Rheumatology International (2019) 39:469–478
https://doi.org/10.1007/s00296-018-4190-2

Rheumatology
INTERNATIONAL

OBSERVATIONAL RESEARCH

Circulating S100 proteins effectively discriminate SLE patients from healthy controls: a cross-sectional study

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Received: 6 September 2018 / Accepted: 29 October 2018 / Published online: 3 November 2018

Abstract

S100 proteins are currently being investigated as potential diagnostic and prognostic biomarkers of several cancers and inflammatory diseases. The aims of this study were to analyse the plasma levels of S100A4, S100A8/9 and S100A12 in patients with incomplete systemic lupus erythematosus (iSLE), in patients with established SLE and in healthy controls (HCs) and to investigate the potential utility of the S100 proteins as diagnostic or activity-specific biomarkers in SLE. Plasma levels were measured by ELISA in a cross-sectional cohort study of 44 patients with SLE, 8 patients with iSLE and 43 HCs. Disease activity was assessed using the SLEDAI-2K. The mean levels of all S100 proteins were significantly higher in SLE patients compared to HCs. In iSLE patients, the levels of S100A4 and S100A12 but not S100A8/9 were also significantly higher compared to HCs. There were no significant differences in S100 levels between the iSLE and SLE patients. Plasma S100 proteins levels effectively discriminated between SLE patients and HCs. The area under the curve (AUC) for S100A4, S100A8/9 and S100A12 plasma levels was 0.989 (95% CI 0.976–1.000), 0.678 (95% CI 0.563–0.792) and 0.807 (95% CI 0.715–0.899), respectively. S100 levels did not differentiate between patients with high and low disease activity. Only the S100A12 levels were significantly associated with SLEDAI-2K and with cSLEDAI-2K. S100 proteins were significantly higher in SLE patients compared HCs and particularly S100A4 could be proposed as a potential diagnostic biomarker for SLE.

Keywords Biomarkers · SLE · S100 proteins · Disease activity

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Abbreviations

ANA	Anti-nuclear antibodies
anti-dsDNA	Anti-double-stranded DNA antibody
BILAG	British Isles Lupus Assessment Group disease activity index
CI	Confidence interval
c-SLEDAI-2 K	Systemic lupus erythematosus Disease Activity Index 2000 clinical items
DAMPs	Damage-associated molecular patterns
ELISA	Enzyme-linked immunosorbent assay
EMT	Epithelial–mesenchymal transition
GC	Glucocorticoids
HCs	Healthy controls
IF	Immunofluorescence
IS	Immunosuppressants
LIA	Line immunoassay
ROC	Receiver operating characteristic
SD	Standard deviation
SLE	Systemic lupus erythematosus
SLEDAI-2K	Systemic Lupus Erythematosus Disease Activity Index 2000
SLICC/ACR	Systemic Lupus International Collaborating Clinics/American College of Rheumatology
RA	Rheumatoid arthritis
RAGE	The receptor for advanced glycation end product

Introduction

Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disease with diverse clinical symptoms and serological abnormalities. The hallmarks of SLE, such as the production of autoantibodies generated by hyper-reactive B cells, deposition of immune complexes in tissues, excessive complement activation and impaired clearance of apoptotic cells, are generally thought to be consequences of immune dysregulation [1].

The combination of heterogeneous clinical manifestations and unpredictable disease course makes SLE a challenging autoimmune disease both for investigators and physicians. Although there has been significant progress in the management of patients with SLE, there is an unmet need for specific early diagnostic, sub-classifying, monitoring and predictive biomarkers for routine clinical use [2–4]. Despite our increasing knowledge regarding the pathogenesis of SLE, few lupus disease activity biomarkers such as the erythrocyte sedimentation rate, dsDNA or complement protein levels have been used in clinical care, and even these biomarkers are not reliable and accurate [5].

The S100A protein family represents a large calcium-binding subfamily with a regulatory role in a wide range of cellular functions. Members of this family act mainly as damage-associated molecular patterns (DAMPs) of innate immune processes. These small (10–12 kDa) acidic proteins can be found in a broad spectrum of cells and tissues. They play important roles in the regulation of homeostasis, the cell cycle, migration, phosphorylation and the secretion of several proteins via their interactions with several effector proteins [6]. Four of the most prominent members, S100A8, S100A9, S100A12 and S100A4, have been intensively studied in cancer [7, 8] and autoimmune rheumatic diseases [9–14]. These proteins are mainly expressed and secreted by activated or dying phagocytic cells, although their expression can also be induced in other cell types [15, 16]. The principal mechanism of action of S100 proteins is mediated by their binding to Toll-like receptors (TLR-4 and TLR-9) and receptor for advanced glycation end products (RAGE) on the surface of leukocytes and endothelial cells, which is a process leading to the release of main pro-inflammatory cytokines such as tumour necrosis factor (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) [17, 18].

It is important to mention a unique role of S100A4 (also known as fibroblast specific protein 1 or metastasin) in the pathogenesis of fibrotic diseases [19, 20] distinguishing S100A4 from other members of the S100 family. Recent data showed an elevation of serum S100A8/9 and its correlation with disease activity in patients with SLE [21]. In addition, S100A8/9, but not S100A12, has been shown to be up-regulated in the serum of SLE patients with inactive disease compared to healthy individuals [22]. To the best of our knowledge, S100A4 levels were only studied in serum and urine samples of patients with childhood-onset SLE [23]. Therefore, the aim of our study was to measure the levels of S100A8/9, S100A12 and S100A4 in the plasma of SLE patients and to investigate their potential roles as diagnostic or activity-specific biomarkers in SLE.

Materials and methods

Subjects and data collection

Forty four patients with SLE, 8 patients with "incomplete" SLE (iSLE) attending the Institute of Rheumatology in Prague, and 43 healthy controls (HCs) were enrolled in our study. The SLE diagnosis was based on the American College of Rheumatology (ACR) 1997 revised classification criteria for SLE [24]. Disease activity was assessed using the SLEDAI-2K (Systemic Lupus Erythematosus Disease Activity Index 2000) [25]. The study was approved by the Ethics Committee of the Institute of Rheumatology, and

informed consent was obtained from all patients prior to initiation of the study.

Definitions

Active disease was defined as a SLEDAI-2K score ≥ 6 [26]. For analytical purposes, SLEDAI-2K items describing the involvement of one organ or tissue were consolidated into a single SLEDAI-2K domain (neuropsychiatric features = seizure, psychosis, organic brain syndrome, cranial nerve disorder, lupus headache, cerebrovascular accident; renal features = haematuria, proteinuria, pyuria, urinary casts; serositis = pleurisy, pericarditis; haematological features = thrombocytopenia, leukopenia). Scores for the clinical items of the SLEDAI-2K (c-SLEDAI-2K) were calculated by subtracting the contribution of hypocomplementaemia and anti-dsDNA positivity from the total SLEDAI-2K score. Incomplete SLE patients were required to be ANA positive, and to fulfil further 1–2 of the 1997 ACR classification criteria.

Laboratory analyses

Blood samples were collected from patients with SLE and HCs, immediately centrifuged and stored at − 80 °C until being analysed. Plasma levels of S100A8/9 and S100A12 were measured by enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's protocol (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland; Cyclex Co., Ltd., Nagano, Japan, respectively). Absorbance was detected using the Sunrise ELISA reader (Tecan, Salzburg, Austria) with 450 nm as the primary wavelength. The intra- and inter-assay coefficients of variation for the MRP8/14 (S100A8/9) Elisa kit were 4.3% and 5.8%, respectively, and for the S100A12/EN-RAGE ELISA Kit, they were 4.3% and 5.4%, respectively. The sensitivity for the MRP8/14 kit was better than 56 pg/ml of the sample. The sensitivity for the S100A12/EN-RAGE ELISA Kit was far below 0.4 µg/ml of sample. Plasma levels of S100A4 were measured by homemade ELISA as previously described by Klingelhöfer et al. [11] and by Zibert et al. [27]. Routine laboratory and immunological measurements needed for the calculation of the SLEDAI-2K and other routine immunological tests [anti-nuclear antibodies (ANA), ANA line immunoassay (LIA) and anti-nucleosome antibodies] were measured at the baseline visit. ANA antibodies were detected by indirect immunofluorescence (Immuno Concepts, Sacramento, CA, USA) and further characterized by the LIA method (IMTEC, Wiesbaden, Germany). Anti-dsDNA antibodies were detected by immunofluorescence (Immuno Concepts); normal was defined as a threshold titre < 1:10. Anti-nucleosome antibodies were measured by ELISA (EUROIMMUN, Lübeck, Germany); normal was defined as 0–24 U/ml. Complement levels were measured using the AU system with reagents (Beckman Coulter, Brea, CA, USA). The reference range for C3 in the serum was 0.9–1.8 g/l, and for C4, it was 0.1–0.4 g/l.

Statistical analysis

Continuous variables were expressed as a median value with interquartile range (IQR). Categorical data were summarized as absolute frequencies and percentages. Normality of data across analysed subgroups was tested via Shapiro–Wilk test with 5% level of statistical significance. We compared baseline serum S100 proteins levels across different subgroups by the Mann–Whitney test with Bonferroni correction. We used univariate linear regression analyses to assess the association between the baseline serum S100 proteins levels and clinical and laboratory manifestations of SLE. Receiver operating characteristic (ROC) curve analysis was performed to establish the optimal discriminatory threshold to identify patients with SLE from HCs. A two-tailed p value < 0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS version 22 software (IBM SPSS, Armonk, NY, USA).

Results

Demographic and clinical characteristics of patients with SLE and iSLE

SLE patients were divided based on their disease activity as follows: SLE patients with high disease activity (SLEDAI ≥ 6, 20.5%) and SLE patients with low disease activity (SLEDAI ≤ 6, 79.5%). The cohort of 44 SLE patients consisted of 93.2% women with a median (IQR) age of 37 (26) years; 97.7% of SLE patients were ANA-positive, 51.2% were anti-nucleosome antibody-positive, and 52.3% had low serum complement (C3, C4 or both). The median (IQR) SLEDAI-2K was 3.5 (3.0); the disease duration was 4.0 (6.5), and 83.0% of patients were using oral glucocorticoids (GC), 86.4% anti-malarials and 56.8% immunosuppressants (IS). 29.2% of patients were using azathioprine, 13.6% methotrexate, 11.4% mycophenolate mofetil, 2.3% cyclosporine and 2.3% leflunomide. The median (IQR) dose of prednisone (or equivalent) was 7.5 (7.5) mg/day. The group of 8 iSLE patients consisted of 75.0% women with a median (IQR) age of 59 (25); 100% of iSLE patients were ANA-positive, 12.5% were anti-nucleosome antibody-positive and 12.5% had low serum complement. The median (IQR) SLEDAI-2K was 0.0 (1.5); 50.0% of patients were using oral GC, 62.5% antimalarials and 25.0% IS. The baseline demographic characteristics of the patients and HCs are summarized in Table 1.

Table 1 Baseline demographic and clinical characteristics of patients with systemic lupus erythematosus (SLE) and healthy controls

	SLE ($n=44$)[b]	Incomplete SLE ($n=8$)	Healthy controls ($n=43$)
Female	41 (93.2%)	6 (75.0%)	38 (88.4%)
Age (years)	37 (26)	59 (25)	45 (18)
Caucasian	44 (100%)	8 (100%)	43 (100%)
Disease duration (years)	4.0 (6.5)	–	
SLICC/ACR damage index	0.0 (1.0)	0.0 (0.0)	
SLEDAI-2K	3.5 (3.0)	0.0 (1.5)	
cSLEDAI-2K (only clinical SLEDAI items)	0.0 (2.0)	0.0 (0.5)	
SLEDAI-2K ≥ 6	9 (20.5%)	0 (0.0%)	
ANA+	43 (97.7%)	8 (100%)	
Anti-dsDNA IF +	20 (45.5%)	0 (0.0%)	
Any SLEDAI clinical features	15 (34.1%)	2 (25%)	
Neurological features[a]	0 (0.0%)	0 (0.0%)	
Vasculitis[a]	1 (2.3%)	0 (0.0%)	
Arthritis[a]	5 (11.4%)	0 (0.0%)	
Myositis[a]	0 (0.0%)	0 (0.0%)	
Renal features[a]	5 (11.4%)	0 (0.0%)	
Rash[a]	6 (13.6%)	0 (0.0%)	
Alopecia[a]	1 (2.3%)	0 (0.0%)	
Mucosal ulcers[a]	1 (2.3%)	0 (0.0%)	
Serositis[a]	0 (0.0%)	1 (12.5%)	
Haematological features[a]	3 (6.8%)	1 (12.5%)	
Fever[a]	0 (0.0%)	0 (0.0%)	
Increased DNA binding[a]	22 (50.0%)	0 (0.0%)	
Low complement[a]	23 (52.3%)	1 (12.5%)	
Anti-nucleosome antibodies +	21 (51.2%)	1 (12.5%)	
Oral glucocorticoids	39 (83.0%)	4 (50.0%)	
Antimalarials (Hydroxychloroquine)	38 (86.4%)	5 (62.5%)	
Immunosuppressants	25 (56.8%)	2 (25.0%)	

ANA anti-nuclear antibodies. IF immunofluorescence. SLEDAI-2K Systemic Lupus Erythematosus Disease Activity Index 2000. SLICC/ACR Systemic Lupus International Collaborating Clinics/American College of Rheumatology. SLE systemic lupus erythematosus

[a]According to SLEDAI-2K definitions; renal. haematological. serositis and neuropsychiatric SLEDAI-2K features were merged into one item (see "Definitions" section in text)

[b]Counts and percentages are computed only from valid cases ($N=44$ for SLEDAI parameters. $N=42$ for anti-nucleosome antibodies). Data are presented as number and percentage for categorical parameters and median with interquartile range (IQR) for continuous parameters

Comparison of plasma levels of S100 proteins between SLE patients and HCs

In our SLE cohort, the plasma levels of S100A4, S100A8/9 and S100A12 were significantly increased compared to HCs ($p<0.001$, $p<0.05$, $p<0.001$, respectively) (Fig. 1a–c; Table 2). In iSLE patients, the levels of S100A4 and S100A12 but not S100A8/9 were significantly higher compared to HCs ($p<0.001$, $p<0.05$, $p=$ ns, respectively) (Fig. 1a–c; Table 2). There were no significant differences in the S100 proteins levels between the iSLE and SLE patients (Fig. 1a–c; Table 2). The levels of S100 proteins were not associated with age, sex or disease duration and were not significantly affected by the corticosteroid dosing (data not shown).

Plasma S100 levels discriminate between SLE patients and HCs

To test for discriminative properties of S100 proteins levels, we performed a ROC curve analysis. The area under the curve (AUC) for the S100A4 levels was 0.989 with a sensitivity of 95.5% and a specificity of 93.0% (Fig. 2a). The AUC for the S100A8/9 levels was 0.678 with a sensitivity

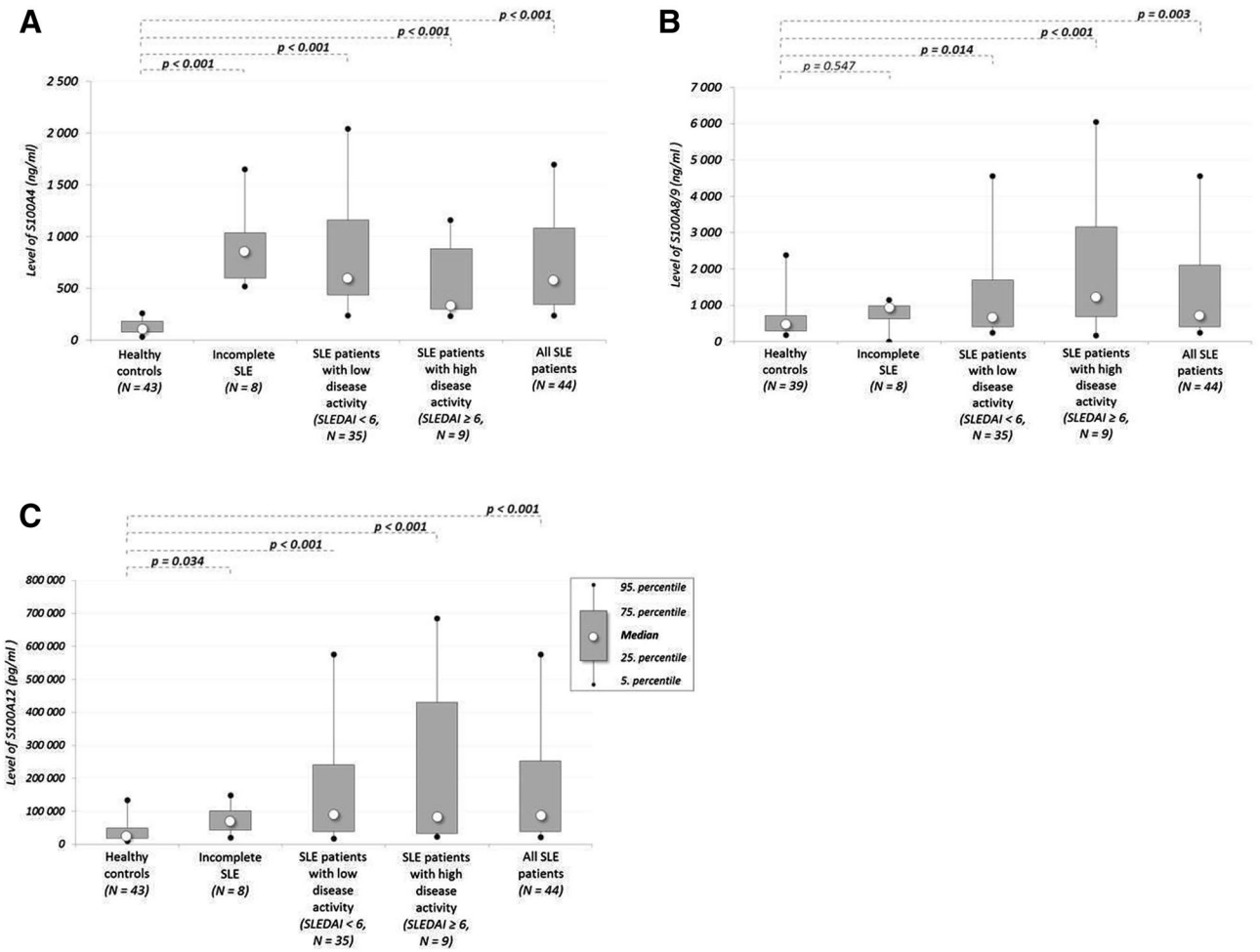

Fig. 1 Comparison of plasma S100A4 (**a**), S100A8/9 (**b**) and S100A12 (**c**) levels in patients with incomplete systemic lupus erythematosus (SLE), low disease activity, and high disease activity in all SLE patients and healthy control subjects. *ns* not significant

Table 2 Cross-sectional association between plasma S100 proteins levels of patients with SLE and healthy control

		Healthy controls ($N=43$)	Incomplete SLE ($N=8$)	SLE patients with low disease activity (SLEDAI < 6, $N=35$)	SLE patients with high disease activity (SLEDAI ≥ 6, $N=9$)	SLE patients—all ($N=44$)
S100A4 (ng/ml)	Median (IQR)	101.8 (105.9)	866.8*** (433.7)	602.4*** (725.6)	334.3*** (580.2)	581.3*** (10 737.9)
S100A8/9 (ng/ml)	Median (IQR)	491.9 (428.6)	960.5^{ns} (354.9)	686.2^{ns} (1 290.2)	1 257.4* (2 765.6)	733.8* (1 689.0)
S100A12 (pg/ml)	Median (IQR)	25 994.9 (30 391.7)	71 054.1* (57 593.0)	91 397.6*** (202 727.0)	84 431.7* (396 987.0)	88 887.1*** (214 273.0)

Differences in S100 proteins levels were tested via Mann–Whitney test with Bonferroni correction

ns not significant

*$p<0.05$ for comparisons with healthy controls

**$p<0.01$ for comparisons with healthy controls

***$p<0.001$ for comparisons with healthy controls; ns for comparisons with healthy controls

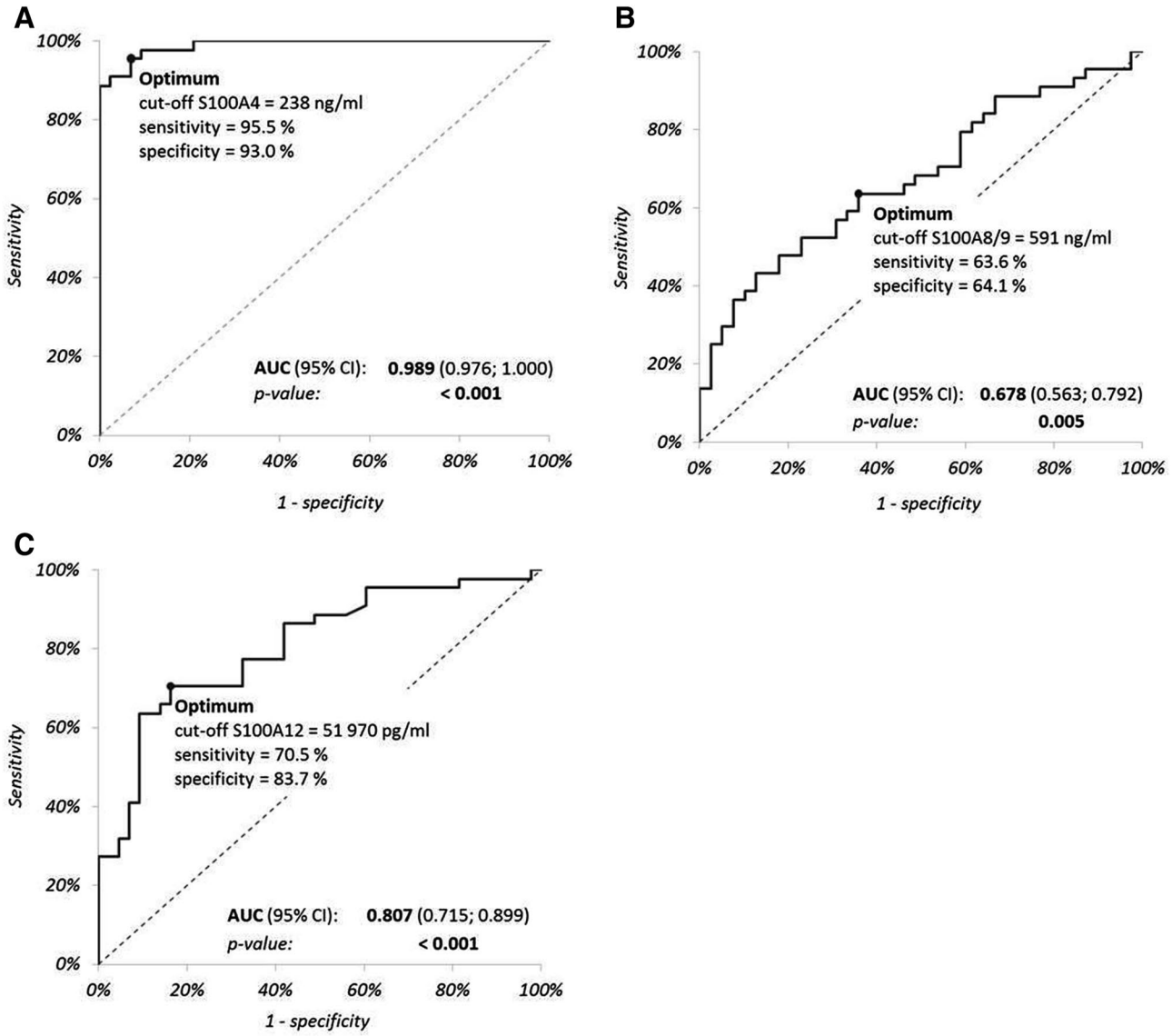

Fig. 2 Receiver operating characteristic curve analysis of S100A4 (**a**), S100A8/9 (**b**), S100A12 (**c**) plasma levels as diagnostic biomarkers of systemic lupus erythematosus (SLE). The area under the curve discriminates between all SLE patients and healthy control subjects

of 63.6% and a specificity of 64.1% (Fig. 2b). The AUC for S100A12 was 0.807 with a sensitivity of 70.5% and a specificity of 83.7% (Fig. 2c).

Association between circulating levels of S100 proteins and measures of disease activity

The S100 levels were not significantly different between the patients with high and low disease activity (Fig. 1a–c; Table 2). There were no significant associations between the S100A4 or S100A8/9 levels and the SLEDAI-2K scores. However, we found a significantly positive association between the S100A12 levels and SLEDAI-2K ($p = 0.035$) and cSLEDAI-2K ($p = 0.038$). Both S100A8/9 and S100A12 levels were associated with the presence of arthritis ($p = 0.011$, $p = 0.022$, respectively). There were no other associations between the S100 levels and various organ domains according to the SLEDAI such as renal features, rash, alopecia and mucosal ulcers (Table 3).

Association between circulating levels of S100 proteins and conventional laboratory parameters

Only S100A4 levels were associated with the presence of anti-nucleosomal antibodies ($p = 0.042$); there were no other associations between S100 proteins and other conventional

Table 3 Cross-sectional associations between plasma S100 protein levels and clinical and laboratory parameters of SLE patients—univariate regression analyses

	S100A4		S100A8/9		S100A12	
	β[a] (95% CI)	*p* value	β[a] (95% CI)	*p* value	β[a] (95% CI)	*p* value
Categorical variables						
Any SLEDAI clinical features (yes vs. no)	− 152.7 (− 472.7; 167.3)	0.341	35.2 (− 1108.0; 1178.5)	0.951	82,210.5 (− 42,043.8; 206,464.8)	0.189
Neuropsychiatric clinical features[a] (yes vs. no)	–	–	–	–	–	–
Vasculitis[a] (yes vs. no)	− 470.0 (− 1488.5; 548.5)	0.357	2844.8 (− 682.0; 6371.5)	0.111	260,679.7 (− 134,575.9; 655 935.4)	0.190
Arthritis[a] (yes vs. no)	− 408.1 (− 874.2; 58.1)	0.085	2088.6 (509.7; 3667.4)	**0.011**	209,311.5 (31,393.6; 387,229.5)	**0.022**
Myositis[a] (yes vs. no)	–	–	–	–	–	–
Renal features[a] (yes vs. no)	− 307.5 (− 781.1; 166.1)	0.197	461.4 (− 1 240.1; 2 163.0)	0.587	56,672.2 (− 131,985.3; 245,329.8)	0.548
Rash[a] (yes vs. no)	− 19.6 (− 466.4; 427.2)	0.930	− 1 152.7 (− 2 690.5; 385.1)	0.138	− 38,712.1 (− 213,529.9; 136,105.8)	0.657
Alopecia[a] (yes vs. no)	− 470.0 (− 1488.5; 548.5)	0.357	2 844.8 (− 682.0; 6 371.5)	0.111	260,679.7 (− 134,575.9; 655,935.4)	0.190
Mucosal ulcers[a] (yes vs. no)	− 470.0 (− 1488.5; 548.5)	0.357	2 844.8 (− 682.0; 6 371.5)	0.111	260,679.7 (− 134,575.9; 655,935.4)	0.190
Serositis[a] (yes vs. no)	–	–	–	–	–	–
Haematological features[a] (yes vs. no)	502.8 (− 216.6; 1222.1)	0.166	− 706.8 (− 3 299.2; 1 885.6)	0.585	198,751.1 (− 83,234.8; 480,737.0)	0.162
Fever[a] (yes vs. no)	–	–	–	–	–	–
Anti-dsDNA ab. IF (positive vs. negative)	− 65.9 (− 373.2; 241.4)	0.667	563.7 (− 510.4; 1 637.8)	0.296	11,933.3 (− 108,780.5; 132,647.1)	0.843
Complement C3/C4 (low vs. normal)	104.9 (− 212.7; 422.5)	0.509	− 475.0 (− 1598.5; 648.5)	0.398	94,462.1 (− 27,076.0; 216,000.2)	0.124
Anti-nucleosome ab. (positive vs. negative)	**− 315.5 (− 619.3; − 11.8)**	**0.042**	396.4 (− 635.5; 1428.2)	0.442	102,306.1 (− 13,028.9; 217,641.2)	0.081
Continuous variables						
SLEDAI-2K	− 19.9 (− 47.5; 7.6)	0.152	72.3 (− 24.9; 169.5)	0.141	11 319.6 (822.2; 21,817.0)	**0.035**
cSLEDAI-2K (only clinical SLEDAI items)	− 25.0 (− 56.0; 6.0)	0.111	97.4 (− 11.4; 206.2)	0.078	12 620.9 (721.4; 24,520.5)	**0.038**
C3 (g/l)	− 271.5 (− 796.3; 253.2)	0.302	1 048.0 (− 810.1; 2 906.1)	0.261	− 126 826.8 (− 331 313.8; 77 660.1)	0.217
C4 (g/l)	− 530.1 (− 1662.7; 602.5)	0.350	3 119.4 (− 823.5; 7062.3)	0.118	− 19 815.2 (− 468,437.7; 428 807.4)	0.929
Anti-dsDNA ab. (titre)	70.2 (− 28.7; 169.0)	0.153	8.4 (− 411.5; 428.4)	0.967	31,137.9 (− 18,133.2; 80,409.0)	0.200
Anti− nucleosome ab. (units)	− 0.2 (− 1.6; 1.1)	0.705	4.3 (− 0.7; 9.2)	0.089	220.1 (− 678.0; 1118.2)	0.613

The regression coefficient β corresponds to the difference in the respective S100 protein level between groups (when assessing categorical variables) or to the change in the respective S100 protein associated with a 1 unit increase in the assessed variable (when assessing continuous variables).

Statistically significant *p* values are shown in bold

IF immunofluorescence, *ab.* antibodies, *anti-dsDNA* anti-double-stranded DNA

[a]According to SLEDAI definitions (renal, haematological, serositis and neuropsychiatric SLEDAI features were merged into one item—see definitions)

markers such as C3 or C4 and the presence of antibodies against anti-double stranded DNA (Table 3). We found a significant association between plasma levels of S100A8/9 and S100A12 ($r = 0.383$, $p = 0.010$). There was no

association between the levels of S100A4 and the remaining two S100 proteins.

Discussion

In this cross-sectional study, we found that the S100A8/9, S100A12 and S100A4 proteins were markedly increased in SLE patients compared to HCs. Among the studied S100 proteins, S100A4 showed the highest discriminative value for the distinction between patients with SLE (including incomplete SLE) and HCs.

S100A4 plays important role in cancer biology [28, 29]. It promotes tumor invasion and metastasis via induction of epithelial–mesenchymal transition (EMT), dysregulation of cell death and enhancement of cell motility [30–35]. Numerous cancer studies have described S100A4 as a potential metastasis and prognostic biomarker [36–38]. Moreover, S100A4 is also known to be involved in the pathogenesis of several fibrotic, inflammatory and autoimmune diseases [12, 13, 18–20]. Some of the mentioned properties of S100A4 (dysregulation of cell death, enhancements of cell motility and mediation of fibrotic processes) could be important in the pathogenesis of SLE. Indeed, S100A4 has been recently reported as a potential urine lupus nephritis marker [23].

We found that S100A4 levels were elevated both in patients with complete as well as incomplete SLE. Therefore, S100A4 appears to be a more general marker discriminating healthy individuals from patients with lupus–like connective tissue disease rather than SLE-specific markers (in a manner similar, for example, to elevated levels of D-dimers in patients with suspected thromboembolism).

Our finding of systemic elevation of S100A8/9 and S100A12 in SLE patients is consistent with previous reports [21, 39–41]. To the best of our knowledge, we are the first to show an up-regulation of S100A4 in the plasma of adult SLE patients. In our study, an established homemade ELISA with highly specific antibody [27] was used to analyse the S100A4 protein in plasma. This could explain the discrepancy between our results and the data of Turnier et al. [23] and potentially highlight the better performance of our assay.

Defective clearance of apoptotic and necrotic cells probably represents one of the key event in the initiation of the autoimmune processes in some patients with SLE [42–45]. The aberrant clearance of dying cells results in the accumulation of apoptotic remnants, including nuclear debris, which binds immunoglobulin to form circulating immune complexes (IC). These IC can promote the production of autoantibodies by germinal B cells [46], damage target tissues [47] or stimulate the release of INF-α by plasmacytoid dendritic cells (pDCs) [48].

According to Lood et al., IC stimulate secretion of S100A8/9 by pDCs [49] and thus may contribute to the systemic elevation of S100A8/9 in patients with SLE. In this regard, it is worth mentioning that S100A4 can maintain cell fate of mature pDCs [50].

In particular relevance, the dying cells can synthetize and release an increased amount of the DAMPs [51] and therefore, may be a relevant source of S100 proteins in SLE. Upon binding to specific receptors, these dangerous molecules stimulate the expression of adhesion molecules on the surface of endothelial cells, recruit inflammatory cells to the sites of tissue damage, stimulate the production of pro-inflammatory cytokines and chemokines and also further induce apoptosis [32, 52].

Neutrophils play an important role in the pathogenesis of SLE [53, 54]. These cells can extrude decondensed chromatin decorated with antimicrobial proteins forming neutrophil extracellular traps (NETs) to kill pathogens [55]. NETs also contribute to the inflammation [56, 57] and represent a potential source of autoantigens and DAMPs in SLE [58]. Of note, S100A8 and S100A9 were identified as two major neutrophil extracellular traps (NETs)-associated proteins [59].

The levels of S100A4 and S100A8/9 were more markedly up-regulated in the group of SLE patients with high disease activity. This may be caused by increased apoptosis of both myeloid and lymphoid cells and its association with the disease activity of SLE patients [43, 60].

We observed a statistically significant association of S100A8/9 and S100A12 levels with the presence of arthritis in SLE patients. This is in line with the findings of Haga et al. [39] on S100A8/9. Moreover, the up-regulation of S100A8/9 and S100A12 and their association with disease activity have already been well-described in RA [9, 10]. It is important to mention that the significance of these results might be biased by the multiple comparisons.

There are some limitations to our study. Our study was cross-sectional with a relatively small number of participants, especially in the group of patients with incomplete SLE. Therefore, a larger validation cohort of SLE patients is needed to confirm our results. Also, the sensitivity and specificity of the studied S100 proteins should be assessed on patients with other connective tissue diseases. Lastly, the clinical performance of the S100A4 immunoassay need to be further evaluated.

In conclusion, all assessed S100 proteins were significantly higher in SLE patients compared to healthy subjects. As levels of S100A4 measured by our home made ELISA showed outstanding ability to discriminate between healthy subjects and patients (both with SLE and lupus-like undifferentiated connective tissue disease), S100A4 may prove to be a clinically useful non-specific biomarker to exclude SLE-related diseases.

Acknowledgements We would like to thank Milada Lösterová for the excellent work as a study nurse in the management of the study.

Author contributions Substantial contributions to the conception or design of the work: BS, MG, JZ, LS, LAC, KP, JV. Substantial contributions to the acquisition, analysis, or interpretation of data for the work: BS, HH, JZ, LS, LSz, LN, MU, LAC, JV. Drafting the work or revising it critically for important intellectual content: BS, HH, MG, LS, JZ, LSz, LN, MU, LAC, KP, JV. Final approval of the version to be published: BS, HH, MG, JZ, LS, LSz, LN, MU, LAC, KP, JV. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: BS, HH, MG, JZ, LS, LSz, LN, MU, LAC, KP, JV.

Funding This study was supported by the project of the Ministry of Health of the Czech Republic for conceptual development of research organization [Grant no. 00023728].

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and bits later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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