22.4)</td>
<td>10.9 (7.8–19.9)</td>
<td>12.1 (6.2–24.6)</td>
</tr>
</tbody>
</table>

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, Haemoglobin A1c, Chol, total cholesterol, hsCRP, high-sensitivity C-reactive protein.

*aHbA1c 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.
(1.3–5.7) ng/mL, P = 0.29; or between those with CVD 3.0(1.3–5.5) and without CVD 3.2(1.3–5.8) ng/mL, P = 0.67. However, whereas S100A12 (Figure 2A) and sRAGE (Figure 2B) concentrations were found to be markedly increased in the CKD 5 patients as compared with controls, there were no statistically significant differences between the subgroups of patients with DM only (n = 28), CVD only (n = 39), DM + CVD (n = 39), or none of these comorbidities (n = 94).

In a multivariate logistic regression analysis, S100A12 > 40.2 ng/mL, the cut-off value of S100A12 as predictor of all-cause mortality as defined by the ROC curve, was 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 (Table 2).

sRAGE. In CKD 5 patients, sRAGE correlated negatively with GFR (p = −0.26; P < 0.01) and positively with serum creatinine (p = 0.25; P < 0.001) while other associations observed for S100A12 (see above) were not significant.

HMGB1, S100A12 and sRAGE in inflamed and non-inflamed patients. Data on HMGB1 were available in a limited number of investigated patients and controls. The associations of S100A12 and sRAGE, respectively, with HMGB1 among the CKD patients appeared to be related to presence or not of inflammation (as defined by hsCRP > 10 mg/L). In non-inflamed CKD 3–4 patients (n = 28), but not among inflamed CKD 3–4 patients, HMGB1 was positively associated with s100A12 (p = 0.41; P = 0.02). In inflamed CKD 5 patients (n = 11), but not among non-inflamed CKD 5 patients, HMGB1 correlated inversely with sRAGE (p = −0.69; P = 0.02). In control subjects, HMGB1 was not significantly associated with sRAGE or S100A12.

Table 2. Multivariate logistic models examining predictors of cardiovascular disease in 200 CKD Stage 5 patients

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>–</td>
</tr>
<tr>
<td>Age &lt;55 years, ref</td>
<td>4.64 (2.31–9.32)</td>
</tr>
<tr>
<td>Sex, female ref</td>
<td>2.88 (1.45–5.71)</td>
</tr>
<tr>
<td>S100A12, &gt;40.2 ng/mL, ref</td>
<td>1.80 (0.95–3.48)</td>
</tr>
</tbody>
</table>

According to analysis by the 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 >40.2 ng/mL were 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 (Table 2).

Pseudo r² = 0.14, OR, odds ratio; CI, confidence interval.
S100A12 and sRAGE levels and risk of mortality in CKD 5 patients

Fifty-three (27%) of the CKD5 patients died during a median follow-up of 23 (10–37) months. The main causes of death were CVD (n = 22; 42%) and infections (n = 13; 25%). In multivariate Cox regression models (Table 3), plasma sRAGE was not associated with all-cause mortality risk. In contrast, a 1-SD higher level of S100A12 was associated with increased all-cause mortality risk with a crude HR of 1.34 (95% CI; 1.06–1.69; P = 0.01). The association remained significant after adjusting for age and sex (Model 1, HR 1.36 (1.08–1.71); P = 0.008), plus also DM and CVD [Model 2, HR 1.36 (1.06–1.74), P = 0.015], and when also including nutritional status, serum albumin and hsCRP [Model 3; HR 1.32 (1.01–1.73); P = 0.044].

DISCUSSION

Plasma concentrations of S100A12 and sRAGE, and the ratio S100A12/sRAGE, were markedly elevated in CKD Stage 5 patients starting on dialysis, CKD Stage 3–4 patients, and prevalent PD and HD patients suggesting that these alterations are typical for moderate and advanced stages of CKD and not much influenced by dialysis therapy. In CKD 5 patients, plasma S100A12 was associated with inflammation and presence of CVD and DM, and a 1-SD higher level of S100A12 associated with 32% higher all-cause mortality risk. Plasma sRAGE correlated inversely with GFR but did not associate with inflammation, comorbidities or mortality risk. To the best of our knowledge, this is the first study exploring the association of S100A12 and sRAGE levels in relation to mortality risk in CKD Stage 5 patients starting on dialysis.

In non-CKD populations, low plasma sRAGE and high plasma S100A12 may indicate increased disease activity, and, in inflammatory conditions such as Kawasaki’s disease [34] and in Familial Mediterranean fever, inflammatory bowel disease and rheumatoid arthritis, S100A12 concentrations may be 5–10 times higher than in healthy subjects [35–37]. Likewise, in the current study, S100A12 was four times higher in level in CKD 5 patients than in controls and increased with increasing hsCRP levels.

Among CKD patients, increased levels of serum S100A12 were reported to decrease in glomerulonephritis patients with myeloperoxidase anti-neutrophil cytoplasmic antibodies following treatment by prednisolone [38]. In patients with CKD Stages 2–4, serum S100A12 levels increased with declining renal function [19]. In the current study, S100A12 levels were not associated with GFR but correlated with hsCRP similar to the results of a previous study of patients with various degrees of CKD including HD patients [20].

These associations are illustrated by spline curves depicting hazard ratios for all-cause mortality in relation to plasma S100A12 (Figure 3A) and sRAGE levels (Figure 3B).

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

<table>
<thead>
<tr>
<th></th>
<th>S100A12 HR (95% CI)</th>
<th>P-value</th>
<th>sRAGE HR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>1.34 (1.06–1.69)</td>
<td>0.01</td>
<td>1.19 (0.74–1.92)</td>
<td>0.47</td>
</tr>
<tr>
<td>Model 1: adjusted for age and sex</td>
<td>1.36 (1.08–1.71)</td>
<td>0.008</td>
<td>1.22 (0.78–1.92)</td>
<td>0.38</td>
</tr>
<tr>
<td>Model 2: adjusted for age, sex, DM and CVD</td>
<td>1.36 (1.06–1.74)</td>
<td>0.015</td>
<td>1.21 (0.77–1.90)</td>
<td>0.41</td>
</tr>
<tr>
<td>Model 3: adjusted for age, sex, DM, CVD, SGA, S-albumin and hsCRP</td>
<td>1.32 (1.01–1.73)</td>
<td>0.044</td>
<td>1.23 (0.76–2.01)</td>
<td>0.40</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; DM, diabetes mellitus; CVD, cardiovascular disease; SGA, subjective global assessment; hsCRP, high-sensitivity C-reactive protein.
the increase of sRAGE may not have been significant in uraemia [22]. The results of the current study suggest that the counter-regulatory system against vascular endothelial damage may thus indicate enhanced RAGE expression within a setting in the uraemic milieu [21]. An increased sRAGE level response against increased renal function, as reported also in previous studies [21, 41]. Decreased renal function seems to be a more important determinant of sRAGE levels than the dialysis technique; whereas differences in plasma S100A12 and sRAGE levels between different categories of CKD patients are likely to be influenced by multiple factors, the inflammatory state (for S100A12) and residual renal function (for sRAGE) seems to be of major importance. Furthermore, concurrent medication such as statins may also matter as blockade of RAGE-S100A12 by statins was reported to prevent inflammation [27, 39]. In our study, 72 patients (36%) were on statin treatment but use of statins were not associated with S100A12 levels, 29.8(15.5–104.7) versus 34.7(13.1–147.2) ng/mL; P = 0.34, in patients with and without statins respectively. As noted in our study and in other studies, S100A12 levels were strongly associated with inflammation [20, 34–37], possibly due to increased synthesis and/or release of S100A12 by activated leukocytes which may be up-regulated by cytokines and other molecules in uraemia [11]. Whereas S100A12 levels in the current study did not differ significantly between the dialysed and non-dialysed CKD 5 patients, or between those treated with HD and PD, comorbidities such as DM and CVD are known contributors to elevated S100A12 levels; excessive expression of the S100A12 gene in uraemic leukocytes in CKD Stage 4–5 patients, particularly in those with CVD, have been reported [40]. Other factors that could potentially influence the plasma S100A12 level in CKD remain unclear; the metabolism and excretion of S100A12 in mammals has to the best of our knowledge not been reported.

In our study, increasing plasma sRAGE correlated with declining renal function, as reported also in previous studies [21, 41] and sRAGE decreases after renal transplantation [21, 41]. Decreased renal function seems to be a more important determinant of sRAGE levels than the dialysis technique; in our study and in a previous study [21], sRAGE levels did not differ between HD and PD patients. In addition, increased levels of circulating sRAGE in CKD might reflect a protective response against inflammation, oxidative stress and toxic substrates in the uraemic milieu [21]. An increased sRAGE level may thus indicate enhanced RAGE expression within a counter-regulatory system against vascular endothelial damage in uraemia [22]. The results of the current study, suggest that the increase of sRAGE may not have not be sufficient to counteract the putative deleterious effects of the more pronounced increase of S100A12.

Low sRAGE was reported to be linked to higher risk for cardiovascular complications in patients with end-stage renal disease [23] but in the current study, and in a previous study in prevalent HD patients [25], sRAGE was not associated with CVD or mortality. Interestingly, according to the spline curve analysis (Figure 3), there was a trend—although not statistically significant—towards increased mortality risk associated with a high rather than low sRAGE level contrasting with the findings of Selvin et al. [10] who observed the opposite relation in 1201 participants in the Atherosclerosis Risk in Communities study. These discrepancies might at least partly be due to the association, observed in the current study, between reduced renal residual renal function, a powerful predictor of mortality risk, and increased plasma sRAGE; one may speculate that this could have disguised a putative beneficial influence of increased sRAGE.

In the current study, we compared S100A12 and sRAGE concentrations in CKD Stage 5 patients initiating dialysis therapy with data from other cohorts of CKD patients, including CKD 3–4 patients and prevalent CKD 5 patients who had been on dialysis with PD or HD for 1 year. These results show that whereas S100A12 and sRAGE concentrations in general were higher in CKD 5 as compared with CKD 3–4 patients, dialysis treatment over 1 year did not seem to affect S100A12 and sRAGE concentrations. Considering the assumed pro-inflammatory and anti-inflammatory properties of S100A12 and sRAGE respectively, one would expect that patients surviving on dialysis would be those with lower S100A12 and higher sRAGE levels; however, the S100A12/sRAGE ratio did not differ between the CKD cohorts (Table 1). Furthermore, among the 200 CKD Stage 5 patients, the mortality predictive role of combinations of S100A12 and sRAGE levels (lower than median S100A12 and higher than median sRAGE levels compared with the three other possible patterns of S100A12 and sRAGE) did not differ in the current study. Thus, we were not able to detect any protective influence of the elevated plasma concentrations of sRAGE in the CKD Stage 5 patients. The observation in the current study that HMGB1—a pro-inflammatory mediator of tissue injury—correlated inversely with sRAGE in inflamed but not in non-inflamed CKD 5 patients could perhaps be interpreted as indicating a failed protective response of sRAGE in response to elevated HMGB1 levels.

Several limitations of the present study should be acknowledged. As we studied cross-sectional cohorts each with a relatively small number of subjects, we cannot draw conclusions regarding causality or to what extent levels of S100A12 and sRAGE are affected by renal replacement therapy. On the other hand, detailed phenotyping, including biomarkers of inflammation and nutritional status and comorbidities, strengthens the assessment of mortality risk in the current study.

In summary, plasma S100A12 and sRAGE concentrations, and the ratio S100A12/sRAGE, are markedly elevated in CKD 5 patients starting on dialysis. Similar changes were observed in CKD 3–4 patients and in prevalent dialysis (PD or HD) patients suggesting that these alterations are typical features of moderate and advanced CKD that do not seem to be much modified by dialysis therapy. In the CKD 5 patients, S100A12 positively associated with inflammation and presence of CVD and DM, and was an independent predictor of increased all-cause mortality. Plasma sRAGE was inversely related with residual renal function and was not associated with inflammation, comorbidities or mortality. Considering that the ratio S100A12/sRAGE was markedly elevated, we speculate that the putative protective role of sRAGE was not sufficient to counteract the apparent deleterious effects of the very high
S100A12 levels in this patient population. Overall these results indicate that S100A12 may identify CKD Stage 5 patients at high mortality risk while sRAGE does not seem to be a valid risk marker in this patient population. Possible therapeutic strategies targeting S100A12 should be considered.

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CONFLICT OF INTEREST STATEMENT

Bengt Lindholm is affiliated with Baxter Healthcare. None of the other authors have declared any conflicts of interest. The results presented in this paper have not been published previously in whole or part, except in abstract format.

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Rituximab-associated agranulocytosis in children with refractory idiopathic nephrotic syndrome: case series and review of literature

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ABSTRACT

Background. Agranulocytosis has been reported as a delayed-onset complication of rituximab treatment. However, the exact incidence and risk factors of this complication in patients with nephrotic syndrome remain unknown.

Methods. Records of 213 rituximab treatments for 114 patients with refractory nephrotic syndrome between February 2006 and April 2013 were reviewed to identify episodes of agranulocytosis (defined as an absolute neutrophil count of <500 mm$^{-3}$).

Results. Eleven episodes of agranulocytosis were detected in 11 patients. Median time of onset of agranulocytosis was 66 days (range, 54–161 days) after rituximab treatment. Nine patients experienced acute infections and received antibiotics. All but one patient received granulocyte colony-stimulating factor. Agranulocytosis resolved in all cases within a median of 3 days. The incidence of agranulocytosis was 9.6% in total patients and 5.2% in all treatments. Median age of the 11 patients who developed agranulocytosis was 6.4 years at the first rituximab treatment, significantly younger than the median age of the 103 patients who did not (median, 12.5 years; P = 0.0009). Five patients received re-treatment with rituximab. No recurrence of agranulocytosis was observed in any patient.

Conclusions. It is important to pay extra attention to this clinically serious delayed-onset complication as it may be accompanied by life-threatening infections such as sepsis. Further clinical studies are needed to clarify its pathogenesis.

Keywords: agranulocytosis, B cell, infection, nephrotic syndrome, rituximab

INTRODUCTION

Rituximab is a chimeric monoclonal antibody directed against the cell surface antigen CD20 expressed on B lymphocytes. As