RHEUMATOLOGY

Rheumatology 2020;59:2237–2249
doi:10.1093/rheumatology/kez528
Advance Access publication 13 November 2019

Original article

ALCAM and VCAM-1 as urine biomarkers of activity and long-term renal outcome in systemic lupus erythematosus

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Abstract

Objectives. We investigated the cell adhesion molecules (CAMs) Vascular CAM 1 (VCAM-1) and Activated Leucocyte CAM (ALCAM) as urinary biomarkers in SLE patients with and without renal involvement.

Methods. Female SLE patients ($n = 111$) and non-SLE population-based controls ($n = 99$) were enrolled. We measured renal activity using the renal domain of the BILAG index and urine (U) and plasma (P) concentrations of soluble (s)VCAM 1 and U-sALCAM using ELISA. U-sCAM levels were next corrected by U-creatinine.

Results. U-sVCAM-1/creatinine and U-sALCAM/creatinine ratios were higher in SLE patients *vs* non-SLE controls ($P < 0.001$ for both), as well as in patients with active/low-active (BILAG A–C; $n = 11$) *vs* quiescent (BILAG D; $n = 19$) LN ($P = 0.023$ and $P = 0.001$, respectively). U-sALCAM/creatinine but not U-sVCAM-1/creatinine ratios were higher in patients with nephritis history (BILAG A–D; $n = 30$) *vs* non-renal SLE (BILAG E; $n = 79$) ($P = 0.014$). Patients with baseline U-sVCAM-1/creatinine ratios $\geq$75th percentile showed a 23-fold increased risk of a deterioration in estimated glomerular filtration rate by $\geq$25% during a 10-year follow-up (odds ratio: 22.9; 95% CI: 2.8, 189.2; $P = 0.004$); this association remained significant after adjustments for age, disease duration and organ damage. Traditional markers including anti-dsDNA antibodies did not predict this outcome.

Conclusion. While high U-sVCAM-1 levels appear to reflect SLE disease activity, sALCAM might have particular importance in renal SLE. Both U-sVCAM-1 and U-sALCAM showed ability to distinguish SLE patients with active renal involvement from patients with quiescent or no prior nephritis. High U-sVCAM-1 levels may indicate patients at increased risk for long-term renal function loss.

Key words: systemic lupus erythematosus, lupus nephritis, biomarkers, urinary biomarkers, adhesion molecules

CLINICAL SCIENCE

Rheumatology key messages

- Urinary soluble activated leucocyte cell adhesion molecule was associated with LN history in patients with SLE.
- Urinary soluble activated leucocyte cell adhesion molecule and soluble vascular cell adhesion molecule 1 levels were elevated in patients with active *vs* quiescent LN.
- Unlike traditional markers, high urine soluble vascular cell adhesion molecule 1 levels predicted long-term renal function deterioration in lupus patients.

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Submitted 7 May 2019; accepted 6 September 2019

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Introduction

Renal involvement is one of the main causes of increased morbidity and mortality in patients with SLE [1]. The diagnosis of LN and choice of therapeutic interventions are mainly based on histological evaluation. Recent research has focussed on the identification of non-invasive assessment tools that accurately track renal activity and portend long-term prognosis [2–4]. Urinary biomarkers are attractive candidates since they

are directly excreted from the kidney and readily accessible for examination.

Cell adhesion molecules (CAMs) are known to be important components in the inflammatory process. They are expressed on the vascular endothelium and act as counter-receptors for leucocyte integrins, enabling leucocyte adhesion and rolling along endothelial cell surfaces, a process that eventually leads to extravasation of leukocytes into adjacent tissue [5, 6]. Vascular CAM 1 (VCAM-1), or cluster of differentiation 106 (CD106), is the most abundant circulating CAM in the periphery. It is constitutively expressed in endothelial cells and glomerular parietal epithelial cells [7], and has been shown to be substantially elevated in active SLE, during renal allograft rejection and in septic shock [8, 9]. In healthy renal tissue, VCAM-1 expression is typically restricted to parietal epithelial cells of the Bowman's capsule, while in vasculitis and LN, VCAM-1 expression has also been observed on proximal tubular cells [10, 11]. Upregulated expression of VCAM-1 has been seen in the glomerular mesangium and endothelium in lupus-like murine glomerulonephritis [11], as well as in skeletal muscle [12] and endothelial cells from non-lesional skin [13] in SLE patients. Previous studies have suggested a role of VCAM-1 in LN development [11, 14–16]. Elevated urine levels of soluble (s)VCAM-1 have also been observed in SLE patients compared with controls [17], and in LN patients with advanced renal damage [18].

Activated leucocyte CAM (ALCAM), also known as CD166, is the only known ligand for the type 1 transmembrane glycoprotein CD6 on activated leukocytes [19]. ALCAM is expressed at high levels on antigen-presenting cells, and plays an important role in the co-stimulation of T cells [20–23] with predominantly ALCAM–CD6 interactions forming the immunological synapse at the T cell–antigen-presenting cell interface, eliciting sustained T cell activation [23]. ALCAM plays a critical role in mediating the transmigration of T cells and monocytes across the endothelium and blood–brain barrier [24, 25]. Increased expression has been observed in renal tissue from MRL/lpr lupus-like glomerulonephritis mouse strains [26]. In diabetic nephropathy, serum concentrations of sALCAM have been shown to be elevated and inversely correlated with renal function, while ALCAM expression was upregulated both in glomeruli and tubules, mainly in podocytes [27].

Recent semi-unbiased high-throughput proteomic approaches have revealed a potential for sVCAM-1 and sALCAM as urinary biomarkers in LN [28, 29]. The aim of the present study was to investigate the potential usefulness of urinary sVCAM-1 and sALCAM as non-invasive biomarkers of activity and long-term renal prognosis in Caucasian patients with SLE with and without renal involvement.

Methods

Patients with SLE ($n = 111$) and non-SLE population-based controls ($n = 99$) of similar age distributions from the Karolinska lupus cohort were included in this longitudinal retrospective study, and followed for up to 10 years. All study participants were female and of Caucasian origin. Patient and control characteristics, including immunosuppressive treatments prior to and at the time of enrolment in the study, are presented in Table 1. The patients' rights, safety and well-being were protected in compliance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrolment. The study protocol was approved by the regional ethics review board in Stockholm, Sweden.

Clinical assessment

All patients fulfilled the 1982 revised ACR [30] and the 2012 SLICC [31] SLE classification criteria. Based on their medical history, SLE patients were stratified into patients with or without a history of LN. All but three patients with a history of renal involvement had undergone one or more renal biopsies prior to enrolment.

Global SLE disease activity at the time of enrolment was assessed using the SLEDAI-2K [32]. Organ damage was assessed using the SLICC/ACR Damage Index [33]. Renal activity was assessed using the renal descriptors of the SLEDAI-2K (urinary casts, haematuria, proteinuria, pyuria) and the renal domain of the BILAG 2004 index [34]. Renal BILAG was not calculated in patients with end-stage renal disease at baseline ($n = 2$).

Assessment of sCAM and autoantibody levels

Urine and plasma samples from the patients and the controls were collected at the time of enrolment, and stored at –80°C until the analysis. ELISA kits from R&D Systems (Minneapolis, MN, USA) were used to measure levels of sALCAM (catalogue number: DY656) and sVCAM-1 (catalogue number: DY809) according to the manufacturer's protocols. Briefly, a 96-well microplate was coated with capture antibody and incubated overnight at room temperature. The plate was then blocked with reagent diluent for 1 h before 1:50 diluted urine samples were added and incubated for 2 h; this was followed by incubation with biotinylated detection antibody for another 2 h. Subsequently, streptavidin-horseradish peroxidase conjugate and substrate solution were added in succession and were incubated for 20 min each. Following addition of stop solution, optical density was determined at 450 nm using an ELx808 microplate reader from BioTek Instruments (Winooski, VT, USA). Sample concentrations were calculated based on standard curves. All measurements were assayed in duplicate.

To normalize the concentrations of urinary (U)-sVCAM-1 and U-sALCAM, U-creatinine concentrations from the same sampling occasions were determined using the Creatinine Parameter Assay Kit from R&D Systems (catalogue number: KEG005). Urine samples

TABLE 1 Baseline characteristics

	SLE patients			Non-SLE controls, *N* = 99
	All patients, *N* = 111	LN history, *N* = 30	Never LN, *N* = 79	
Age (years)	51.6 (36.2–61.7)	48.6 (38.1–56.9)	54.9 (35.6–62.7)	54.1 (43.4–61.7)
Women	111 (100)	30 (100)	79 (100)	99 (100)
Ethnicity				
Caucasian	111 (100)	30 (100)	79 (100)	99 (100)
SLE duration (years)	13.6 (6.4–24.4)	15.7 (9.4–29.6)	12.9 (5.4–23.8)	N/A
SLEDAI-2K	4 (0–8)	4 (0–13)	4 (0–6)	N/A
Renal SLEDAI-2K[a]	0 (0–4)	4 (0–4)	0 (0–0)	N/A
BILAG index				
A	2	2	N/A	N/A
B	3	3	N/A	N/A
C	6	6	N/A	N/A
D	19	19	N/A	N/A
E	79	N/A	79	N/A
ESRD	2	N/A	N/A	0
Hypertension	30 (27.0)	10 (33.3)	18 (22.8)	22 (22.2)
Systolic BP	125 (110–140); N=110	130 (120–136)	125 (107–141); N=78	120 (110–140)
Diastolic BP	77 (70–85); N=110	80 (70–83)	75 (70–85); N=78	80 (70–85)
Use of prednisone or equivalent	64 (57.7)	22 (73.3)	40 (50.6)	N/A
Prednisone equivalent dose (mg/day)	2.5 (0.0–7.5)	5.0 (0.0–7.5)	0.5 (0.0–7.5)	N/A
Antimalarial agents	32 (28.8)	7 (23.3)	25 (31.6)	N/A
IS[b] at enrolment	30 (27.0)	13 (43.3)	17 (21.5)	N/A
Azathioprine	13 (11.7)	4 (13.3)	9 (11.4)	N/A
MTX	7 (6.3)	1 (3.3)	6 (7.6)	N/A
CYC	7 (6.3)	6 (20.0)	1 (1.3)	N/A
MMFI	4 (3.6)	3 (10.0)	1 (1.3)	N/A
Rituximab	2 (1.8)	2 (6.7)	0 (0.0)	N/A
IS[b] until enrolment	57 (51.4)	26 (86.7)	29 (36.7)	N/A
Azathioprine	42 (37.8)	19 (63.3)	22 (27.8)	N/A
MTX	20 (18.0)	4 (13.3)	14 (17.7)	N/A
CYC	20 (18.0)	16 (53.3)	4 (5.1)	N/A
MMF	7 (6.3)	5 (16.7)	2 (2.5)	N/A
Rituximab	4 (3.6)	3 (10.0)	1 (1.3)	N/A
ACE inhibitors	12 (10.8)	7 (23.3)	3 (3.8)	N/A
ARBs	5 (4.5)	2 (6.7)	2 (2.5)	N/A

Data are presented as medians (interquartile range) or numbers (percentage). The number of observations is indicated in cases of missing values. [a]Urinary casts, haematuria, proteinuria, pyuria. [b]Excluding antimalarial agents. SLEDAI-2K: SLEDAI 2000; ESRD: end-stage renal disease; BP: blood pressure; IS: immunosuppressive agents; ACE: angiotensin-converting enzyme; ARBs: angiotensin II receptor blockers; N/A: not applicable or not available.

were 1:20 diluted and incubated with alkaline picrate solution for 30 min at room temperature. Optical densities were determined using the microplate reader set to 490 nm. The fractional sVCAM-1 excretion ratio was calculated using the following formula:

$$\text{Fractional sCAM excretion ratio} = \frac{\text{urine sCAM} \times \text{serum creatinine}}{\text{plasma sCAM} \times \text{urine creatinine}}$$

Serum IgG anti-dsDNA, anti-Smith (anti-Sm) and anti-nucleosome antibody levels were measured using multiplex immunoassay technology (BioPlex 2200 System, Bio-Rad Laboratories, Inc., Hercules, CA, USA). Levels of IgG antibodies targeting complement component 1q (anti-C1q) were determined using ELISA (Alegria, ORGENTEC Diagnostika GmbH, Mainz, Germany). Serum (S) levels of C3 and C4 complement components were measured using nephelometry. We also analysed IgG, IgM and IgA anticardiolipin and anti-β_2-glycoprotein I antibodies, and the lupus anticoagulant (see Supplementary Material, available at *Rheumatology* online).

The lower detection limit of the assay was 5 IU/ml for IgG anti-dsDNA, and 1 IU/ml for IgG anti-Sm and anti-nucleosome antibodies. The upper detection limit was 300 IU/ml for IgG anti-dsDNA and 8 IU/ml for IgG anti-Sm and anti-nucleosome antibodies. Values under the

lower detection limit were set to half the lower limit value, and values over the upper detection limit were set to twice the upper limit value before statistical analysis.

Assessment of renal function

We assessed SLE patients' renal function at the time of enrolment and at the 10-year follow-up visit using the plasma creatinine concentration (μmol/l) and the creatinine-based estimated glomerular filtration rate (eGFR). We calculated the eGFR using the revised Lund-Malmö equation [35], and stratified the patients into the corresponding chronic kidney disease (CKD) stages [36]. For patients on dialysis, we considered the last creatinine value prior to initiation of dialysis. Creatinine values calculated before the method shift to isotope dilution mass spectrometry were suppressed by a factor 175/186. Unfavourable long-term renal outcome was defined as a worsening of eGFR by ≥25% through the 10-year follow-up, in line with definitions by the Renal Disease Subcommittee of the ACR Ad hoc Committee on SLE response criteria [37]. Data were retrieved from the electronic medical charts at the Karolinska University Hospital.

Statistics

Data are presented as medians (interquartile ranges, IQRs) or numbers (percentages, %). We used the non-parametric Wilcoxon signed-rank test for pairwise comparisons between baseline and the 10-year follow-up and the Mann–Whitney *U* test for comparisons between unrelated samples, e.g. between SLE patients and non-SLE controls. Receiver operating characteristic (ROC) curves were constructed for illustrative purposes, and coordinate points were examined to determine optimal threshold values. Logistic regression was used for further evaluation of U-sCAM levels as predictors of long-term renal function deterioration; adjustments for potential confounding factors were made as appropriate. For correlations, we used the non-parametric Spearman's rank correlation coefficient. *P*-values < 0.05 were considered statistically significant. Statistical analyses were performed using the IBM SPSS Statistics 25 software (IBM Corp., Armonk, NY, USA).

Results

Thirty-two of the 111 SLE patients (28.8%) had a history of LN while 79 patients (71.2%) had no renal involvement until the time of enrolment. Of the 32 patients with LN history, 11 patients had renal BILAG A–C at the time of enrolment and were designated 'active renal SLE', 19 patients had no renal activity (renal BILAG D) and 2 patients had end-stage renal disease and were therefore not assessed with BILAG (Table 1). Of the 11 patients with renal BILAG A–C, 4 patients had a World Health Organization (WHO) class III glomerulonephritis in the most recent active renal biopsy, together with a membranous class V pattern in 1 of them, and 7 patients had a WHO class IV LN; in 2 of the latter patients with class IV LN, the proliferative histological pattern had switched to membranous class V nephritis in subsequent renal biopsies. Of the 19 patients with renal BILAG D (previously active, currently quiescent renal SLE), 3 patients had a WHO class II nephritis in the most recent renal biopsy, 1 patient had a WHO class III nephritis, 8 patients had a class IV nephritis that switched to a membranous WHO class V pattern in a subsequent biopsy in one of these cases, 2 patients had a pure membranous class V LN, 3 patients had not undergone renal biopsy, and in the last 2 cases we were unable to retrieve the renal biopsy report.

The median baseline eGFR in the entire SLE patient group was 80.9 ml/min/1.73 m^2 (IQR: 68.7–95.5 ml/min/1.73 m^2), and in the non-SLE controls it was 85.3 ml/min/1.73 m^2 (IQR: 74.9–94.4 ml/min/1.73 m^2) ($P = 0.117$). Detailed information about eGFR levels in the different subgroups of the patients as well as other clinical and laboratory assessments, including autoantibody levels, are presented in Tables 1–3.

For the subanalysis with regard to antiphospholipid antibodies, see the Supplementary Material, available at *Rheumatology* online.

SLE patients *vs* non-SLE controls

U-sVCAM-1 and U-ALCAM levels correlated with each other, both in SLE patients ($r = 0.63$; $P < 0.001$) and non-SLE controls ($r = 0.49$; $P < 0.001$). In comparative analysis between SLE patients and controls, we observed higher U-sVCAM-1 levels ($P = 0.001$) and a trend towards higher plasma (P)-sVCAM-1 concentrations ($P = 0.051$) in SLE patients, but urine levels of sALCAM did not differ between the two groups ($P = 0.948$) (Table 2). After creatinine-adjustment, both U-sVCAM-1/creatinine and U-sALCAM/creatinine ratios were higher in SLE patients than in controls ($P < 0.001$ for both) (Fig. 1). Moreover, the fractional sVCAM-1 excretion ratio was also higher in SLE patients than in controls ($P < 0.001$) (Table 2). Results from comparisons of autoantibody and complement levels are presented in Table 2.

Patients with LN history *vs* non-renal SLE patients

We next conducted a comparative analysis between SLE patients with current or previous LN at the time of enrolment (renal BILAG A–D) and patients with no renal involvement from the diagnosis of SLE until enrolment (renal BILAG E). In this analysis, P-sVCAM-1, U-sVCAM-1 and U-sALCAM levels did not differ between the two groups, with the only exception of the U-sALCAM/creatinine ratio being higher in SLE patients with a history of LN ($P = 0.014$).

In this analysis, anti-dsDNA and anti-C1q levels were higher in SLE patients with a history of LN ($P = 0.003$ and $P = 0.005$, respectively) and serum C4 levels were lower ($P = 0.016$) compared with non-renal SLE patients (Table 2).

Table 2 SLE patients *vs* non-SLE controls, and with *vs* without a history of LN

Comparisons between SLE patients *vs* non-SLE controls			
	SLE patients, $N = 111$	Non-SLE controls, $N = 99$	*P*-values
P-sVCAM-1 (ng/ml)	379.0 (309.0–500.4); $N = 106$	351.6 (297.7–421.1); $N = 96$	0.051
U-sVCAM-1 (ng/ml)	9.4 (2.8–26.3)	4.4 (1.8–8.8)	**0.001**
U-sALCAM (ng/ml)	6.2 (2.3–13.8)	5.2 (2.8–11.8)	0.948
U-sVCAM-1 adjusted	1.6×10^{-5} (0.5×10^{-5}–5.2×10^{-5})	0.5×10^{-5} (0.2×10^{-5}–1.0×10^{-5})	**<0.001**
U-sALCAM adjusted	1.2×10^{-5} (0.5×10^{-5}–2.4×10^{-5})	0.6×10^{-5} (0.4×10^{-5}–1.0×10^{-5})	**<0.001**
sVCAM-1 fractional excretion	2.8×10^{-2} (0.8×10^{-2}–9.4×10^{-2}); $N = 106$	1.0×10^{-2} (0.4×10^{-2}–1.9×10^{-2}); $N = 96$	**<0.001**
S-creatinine (μmol/l)	67.7 (60.0–80.0)	67.0 (59.0–73.0)	0.206
P-albumin (g/l)	40 (37–42)	42 (41–44)	**<0.001**
eGFR (ml/min/1.73 m^2)	80.9 (68.7–95.5)	85.3 (74.9–94.4)	0.117
S-C3 (g/l)	0.93 (0.71–1.08)	1.03 (0.92–1.20)	**<0.001**
S-C4 (g/l)	0.15 (0.10–0.21)	0.21 (0.18–0.25)	**<0.001**
S-C1q (%)	106 (88–121)	N/A	N/A
Anti-dsDNA (IU/ml)	2.5 (2.5–14.0); N=109	2.5 (2.5–2.5)	**<0.001**
Anti-C1q (IU/ml)	0.8 (0.8–4.6)	N/A	N/A
Anti-Sm (U/ml)	0.5 (0.5–0.5)	0.5 (0.5–0.5)	**<0.001**
Anti-nucleosome (U/ml)	0.5 (0.5–2.6)	0.5 (0.5–0.5)	**<0.001**
U-albumin/creatinine ratio (mg/mmol)[a]	8.5 (5.4–11.9); $N = 101$	9.7 (6.3–12.4); $N = 96$	0.291
Comparisons between patients with *vs* without current or previous LN			
	LN history BILAG A–D, $n = 30$	Never LN BILAG E, $n = 79$	*P*-values
P-sVCAM-1 (ng/ml)	442.8 (317.1–525.2); $N = 28$	362.0 (292.7–482.2); $N = 77$	0.180
U-sVCAM-1 (ng/ml)	9.1 (2.0–20.2)	8.8 (2.8–26.3)	0.630
U-sALCAM (ng/ml)	7.6 (3.0–14.7)	5.2 (2.2–11.9)	0.322
U-sVCAM-1 adjusted	2.6×10^{-5} (0.4×10^{-5}–5.5×10^{-5})	1.3×10^{-5} (0.6×10^{-5}–3.7×10^{-5})	0.528
U-sALCAM adjusted	1.7×10^{-5} (1.1×10^{-5}–6.4×10^{-5})	1.0×10^{-5} (0.5×10^{-5}–2.1×10^{-5})	**0.014**
sVCAM-1 fractional excretion	4.1×10^{-2} (0.5×10^{-2}–11.3×10^{-2}); $N = 28$	2.6×10^{-2} (0.9×10^{-2}–8.6×10^{-2}); $N = 77$	0.674
S-creatinine (μmol/l)	70.6 (60.6–81.6)	66.8 (60.0–79.0)	0.365
P-albumin (g/l)	38 (34–41)	40 (38–42)	**0.012**
eGFR (ml/min/1.73 m^2)	81.0 (72.0–97.0)	81.5 (68.7–95.5)	0.889
S-C3 (g/l)	0.86 (0.54–1.08)	0.93 (0.75–1.11)	0.162
S-C4 (g/l)	0.12 (0.03–0.19)	0.16 (0.12–0.22)	**0.016**
S-C1q (%)	107 (65–121)	105 (89–120)	0.354
Anti-dsDNA (IU/ml)	9.0 (2.5–28.0); $N = 29$	2.5 (2.5–8.3); $N = 78$	**0.003**
Anti-C1q (IU/ml)	2.7 (0.8–16.5)	0.8 (0.8–1.1)	**0.005**
Anti-Sm (U/ml)	0.5 (0.5–2.6)	0.5 (0.5–0.5)	**0.011**
Anti-nucleosome (U/ml)	1.8 (0.5–6.2)	0.5 (0.5–2.3)	0.073
U-albumin/creatinine ratio (mg/mmol)[a]	7.6 (5.5–13.1); $N = 28$	8.8 (5.4–11.8); $N = 71$	0.680

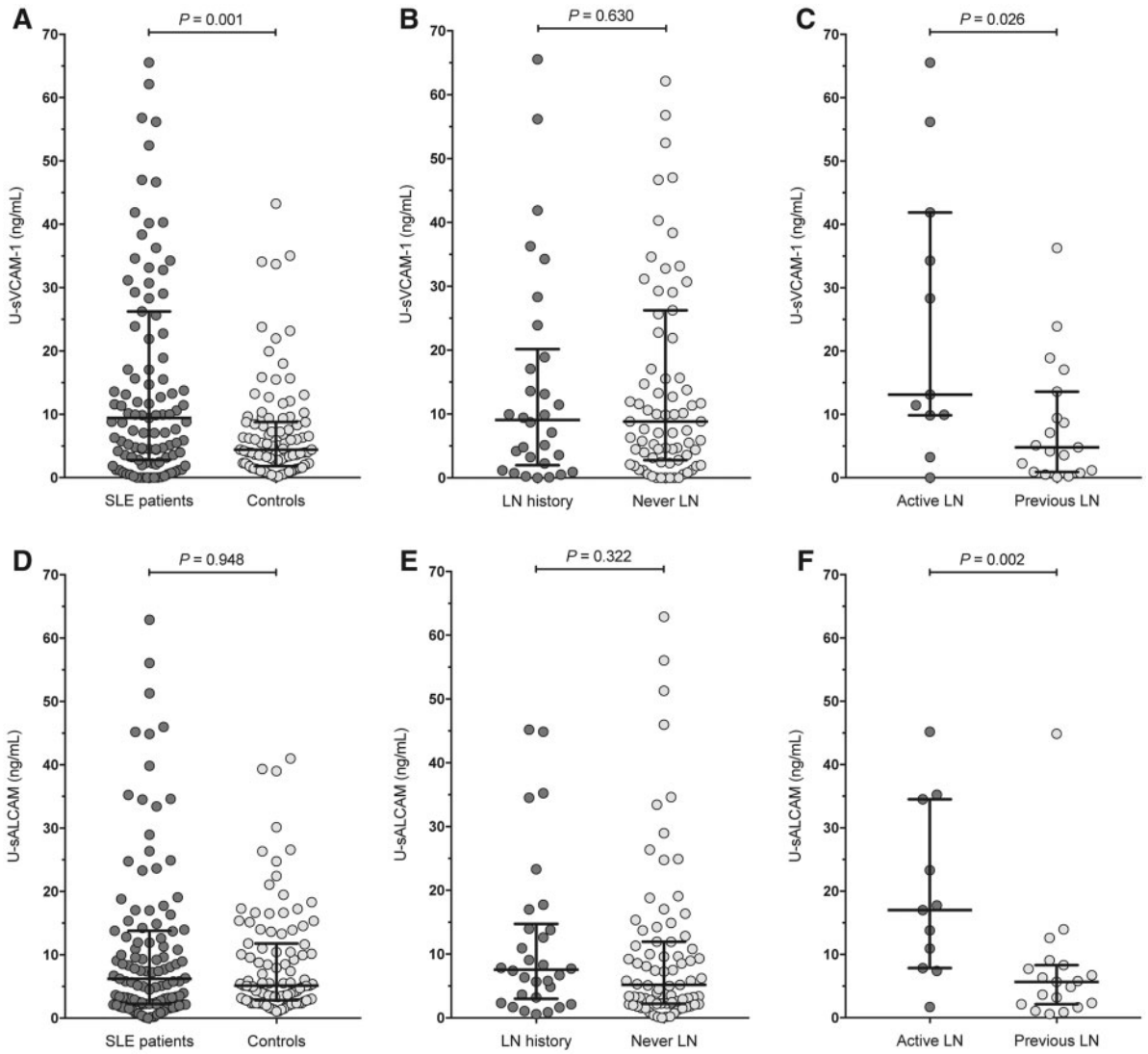
Data are presented as medians (interquartile range). The number of observations is indicated in cases of missing values. Statistically significant *P*-values are in bold. [a]The corresponding 24-h albumin excretion (g/day) can be approximated by multiplying the ratio with a factor of 10. VCAM-1: vascular cell adhesion molecule 1; ALCAM: activated leucocyte cell adhesion molecule; eGFR: estimated glomerular filtration rate; P: plasma; U: urine; S: serum; s: soluble; Sm: Smith; N/A: not applicable or not available.

Patients with active/low-active *vs* quiescent LN

Finally, we compared patients with renal activity at the time of enrolment (renal BILAG A–C), including low-grade renal activity (renal BILAG C), with SLE patients who had at least one LN flare prior to enrolment but no current renal activity (renal BILAG D). In this analysis, U-sVCAM-1 and U-sALCAM were higher in patients with active *vs* inactive LN ($P = 0.026$ and $P = 0.002$, respectively) (Fig. 1), including creatinine-adjusted urinary ratio levels (Table 3). P-sVCAM-1 concentrations were also higher ($P = 0.019$), but fractional sVCAM-1 excretion ratio levels did not differ between the two groups ($P = 0.156$).

Serum levels of IgG anti-dsDNA were higher in patients with active LN ($P = 0.024$) and C3 and C4 levels

Fig. 1 U-sCAM levels in SLE patients and controls



The scatterplots illustrate differences in urine levels of soluble sVCAM-1 (**A**–**C**) and sALCAM (**D**–**F**) between (i) SLE patients and non-SLE population-based controls (A, D), (ii) SLE patients with a history of LN (renal BILAG A–D) and SLE patients with no evidence of renal involvement until the time of enrolment (renal BILAG E) (B, E), and (iii) SLE patients with currently active/low-active renal disease (renal BILAG A–C) and SLE patients with previously active but currently quiescent LN (renal BILAG D) (C, F). *P*-values are derived from Mann–Whitney *U* tests. Lines and whiskers denote medians and the 25th and 75th percentiles. Outliers may be omitted due to scaling. CAM: cell adhesion molecule; VCAM-1: vascular cell adhesion molecule 1; ALCAM: activated leucocyte cell adhesion molecule; U: urine; s: soluble.

were lower ($P = 0.004$ and $P = 0.005$, respectively), while the difference in anti-C1q levels did not reach statistical significance ($P = 0.070$). Levels of anti-Sm and anti-nucleosome antibodies did not differ between the two groups (Table 3).

Correlations with CKD stages

Within the SLE patients, we observed moderate correlations between CKD stages and U-sVCAM-1 levels, both before ($r = 0.32$; $P = 0.001$) and after ($r = 0.34$; $P < 0.001$) U-creatinine adjustment, as well as between CKD stages and fractional sVCAM-1 excretion rates ($r = 0.40$; $P < 0.001$). A weaker correlation was observed between U-sALCAM levels and CKD stages, reaching statistical significance only after U-creatinine adjustment ($r = 0.22$; $P = 0.020$). No positive correlation was seen between CKD stages and traditional markers, including anti-dsDNA, anti-C1q, S-C3, S-C4 or U-albumin/creatinine ratio ($r < 0.15$ for all).

In SLE patients with a history of LN (renal BILAG A–D), we observed more prominent correlations between

Table 3 Comparisons between patients with active/low-active *vs* quiescent LN

	Active/low-active LN: BILAG A–C, $n = 11$	Previous LN: BILAG D, $n = 19$	*P*-values
P-sVCAM-1 (ng/ml)	512.2 (429.2–687.1); $N = 9$	344.6 (303.7–476.3)	**0.019**
U-sVCAM-1 (ng/ml)	13.1 (9.9–41.9)	4.8 (0.9–13.6)	**0.026**
U-sALCAM (ng/ml)	17.0 (7.9–34.5)	5.7 (2.1–8.3)	**0.002**
U-sVCAM-1 adjusted	5.4×10^{-5} (3.6×10^{-5}–9.7×10^{-5})	0.8×10^{-5} (0.3×10^{-5}–3.1×10^{-5})	**0.023**
U-sALCAM adjusted	7.9×10^{-5} (1.9×10^{-5}–15.9×10^{-5})	1.5×10^{-5} (0.5×10^{-5}–1.7×10^{-5})	**0.001**
sVCAM-1 fractional excretion	10.8×10^{-2} (3.1×10^{-2}–15.0×10^{-2}); $N = 9$	2.7×10^{-2} (0.5×10^{-2}–$7.5 \times \times 10^{-2}$)	0.156
S-creatinine (μmol/l)	72.5 (50.0–100.7)	69.6 (63.0–80.0)	0.966
P-albumin (g/l)	34 (30–38)	39 (38–42)	**0.007**
eGFR (ml/min/1.73 m^2)	86.1 (54.4–98.3)	80.5 (72.3–95.4)	0.899
CKD; *n* (%)			
Stage 1	5 (45.5)	6 (31.6)	N/A
Stage 2	3 (27.3)	10 (52.6)	N/A
Stage 3	1 (9.1)	3 (15.8)	N/A
Stage 4	2 (18.2)	0 (0.0)	N/A
S-C3 (g/l)	0.52 (0.47–0.92)	0.96 (0.71–1.16)	**0.004**
S-C4 (g/l)	0.07 (0.02–0.13)	0.15 (0.08–0.23)	**0.005**
S-C1q (%)	73 (33–115)	109 (97–123)	**0.037**
Anti-dsDNA (IU/ml)	70.5 (2.5–375.0); $N = 10$	7.0 (2.5–16.0)	**0.024**
Anti-C1q (IU/ml)	13.0 (0.8–70.0)	0.8 (0.8–8.1)	0.070
Anti-Sm (U/ml)	0.5 (0.5–2.6)	0.5 (0.5–2.0)	1.000
Anti-nucleosome (U/ml)	2.5 (0.5–16.0)	1.3 (0.5–2.4)	0.216
U-albumin/creatinine ratio (mg/mmol)[a]	6.2 (4.1–14.7)	7.8 (5.6–12.1); $N = 17$	0.853

Data are presented as medians (interquartile range) or numbers (percentage). The number of observations is indicated in cases of missing values. Statistically significant *P*-values are in bold. [a]The corresponding 24-h albumin excretion (g/day) can be approximated by multiplying the ratio with a factor of 10. VCAM-1: vascular cell adhesion molecule 1; ALCAM: activated leucocyte cell adhesion molecule; eGFR: estimated glomerular filtration rate; CKD: chronic kidney disease; P: plasma; U: urine; S: serum; s: soluble; Sm: Smith; N/A: not applicable or not available.

CKD stages and U-sVCAM-1 levels ($r = 0.39$; $P = 0.036$), and between CKD stages and fractional sVCAM-1 excretion rates ($r = 0.50$; $P = 0.006$). In contrast, U-sALCAM ($r = 0.20$; $P = 0.284$) and traditional markers (anti-dsDNA, anti-C1q, S-C3, S-C4 and U-albumin/creatinine ratio; $r < 0.15$ for all) did not correlate with CKD stages.

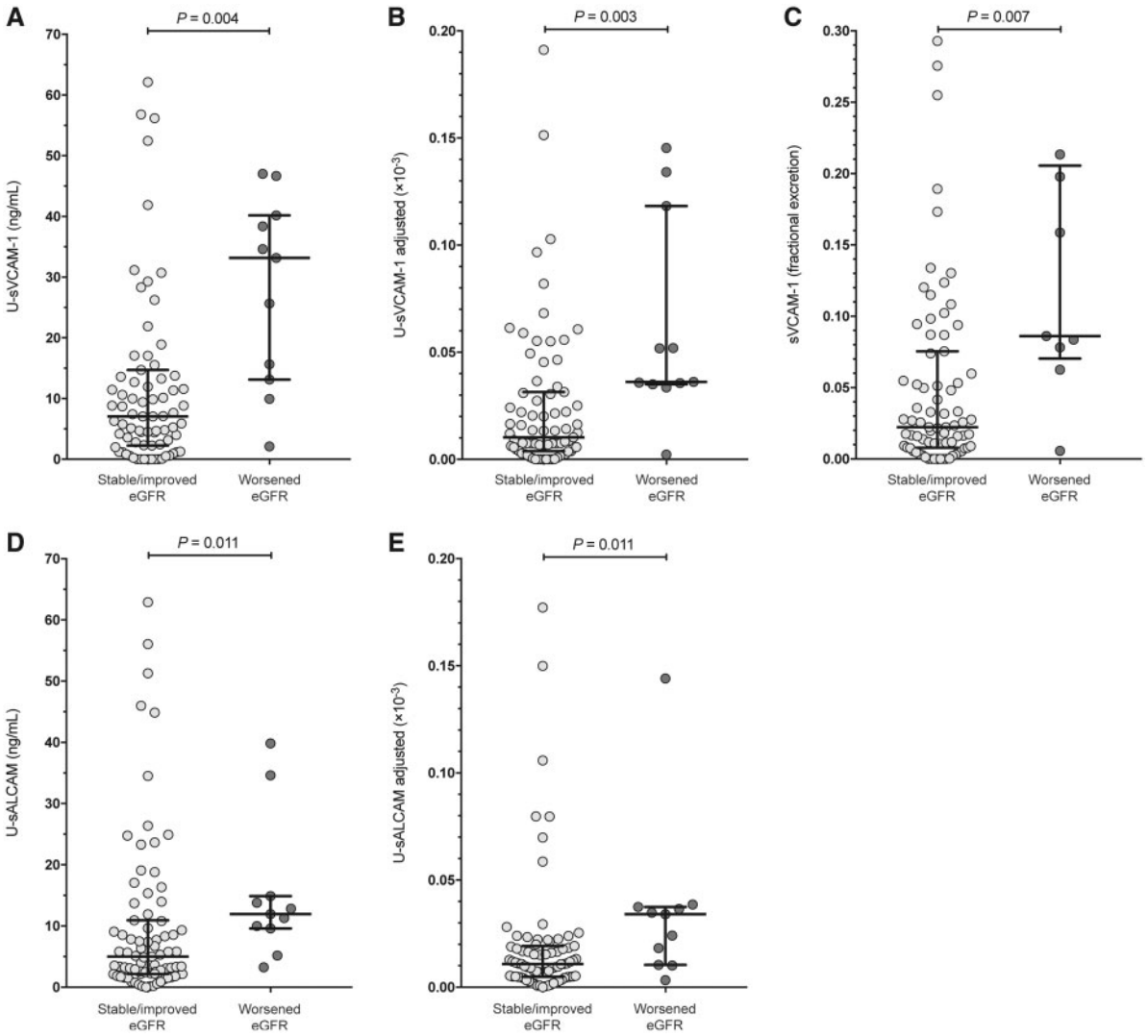
Long-term renal outcome

We assessed the renal outcome of the patients 10 years after enrolment in the study. Since renal involvement may occur at any time during the course of SLE, this analysis was not restricted to patients with LN prior to enrolment. Data were available in 90 of the 111 patients initially included; of the remaining 21 patients, 12 patients had died due to non-renal causes, 6 patients were lost to follow-up and 3 patients had not reached the 10-year follow-up. Of the 90 patients assessed, eGFR had deteriorated by ≥25% in 11 patients (12.2%); 1 of those patients was assessed as having a BILAG C score at baseline, 9 had a BILAG E score and 1 had end-stage renal disease. Of the 79 SLE patients with renal BILAG E at the time of enrolment, i.e. no renal involvement until baseline, 4 patients developed renal involvement during the retrospectively retrievable follow-up; the renal histopathology revealed WHO class IV LN in 2 of these patients, and focal necrotizing glomerulonephritis with crescentic features and scarce immune deposits consistent with vasculitis in the 2 other cases.

Baseline levels of both U-sVCAM-1 and U-sALCAM showed ability to distinguish patients who worsened in eGFR from patients who did not ($P = 0.004$ and $P = 0.011$, respectively) (Fig. 2). Likewise, similar ability was shown for the corresponding creatinine-adjusted values, as well as for the fractional sVCAM-1 excretion ratio (Fig. 2). In contrast, no predictive ability was implicated for P-sVCAM-1 concentrations ($P = 0.650$).

We next conducted ROC curve analysis for creatinine-adjusted levels of U-sVCAM-1 and U-sALCAM as predictors of renal function deterioration at the 10-year follow-up, defined as a decline in eGFR by ≥25% (Fig. 3). For the purpose of comparison, we also created the corresponding ROC curves for baseline serum levels of anti-dsDNA and anti-C1q antibodies, which showed no discriminatory ability (Fig. 3). The optimal threshold value for U-sVCAM-1/creatinine was 0.32×10^{-4}, which yielded a sensitivity of 90.9% and a specificity of

FIG. 2 Baseline sCAM levels and long-term renal prognosis



The scatterplots display comparisons of baseline urine and plasma levels of sVCAM-1 and urine levels of sALCAM between SLE patients who showed a worsening in estimated glomerular filtration rate by ≥25% from baseline through the 10-year follow-up and SLE patients who did not. *P*-values are derived from Mann–Whitney *U* tests. Lines and whiskers denote medians and the 25th and 75th percentiles. Outliers may be omitted due to scaling. CAM: cell adhesion molecule; VCAM-1: vascular cell adhesion molecule 1; ALCAM: activated leucocyte cell adhesion molecule; U: urine; P: plasma; s: soluble.

75.9%, and for U-sALCAM/creatinine 0.18×10^{-4}, which yielded a sensitivity of 72.7% and a specificity of 72.2%; both of them were similar to the 75th percentile of the corresponding ratio distribution (0.24×10^{-4} and 0.17×10^{-4}, respectively). Based on this, we dichotomized the ratio distributions into levels ≥75th percentile and levels below this threshold value in order to test the performance of high ratio values in predicting long-term eGFR deterioration.

While the positive predictive value of high baseline U-sVCAM-1/creatinine values (≥75th percentile) in portending eGFR deterioration by ≥25% through the 10-year follow-up was poor (29.4%; 95% CI: 22.1%, 37.9%), the negative predictive value was high (98.2%; 95% CI: 89.4%, 99.7%), yielding an overall diagnostic accuracy of 72.2% (95% CI: 61.78%, 81.15%). Similarly, the positive predictive value of baseline U-ALCAM/creatinine values ≥75th percentile in portending eGFR deterioration was 25.0% (95% CI: 16.9%, 35.3%) while the negative predictive value was 94.8% (95% CI: 87.4%, 98.0%), yielding a diagnostic accuracy of 70.0% (95% CI: 59.43%, 79.21%).

In univariate logistic regression analysis, patients with baseline U-sVCAM-1/creatinine values ≥75th percentile showed a 23-fold increased risk to deteriorate in eGFR by ≥25% [odds ratio (OR): 22.9; 95% CI: 2.8, 189.2;

$P = 0.004$]. The corresponding OR for baseline U-sALCAM/creatinine values ≥75th percentile was 6.1 (95% CI: 1.5, 25.1; $P = 0.012$) (Fig. 3). We next created regression models for possible confounding factors; these included age at baseline, SLE disease duration from the diagnosis until enrolment in the study, baseline SLICC/ACR Damage Index scores, baseline eGFR and a history of LN until baseline (current or previous LN at the time of enrolment defined as renal BILAG A–D). High age, long SLE duration and low eGFR at enrolment were associated with eGFR deterioration, while SLICC/ACR Damage Index scores and LN history displayed no association (Fig. 3). We also created models for hypertension and use of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers at the time of enrolment, baseline U-albumin/creatinine ratios, baseline serum IgG anti-dsDNA and anti-C1q levels, and baseline serum C3 and C4 levels; none of these was associated with the long-term renal outcome (data not shown).

Finally, we created multivariate logistic regression models in order to assess priority and independence. The low number of patients fulfilling the criteria of the outcome (eGFR deterioration) limited the multivariate analyses to models containing a maximum of two variables. In this analysis, only U-sVCAM-1/creatinine values ≥75th percentile were shown to significantly impact the risk for eGFR deterioration through the 10-year follow-up after the corresponding adjustments, i.e. age, SLE disease duration and baseline eGFR (Fig. 3).

Interestingly, three of the four patients with renal BILAG E at the time of enrolment who later developed renal involvement (one with WHO class IV LN and two with focal sclerosing glomerulonephritis in renal histopathology) had baseline levels of both U-sVCAM-1/creatinine and U-sALCAM/creatinine ratios above the respective 75th percentile. The other patient who developed a WHO class IV glomerulonephritis had a baseline U-sVCAM-1/creatinine ratio of 0.7×10^{-5}, i.e. similar to the respective median value (0.7×10^{-5}) (IQR: 0.3×10^{-5} to 3.4×10^{-5}), and a U-sALCAM/creatinine ratio of 0.3×10^{-5} (median: 0.9×10^{-5}; IQR: 0.4×10^{-5} to 1.7×10^{-5}).

Discussion

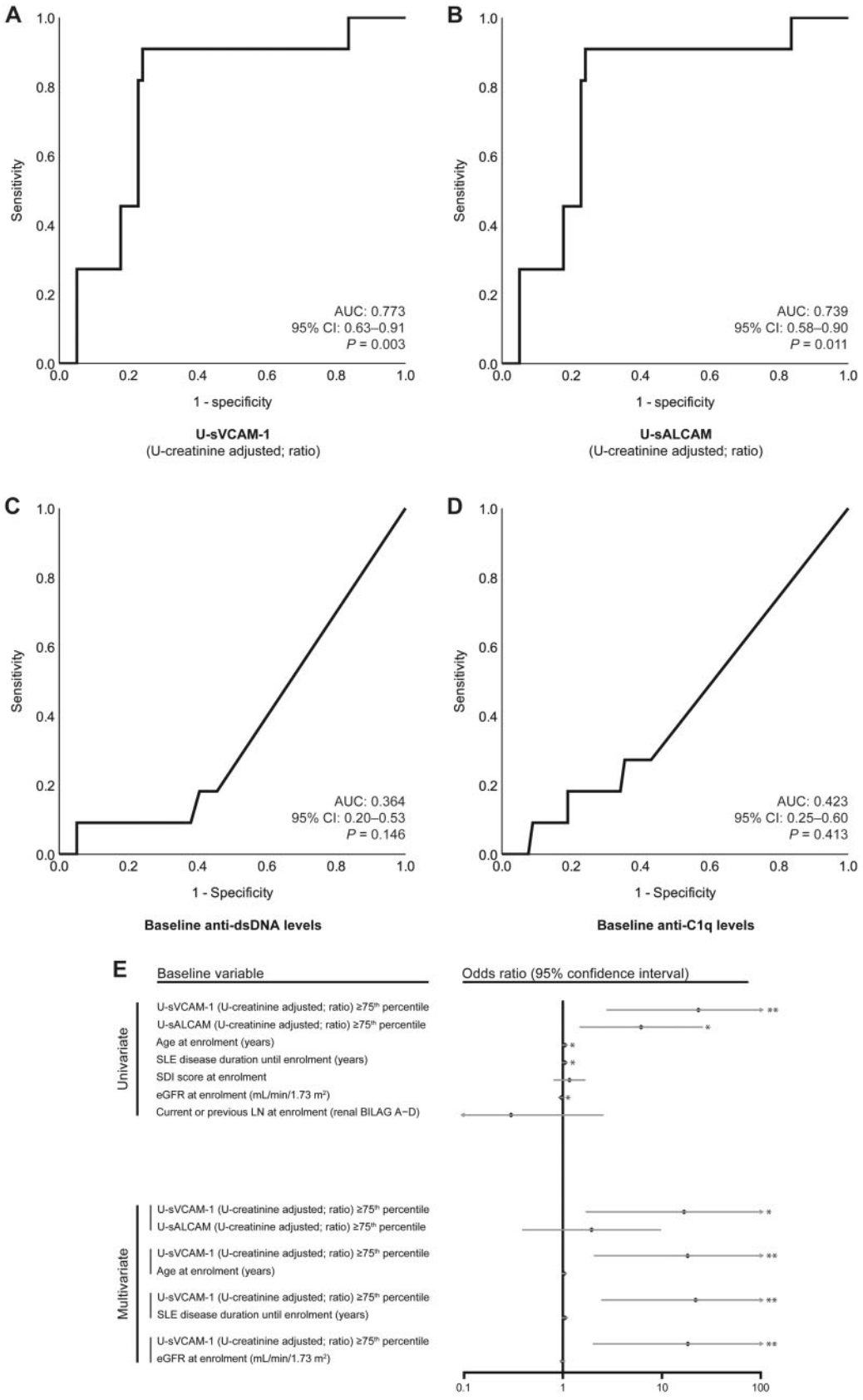
We demonstrated higher U-sVCAM-1 levels in SLE patients than in population-based non-SLE controls, comparable levels in SLE patients with and without a history of renal involvement, and higher levels in patients with active/low-active LN compared with SLE patients with previously active, currently quiescent nephritis. In contrast, creatinine-adjusted U-sALCAM levels were higher not only in SLE patients *vs* controls, but also in patients with an LN history *vs* patients with no manifest LN until the time of enrolment. Like U-sVCAM-1, U-sALCAM showed ability to distinguish active from quiescent LN. Importantly, high baseline U-sVCAM-1/creatinine levels were found to be a strong predictor of long-term deterioration of the renal function, unlike traditional markers of renal involvement including proteinuria, anti-dsDNA and anti-C1q levels.

Urine and plasma levels of sVCAM-1 were elevated in SLE patients compared with population-based non-SLE controls. After adjustment by U-creatinine concentrations, U-sALCAM was also elevated in SLE patients compared with controls. Moreover, both U-sVCAM-1 and U-sALCAM levels were significantly elevated in patients with active or low-active LN compared with SLE patients with previous, currently quiescent renal involvement. Although there was a clear association between chronic (SLE disease) or acute (active LN) inflammatory state and elevated sCAM levels, it remains unclear whether the elevated levels merely represent a reactive response to the inflammatory milieu or if they contribute to disease development. Elevated P-sVCAM-1 levels have been shown to be associated with cardiovascular events and shorter time to their development [38], as well as with cardiovascular mortality [39], underscoring the importance of endothelial activation in cardiovascular comorbidities in patients with SLE.

These findings should be interpreted in light of the equilibrium between the membrane-bound CAMs and their soluble counterparts. Shedding of VCAM-1 and ALCAM from the cell membrane is mainly mediated by the TNF-α converting enzyme (also known as ADAM17), and regulated by the tissue inhibitor of metalloproteinase 3 [40, 41]. Inflammatory and haematopoietic cell mobilization mediators such as TNF-α and growth factors of the myeloid lineage have been shown to increase the expression [42] and the shedding of CAMs [43, 44]. Pro-inflammatory cytokine-mediated downregulation of tissue inhibitor of metalloproteinase 3 results in impeded inhibition of ADAM-17, and thus enhanced CAM shedding. Based on this, the increased U-sVCAM-1 and U-sALCAM levels observed in the present study may reasonably reflect a state of inflammation, resulting in increased CAM production and shedding. The abundance of the molecules in urine from patients with active LN may at least in part reflect the local inflammatory process in renal tissue. Interestingly, unlike U-sVCAM-1, creatinine-adjusted U-sALCAM levels could also discriminate between patients with LN history and patients without evidence of previous or ongoing LN. This discrepancy may imply that although both molecules may reflect inflammation in SLE, ALCAM might be of particular importance in LN.

The potential contribution of ALCAM to the pathogenesis of LN is not fully clarified, but several mechanistic attributes of the molecule might be important. First, ALCAM on antigen-presenting cells plays a critical role in co-stimulating T cells via CD6, and elevated expression may contribute to a breach of T cell tolerance [19–23, 45]. Second, ALCAM is expressed on endothelial cells where it plays a role in recruiting activated monocytes and T cells via ALCAM–ALCAM and ALCAM–CD6 interactions, and facilitates extravasation of activated leukocytes into inflamed tissues such as the kidney in LN [24, 25]. Finally, at least three cell types may

FIG. 3 Baseline U-sCAM levels as predictors of long-term renal function deterioration



potentially express ALCAM within glomerular and tubulointerstitial areas in renal tissue, including macrophages, endothelial cells and podocytes [27], and S100B, a protein with danger-associated molecular pattern activity, has been shown to engage ALCAM and trigger inflammation via NF-κB activation [46]. Our results imply that sALCAM is increased both in active and quiescent renal SLE, and urine samples may be utilized to capture this upregulation.

Traditional markers such as anti-dsDNA, anti-C1q, hypocomplementaemia and albuminuria measured at the time of enrolment were not found to portend long-term renal function deterioration. Neither was aPL positivity found to have any impact, in conformity with a recent study that showed no association between the presence of aPL and long-term renal function impairment in patients with LN but without histological findings consistent with aPL-associated nephropathy, such as thrombotic microangiopathy [47]. In contrast, levels of U-sVCAM-1/creatinine were found to be a strong predictor of long-term renal function deterioration, with levels ≥75th percentile yielding a 23-fold increased risk of eGFR deterioration by ≥25% over a 10-year follow-up period. This has to be seen against the background of the yearly eGFR decline in the general population, which has been estimated to be 0.6–1.2 ml/min/1.73 m^2 or 0.47% in different studies [48–50]. Importantly, since renal involvement may occur at any time during the course of SLE, this analysis was not restricted to patients with a history of LN prior to enrolment. The finding is in line with previous literature where U-sVCAM-1 levels were increased in LN patients showing histological evidence of renal damage [18], and merits further mechanistic study in order to clarify whether the elevated levels of sVCAM-1 are a consequence of a sustained inflammatory state that results in chronic damage or represent a causal relationship between VCAM-1 and the development of renal injury and damage. Interestingly, three of four patients with no history of renal involvement at the time of enrolment who later developed renal disease had high baseline U-sVCAM-1/creatinine and U-sALCAM/creatinine ratios.

The limitations of this study included the relatively low number of participants, especially regarding patients with active LN and patients showing deterioration of renal function. Strengths included the structured longitudinal long-term evaluation of renal function, and the control group consisting of well-characterized population-based non-SLE individuals. The study provides implications that merit validation in larger SLE and LN cohorts, as well as mechanistic studies to clarify the role of CAMs during inflammation and in fibrosis at the level of the end-organ.

In summary, U-sVCAM-1 appears to reflect active SLE disease, whereas sALCAM may also have a particular role in renal affliction. Urine levels of both sVCAM-1 and sALCAM showed ability to distinguish between SLE patients with active renal involvement compared with SLE patients with quiescent nephritis or no nephritis history. High U-sVCAM-1 levels may be a marker of increased risk for renal function deterioration, justifying attentive surveillance and conscientious renoprotective interventions.

Acknowledgements

We express our gratitude to Eva Jemseby and Julia Norkko for managing the urine and serum samples. The datasets generated and analysed during the current study are available from the corresponding author on reasonable request. Study conception and design: I.P., I.G. and C.M.; acquisition of data: I.P., S.G., A.Z., K.V., T.Z., D.H., J.B., C.S.A., P.G., A.L., E.S., I.G. and C.M.; interpretation of data: I.P., S.G., T.Z., A.L., E.S., I.G. and C.M.; manuscript draft: I.P., T.Z., I.G. and C.M. All authors revised the manuscript critically and read and approved the final version of the manuscript prior to submission.

Funding: This work was supported by grants from the Lupus Research Alliance; Swedish Research Council; Swedish Rheumatism Association (R-859621); Professor Nanna Svartz Foundation (2018–00250); Ulla and Roland Gustafsson Foundation (2019–12); King Gustaf V's 80-year Foundation; Swedish Society of Medicine; Ingegerd Johansson Donation; ALF funding from Region Stockholm; Karolinska Institutet Foundations and the National Institutes of Health (R01AR074096).

Disclosure statement: The authors have declared no conflicts of interest.

Fig. 3 Continued
The ROC curves illustrate the performance of baseline creatinine-adjusted U-sVCAM-1 and U-sALCAM as predictors of long-term renal function deterioration, defined as a worsening in eGFR by ≥25% through the 10-year follow-up (**A**, **B**); ROC curves for baseline serum anti-dsDNA (**C**) and anti-C1q (**D**) are shown for the purpose of comparison. The coordinates were examined to determine optimal threshold values. For U-sVCAM-1/creatinine, 0.32×10^{-4} yielded a sensitivity of 90.9% and a specificity of 75.9%. For U-sALCAM/creatinine, 0.18×10^{-4} yielded a sensitivity of 72.7% and a specificity of 72.2%. Both cut-offs were similar to the 75th percentile of the corresponding ratio distribution (0.24×10^{-4} and 0.17×10^{-4}, respectively). We next tested the performance of levels ≥75th percentile in predicting long-term eGFR deterioration. The forest plot (**E**) summarizes results from logistic regression analysis. High baseline U-sVCAM-1/creatinine values were associated with an increased risk to deteriorate in eGFR. SDI: SLICC/ACR Damage Index; ROC: receiver operating characteristics; AUC: area under the curve; eGFR: estimated glomerular filtration rate; CAM: cell adhesion molecule; VCAM-1: vascular cell adhesion molecule 1; ALCAM: activated leucocyte cell adhesion molecule; U: urine; s: soluble.

Supplementary data

Supplementary data are available at *Rheumatology* online.

References

1 Cervera R, Khamashta MA, Font J *et al*. Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. Medicine (Baltimore) 2003;82:299–308.

2 Reyes-Thomas J, Blanco I, Putterman C. Urinary biomarkers in lupus nephritis. Clin Rev Allergy Immunol 2011;40:138–50.

3 Mok CC, Soliman S, Ho LY *et al*. Urinary angiostatin, CXCL4 and VCAM-1 as biomarkers of lupus nephritis. Arthritis Res Ther 2018;20:6.

4 Wu T, Xie C, Wang HW *et al*. Elevated urinary VCAM-1, P-selectin, soluble TNF receptor-1, and CXC chemokine ligand 16 in multiple murine lupus strains and human lupus nephritis. J Immunol 2007;179:7166–75.

5 Springer TA. Adhesion receptors of the immune system. Nature 1990;346:425–34.

6 Brady HR. Leukocyte adhesion molecules and kidney diseases. Kidney Int 1994;45:1285–300.

7 Rabb H, O'Meara YM, Maderna P, Coleman P, Brady HR. Leukocytes, cell adhesion molecules and ischemic acute renal failure. Kidney Int 1997;51:1463–8.

8 Gearing AJ, Newman W. Circulating adhesion molecules in disease. Immunol Today 1993;14:506–12.

9 Zonneveld R, Martinelli R, Shapiro NI *et al*. Soluble adhesion molecules as markers for sepsis and the potential pathophysiological discrepancy in neonates, children and adults. Crit Care 2014;18:204.

10 Seron D, Cameron JS, Haskard DO. Expression of VCAM-1 in the normal and diseased kidney. Nephrol Dial Transplant 1991;6:917–22.

11 Wuthrich RP, Snyder TL. Vascular cell adhesion molecule-1 (VCAM-1) expression in murine lupus nephritis. Kidney Int 1992;42:903–14.

12 Pallis M, Robson DK, Haskard DO, Powell RJ. Distribution of cell adhesion molecules in skeletal muscle from patients with systemic lupus erythematosus. Ann Rheum Dis 1993;52:667–71.

13 Belmont HM, Buyon J, Giorno R, Abramson S. Up-regulation of endothelial cell adhesion molecules characterizes disease activity in systemic lupus erythematosus. The Shwartzman phenomenon revisited. Arthritis Rheum 1994;37:376–83.

14 Chen X, Xu Q, Tang L. [Expression of intercellular adhesion molecule-1 and vascular adhesion molecule-1 in kidney of patients with lupus nephritis and membrano-proliferative glomerulonephritis]. Zhonghua Bing Li Xue Za Zhi 1995;24:149–51.

15 Ilic T, Mitic I, Durdevic-Mirkovic T *et al*. [Correlation between sera levels of sICAM-1 and sVCAM-1 and severity of kidney lesions in patients with lupus nephritis]. Med Pregl 2007;60(Suppl 2):128–32.

16 Sabry A, Sheashaa H, El-Husseini A *et al*. Intercellular adhesion molecules in systemic lupus erythematosus patients with lupus nephritis. Clin Rheumatol 2007;26:1819–23.

17 Molad Y, Miroshnik E, Sulkes J *et al*. Urinary soluble VCAM-1 in systemic lupus erythematosus: a clinical marker for monitoring disease activity and damage. Clin Exp Rheumatol 2002;20:403–6.

18 Abd-Elkareem MI, Al Tamimy HM, Khamis OA, Abdellatif SS, Hussein MR. Increased urinary levels of the leukocyte adhesion molecules ICAM-1 and VCAM-1 in human lupus nephritis with advanced renal histological changes: preliminary findings. Clin Exp Nephrol 2010;14:548–57.

19 Bowen MA, Patel DD, Li X. Cloning, mapping, and characterization of activated leukocyte-cell adhesion molecule (ALCAM), a CD6 ligand. J Exp Med 1995;181:2213–20.

20 Gimferrer I, Calvo M, Mittelbrunn M *et al*. Relevance of CD6-mediated interactions in T cell activation and proliferation. J Immunol 2004;173:2262–70.

21 Hassan NJ, Barclay AN, Brown MH. Frontline: optimal T cell activation requires the engagement of CD6 and CD166. Eur J Immunol 2004;34:930–40.

22 Singer NG, Richardson BC, Powers D *et al*. Role of the CD6 glycoprotein in antigen-specific and autoreactive responses of cloned human T lymphocytes. Immunology 1996;88:537–43.

23 Zimmerman AW, Joosten B, Torensma R *et al*. Long-term engagement of CD6 and ALCAM is essential for T-cell proliferation induced by dendritic cells. Blood 2006;107:3212–20.

24 Cayrol R, Wosik K, Berard JL *et al*. Activated leukocyte cell adhesion molecule promotes leukocyte trafficking into the central nervous system. Nat Immunol 2008;9:137–45.

25 Masedunskas A, King JA, Tan F *et al*. Activated leukocyte cell adhesion molecule is a component of the endothelial junction involved in transendothelial monocyte migration. FEBS Lett 2006;580:2637–45.

26 Teramoto K, Negoro N, Kitamoto K *et al*. Microarray analysis of glomerular gene expression in murine lupus nephritis. J Pharmacol Sci 2008;106:56–67.

27 Sulaj A, Kopf S, Gröne E *et al*. ALCAM a novel biomarker in patients with type 2 diabetes mellitus complicated with diabetic nephropathy. J Diabetes Complications 2017;31:1058–65.

28 Stanley S, Ding H, Pedroza C *et al*. Comprehensive aptamer-based screening of 1129 proteins reveals novel urinary biomarkers of lupus nephritis. Arthritis Rheumatol 2016;68:3778.

29 Landolt-Marticorena C, Prokopec SD, Morrison S *et al*. A discrete cluster of urinary biomarkers discriminates between active systemic lupus erythematosus patients with and without glomerulonephritis. Arthritis Res Ther 2016;18:218.

30 Tan EM, Cohen AS, Fries JF *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.

31 Petri M, Orbai AM, Alarcón GS *et al*. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum 2012;64:2677–86.

32 Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index. J Rheumatol 2002 2000;29:288–91.

33 Gladman D, Ginzler E, Goldsmith C *et al*. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. Arthritis Rheum 1996;39:363–9.

34 Isenberg DA, Rahman A, Allen E *et al*. BILAG 2004. Development and initial validation of an updated version of the British Isles Lupus Assessment Group's disease activity index for patients with systemic lupus erythematosus. Rheumatology (Oxford) 2005;44:902–6.

35 Nyman U, Grubb A, Larsson A *et al*. The revised Lund-Malmo GFR estimating equation outperforms MDRD and CKD-EPI across GFR, age and BMI intervals in a large Swedish population. Clin Chem Lab Med 2014;52: 815–24.

36 Levey AS. A decade after the KDOQI CKD guidelines. Am J Kidney Dis 2012;60:683–5.

37 Renal Disease Subcommittee of the American College of Rheumatology Ad Hoc Committee on Systemic Lupus Erythematosus Response Criteria. The American College of Rheumatology response criteria for proliferative and membranous renal disease in systemic lupus erythematosus clinical trials. Arthritis Rheum 2006;54:421–32.

38 Gustafsson J, Gunnarsson I, Börjesson O *et al*. Predictors of the first cardiovascular event in patients with systemic lupus erythematosus - a prospective cohort study. Arthritis Res Ther 2009;11:R186.

39 Gustafsson JT, Simard JF, Gunnarsson I *et al*. Risk factors for cardiovascular mortality in patients with systemic lupus erythematosus, a prospective cohort study. Arthritis Res Ther 2012;14:R46.

40 Garton KJ, Gough PJ, Philalay J *et al*. Stimulated shedding of vascular cell adhesion molecule 1 (VCAM-1) is mediated by tumor necrosis factor-alpha-converting enzyme (ADAM 17). J Biol Chem 2003;278:37459–64.

41 Singh RJ, Mason JC, Lidington EA *et al*. Cytokine stimulated vascular cell adhesion molecule-1 (VCAM-1) ectodomain release is regulated by TIMP-3. Cardiovasc Res 2005;67:39–49.

42 Levesque MC, Heinly CS, Whichard LP, Patel DD. Cytokine-regulated expression of activated leukocyte cell adhesion molecule (CD166) on monocyte-lineage cells and in rheumatoid arthritis synovium. Arthritis Rheum 1998;41:2221–9.

43 Lévesque JP, Takamatsu Y, Nilsson SK, Haylock DN, Simmons PJ. Vascular cell adhesion molecule-1 (CD106) is cleaved by neutrophil proteases in the bone marrow following hematopoietic progenitor cell mobilization by granulocyte colony-stimulating factor. Blood 2001;98:1289–97.

44 Ulyanova T, Scott LM, Priestley GV *et al*. VCAM-1 expression in adult hematopoietic and nonhematopoietic cells is controlled by tissue-inductive signals and reflects their developmental origin. Blood 2005;106:86–94.

45 Hassan NJ, Simmonds SJ, Clarkson NG *et al*. CD6 regulates T-cell responses through activation-dependent recruitment of the positive regulator SLP-76. Mol Cell Biol 2006;26:6727–38.

46 von Bauer R, Oikonomou D, Sulaj A *et al*. CD166/ ALCAM mediates proinflammatory effects of S100B in delayed type hypersensitivity. J Immunol 2013;191: 369–77.

47 Parodis I, Arnaud L, Gerhardsson J *et al*. Antiphospholipid antibodies in lupus nephritis. PLoS One 2016;11:e0158076.

48 Halbesma N, Kuiken DS, Brantsma AH *et al*. Macroalbuminuria is a better risk marker than low estimated GFR to identify individuals at risk for accelerated GFR loss in population screening. J Am Soc Nephrol 2006;17:2582–90.

49 Matsushita K, Selvin E, Bash LD *et al*. Change in estimated GFR associates with coronary heart disease and mortality. J Am Soc Nephrol 2009;20:2617–24.

50 Kronborg J, Solbu M, Njolstad I *et al*. Predictors of change in estimated GFR: a population-based 7-year follow-up from the Tromso study. Nephrol Dial Transplant 2008;23:2818–26.