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Evaluating S100B as a serum biomarker for central neurosarcoidosis

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ABSTRACT

Background: S100B is a calcium-binding protein found primarily in glial cells. In the setting of neuronal injury and disruption of the blood brain barrier, S100B can leak into the cerebrospinal fluid and systemic circulation.
Objectives: To determine if serum S100B distinguishes patients with central neurosarcoidosis (NS) from patients with extra-neurologic sarcoidosis (ENS) and healthy controls, and if S100B levels correlate with MRI measures of disease burden.
Methods: Patients were enrolled from the Cleveland Clinic Sarcoidosis Center. Patients with traumatic brain injury, central nervous system (CNS) infections, CNS malignancy, neurodegenerative disorders, schizophrenia, bipolar disorder, or melanoma were excluded. S100B levels were compared between patients with NS, ENS, and healthy controls, and between NS patients with varying degrees of post-contrast enhancement on MRI.
Results: Median (interquartile range) S100B levels were 101 pg/mL (92, 136) for 11 NS patients, 89 pg/mL (73, 107) for 11 ENS patients, and 60 pg/mL (39, 74) for 26 healthy controls. There was a significant difference between NS and control groups ($p = 0.01$). The difference between NS and ENS groups did not rise to the level of statistical significance ($p = 0.178$). S100B levels were significantly different between NS patients with varying degrees of enhancement on MRI ($p = 0.04$).
Conclusions: S100B deserves additional study as a biomarker for CNS injury in NS. It may be useful as a longitudinal measure of disease activity.

1. Background

Sarcoidosis is a chronic inflammatory disease characterized by the formation of non-necrotizing granulomas. As many as 15% of sarcoidosis cases have neurological complications, commonly affecting the cranial nerves, meninges, brain parenchyma, and spinal cord [1]. Management of central nervous system (CNS) complications are particularly challenging because clinical symptoms may not reflect disease activity. Currently, monitoring requires expensive or invasive tests, like magnetic resonance imaging (MRI) or lumbar puncture.

S100B is a calcium-binding protein found primarily in glial cells, although it has been detected in other extraneural cell types [2]. In the setting of neuronal injury and disruption of the blood brain barrier (BBB), S100B can leak into the cerebrospinal fluid (CSF) and systemic circulation [2]. In multiple sclerosis (MS), serum levels correlate with the degree of inflammation and response to treatment [3,4].

We compared S100B levels between neurosarcoidosis (NS) patients, extra-neurologic sarcoidosis (ENS) patients, and healthy controls, and among NS patients with varying degrees of post-contrast enhancement on MRI. This is the first study of serum S100B as a biomarker in NS.

2. Methods

Consecutive patients from the Cleveland Clinic were enrolled. All patients were adults 18 years of age or older with a diagnosis of sarcoidosis meeting the American Thoracic Society, European Respiratory Society, and World Association of Sarcoidosis and Other Granulomatous Disorders criteria [5,6]. All NS patients met A Case Control Etiologic Study of Sarcoidosis (ACCESS) criteria for definite neurologic involvement [7]. All NS patients had evidence of CNS inflammation on MRI (leptomeningeal enhancement, enhancing intracranial parenchymal lesions, cranial nerve enhancement, enhancing spinal cord

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lesions) or CSF studies (pleocytosis, increased IgG synthesis, or CSF-specific oligoclonal bands with or without elevated protein or low glucose) consistent with NS. Blood was drawn within 6 weeks of the relevant diagnostic test prior to change in therapy. Among patients with post-contrast enhancement on MRI but no CSF studies, causes of CNS inflammation other than NS were clinically excluded. Patients with traumatic brain injury, CNS infections, CNS malignancy, neurodegenerative disorders, schizophrenia, bipolar disorder, or melanoma were excluded from the study, as these are potential confounders. Patients with obesity and positive screening for clinical depression, also potential confounders, were included, but their influence on the study results was evaluated in post-hoc analyses. Patients with neuropathy were excluded to focus on CNS disease.

2.1. Study assessments

Patients were assessed at baseline for age, sex, race, organ involvement, treatment status, body mass index (BMI), malignancy, depression symptoms, and clinical diagnosis of migraine. Depression could not be excluded, but depression severity was evaluated by the Patient Health Questionnaires (PHQ)-9 [8].

For the S100B analysis, blood samples were collected in red cap Vacutainer sterile tubes (BD Bioscience) and the serum was separated by centrifugation (2000 RPM, 10 min). Samples were de-identified and assigned an internal ID matching other testing. Serum samples were then stored at −80° C. Serum ID matched consent forms. S100B measurements were performed using an ELISA kit (98 wells, anti-human S100B, Diasorin) and reading was done using a multi-plate fluorescent reader. Fluorescent signals were converted into ng/ml as per standard curve concentrations [9].

2.2. Statistical methods and data analysis

Statistical calculations were performed with R software [10]. Baseline demographics, disease characteristics, and key comorbidities were reported with descriptive statistics using median and interquartile range for continuous variables and frequency and percentage for categorical variables. The Kruskal-Wallis test was used for comparisons between multiple groups. Pairwise comparisons were made using the Wilcoxon rank-sum test with continuity correction.

2.3. Post-hoc analyses

To get a rough approximation of whether S100B levels correlate with disease burden for NS patients, we compared S100B levels among groups with varying degrees of post-contrast enhancement on MRI. For the purposes of this study, we graded the extent of enhancement as none, mild, moderate, or severe based on the number of anatomical sites involved. Patients were assigned 1 point for each of the following: supratentorial meningeal enhancement, infratentorial meningeal or cranial nerve enhancement, intracranial parenchymal enhancing lesions, enhancing lesions in the cervical spine, enhancing lesions in the thoracic spine, and enhancement of the lumbar spine or cauda equina. A score of 1–2 points was graded as mild. A score of 3–4 points was graded as moderate. A score of 5 or more points was graded as severe. See Table 1.

Some NS patients had outside MRIs whose images were not available for review at the time of the post-hoc analysis. For these patients, if an on-site MRI was obtained within 90 days of the blood draw, it was included in the analysis, as long as there were no changes in treatment during the interval.

To determine if depression influenced the study results, S100B levels were compared between NS, ENS, and control patients after excluding depression. It was not necessary to repeat the analysis excluding patients with obesity, because there was no significant difference between groups at baseline.

2.4. Standard protocol approvals, registrations, and patient consents

All patients participated voluntarily and provided written informed consent. The study was approved by the institutional review board (IRB) for Cleveland Clinic (IRB# 12–616, 18–698).

3. Results

We recruited 26 healthy controls, 11 patients with ENS, and 11 patients with NS. Baseline demographics are summarized in Table 2. Only differences in age ($p = 0.006$), depression severity ($p < 0.001$), and history of migraine headaches ($p = 0.030$) were statistically significant between the groups. ENS patients were older, had higher levels of depression, and were more likely to have migraine headaches compared to NS patients and controls. 8 ENS patients were on immunosuppressive therapy compared to 7 NS patients. Disease characteristics and treatment status for ENS and NS patients are summarized in Table 3.

S100B levels by group are shown in Fig. 1. Median S100B levels with interquartile range (IQR) were 101 pg/mL (92, 136) for NS patients, 89 pg/mL (73, 107) for ENS patients, and 60 pg/mL (39, 74) for healthy controls. There was a statistically significant difference between the NS and control groups ($p = 0.010$) and ENS and control groups ($p = 0.001$). Although there was a difference between S100B levels in the ENS and NS groups, it did not rise to the level of statistical significance ($p = 0.178$).

Table 1
Extent of post-contrast enhancement for neurosarcoidosis patients based on the number of anatomical sites involved.

ID	MRI[a]	Supra-tentorial	Infra-tentorial[b]	Paren-chymal	C-spine	T-spine	L-spine[c]	Total Sites	Extent of Enhancement
1	Yes	**Yes**	**Yes**	No	No	No	No	2	Mild
2[d]	No	–	–	–	–	–	–	–	–
3	Yes	No	No	No	**Yes**	No	No	1	Mild
4[e]	Yes	No	No	No	No	No	No	0	None
5	Yes	No	**Yes**	No	**Yes**	**Yes**	No	3	Moderate
6	Yes	No	**Yes**	No	**Yes**	**Yes**	**Yes**	4	Moderate
7[e]	No	–	–	–	–	–	–	–	–
8	Yes	No	**Yes**	No	**Yes**	No	No	2	Mild
9	Yes	No	**Yes**	No	**Yes**	No	No	2	Mild
10[e]	Yes	No	No	No	No	No	No	0	None
11	Yes	**Yes**	**Yes**	**Yes**	No	No	No	3	Moderate

ID: Patient Identifier.
[a] MRIs with images available for review.
[b] Includes cranial nerve enhancement.
[c] Includes cauda equina enhancement.
[d] Outside MRI.
[e] Cerebrospinal fluid evidence of disease activity.

Table 2
Baseline demographics in patients with neurosarcoidosis, extra-neurologic sarcoidosis, and controls.

	Neurosarcoidosis	Extra-Neurologic Sarcoidosis	Controls	P
n	11	11	26	
Age (median [IQR])	46.0 [41.0, 50.0]	57.0 [53.0, 58.0]	45.0 [37.0, 53.5]	0.006
Female (%)	6 (54.5)	8 (72.7)	17 (65.4)	0.667
Race (%)				0.406
Asian	0 (0.0)	0 (0.0)	2 (7.7)	
African American	3 (27.3)	1 (9.1)	5 (19.2)	
Caucasian	8 (72.7)	10 (90.9)	16 (61.5)	
Other	0 (0.0)	0 (0.0)	3 (11.5)	
BMI (median [IQR])	31.6 [30.0, 35.5]	28.0 [24.3, 35.5]	28.4 [24.1, 31.0]	0.123
Depression (%)[a]				<0.001
None	1 (9.1)	5 (45.5)	21 (80.8)	
Mild	8 (72.7)	2 (18.2)	5 (19.2)	
Moderate	1 (9.1)	3 (27.3)	0 (0.0)	
Moderately Severe	1 (9.1)	1 (9.1)	0 (0.0)	
Migraines (%)	0 (0.0)	2 (18.2)	0 (0.0)	0.03

IQR: interquartile range; BMI: body mass index.
[a] Depression was measured by the Patient Health Questionnaire-9. Scores were divided into the following categories: 0–4 (None), 5–9 (Mild), 10–14 (Moderate), 15–19 (Moderately Severe), and 20–27 (Severe).

Table 3
Comparison of disease characteristics and treatment status in patients with neurosarcoidosis and extra-neurologic sarcoidosis.

	Neurosarcoidosis	Extra-Neurologic Sarcoidosis
n	11	11
Immunosuppressive Treatment (%)		
Prednisone	7 (63.6)	4 (36.4)
Mycophenolate	1 (9.1)	0 (0.0)
Methotrexate	0 (0.0)	5 (45.5)
Azathioprine	2 (18.2)	0 (0.0)
Infliximab	0 (0.0)	1 (9.1)
No treatment	4 (36.4)	3 (27.3)
Other Organ Involvement (%)		
Lung	9 (81.8)	9 (81.8)
Skin	1 (9.1)	3 (27.3)
Liver	0 (0.0)	2 (18.2)
Eye	1 (9.1)	2 (18.2)
Spleen	0 (0.0)	0 (0.0)
Bone Marrow	0 (0.0)	0 (0.0)
Kidney	0 (0.0)	0 (0.0)
Cardiac	1 (9.1)	0 (0.0)
Joint	0 (0.0)	1 (9.1)
Extrathoracic Lymph Node	0 (0.0)	0 (0.0)
Parotid/Salivary Gland	0 (0.0)	0 (0.0)
Muscle	0 (0.0)	0 (0.0)

3.1. Post-hoc analyses

S100B levels by extent of enhancement are summarized in Fig. 2. There was a significant difference between S100B levels among NS patients with no, mild, or moderate enhancement on MRI ($p = 0.04$). Median (IQR) S100B levels were 200 pg/mL (170, 280) for moderate, 110 pg/mL (100, 110) for mild, and 90 pg/mL (80, 90) for no enhancement on MRI.

A scatterplot matrix was run to estimate whether there was a linear relationship between the variables of interest (subject type, S100B levels, age, sex, race, BMI, depression severity, history of migraines, and active treatment). Only depression severity was correlated with S100B levels. The analysis was repeated excluding all 8 patients with depression with no change in the results.

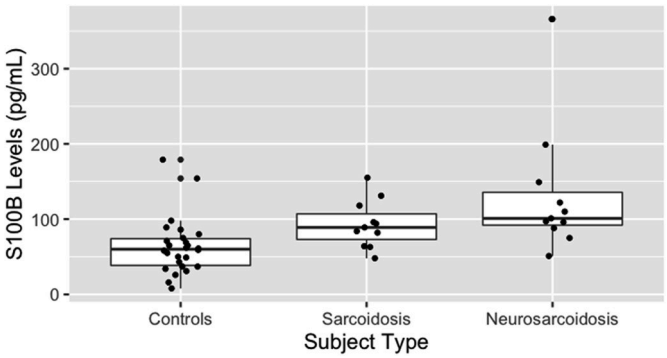

Fig. 1. S100B levels by subject type.

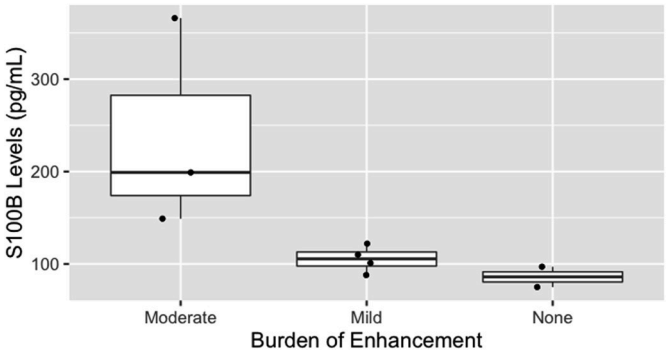

Fig. 2. S100B levels by extent of enhancement on MRI

4. Discussion

A variety of biomarkers have been investigated for sarcoidosis, including angiotensin-converting enzyme, soluble interleukin-2 receptor, C-reactive protein, and serum lysozyme. These tests may have some utility for monitoring systemic disease, but they have been insufficiently sensitive and specific in both CSF and serum for monitoring patients with CNS disease [11]. S100B has emerged as a potential biomarker for CNS injury.

In this study, S100B levels were significantly higher in NS patients compared to healthy controls. There was a difference between S100B levels in NS and ENS patients, but it fell short of statistical significance. We also found significantly higher S100B levels among NS patients with a greater number of enhancing sites on MRI, suggesting a relationship between S100B and the extent of CNS inflammation.

The major limitation of our study was its small sample size. This decreased the power of the study and may have contributed to the lack of statistical significance in the primary outcome. Although all patients in the study were screened for neurologic symptoms, it is possible that some of the patients in the ENS group had subclinical neurologic disease. Unascertained CNS sarcoidosis may have further reduced our ability to detect a difference between the two groups. In addition, S100B is expressed in some extraneural cell types, including lymphocytes and bone marrow cells [2]. This may explain why there was a difference in S100B levels between ENS patients and healthy controls. Differences in age, history of migraine headaches, and treatment status between the ENS and NS groups could have contributed to the lack of statistical significance as well.

We were unable to exclude patients with depression and obesity, which are potential confounders. However, there was no correlation between BMI and S100B levels, and when the analysis was repeated excluding patients with depression, there was no change in study results.

Treatment with prednisone can also influence permeability of the blood-brain barrier but would be expected to decrease rather than increase neuronal injury leading to the release of S100B.

5. Conclusion

Our findings support further investigation of S100B as a serum biomarker for disease activity in NS. Given the small absolute differences in S100B levels between NS patients, ENS patients, and healthy controls, S100B may have more utility as a longitudinal measure of disease activity at an individual level than as a screening test for NS. Future studies should evaluate whether S100B levels distinguish NS from ENS patients in a larger cohort, and evaluate the longitudinal collection of S100B in NS patients in relation to other markers of disease activity, including MRI metrics and clinical relapse.

6. Financial disclosures

Dr. Brandon Moss has received personal fees as a speaker for Sanofi Genzyme and a consultant for Genentech and has stock in Pfizer. He is supported by a grant from the Anne Theodore Foundation.

Dr. Patel has no relevant disclosures.

Dr. Tavee has no relevant disclosures.

Dr. Culver has no relevant disclosures.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Moss has received personal fees as a speaker for Genzyme and a consultant for Genentech and has stock in Pfizer. He is supported by a grant from the Anne Theodore Foundation. Dr. Patel has no relevant disclosures. Dr. Tavee has no relevant disclosures. Dr. Culver has no relevant disclosures.

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