S100A11 (calgizzarin) is released by circulating mononuclear cells and its elevated plasma levels distinguish systemic lupus erythematosus patients from healthy individuals

Sirs,

Systemic lupus erythematosus (SLE) is a chronic autoimmune condition with complex immunological pathogenesis and diverse clinical features, as a consequence of multi-system inflammation (1). Although there has been a significant progress in the management of patients with SLE, there is an unmet need for specific diagnostic or predictive biomarkers for routine clinical use. Certain members of S100 protein family such as S100A8/9 and S100A12 are up-regulated in several autoimmune inflammatory disorders, including SLE (2-4), and their potential as diagnostic or prognostic biomarkers is emerging. S100A11 (calgizzarin) is a less known S100 protein that has been extensively studied in cancer (5). Very recently, our group showed an implication of S100A11 in the pathogenesis of rheumatoid arthritis (RA) and thereby its potential role in autoimmune diseases (6). We described a local accumulation of S100A11 protein in the synovial tissues and fluids of patients with RA and its association with inflammation and disease activity (6). Altogether, these findings prompted our present study focusing on S100A11 in SLE.

Plasma was obtained from 44 patients with SLE (44 females; mean age ± SD: 40±16 years), 40 patients with RA (27 females; 13 males; mean age ± SD: 55±14 years), 38 patients with idiopathic inflammatory myopathies IIM (28 females; 10 males; mean age ± SD: 53±14 years), 40 patients with systemic sclerosis SSc (35 females; 5 males; mean age ± SD: 54±11 years) and 41 healthy controls (HC) (35 females and 6 males; mean age ± SD: 44±11 years). SLE patients fulfilled the revised 1997 American College of Rheumatology classification criteria for SLE (7). Patients with IIM, SSc and RA met their classification criteria (8-11). SLE patients' disease activity was assessed using the SLEDAI-2K (12). Baseline characteristics of patients with SLE are given in Table 1. Peripheral blood mononuclear cells (PBMCs) were isolated from 7 SLE patients (7 females; mean age ± SD: 34.7±10.5 years) and 6 HC (6 females; mean age ± SD: 36.2±11.1 years) and incubated for 24 hours without treatment as previously described (6). After 24 hours the cells were lysed using RLT lysis buffer and the cell culture supernatants were collected. RNA isolation, reverse transcription and TaqMan Real-Time PCR were performed as previously described (13). Data were analysed using the ddCt method for relative quantification, and beta-actin was used as an endogenous control. S100A11 levels were measured using a commercially available ELISA kit (BioVendor, Brno, Czech Republic). Informed consents were obtained from all participants. Statistical analyses were performed using IBM SPSS v. 22 software (IBM SPSS, Armonk, NY, USA). Data were expressed as median (IQR).

Systemic levels of S100A11, alike of other S100 proteins (2-4), were significantly elevated in all SLE patients in contrast to HC (7.2 [2.9; 16.6] *vs*. 2.4 [1.5; 9.9], $p<0.0001$) (Fig. 1A). When compared to other systemic rheumatic diseases, circulating S100A11 was higher in SLE patients in contrast to patients with IIM ($p<0.0001$), SSc ($p<0.0001$) or RA ($p<0.01$) (Fig. 1A). No difference between the SLE patients with low disease ac-

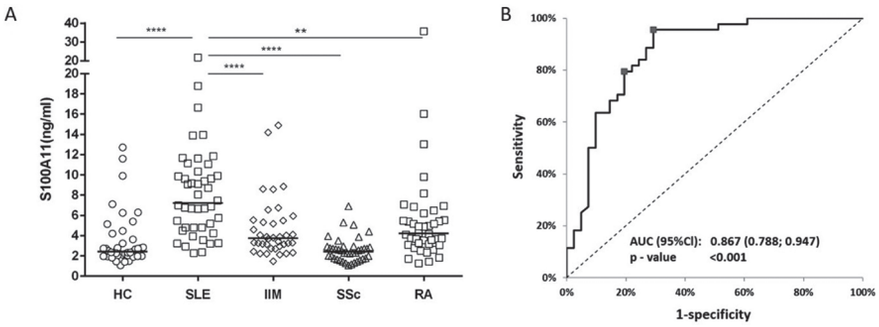

Fig. 1. S100A11 plasma levels are higher in patients with SLE than in other systemic rheumatic diseases and discriminate SLE patients from healthy controls.

S100A11 protein is up-regulated in plasma of patients with systemic lupus erythematosus (SLE) compared to healthy controls (HC), patients with idiopathic inflammatory myopathies (IIM), systemic sclerosis (SSc) or rheumatoid arthritis (RA), (**A**). Receiver-operator characteristic (ROC) curves of plasma S100A11 for distinguishing patients with SLE from HC (area under curve defined as AUC (95% CI): 0.867 (0.788; 0.947), $p<0.001$; optimal cut-off point for S100A11 was 2.88 ng/ml), (**B**). The horizontal bar represents median; $^{**}p<0.01$, $^{****}p<0.0001$.

Table I. Baseline characteristics of the SLE patients and associations of S100A11 with clinical and laboratory parameters of SLE at baseline.

	Baseline characteristics of patients with SLE (n=44)	Associations of baseline S100A11 levels with selected parameters of SLE*	
	n (%) or mean (SD)	β* (95% CI)	*p*-value
S100A11 (ng/ml)	8.1 (4.4)	-	-
Gender (female)	41 (93.2 %)	-1.8 (-7.1; 3.5)	0.497
Age (years)	40 ± 16	0.0 (-0.1; 0.1)	0.504
Disease duration (years)	6.1 (6.5)	0.0 (-0.2; 0.3)	0.800
BMI	24.7 (5.2)	0.0 (-0.3; 0.3)	0.993
SLICC/ACR Damage Index	0.4 (0.8)	1.8 (0.0; 3.6)	0.044
SLEDAI-2K	3.9 (5.5)	0.0 (-0.2; 0.3)	0.886
cSLEDAI-2K (only clinical SLEDAI items)	1.9 (4.9)	0.0 (-0.3; 0.3)	0.865
SLEDAI-2K ≥6	9 (20.5%)	0.7 (-2.9; 4.2)	0.709
ANA+	43 (97.7%)	8.0 (-3.4; 19.4)	0.165
Anti-nucleosome antibodies +	21 (51.2%)	-0.1 (-2.7; 2.5)	0.951
Increased DNA binding	22 (50.0%)	-0.5 (-3.6; 2.5)	0.718
Low complement	23 (52.3%)	0.0 (-3.0; 3.0)	0.996
Clinical manifestations			
Any SLEDAI clinical manifestations	15 (34.1%)	-0.2 (-3.2; 2.8)	0.876
Alopecia	1 (2.3%)	-1.1 (-11.0; 8.8)	0.821
Arthritis	5 (11.4%)	1.3 (-3.5; 6.0)	0.592
Mucosal ulcers	1 (2.3%)	-1.1 (-11.0; 8.8)	0.821
Myositis	0 (0.0%)	-	-
Neurologic manifestations	0 (0.0%)	-	-
Rash	6 (13.6%)	-2.9 (-7.1; 1.3)	0.174
Renal manifestations	5 (11.4%)	1.5 (-3.2; 6.3)	0.513
Serositis	0 (0.0%)	-	-
Vasculitis	1 (2.3%)	-1.1 (-11.0; 8.8)	0.821
Treatment			
Oral glucocorticoids	39 (83.0%)	4.1 (0.6; 7.6)	0.673
Hydroxychlorochine	38 (86.4%)	-0.8 (-4.2; 2.5)	0.617
Immunosupressive drugs	25 (56.8%)	1.7 (-1.0; 4.4)	0.219

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

Data are presented as number and percentage or mean and standard deviation.

*univariate regression analyses adjusted for age and sex.

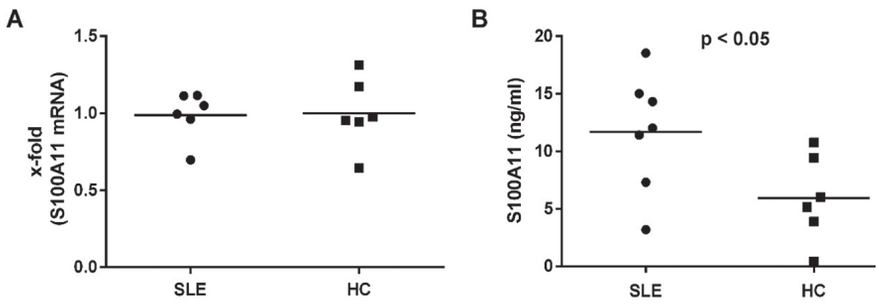

Fig. 2. Expression and release of S100A11 by PBMCs.
Expression of S100A11 mRNA in PBMCs from patients with SLE and HC (**A**). PBMCs from SLE patients spontaneously release significantly higher levels of S100A11 compared to the cells from HC (**B**). The horizontal bar represents mean.

tivity (SLEDAI <6; n=35) and high disease activity (SLEDAI ≥6; n=9) was observed (6.9 [2.4; 18.8] *vs*. 8.7 [2.9;16.6], p=0.709). Of interest, we found no significant association of circulating S100A11 with laboratory parameters and clinical manifestations of SLE, although it slightly correlated with accumulated damage/organ failure over time (Table I). This is in line with our findings in RA, where only the local but not the systemic levels of S100A11 are related to the disease activity and inflammation (6). Furthermore, treatment did not affect S100A11 levels (Table I). Using receiver operating characteristic (ROC) curve analysis, we also show that systemic levels of S100A11 discriminate patients with SLE from HC (area under curve defined as AUC (95% CI): 0.867 (0.788; 0.947), p<0.001; optimal cut-off point for S100A11 was 2.88 ng/ml), (Fig. 1B). Furthermore, despite no difference in the S100A11 mRNA expression in PBMCs from patients with SLE and HC (Fig. 2A), we could demonstrate increased spontaneous secretion of S100A11 protein by PBMCs from SLE patients compared with HC (12.0 [7.3; 15.0] *vs*. 5.6 [3.0; 9.8], p<0.05), (Fig. 2B). These findings indicate that PBMCs could be a possible source of the S100A11 protein to the circulation of SLE patients. Given the fact that SLE patients show abnormalities in cell death at several levels, including apoptosis (14) and that S100A11 interacts with annexin I (15), molecule which mediates the apoptotic cells clearance (16), our results may suggest that the increase of S100A11 in plasma of SLE patients could be related to the apoptotic processes in SLE.

Recently, certain S100 proteins have been related to cardiovascular diseases and atherosclerosis (2, 17). Typical cardiovascular outcomes such as angina, myocardial infarction or stroke collected through SLICC index were not present in our cohort, probably due to the low mean age of the SLE patients. In addition, measures of subclinical atherosclerosis were not available, which could be considered as a limitation of our study.

In conclusion, elevated levels of S100A11 in plasma of patients with SLE, probably originating from mononuclear cells, discriminate between SLE patients and HC and may have potential diagnostic implications.

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This study was funded by grant 15-34065A of the Agency for Healthcare Research of the Czech Republic.

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Competing interests: none declared.

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