Cytokine 116 (2019) 13–20

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Pro-inflammatory S100A11 is elevated in inflammatory myopathies and reflects disease activity and extramuscular manifestations in myositis

Lucie Andrés Cerezo[a], Hana Hulejová[a], Barbora Šumová[a], Tereza Kropáčková[a], Olga Kryštůfková[a], Martin Klein[a], Heřman F. Mann[a], Josef Zámečník[b], Ondřej Pecha[c], Karel Pavelka[a], Jiří Vencovský[a], Ladislav Šenolt[a,*]

[a] Institute of Rheumatology and Department of Rheumatology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic
[b] Department of Pathology and Molecular Medicine, 2nd Medical School and University Hospital Motol, Charles University, Prague, Czech Republic
[c] Technology Centre of the Czech Academy of Sciences, Prague, Czech Republic

ARTICLE INFO

Keywords:
Calgizzarin
S100 proteins
Myositis
Inflammation
Disease activity

ABSTRACT

Background: S100A11 (calgizzarin), a member of the S100 family, is associated with oncogenesis, inflammation and myocardial damage. Our aim was to analyse S100A11 in idiopathic inflammatory myopathies (IIMs) and its association with disease activity features and cancer development.

Methods: S100A11 in muscle was determined by immunohistochemistry in polymyositis (PM), dermatomyositis (DM), myasthenia gravis (MG) and in subjects without autoimmune inflammatory disease (HC). S100A11 in plasma was measured in 110 patients with IIMs (PM, DM, and cancer associated myositis (CAM) patients) and in 42 HC. Disease activity was assessed by myositis disease activity assessment (MYOACT), muscle enzymes and C-reactive protein (CRP) were measured by routine laboratory techniques; autoantibodies by immunoprecipitation or by immunoblot.

Results: We observed an accumulation of S100A11 in the cytoplasm of regenerating and necrotizing muscle fibres of PM and DM patients. S100A11 was increased in plasma of all myositis patients compared to HC (3.8 (1.5–16.8) vs 2.8 (1.7–11.2) ng/ml, $p = 0.011$) and in DM and CAM patients compared to HC (4.0 (2.2–14.9) and 4.5 (1.5–9.1) vs 2.8 (1.7–11.2) ng/ml, $p < 0.001$ and $p = 0.022$, respectively). In all myositis patients, S100A11 correlated with the levels of lactate dehydrogenase ($r = 0.256$, $p = 0.011$), aspartate aminotransferase (AST) ($r = 0.312$, $p = 0.002$), CRP ($r = 0.254$, $p = 0.022$) and MYOACT ($r = 0.245$, $p = 0.022$). S100A11 was associated with MYOACT ($r = 0.377$, $p = 0.030$) and pulmonary and cutaneous disease activity in DM patients ($r = 0.408$, $p = 0.017$ and $r = 0.417$, $p = 0.01$, respectively). S100A11 was related to the levels of AST ($r = 0.412$, $p = 0.027$) in PM and to the levels of creatine phosphokinase ($r = 0.432$, $p = 0.028$) in CAM patients.

Conclusions: We show for a first time a potential implication of S100A11 in the local inflammatory and tissue remodelling processes in myositis and an association of circulating S100A11 with disease activity and extra muscular manifestations in DM.

1. Introduction

Idiopathic inflammatory myopathy (myositis) is a heterogeneous group of chronic muscle disorders that are clinically characterized by symmetric proximal skeletal muscle weakness, fatigue and elevated muscle enzymes in serum. Typical histopathological changes are mononuclear cell infiltration and myofibre degeneration/fibrosis [1]. Immunological features include autoantibodies and autoreactive lymphocytes, with unusual over-expression of major histocompatibility complex (MHC) class I molecules on the surface of the affected myofibres [2]. Distinct categories of myositis, including polymyositis (PM), dermatomyositis (DM), inclusion body myositis (IBM), and immune-mediated necrotizing myopathy (IMNM) are recently distinguished [1–5]. Since the muscle microenvironment is very complex, the underlying mechanisms that mediate muscle damage and dysfunction in myositis are not yet fully understood. A significant number of mediators synthesized within the muscle by different cells such as myocytes, fibroblasts, endothelial or inflammatory cells can participate in the

* Corresponding author at: Institute of Rheumatology, Na Slupi 4, 12850 Prague 2, Czech Republic.
E-mail address: senolt@revma.cz (L. Šenolt).

https://doi.org/10.1016/j.cyto.2018.12.023
Received 20 April 2018; Received in revised form 8 November 2018; Accepted 28 December 2018
Available online 23 January 2019

Table 1
Characteristics of patients with idiopathic inflammatory myopathies (IIMs) and healthy controls (HC).

	Idiopathic inflammatory myopathies (IIMs)	Dermatomyositis (DM)	Polymyositis (PM)	Cancer-associated myositis (CAM)	Healthy controls (HC)
Number	110	40	40	30	42
Gender (female/male)	79/31	27/13	28/12	24/6	30/12
Age (years)	58 (25–82)	56 (27–73)	56 (25–74)	63 (36–82)	55 (25–79)
Disease duration (years)	1.2 (0.1–37.9)	1.7 (0.2–37.9)	1.2 (0.3–19.0)	0.4 (0.1–14.8)	NA
Biochemical markers					
CRP (mg/L)	2.6 (0.3–102.4)	2.4 (0.3–30.1)	2.8 (0.3–102.4)	3.9 (0.3–96.6)	NA
CK (ukat/L)	2.3 (0.2–105.4)	1.1 (0.2–105.4)	4.4 (0.3–64.4)	2.7 (0.2–46.2)	NA
LD (ukat/L)	4.6 (0.9–32.0)	4.2 (0.9–32.0)	4.4 (2.3–31.8)	5.6 (1.2–17.7)	NA
AST (ukat/L)	0.6 (0.3–6.5)	0.6 (0.3–6.5)	0.6 (0.3–4.4)	0.8 (0.3–3.2)	NA
Clinical features (number)					
Mechanics hands	37	19	9	9	NA
Raynaud's phenomenon	29	13	11	5	NA
Arthritis	30	10	15	5	NA
Interstitial lung disease	45	14	22	9	NA
Cardiac Involvement	19	6	8	5	NA
Dysphagia	49	22	15	12	NA
Alopecia	15	6	3	6	NA
Calcinosis	6	5	0	1	NA
Ulcerations	9	5	0	4	NA
Disease activity					
MYOACT	0.07 (0–0.34)	0.07 (0–0.27)	0.06 (0–0.34)	0.09 (0–0.30)	NA
MITAX	0.14 (0–0.63)	0.13 (0–0.47)	0.16 (0.02–0.63)	0.13 (0–0.44)	NA
Constitutional DA (VAS)	5 (0–60)	4.5 (0–40)	8.5 (0–60)	2.5 (0–43)	NA
Cutaneous DA (VAS)	5 (0–70)	17 (0–52)	0 (0–25)	18 (0–70)	NA
Skeletal DA (VAS)	0 (0–63)	0 (0–37)	0 (0–63)	0 (0–28)	NA
Gastrointestinal DA (VAS)	0 (0–81)	0 (0–57)	0 (0–38)	0 (0–81)	NA
Pulmonary DA (VAS)	5 (0–65)	3.5 (0–42)	8 (0–65)	5 (0–28)	NA
Cardiac DA (VAS)	0 (0–41)	0 (0–10)	0 (0–41)	0 (0–14)	NA
Muscle DA (VAS)	24.5 (0–94)	19 (0–85)	25 (2–93)	28 (0–94)	NA
Physician's global disease assessment (VAS)	24 (0–90)	24 (0–74)	25 (1–90)	23 (0–68)	NA
Patient's global disease assessment (VAS)	50 (4–100)	24 (0–90)	50 (5–88)	46 (8–91)	
HAQ	0.9 (0–3.0)	0.9 (0–2.6)	0.9 (0–2.9)	1.1 (0–3.0)	NA
MMT8	65 (0–80)	60 (40–80)	69 (0–79)	63 (44–79)	NA
Treatment					
Daily GC dosage (mg prednisone equivalent)	20 (0–100)	15 (0–80)	20 (0–100)	25 (0–100)	NA
Immunosupressive drugs (number of patients)	MTX (26), AZA (3), Plaquenil (3), CyA (4), CPA (2), CPA + CyA (1), CPA + MTX (1), MTX + AZA + CyA (2), MTX + SAS (1), MTX + Plaquenil (1), MTX + CyA + Plaquenil (1), MTX + CyA (1), MTX + AZA (1), no immunosupressive drugs at the time of blood withdrawal (63)	MTX (13), MTX + AZA + CyA, (1) CyA (1), AZA (1), Plaquenil (2), no immunosupressive drugs at the time of blood withdrawal (23)	MTX (7), AZA (2), Plaquenil (1), CyA (2), CPA (2), CPA + CyA (1), CPA + MTX (1). MTX + AZA + CyA (1), MTX + SAS (1), MTX + Plaquenil (1), MTX + CyA + Plaquenil (1), no immunosupressive drugs at the time of blood withdrawal (19)	MTX (6), MTX + CyA (1), MTX + AZA, CyA (1), no immunosupressive drugs at the time of blood withdrawal (21)	NA

Data are presented as number of patients or median (min-max). AST, aspartate aminotransferase; AZA, azathioprine; CK, creatinine phosphokinase; CRP, C-reactive protein; CyA, cyclosporine A; CPA, cyclophosphamide; DA, disease activity; HAQ, health assessment questionnaire; LD, lactate dehydrogenase; MITAX, myositis intention to treat index; MMT, manual muscle testing; MTX, methotrexate; MYOACT, myositis disease activity assessment; NA, not applicable; SAS, sulfasalazine; VAS, visual analogue scale.

triggering or perpetuation of chronic autoimmune attacks on the muscle.

The S100 family of calcium-binding proteins comprises more than 20 members that carry out broad range of intracellular and extracellular functions [6]. Certain S100 proteins have been previously linked to the damage-repair-regeneration cascade of processes within the muscle tissue [7–11]. S100A11 (S100C, calgizzarin or MLN70), represents a less recognized member of the S100 family. First identified in 1991 in chicken gizzard smooth muscle [12], S100A11 has since been purposed to exert different functions such as enzyme activity regulation, exo/endocytosis, cell proliferation, apoptosis, transcriptional regulation and cell differentiation [13–18]. Modulated expression of S100A11 has been reported in tumours and linked to metastasis [19]. Recent studies revealed a potential link between the S100A11 protein and inflammation [20,21]. The implication of S100A11 in low-grade inflammation was documented in osteoarthritis (OA) [20]. Our group showed significant accumulation of S100A11 in the synovium and in the synovial fluid of patients with rheumatoid arthritis (RA) and

an association with inflammation and disease activity in RA [21]. Similar to other S100 proteins [9,22], S100A11 exhibits activities in cardiac diseases [23,24]. Up-regulated gene and protein expression of S100A11 was reported in the circulation of patients with infectious endocarditis, and the potential of S100A11 as a diagnostic marker of infectious endocarditis was suggested [23]. As demonstrated in a cardiotoxicity animal model, S100A11 is increased in hypertrophic cardiomyocytes and is possibly involved in the myocardial damage-regeneration process [24].

However, there is a lack of knowledge about the expression and biological function of S100A11 within the skeletal muscle microenvironment and during pathological conditions. Given the recently discovered implication of S100A11 in the inflammation and damage-regeneration processes, the purpose of this study was to address the role of S100A11 in the pathogenesis of autoimmune skeletal muscle diseases.

2. Methods

2.1. Patient characteristics and disease activity assessment

The study group comprised 110 patients with myositis (40 with DM, 40 with PM and 30 with CAM) and 42 healthy controls (HC). The group of patients with CAM included 23 patients with DM (CDM) and 7 patients with PM (CPM). Myositis patients were recruited from a single centre of the inpatient and outpatient departments of the Institute of Rheumatology in Prague. Disease duration from the first symptoms ranged from 0 to 38 months. None of the patients were treated for myositis associated overlap syndromes. Patients with PM and DM fulfilled Bohan and Peter diagnostic criteria [25,26]. CAM was defined as cancer occurring in IIMs patients within 3 years of the disease onset. Informed consent was obtained from each subject and the study was conducted with the approval of the Ethics Committee of the Institute of Rheumatology in Prague.

Clinical disease status was assessed by the myositis disease activity assessment (MYOACT), myositis intention to treat index (MITAX) and myositis damage index (MDI), physician and patient global activity using visual analogue scales (VAS), manual muscle testing of 8 muscle groups (MMT-8). Extra-muscular involvement (constitutional, cardiac, pulmonary, gastrointestinal, skeletal, and cutaneous) was also evaluated using a composite score (composite extra-muscular VAS score).

The myositis patients in our study were also suffering from Raynaud's syndrome (26%), arthritis (27%), dysphagia (45%) or mechanic's hands (34%). In addition, 41% of myositis patients were diagnosed with interstitial lung disease and 17% with cardiac involvement. Out of 110 myositis patients, 27 patients were treatment-naive at the time of examination and 96 patients received glucocorticoids (the median dose was 20 mg of prednisone or its equivalent per day ranging from 1.25 to 100 mg per day). In addition, thirty-two patients were treated with methotrexate, four patients with cyclosporine A, three with azathioprine, three with hydroxychloroquine and two with cyclophosphamide. Patient characteristics are summarized in Table 1. Autoantibodies detected in patients with IIMs are listed in Additional file 1.

2.2. Laboratory measurements

Peripheral blood samples were collected and immediately processed. Plasma aliquots were stored at −80 °C until use. S100A11 levels in plasma were quantified using a commercially available ELISA kit (BioVendor, Brno, Czech Republic). This assay has a detection limit of 0.01 ng/ml. The analyses were performed using a SUNRISE ELISA reader (Tecan, Salzburg, Austria) at 450 nm.

Serum levels of muscle enzymes including creatine phosphokinase (CK), lactate dehydrogenase (LD), aspartate aminotransferase (AST) and C-reactive protein (CRP) were analysed by routine laboratory techniques. Myositis specific and associated autoantibodies were detected by immunoprecipitation as previously described [27] or by immunoblot (Euroimmun, Lübeck, Germany).

2.3. Immunohistochemistry

Muscle tissue specimens were obtained from patients with PM (n = 5; 1 male and 4 females, mean age ± SD: 58.6 ± 11.9) and DM (n = 6; 1 male and 5 females, mean age ± SD: 45.8 ± 21.0). Control muscle tissue was obtained from patients with autoimmune non-inflammatory disease myasthenia gravis (MG; n = 5; 2 males and 3 females, mean age ± SD: 48.2 ± 12.4) and from healthy controls (HC; n = 5; 2 males and 3 females, mean age ± SD: 48.8 ± 7.8) – individuals without any proven autoimmune inflammatory disease. All myositis subjects underwent diagnostic muscle biopsy from a biopsy site selected using MRI [28] under local anaesthesia using the open biopsy technique from vastus lateralis (or medialis) muscle.

Patients with PM/DM fulfilled Bohan and Peter diagnostic criteria [25,26]. All patients gave their informed consent to participate, and the study was approved by the local ethics committee at the Institute of Rheumatology.

Serial cryostat sections of muscle tissue were fixed in cold acetone, washed in phosphate buffered saline (PBS), blocked in Dual Endogenous Enzyme Block (Dako, Glostrup, Denmark), washed in PBS and blocked in PBS supplemented with 2% bovine serum for 1 h at room temperature (RT). Consequently, sections were incubated with monoclonal rabbit anti-S100A11 antibody 1:100 (Abcam, Cambridge, UK) overnight at 4 °C, rinsed again in PBS buffer and incubated for 1 h at RT with HRP-conjugated mouse anti-rabbit secondary antibody diluted 1:200 (Dako, Glostrup, Denmark). The visualization was achieved by the DAKO Liquid DAB + Substrate Chromogen System (Dako, Glostrup, Denmark). Sections were counterstained with Mayeŕs Haematoxylin. Isotype-specific antibodies were used as negative controls. The samples were then analysed using a BX53 microscope with a DP80 Digital Microscope Camera and CellSens Standard Software (Olympus, Pennsylvania, USA).

2.4. Cell isolation, stimulation experiments and protein analysis

Peripheral blood mononuclear cells (PBMCs) from patients with IIMs (n = 5) and healthy controls (HC, n = 5) were isolated and cultured as previously described [21]. Cells were incubated without treatment or treated with recombinant S100A11 (10–1000 ng/ml S100A11) (Biovendor, Brno, Czech Republic) or LPS (10 ng/ml, Sigma-Aldrich, St Louis, MO, USA). The cell culture supernatants were collected after 24 h and stored at −80 °C. The levels of selected cytokines (TNF-α and IL-6) released into the supernatant were analysed by ELISA kits (Ray Biotech, Norcross, GA, USA).

2.5. Statistical analysis

Data were tested for normality using the Kolmogorov-Smirnov test in total sample as well as within each group of patients (PM, DM, CAM). Differences in S100A11 levels between the groups were analysed using the Mann-Whitney U test. Since most of the variables were not normally distributed, descriptive statistics are presented as median (min-max). The degree of relationships between variables was evaluated using the Spearman's rank-order correlations. Obtained correlation coefficients were further adjusted for age, disease duration, and daily dose of glucocorticoids using the partial correlation technique. The control (confounding) variables were considered simultaneously in the partial correlation analysis. The Wilcoxon test was used for comparisons between two variables in the stimulation experiments. All computations were accomplished using the software SPSS (version 17.0).

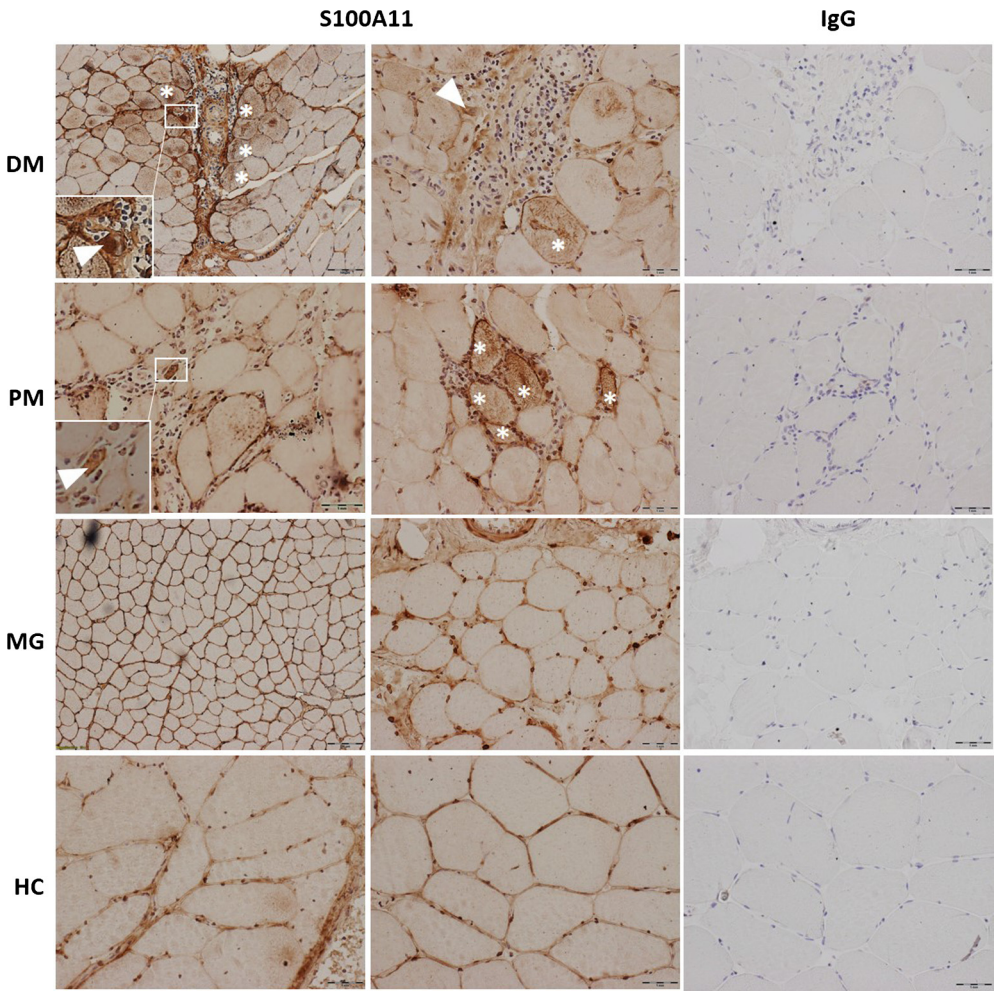

Fig. 1. S100A11 in the muscle of patients with poly/dermatomyositis (PM/DM), myasthenia gravis (MG), healthy controls (HC). In muscle samples of PM and DM patients outside the focus of inflammation and in non-inflammatory controls weak perimembranous, membranous and nuclear S100A11 staining was observed. At site of inflammatory infiltrate in PM and DM, and in perifascicular regions and around the vessels in DM samples, intense membranous immunopositivity as well as the cytoplasmic positivity were revealed. The cytoplasm was S100A11 positive particularly in necrotising and regenerating myofibers in DM and PM. The inflammatory cells also showed focal S100A11 positivity. Isotype-specific antibodies were used as negative controls. * Represents necrotizing muscle fibre, and the arrow points to regenerating muscle fibre. Representative images are shown at magnification 100×, detail 200×.

3. Results

3.1. S100A11 accumulates in the necrotizing and regenerating muscle fibres of patients with inflammatory myopathies

S100A11 was detected to greater or lesser extent in all studied muscle samples. Weak perimembranous, membranous and nuclear S100A11 staining was observed in controls (non-inflammatory, non-autoimmune), in myasthenia gravis and in myositis samples outside the focus of inflammation. Intense membranous and cytoplasmic positivity in muscle cells was revealed at the site of inflammatory infiltrate in PM/DM and in the perifascicular regions in DM samples. S100A11 accumulation was significantly strong in the cytoplasm of necrotising and regenerating myofibres of both DM and PM samples. In DM, S100A11 was detected particularly in the affected muscle fibres surrounding the vessels, whereas S100A11-positive muscle fibres were located around the inflammatory infiltrates in PM. Inflammatory cells within the affected PM/DM muscle tissues also showed focal S100A11 positivity. The S100A11 staining intensity did not significantly differ between PM and DM muscle specimens (Fig. 1).

3.2. S100A11 is increased in plasma of myositis patients

The concentrations of S100A11 in plasma were higher in all myositis patients compared to healthy individuals (3.8 (1.5–16.8) vs 2.8 (1.7–11.2) ng/ml, $p = 0.011$), Fig. 2A. S100A11 levels were particularly up-regulated in patients with DM and CAM compared to healthy controls (4.0 (2.2–14.9) and 4.5 (1.5–9.1) vs 2.8 (1.7–11.2) ng/ml, $p < 0.001$ and $p = 0.022$, respectively), Fig. 2B. After splitting the patients with CAM into the subgroups, only patients with CDM but not CPM showed significant elevation of S100A11 compared to healthy controls (4.6 (1.5–9.1) vs 2.8 (1.7–11.2) ng/ml, $p = 0.031$), Fig. 2B. There was no significant difference in S100A11 levels between patients with PM and healthy controls (3.0 (1.5–16.8) vs 2.8 (1.7–11.2) ng/ml, p = ns). In all myositis patients, a weak negative association with age ($r = -0.202$, $p = 0.034$) was observed. S100A11 positively correlated with daily dose of prednisone in patients with PM ($r = 0.465$, $p = 0.003$). The levels of S100A11 were not affected by sex, BMI or DMARDs in all myositis patients. Systemic levels of S100A11 did not significantly differ between autoantibody positive and negative myositis patients (see Additional file 2).

3.3. S100A11 is inversely associated with disease duration and age in DM patients

Plasma S100A11 levels negatively correlated with disease duration ($r = -0.359$, $p = 0.025$) and age ($r = -0.420$, $p = 0.007$) in patients with DM (Fig. 3A, B). When divided into two groups based on the disease duration (> 5 years and ≤5 years), S100A11 levels and disease activity (MYOACT) were significantly lower in patients with disease duration > 5 years in contrast to the rest of the patients (S100A11: 5.3 (2.5–14.9) vs. 3.3 (2.2–12.8) ng/ml, $p = 0.014$ and MYOACT: 0.1 (0.01–0.27 vs. 0.01 (0–0.04), $p < 0.001$), Fig. 3C and D. Moreover, patients with disease duration > 5 years had significantly higher age compared to the patients suffering of DM for ≤5 years (67.1 (31.6–73.4) vs. 54.3 (26.6–71.8), $p = 0.005$), (Fig. 3E).

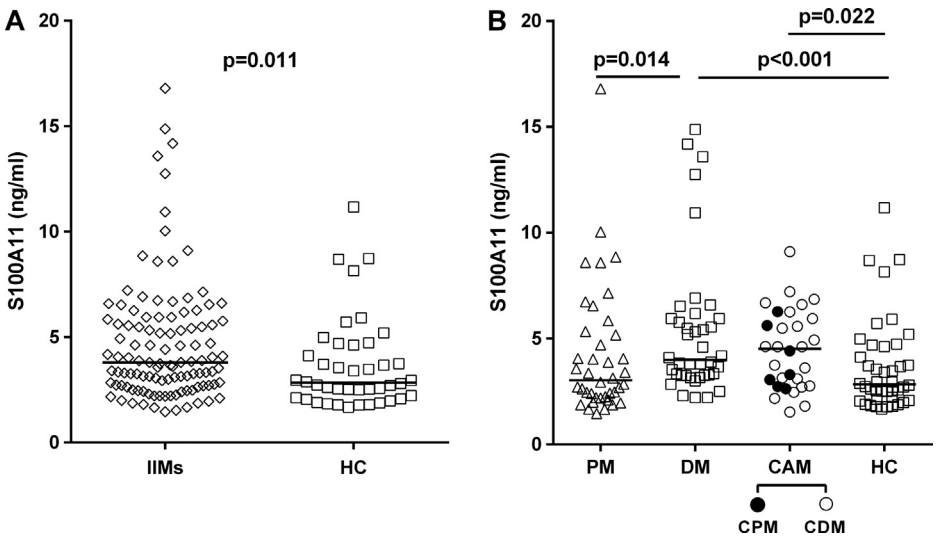

Fig. 2. Systemic levels of S100A11 in patients with myositis. Levels of circulating S100A11 protein are elevated in patients with idiopathic inflammatory myopathies (IIMs) compared to healthy controls (HC), (A) concentrations of S100A11 in patients with dermatomyositis (DM) and cancer associated myositis (CAM) are up-regulated compared to HC (B). The horizontal line represents the median.

3.4. S100A11 is associated with myositis disease activity in DM patients

Circulating S100A11 in patients with DM correlated with CK levels ($r = 0.333$, $p = 0.047$), (Fig. 4A) but only a trend for correlation with AST ($r = 0.318$, $p = 0.059$) was observed. Furthermore, a positive association was found between S100A11 levels and MYOACT score ($r = 0.337$, $p = 0.030$) or between S100A11 and extramuscular disease activity parameters such as pulmonary ($r = 0.408$, $p = 0.017$) and cutaneous ($r = 0.417$, $p = 0.011$) disease activity (Fig. 4B–D). In patients with CAM, a significant correlation was found between S100A11 levels and muscle enzyme CK ($r = 0.432$, $p = 0.028$). The levels of S100A11 did not correlate with the cancer duration in patients with CAM ($r = 0.090$, $p = 0.655$).

Associations of S100A11 with all clinical and laboratory parameters for each subgroup and for all myositis patients are summarized in Table 2.

4. Discussion

In this study, we show for the first time local and systemic accumulation of S100A11 protein in inflammatory myopathies. We disclosed that the S100A11 protein is up-regulated mainly in the regenerating and necrotizing muscle fibres and its systemic levels are associated with levels of muscle enzymes, disease activity and extramuscular manifestations in myositis.

S100A11 represents a multifunctional S100 protein with a diverse spectrum of effects from fundamental and favourable cell housekeeping functions to harmful uncontrolled processes. In addition to its role in oncogenesis [19], S100A11 was recently linked to inflammation and immune system activation in RA [21]. These findings together with the implication of S100A11 in the myocardial damage-regeneration process [24] and its association with inflammation of the endocardium [23] make S100A11 an interesting candidate molecule for investigation in inflammatory myopathies.

Here, we report an accumulation of S100A11 protein in the muscle tissue of patients with myositis, particularly in the regenerating and necrotizing muscle fibres. This finding indicates a possible role of S10011 in muscle tissue remodelling and resembles findings observed previously with S100A4 [8]. Studies on cardiac dysfunction show that S100A11 can be involved in cardiac muscle damage [24] or be a part of cardio protective mechanisms [29]. Dual role of S100A11 in cell growth regulation was also described in keratinocytes [30]. Given the delicate balance between damage and repair process in the skeletal muscle and the apparent functional complexity of S100A11, it is likely that S100A11 exerts both degenerative and regenerative capacities within the myositis muscle. In addition, distinct from other S100 proteins [8,10], S100A11 was detected on membranes and in cell nuclei in all biopsies including those of healthy controls. We believe that this finding corresponds to the physiological role of S100A11 as a regulator of the cell cycle [31] or as a binding partner of annexin [14,15].

We also distinguished specific hallmarks in S100A11 topography, probably related to different pathophysiological processes underlying the two forms of myositis, such as the high occurrence of S100A11 in the affected muscle fibres in proximity to the blood vessels in DM, or an accumulation of S100A11 in the muscle fibres invaded by mononuclear cells in PM.

It is worth mentioning that in our experimental conditions, mononuclear cells from myositis patients show similar basal secretion of cytokines (IL-6 and TNFα) as healthy donors' cells. However, when exposed to high concentrations of S100A11, secretion of IL-6 is significantly up-regulated (see Additional file 3). In this context, we believe that S100A11 released from the necrotic muscle fibres could trigger local inflammation or contribute to the persistence of the inflammatory reaction in the muscle and thereby to the muscle fibre damage.

S100A11 was significantly elevated in the plasma of patients with myositis, in particular in DM when compared to PM patients or healthy individuals. This implies that S100A11 could be of greater relevance in the pathogenesis of DM rather than in PM. It is of interest that S100A11 was increased in patients with CAM compared to controls. Given the high incidence of cancer development in patients with DM [32], it can be speculated that the elevation of S100A11 in CAM in our study is rather related to DM itself than to the cancer development. Moreover, levels of S100A11 do not correlate with the cancer duration and as described before, the role of S100A11 in cancer is type- and stage-specific [19].

Interestingly, circulating S100A11 is inversely associated with age and disease duration in patients with DM. We believe that the age-related decline of muscle mass and physical activity [33] could be one of the factors affecting the systemic levels of S100A11. With respect to the disease duration, it can be speculated that the long-term treatment modulates the levels of S100A11. This is supported by our finding of lower S100A11 levels and disease activity in patients with disease duration > 5 years in contrast to the rest of the patients. Indeed, patients with disease duration > 5 years had significantly higher age compared to the other group. Taken all this together, it can be hypothesized that the long-term treatment and higher age represent inseparable factors affecting the levels of circulating S100A11.

The relationship of circulating S100A11 to CRP and disease activity in myositis patients notes the possible association of S100A11 with

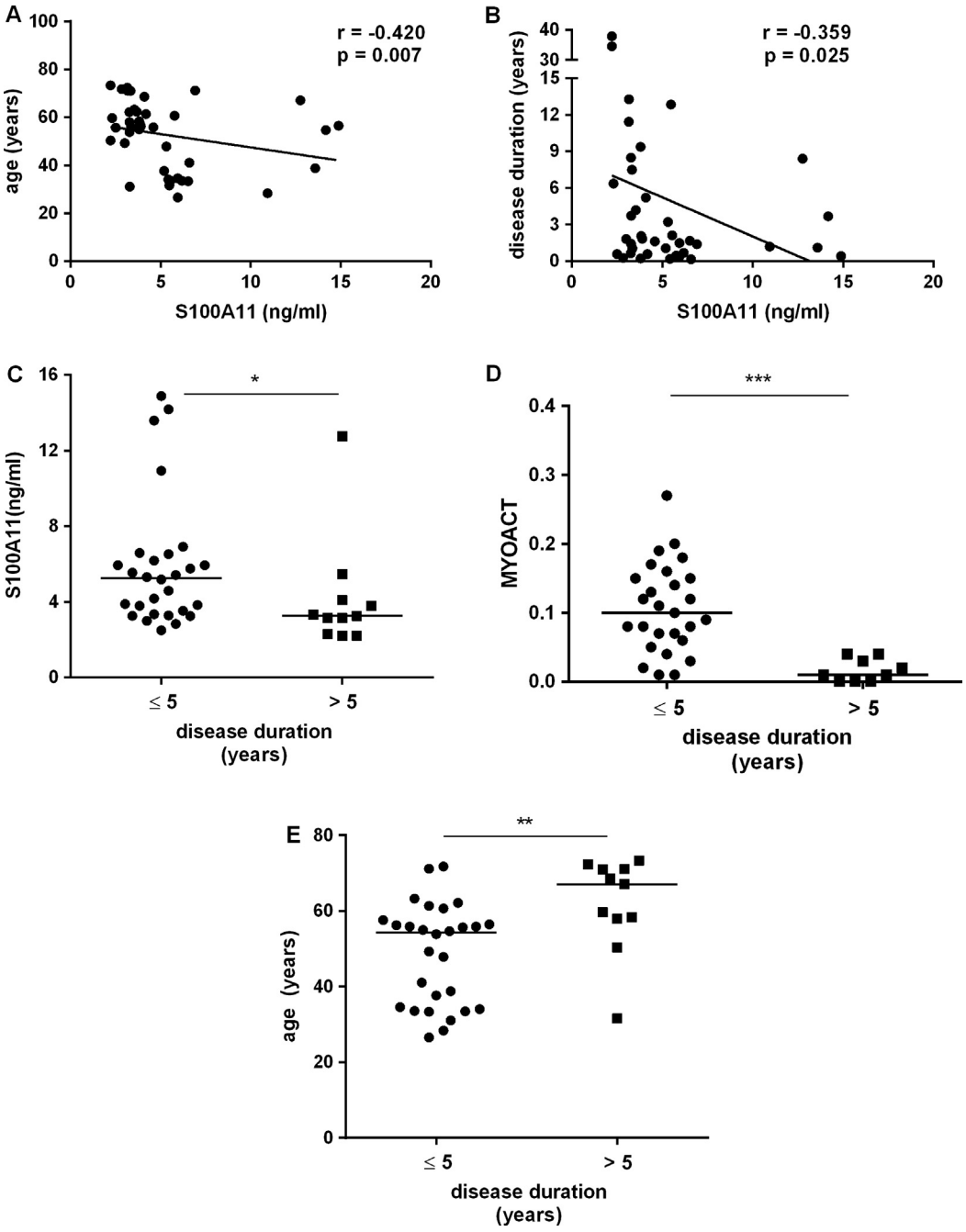

Fig. 3. Circulating S100A11 is related to age and disease duration. S100A11 is inversely associated with age (A) and disease duration (B) in DM patients. Plasma levels of S100A11 and disease activity (MYOACT) are both lower in DM patients with disease duration > 5 years in comparison with patients with the disease duration ≤5 years (C and D). DM patients with disease duration > 5 years had significantly higher age compared to the patients suffering of DM for ≤5 years (E). The horizontal line represents the median. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

immune inflammatory reaction. This is consistent with data reported in RA [21] and together with the elevation of S100A11 in systemic lupus erythematosus (manuscript accepted for publication in Clin Exp Rheumatol) further highlights the link of S100A11 to autoimmune rheumatic diseases. However, the role of circulating S100A11 as an indicator of muscle tissue damage is disputable. There is only a weak association of S100A11 with muscle enzymes. Although S100A11 is overexpressed in necrotizing fibres in both DM and PM biopsies, the systemic elevation was observed only in DM. Thus, circulating S100A11 may not refer to pathological events within the myositis muscle. Indeed, S100A11 significantly correlates with extra muscular involvement in DM. Altogether, it could be speculated that the systemic levels of S100A11 may reflect global disease activity rather than the muscle impairment in myositis.

Interestingly, there is no difference in the levels of S100A11 between myositis patients with and without arthritis. Indeed, according to our results, S100A11 was not related to the levels of anti-Jo-1 (data not shown) autoantibody which is linked with increased rates of arthritis in IIMs [34]. These findings are further supported by the fact that only the local but not the systemic elevation of S100A11 reflected the disease activity and inflammation in RA [21].

This study has some limitations. Muscle biopsies were obtained from patients who were different from those who provided the blood samples. Therefore, the discrepancy between the local and systemic

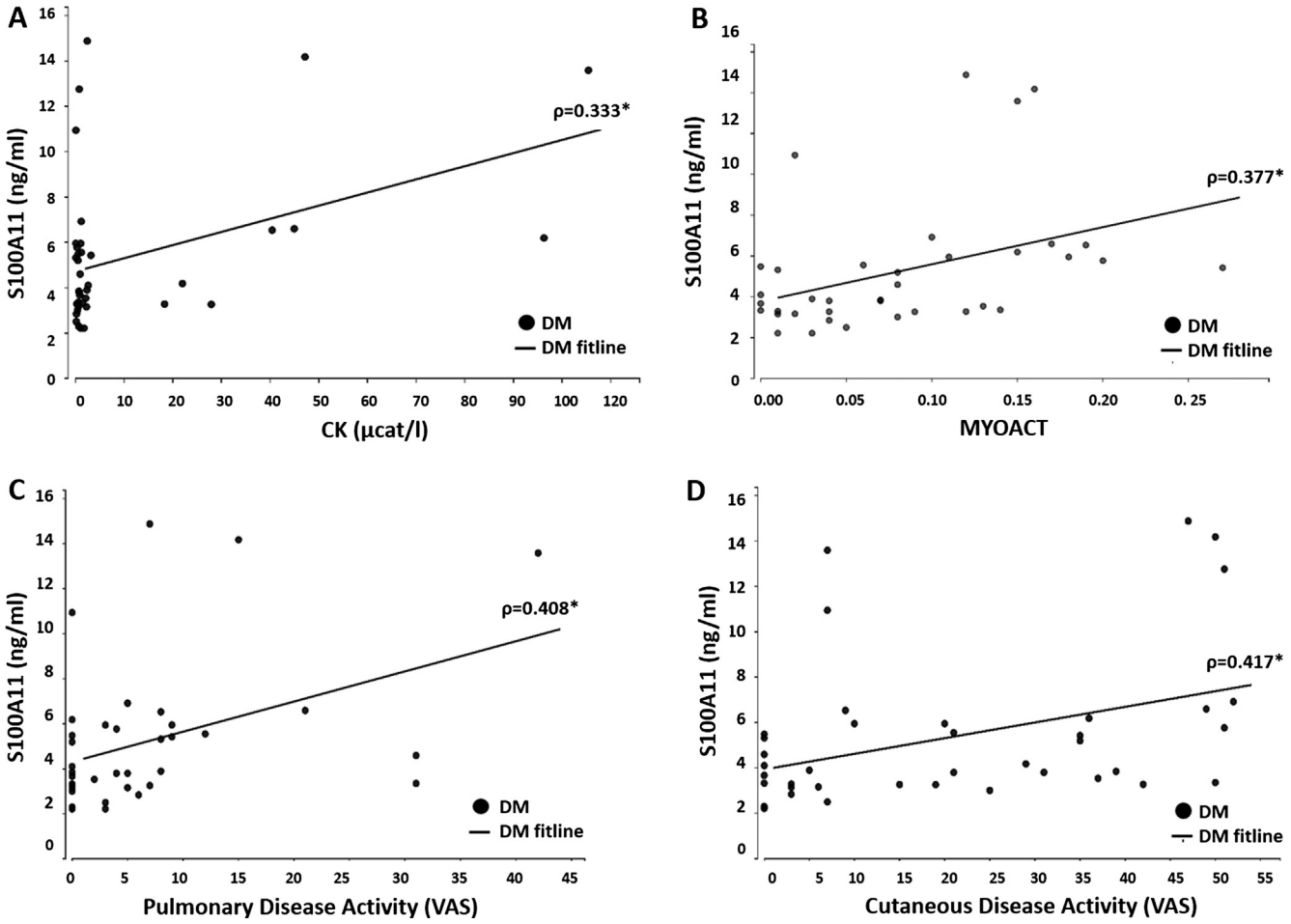

Fig. 4. Association of circulating S100A11 with disease activity parameters and myositis-related clinical features in patients with dermatomyositis. S100A11 in plasma of dermatomyositis (DM) patients is associated with the levels of creatinkinase CK (A) and with the MYOACT score (B). Moreover, systemic levels of S100A11 correlate with the pulmonary (C) and cutaneous (D) disease activity. Data were analysed using Spearman's Rho test; $^{*}p < 0.05$.

Table 2
Association of S100A11 levels with clinical and laboratory parameters of patients with myositis.

		IIMs	DM	PM	CAM
Muscle enzymes	CK	0.167	0.333*	0.174	0.432*
	LD	0.256*	0.048	0.363	0.339
	AST	0.312**	0.318	0.412*	0.338
CRP		0.248*	0.239	0.209	0.290
Disease activity score	MYOACT	0.245*	0.377*	0.258	0.104
	MITAX	0.102	0.169	0.195	0.083
Muscle disease activity		0.153	0.141	0.089	0.172
Extramuscular disease activity	gastrointestinal	0.132	0.334*	0.065	−0.102
	pulmonary	0.091	0.408*	0.018	0.064
	cutaneous	0.309**	0.417*	0.273	0.295
	constitutional	0.120	−0.024	0.241	0.231
	cardiovascular	−0.210*	0.089	−0.257	−0.188
	skeletal	−0.150	−0.154	0.067	−0.353

Data were analysed using Spearman's rho, $^{*}p < 0.05$, $^{**}p < 0.01$. AST, aspartate aminotransferase; CAM, cancer-associated myositis; CK, creatinine phosphokinase; CRP, C-reactive protein; DM, dermatomyositis; IIMs, idiopathic inflammatory myopathies; LD, lactate dehydrogenase; MITAX, myositis intention to treat index; MYOACT, myositis disease activity assessment; PM, polymyositis.

accumulation of S100A11 in PM could not be further studied and explained. Moreover, it would be of extreme interest to include in vitro studies to address the effect of S100A11 on the myofibres. Despite these limitations, this study provides new insight into the implication of S100A11 in autoimmune rheumatic diseases.

5. Conclusions

We demonstrate an accumulation of S100A11 in the affected muscle fibres and inflammatory cells within the muscle and thereby the potential implication of S100A11 in the local inflammation and fibre necrosis/regeneration in inflammatory myopathies. Moreover, a systemic increase in S100A11, particularly related to the extra-muscular disease activity in patients with DM, was documented. Further studies are required to determine the contribution of S100A11 to the pathogenesis of inflammatory myopathies.

Declarations

Ethics approval and consent to participate

The study was approved by the local Ethics Committee of the Institute of Rheumatology in Prague, Czech Republic. Written informed consent was obtained from all patients.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LAC and LŠ were responsible for the study concept and design. HH and TK carried out the ELISAs and analysed the data. LAC and BŠ executed the immunohistochemistry which was further analysed and interpreted by JZ. OK, MK and HFM enrolled patients in the study and provided clinical data for the patients OP and LAC carried out the statistical analysis. LAC was responsible for data interpretation and manuscript preparation. JV, LŠ and KP and revised the manuscript and gave their final approval of the version to be published. All authors read and approved the final manuscript.

Acknowledgements

This study was funded by grant 15-34065A of the Agency for Healthcare Research of the Czech Republic. We thank Adrian Cibula for excellent technical assistance.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2018.12.023.

References

[1] M.C. Dalakas, K. Sivakumar, The immunopathologic and inflammatory differences between dermatomyositis, polymyositis and sporadic inclusion body myositis, Curr. Opin. Neurol. 9 (1996) 235–239.
[2] S. Rayavarapu, W. Coley, T.B. Kinder, K. Nagaraju, Idiopathic inflammatory myopathies: pathogenic mechanisms of muscle weakness, Skelet Muscle 3 (2013) 13.
[3] F.C. Ernste, A.M. Reed, Idiopathic inflammatory myopathies: current trends in pathogenesis, clinical features, and up-to-date treatment recommendations, Mayo Clin. Proc. 88 (2013) 83–105.
[4] A.M. Emslie-Smith, A.G. Engel, Necrotizing myopathy with pipestem capillaries, microvascular deposition of the complement membrane attack complex (MAC), and minimal cellular infiltration, Neurology 41 (1991) 936–939.
[5] L. Christopher-Stine, L.A. Casciola-Rosen, G. Hong, T. Chung, A.M. Corse, A.L. Mammen, A novel autoantibody recognizing 200-kd and 100-kd proteins is associated with an immune-mediated necrotizing myopathy, Arthritis Rheum. 62 (2010) 2757–2766.
[6] R. Donato, B.R. Cannon, G. Sorci, F. Riuzzi, K. Hsu, D.J. Weber, et al., Functions of S100 proteins, Curr. Mol. Med. 13 (2013) 24–57.
[7] L. Pleštilová, H. Mann, L. Andrés Cerezo, O. Pecha, J. Vencovský, L. Šenolt, The metastasis promoting protein S100A4 levels associate with disease activity rather than cancer development in patients with idiopathic inflammatory myopathies, Arthritis Res. Ther. 16 (2014) 468.
[8] L.A. Cerezo, K. Kuncová, H. Mann, M. Tomcík, J. Zámecník, E. Lukanidin, et al., The metastasis promoting protein S100A4 is increased in idiopathic inflammatory myopathies, Rheumatology (Oxford) 50 (2011) 1766–1772.
[9] M. Schneider, S. Kostin, C.C. Strøm, M. Aplin, S. Lyngbaek, J. Theilade, et al., S100A4 is upregulated in injured myocardium and promotes growth and survival of cardiac myocytes, Cardiovasc. Res. 75 (2007) 40–50.
[10] S. Seeliger, T. Vogl, I.H. Engels, J.M. Schröder, C. Sorg, C. Sunderkötter, et al., Expression of calcium-binding proteins MRP8 and MRP14 in inflammatory muscle diseases, Am. J. Pathol. 163 (2003) 947–956.
[11] K. Nistala, H. Varsani, H. Wittkowski, T. Vogl, P. Krol, V. Shah, et al., Myeloid related protein induces muscle derived inflammatory mediators in juvenile dermatomyositis, Arthritis Res. Ther. 15 (2013) R131.
[12] H. Todoroki, R. Kobayashi, M. Watanabe, H. Minami, H.J. Hidaka, Purification, characterization, and partial sequence analysis of a newly identified EF-hand type 13-kDa Ca(2+)-binding protein from smooth muscle and non-muscle tissues, J. Biol. Chem. 266 (1991) 18668–18673.
[13] J. Seemann, K. Weber, V. Gerke, Annexin I targets S100C to early endosomes, FEBS Lett. 11 (413) (1997) 185–190.
[14] W.S. Mailliard, H.T. Haigler, D.D. Schlaepfer, Calcium-dependent binding of S100C to the N-terminal domain of annexin I, J. Biol. Chem. 12 (271) (1996) 719–725.
[15] J. Seemann, K. Weber, V. Gerke, Structural requirements for annexin I-S100C complex-formation, Biochem. J. 319 (1996) 123–129.
[16] X.Q. Zhao, M. Naka, M. Muneyuki, T. Tanaka, Ca(2+)-dependent inhibition of actin-activated myosin ATPase activity by S100C (S100A11), a novel member of the S100 protein family, Biochem. Biophys. Res. Commun. 267 (2000) 77–79.
[17] M. Sakaguchi, M. Miyazaki, Y. Inoue, T. Tsuji, H. Kouchi, T. Tanaka, et al., Relationship between contact inhibition and intranuclear S100C of normal human fibroblasts, J. Cell Biol. 12 (149) (2000) 1193–1206.
[18] E. Makino, M. Sakaguchi, K. Iwatsuki, N.H. Huh, Introduction of an N-terminal peptide of S100C/A11 into human cells induces apoptotic cell death, J. Mol. Med. (Berl). 82 (2004) 612–620.
[19] A.R. Bresnick, D.J. Weber, D.B. Zimmer, S100 proteins in cancer, Nat. Rev. Cancer 15 (2015) 96–109.
[20] D.L. Cecil, K. Johnson, J. Rediske, M. Lotz, A.M. Schmidt, R. Terkeltaub, Inflammation-induced chondrocyte hypertrophy is driven by receptor for advanced glycation end products, J. Immunol. 175 (2005) 8296–8302.
[21] L. Andrés Cerezo, B. Šumová, K. Prajzlerová, D. Veigl, D. Damgaard, C.H. Nielsen, et al., Calgizzarin (S100A11): a novel inflammatory mediator associated with disease activity of rheumatoid arthritis, Arthritis Res. Ther. 19 (2017) 79.
[22] A. Schiopu, O.S. Cotoi, S100A8 and S100A9: DAMPs at the Crossroads between Innate Immunity, traditional Risk Factors, and Cardiovascular Disease, Mediators Inflamm. 2013 (2013) 828354.
[23] F. Thuny, J. Textoris, A.B. Amara, A.E. Filali, C. Capo, G. Habib, et al., The Gene Expression Analysis of Blood Reveals S100A11 and AQP9 as Potential Biomarkers of Infective Endocarditis, PLoS One 7 (2012) e31490.
[24] S. Inamoto, S. Murao, M. Yokoyama, S. Kitazawa, S. Maeda, Isoproterenol-induced myocardial injury resulting in altered S100A4 and S100A11 protein expression in the rat, Pathol. Int. 50 (2000) 480–485.
[25] A. Bohan, J.B. Peter, Polymyositis and dermatomyositis (second of two parts), N. Engl. J. Med. 292 (1975) 403–407.
[26] A. Bohan, J.B. Peter, Polymyositis and dermatomyositis (first of two parts), N. Engl. J. Med. 292 (1975) 344–347.
[27] Z. Betteridge, H. Gunawardena, J. North, J. Slinn, N. McHugh, Anti-synthetase syndrome: a new autoantibody to phenylalanyl transfer RNA synthetase (anti-Zo) associated with polymyositis and interstitial pneumonia, Rheumatology (Oxford) 46 (2007) 1005–1008.
[28] Tomasová Studynková J, Charvát F, Jarosová K, Vencovsky J, The role of MRI in the assessment of polymyositis and dermatomyositis, Rheumatology (Oxford) 46 (2007) 1174–1179.
[29] K. Nakatani, Y. Kawanabe, A. Kato, T. Tanaka, Interaction of propranolol with S100 proteins of the cardiac muscle, Eur. J. Pharmacol. 315 (1996) 335–338.
[30] M. Sakaguchi, H. Sonegawa, H. Murata, M. Kitazoe, J. Futami, K. Kataoka, et al., S100A11, an dual mediator for growth regulation of human keratinocytes, Mol. Biol. Cell 19 (2008) 78–85.
[31] F. Foertsch, N. Teichmann, R. Kob, J. Hentschel, U. Laubscher, et al., S100A11 is involved in the regulation of the stability of cell cycle regulator p21(CIP1/WAF1) in human keratinocyte HaCaT cells, FEBS J. 16 (2013) 3840–3853.
[32] Z.A. Zahr, A.N. Baer, Malignancy in myositis, Curr. Rheumatol. Rep. 13 (2011) 208–215.
[33] L.J. Melton III, S. Khosla, C.S. Crowson, et al., Epidemiology of sarcopenia, J. Am. Geriatr. Soc. 48 (2000) 625–630.
[34] E. Katzap, M.L. Barilla-LaBarca, G. Marder, Antisynthetase syndrome, Curr. Rheumatol. Rep. 13 (2011) 175–181.