S100B Is a Potential Disease Activity Marker in Nonsegmental Vitiligo

Reinhart Speeckaert\(^1,4\), Sofie Voet\(^2,3\), Esther Hoste\(^2,3,4\) and Nanja van Geel\(^1,4\)

Vitiligo is a chronic skin condition characterized by progressive depigmentation of the skin. S100B is a damage-associated molecular pattern protein expressed in melanocytes that has been proposed as a marker of melanocyte cytotoxicity. Although the use of S100B as a biomarker in melanoma is well established, to our knowledge its association with vitiligo activity has not yet been investigated. Here, we show that S100B serum levels were significantly increased in patients with active nonsegmental vitiligo and strongly correlated with the affected body surface area. Prospective follow-up showed a predictive value of serum S100B levels on disease progression. In vitro experiments using repeated freeze-thaw procedures showed an intracellular up-regulation of S100B in normal and vitiligo melanocytes before an extensive release in the environment. This phenomenon may explain the increased S100B serum values in the active phase of vitiligo. In a monobenzone-induced vitiligo mouse model we could show the potential of S100B inhibition as a therapeutic strategy in vitiligo. In conclusion, this report shows the possible use of S100B as a biomarker for disease activity in vitiligo. Our data suggest that this damage-associated molecular pattern protein could play a substantial role in the pathogenesis of vitiligo and may be a potential new target for treatment.


INTRODUCTION

Vitiligo is a common depigmentary skin disorder affecting 1–2% of the world’s population. Current pathophysiologic insights promote the theory that melanocytes are destroyed by the immune system, leaving persistent areas of depigmentation (Iannella et al., 2016). Nonetheless, other theories have been suggested regarding causes for depigmentation, including deficient adhesion proteins, increased presence of oxidative stress, and neurogenic factors (Al’Abadie et al., 1994; Spencer et al., 2007; Wagner et al., 2015). One of the major difficulties in the clinical management of vitiligo is the assessment of disease activity. In contrast to other cutaneous disorders, no erythema or scaling is present in vitiligo. Hypopigmented skin areas with blurred borders and confetti-like depigmentations have been associated with disease activity (Benzekri et al., 2013; Sosa et al., 2015). However, these signs are often subjective and only present in a minority of patients. Therefore, the use of an objective marker for disease activity in vitiligo would help guide possible treatments.

S100B is a member of the family of S100 proteins that comprises a multigene group of 21 low-molecular-weight proteins (Eckert et al., 2004). S100B expression has been reported in a variety of tissues, including melanocytes, astrocytes, oligodendrocytes, neural progenitor cells, Schwann cells, kidney epithelial cells, adipocytes, skeletal myofibers, Langerhans cells, and a subpopulation of lymphocytes. Moreover, in pathological conditions, S100B expression can be induced in cell types that lack basal S100B expression, as has been shown in cardiomyocytes (Sorci et al., 2013).

The use of S100B as a disease activity marker has been widely studied in melanoma, where it is currently one of the most established biomarkers. S100B levels are elevated in metastatic melanoma patients and exhibit prognostic capacity to predict survival after systemic treatment (Weide et al., 2013). S100B levels have been shown to correlate with the cytotoxicity of chemotherapy in melanoma patients (ghanem et al., 2001). However, to our knowledge, the role of S100B in vitiligo has not been studied. Previous research on melanoma and brain injury has shown that pathological events leading to cell death of S100B-positive cells coincide with increasing serum levels of S100B. Therefore, we hypothesized that melanocytic cell death in the active phases of vitiligo could lead to increased S100B values.

RESULTS

Patients

S100B serum levels were obtained from 107 vitiligo patients (89 nonsegmental vitiligo patients and 18 segmental vitiligo patients) and 26 healthy control subjects. Most patients received no treatment 3 months before the blood analysis (62 out of 107 patients), and treated patients used only topical cream therapy (45/107 patients). The clinical characteristics of the patients are summarized in Table 1.

S100B serum levels are increased in patients with active of vitiligo

Disease activity in nonsegmental vitiligo patients was evaluated by the patients using a standardized questionnaire.
Disease activity in the last 6 months was mentioned by 37 out of 85 patients, and 48 patients out of 85 reported no progression in the last 6 months. Highly significant increases in S100B serum levels were found in nonsegmental vitiligo patients with disease activity in the last 6 months compared with patients without progressive disease in the last 6 months (n = 37/85 [43.5%] vs. n = 48/85 [56.5%], respectively; P = 0.005) (Figure 1a). Similarly, evaluation by the physician based on follow-up pictures showed a marked increase in S100B serum levels in vitiligo patients with highly active vitiligo compared with patients with lower activity statuses (n = 7/89 [7.9%] vs. n = 82/89 [92.1%], respectively; P = 0.008) (Figure 1b). Patients who were subjected to topical treatment (corticosteroids/tacrolimus/pimecrolimus) had lower serum S100B levels relative to patients who received no therapy (n = 38 vs. n = 51, P = 0.006) (Figure 1c). S100B values were not significantly different in healthy control subjects compared with nonsegmental vitiligo patients (n = 89 vs. n = 23, respectively) but were higher in segmental vitiligo patients (n = 89 vs. n = 18, respectively; P < 0.001) compared with nonsegmental vitiligo patients. Because most segmental vitiligo patients had no disease activity in the last 6 months (13/18 patients), in-depth analysis according to disease activity was not possible for this group.

We found no correlation between S100B and several soluble CD antigens (sCD25, sCD27, sCD40L) or RANTES, which reflect lymphocyte activation. Also, no correlation was found with CXCL12 used as a surrogate marker for natural killer cell chemotaxis (data not shown). This lack of association suggests that the increased S100B serum levels are unlikely to be due to an activated inflammatory immune infiltrate.

S100B serum levels correlate with the vitiligo-affected body surface area
S100B serum levels correlated with an increased affected body surface area (BSA) in nonsegmental vitiligo patients (n = 87; Pearson correlation: P = 0.003, r = 0.302; Spearman: P = 0.020, ρ = 0.235) (Figure 2a). The correlation between the affected BSA and circulating S100B levels was present only in patients with active vitiligo (n = 47) (Figure 2b), and no correlation between BSA and S100B serum levels could be shown in nonprogressive vitiligo patients (n = 40) (Figure 2c). No correlation between S100B serum levels and the affected body location, presence of halo nevi, age, age of onset, sex, or presence of autoimmune diseases was found.

Analysis of the use of S100B as a prognostic marker in vitiligo
In 64 nonsegmental vitiligo patients, circulating S100B levels were analyzed according to the clinical evolution at a follow-up consultation 6 to 12 months later. During the follow-up period, 20 out of 64 (31.3%) patients received no treatment, 35 out of 64 (54.7%) patients were treated with topical corticosteroids, 5 out of 64 patients (7.8%) were treated with topical immunomodulators, 2 out of 64 (3.1%) patients received both topical corticosteroids and immunomodulators, and 2 out of 64 (3.1%) patients were treated with UVB therapy. Overall, S100B levels in circulation were significantly higher in patients with reported disease progression in the follow-up consultation (n = 26 vs. n = 38, respectively; P = 0.040) (Figure 1d). This was also the case in patients who received no treatment (P = 0.010) in the period between consultations, which limits possible bias due to therapy during the follow-up period. Nonetheless, S100B serum levels obtained from progressing and nonprogressing vitiligo patients showed substantial overlap, thereby limiting their use as a predictive test in clinical practice, as indicated by receiver operating characteristic analysis (data not shown). Combining the serum levels of S100B with soluble CD25 (sCD25), a known biomarker to identify disease activity in vitiligo patients (Speeckaert et al., 2016), resulted in a better separation between progressive and nonprogressive vitiligo. Patients with S100B serum levels of less than 10 pg/ml and sCD25 levels less than 3 ng/ml (29/57 [50.9%]) had only a 20.7% chance of progression, whereas patients with serum levels above these cut-off values (28/57 [49.1%]) had a 66.7% chance of progression (data not shown). We show here that increased S100B and sCD25 serum levels might be used to identify patients with progressive vitiligo.

S100B expression in human skin
To test whether S100B is up-regulated in skin upon actively induced vitiligo, we used our previously validated Koebner induction model (van Geel et al., 2012). Briefly, cryotherapy was applied on a pigmented skin test area in a nonsegmental vitiligo patient with very extensive vitiligo (>50% BSA). Lesional and nonlesional biopsy samples were taken 3 days after cryotherapy. In the lesional biopsy sample, a beginning inflammatory infiltrate with moderate increase in CD1a staining was found, and melanocytes were still residing in the basal layer. A marked up-regulation of S100B was observed in lesional skin 3 days after cryotherapy (Figure 3a and b). S100B-positive cells were mainly found around the basal layer and the mid-epidermal area. Substantial S100B expression was also observed in a biopsy sample of a late inflammatory phase of vitiligo despite the absence of

### Table 1. Clinical characteristics of vitiligo patients included in this study

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generalized vitiligo, n/total (%)</td>
<td>89/107 (83.2)</td>
</tr>
<tr>
<td>Segmental vitiligo, n/total (%)</td>
<td>18/107 (16.8)</td>
</tr>
<tr>
<td>Men, n/total (%)</td>
<td>49/107 (45.8)</td>
</tr>
<tr>
<td>Women, n/total (%)</td>
<td>58/107 (54.2)</td>
</tr>
<tr>
<td>Age in years, mean (median, IQR)</td>
<td>36.8 (35, 23–48)</td>
</tr>
<tr>
<td>Duration of vitiligo in years, mean (median, IQR)</td>
<td>9.3 (6, 3–12)</td>
</tr>
<tr>
<td>% affected BSA, mean (median, IQR)</td>
<td>3.16 (1.0, 0.4–2.9)</td>
</tr>
<tr>
<td>Skin type I–III, n/total (%)</td>
<td>104/107 (97.2)</td>
</tr>
<tr>
<td>Skin type IV–VI, n/total (%)</td>
<td>3/107 (2.8)</td>
</tr>
<tr>
<td>Autoimmune diseases, n/total (%)</td>
<td>21/107 (19.6)</td>
</tr>
<tr>
<td>Therapy during 3-month period before blood sampling, n/total (%)</td>
<td>62/107 (57.9)</td>
</tr>
<tr>
<td>No treatment</td>
<td>Topical corticosteroid</td>
</tr>
<tr>
<td>Topical corticosteroid</td>
<td>24/107 (22.4)</td>
</tr>
<tr>
<td>Topical tacrolimus/pimecrolimus</td>
<td>11/107 (10.3)</td>
</tr>
<tr>
<td>Topical corticosteroids + tacrolimus/pimecrolimus</td>
<td>10/107 (9.4)</td>
</tr>
</tbody>
</table>

Abbreviation: IQR, interquartile range.
Figure 1. **S100B is enhanced in active vitiligo.** S100B serum levels in patients were stratified according to vitiligo activity as scored by (a) self-assessment of the patients and (b) evaluation by a physician. (c) S100B serum levels analyzed in patients receiving topical therapy (corticosteroids/tacrolimus/pimecrolimus) or no treatment. (d) Disease evolution according to S100B serum levels.

Figure 2. **S100B correlates with the affected BSA.** Correlations between S100B serum levels and the affected BSA (a) overall and in patients (b) with active and (c) without active vitiligo. BSA, body surface area.
Figure 3. Tissue expression of S100B during the inflammatory processes of vitiligo. Immunohistochemistry of S100B (IS504, Dako, Heverlee, Belgium), CD1a (IS069, Dako) and melan-A (IS069, Dako). S100B, CD1a, and melan-A are visualized with 3-amino-9-ethylcarbazole (red) and counterstained with hematoxylin.
melan-A+ melanocytes (Figure 3c). A similar up-regulation was seen in a group of halo nevi (n = 10) with marked S100B expression in melanocytes, and in two late stage halo nevi, strong S100B expression was detected in cells surrounding melanocyte nests located at areas of CD1a+ staining (Figure 3d and e). These observations suggest that S100B could be taken up by immune cells during the active stage of vitiligo. Therefore, we performed S100B uptake experiments in which we added recombinant fluorescein-conjugated S100B to the supernatant of human peripheral blood mononuclear cells. After 24 hours, expression of fluorescein-conjugated S100B was found in CD1a+/CD11c+ cells, which are direct precursors of skin Langerhans cells (Ito et al., 1999) and which illustrates their capacity to take up extracellular S100B.

**S100B is up-regulated and subsequently released from melanocytes upon cryogenic stress**

To investigate the capacity of melanocytes to produce or release S100B upon injury conditions, cryogenic stress was induced on melanocyte cultures. Briefly, five melanocyte cultures obtained from five different vitiligo patients and seven healthy control melanocyte cultures were subjected to repeated freeze-thaw, with increasing numbers of freeze-thaw cycles. Flow cytometry confirmed the gradual loss of S100B from annexin V−/live-dead− melanocytes to annexin V+/live-dead− melanocytes and live-dead+ melanocytes (P < 0.001 and P < 0.001 vs. annexin V+/live-dead− melanocytes, respectively) (Figure 4). This points to the passive loss of S100B from melanocytes because of disruption of membranous integrity. Overall, the mean intracellular fluorescence intensity of S100B decreased with the number of freeze-thaw cycles because of the increased amount of necrotic cells (P = 0.001) (Figure 4b–d). However, in both live and annexin V+/live-dead− cells, an up-regulation of S100B after one (P = 0.041 and P = 0.008, respectively) and two freeze-thaw cycles was observed (P = 0.026 and P = 0.004) (Figure 4 e and f). In annexin V+/live-dead+ cells the intracellular S100B levels decreased markedly with each freeze-thaw cycle (P < 0.001) (Figure 4g).

The levels of secreted S100B and intracellular S100B were similar between vitiligo and normal melanocytes. Nonetheless, an increased amount of annexin V−/live-dead− cells was observed in vitiligo melanocytes relative to normal melanocytes (P = 0.031).

**In vivo inhibition of S100B protects from depigmentation in a mouse model of vitiligo**

Vitiligo was induced in 7-week-old C57BL/6j mice using daily application of monobenzone 40% cream for 54 consecutive days. Graying of hairs was monitored for 65 days from the start of treatment on. To test whether S100B is involved in the induction of vitiligo, pentamidine was used as an S100B inhibitor (Esposito et al., 2012). Mice were treated with monobenzone (n = 8), with monobenzone in combination with pentamidine (n = 8), or with pentamidine only (n = 8). At day 65 after starting the treatments, half of the mice (n = 4 out of 8) in the monobenzone-treated group showed graying of the hair at the application site, whereas none of the mice developed depigmentations in the other two experimental groups. S100B serum levels were significantly increased in the monobenzone-treated groups versus mice treated with pentamidine only (Figure 5, P = 0.005).

**DISCUSSION**

This study describes the potential use of S100B as a disease activity marker in vitiligo. In this study, higher circulating S100B levels were found in patients with active vitiligo. Moreover, we were able to show that S100B serum levels correlated strongly with the affected body surface area in vitiligo patients undergoing active depigmentation, whereas this was not the case in patients with stable disease (Figure 1 and 2). Prospective follow-up studies showed an association of S100B serum levels with future episodes of active depigmentation (Figure 1d). Sequential blood sampling could shed more light on the use of S100B as a predictive biomarker, because a substantial overlap in the S100B serum level range exists between vitiligo patients and healthy control subjects (Figure 3b).

Using further in vitro experiments, we could show the extensive release of S100B starting from the early stages of melanocyte cell death. These experiments showed that melanocyte death leads to up-regulation of S100B, which may explain the observed increased serum S100B levels in active vitiligo patients. These results are in agreement with earlier findings, because S100B has been proposed as a biomarker for the cytotoxicity of melanocytes. Increased intra- and extracellular concentrations of S100B were reported after treatments with hydroquinone, retinoic acid, and sodium lauryl sulfate (Cheong et al., 2014). Additionally, intracellular up-regulation of S100B after repetitive freeze-thaw cycles was found in both vitiligo and healthy melanocytes. S100B has been shown to protect cells from apoptosis (Lin et al., 2010). Up-regulation of S100B could therefore represent a cell-protective response. However, this mechanism of elevated intracellular S100B synthesis may, in a subsequent phase of cell death, result in an increased release of S100B in the environment.

Given the association of S100B with several autoimmune and autoinflammatory disorders, S100B might be associated with autoimmunity and inflammation rather than specifically with vitiligo activity. Elevated S100B concentrations have been found in serum of patients with lupus, psoriasis, and...
extensive dermatitis, although the underlying mechanism has not fully been clarified (Guidi et al., 2002; Paradisi et al., 2007; Portela et al., 2002). Additionally, in other autoimmune diseases, such as type I diabetes, increased tissue S100B levels have been found, and autoantibodies against S100B are present in serum of patients with Parkinson disease (Wilhelm et al. 2007; Zimmer et al. 1997). A subset of CD8⁺ T lymphocytes and, to a lesser extent, natural killer cells secrete S100B upon activation (Steiner et al., 2011). Therefore, we investigated soluble CD antigens and RANTES as surrogate markers of lymphocyte activation (Brusko et al., 2009). In the investigated cohort of vitiligo patients, S100B

**Figure 4. Up-regulation and release of S100B during melanocyte stress.** (a) Dot plots and bars (mean ± 95% confidence interval) illustrating the loss of intracellular S100B from viable to annexin V⁺/live-dead⁻ cells and live-dead⁺ melanocytes. (b) Number of live-dead⁻ cells, (c) S100B released in the supernatant, and (d) intracellular S100B according to the number of freeze-thaw cycles. Up-regulation of intracellular S100B in (e) live and (f) annexin V⁺/live-dead⁻ melanocytes, and (g) a down-regulation was observed in live-dead⁺ melanocytes with additional freezing cycles (95% confidence interval). MFI, mean fluorescence intensity.
serum levels showed no correlation with any of the investigated soluble CD antigens (sCD25, sCD27, sCD40L) or RANTES. S100B serum concentrations showed no correlation with CXCL12, which regulates the development and trafficking of natural killer cells. This indicates that the elevated S100B concentrations in active vitiligo patients are unlikely to be derived from increased lymphocytic or natural killer cell activities.

Previously, it has been shown that S100B is one of the most differentially up-regulated genes in vitiligo compared with normal melanocytes using transcriptional analysis (Strömberg et al., 2008). This suggests that S100B could be actively involved in the pathophysiology of vitiligo. S100B is a calcium-binding protein, and aberrant calcium homeostasis has been found in vitiligo (Schallreuter-Wood et al., 1996). S100B is considered as a damage-associated molecular pattern protein exerting several immunological activities. It binds to RAGE, which induces a proinflammatory signaling cascade. RAGE ligands such as S100B also increase the expression of RAGE, which recognizes nucleic acids and promotes their uptake via endosomes, thus lowering the concentration threshold for inflammatory responses resulting from nucleic acids (Sirois et al., 2013). This may be of particular importance in autoimmune diseases such as vitiligo. S100B exerts both pro- and anti-inflammatory activities depending on the cell type. It has been shown that stimulation of macrophages with recombinant S100B resulted in an enhanced release of proinflammatory cytokines such as IL-1β and IL-6 (Fujiya et al., 2014; Niven et al., 2015). Experiments in mice showed that extracellular S100B is taken up by Langerhans cells, illustrating its possible involvement in skin immunity. This may explain why S100B expression is still present in the epidermis of a late phase inflammatory vitiligo lesion deficient of viable melanocytes and in late stage halo nevi (Figure 3a–e).

Our results point to an involvement of S100B in the active phase of vitiligo, and S100B could therefore be a potential target for vitiligo therapies. In a pilot study using a monobenzone-induced depigmentation model, we were able to show that mice receiving monobenzone in combination with the S100B inhibitor pentamidine did not develop hair graying in contrast to mice treated with monobenzone only. These data suggest that S100B can be an interesting target for future therapeutic research. A limitation of this experiment is that possible anti-inflammatory effects of pentamidine independent of S100B inhibition could not be excluded.

In conclusion, this study shows increased S100B serum levels in vitiligo patients with active depigmentation compared with vitiligo patients with stable disease. The clear correlation of S100B serum levels with the affected BSA, especially in patients with limited disease, confirms that is a marker for active depigmentation. In addition, our in vitro experiments point to the release of S100B by stressed melanocytes as the most plausible source of serum S100B. These results provide evidence that S100B could be involved in active vitiligo and may be a potential target for treatment.

MATERIALS AND METHODS

Patients

A total of 89 nonsegmental vitiligo patients, 18 segmental vitiligo patients, and 26 control subjects were enrolled in this study between 2009 and 2016. Blood was drawn and serum isolated, after which samples were stored at −80 °C until analysis. This study was approved by the local ethics committee of the Ghent University Hospital. All patients signed a written informed consent.

Scoring of vitiligo disease activity and BSA involvement

Disease activity was scored by digital images taken during the consultation and by clinical examination. Patients were classified as having very active, mild-moderate active, stable, or repigmenting vitiligo. Very active vitiligo was scored in patients with clear progression or obvious activity signs (hypopigmentation, confluent depigmentations), mild-moderate active in patients with small progression (<1% BSA), stable disease in patients with no difference compared with the previous consultation and repigmenting if signs of repigmentation were present. The self-reported disease activity was assessed by questioning disease activity/stability/repigmentation during the last 3, 6, or 12 months. A modified Vitiligo Disease Activity scale was used (Njoo et al., 1999). To assess a possible
predictive role, the disease evolution was assessed during the next consultation (after 6–12 months). The affected BSA was scored on digital pictures of the patients using the Vitiligo Extent Score (van Geel et al., 2016).

ELISA and multiplex analyses
The ELISA protocol for measuring serum levels of S100B was performed according the manufacturer’s recommendation (Millipore, Billerica, MA). One value with very high S100B concentration was excluded from analysis because this patient was pregnant. sCD25, sCD27, and sCD40L (R&D Systems, Abingdon, UK) were measured in serum using Human Fluorokine MAP Base Kit (R&D Systems). The analyses were performed on a Bioplex 200 System (Bio-Rad, Hercules, CA). RANTES and CXCL12 were measured with ELISA (R&D Systems).

In vitro analyses
Five melanocytes cultures obtained from five different vitiligo patients and seven normative melanocyte cultures obtained from healthy subjects were repeatedly dipped into liquid nitrogen with increasing duration and quickly defrosted at 37 °C in a water bath. In the first cycle a freeze period of 5 seconds was carried out, which was increased in subsequent cycles to 10 and 20 seconds of freezing, respectively. Supernatant was harvested after 2.5 hours, and cells were analyzed with flow cytometric analyses.

Flow cytometry
To assess early signs of necrosis and cell death after freeze-thawing, the cells were stained with annexin V (eBiosciences, San Diego, CA) and a fixable live/dead kit (Life Sciences, Merelbeke, Belgium). The cells were fixed and permeabilized (Foxp3 staining buffer, eBioscience) for intracellular S100B determination. S100B was stained with a primary antibody (612376, BD Biosciences, Erembodegem, Belgium) and visualized with a secondary anti-mouse F(ab)-antibody (Life Sciences).

To investigate the uptake of S100B by peripheral blood mononuclear cells, recombinant S100B (Thermo Fisher Scientific, Erembodegem, Belgium) was conjugated with fluorescein (Innova Biosciences, Cambridge, UK). Fluorescein-labeled S100B was added to peripheral blood mononuclear cells (1 mg/ml) for 24 hours. Subsequently, peripheral blood mononuclear cells were stained with CD1a-PE (BD Biosciences, Erembodegem, Belgium) and CD11c-APC-Cy7 (BD Biosciences) and live/dead staining (Fixable Aqua science) for intracellular S100B determination. S100B was stained with an antibody (RTU, Dako), and an anti-melan-A antibody (RTU, Dako) for 30 minutes.

Animal procedures
Vitiligo was induced in C57BL/6 j mice (Janvier Labs, Le Genest-Saint-Ile, France) using daily topical application of monobenzone 40% cream on shaved back skin (Zhu et al., 2013). The mice were either treated with monobenzone 40% (n = 8), monobenzone and pentamide (n = 8, 4 mg/kg intraperitoneal every other day), or pentamide only (n = 8, 4 mg/kg intraperitoneal every other day). The treatment was initiated in mice of 7 weeks old and continued for 54 days, and depigmentations were monitored daily until day 65. Facial vein bleedings were performed at days 0, 10, 20, 30, 40, and 65 of treatment, and blood was left to clot for 2 hours at room temperature. Blood was centrifuged for 20 minutes at 20,000g, and serum was taken and stored at ~70 °C before analysis. All animal procedures were conducted in accordance with institutional and national regulations. All experiments were approved by the local ethics committee of Ghent University.

Statistical analyses
All statistical analyses were performed using SPSS 22.0 (SPSS Science, Chicago, IL). Comparison of continuous variables was performed by Mann-Whitney or Kruskall-Wallis analysis in cases of two or more groups, respectively. For correlation analysis, both Pearson and Spearman correlation analyses were performed. Receiver operating characteristic curve analysis was used to investigate the sensitivity and specificity of S100B as a biomarker for disease activity. In all cases, P less than 0.05 was considered to indicate statistical significance.

CONFLICT OF INTEREST
The authors state no conflict of interest.

REFERENCES


