

ORIGINAL ARTICLE

Clinically relevant increases in serum neurofilament light chain and glial fibrillary acidic protein in patients with Susac syndrome

Domenico Plantone¹  | Eleonora Sabatelli² | Sara Locci¹ | Mariano Marrodan³  | Sini M. Laakso^{4,5} | Farrah J. Mateen⁶  | Amalia Feresiadou^{7,8} | Tom Buelens⁹ | Assunta Bianco²  | Marcela P. Fiol³  | Jorge Correale^{3,10}  | Pentti Tienari^{11,12}  | Paolo Calabresi^{2,13} | Nicola De Stefano¹ | Raffaele Iorio^{2,13} 

¹Department of Medicine, Surgery and Neuroscience, University of Siena, Siena, Italy

²Neurology Unit, Fondazione Policlinico Universitario 'A.Gemelli' IRCCS, Rome, Italy

³Neurology Department, Fleni, Buenos Aires, Argentina

⁴Clinical Neurosciences, Neurology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

⁵Translational Immunology Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland

⁶Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts, USA

⁷Department of Neurology, Uppsala University Hospital, Uppsala, Sweden

⁸Department of Medical Sciences, Section of Neurology, Uppsala University, Uppsala, Sweden

⁹Department of Ophthalmology, CHU St Pierre and Brugmann, Brussels, Belgium

¹⁰Institute of Biological Chemistry and Biophysics (IQUIFIB) CONICET, University of Buenos Aires, Buenos Aires, Argentina

¹¹Department of Neurology, Neurocenter, Helsinki University Hospital, Helsinki, Finland

¹²Research Program of Translational Immunology, Faculty of Medicine, University of Helsinki, Helsinki, Finland

¹³Department of Neuroscience, Università Cattolica del Sacro Cuore, Rome, Italy

Correspondence

Domenico Plantone, Department of Medicine, Surgery and Neuroscience, University of Siena, Viale Bracci 2, 53100 Siena, Italy.
Email: domenico.plantone@unisi.it

Raffaele Iorio, Department of Neuroscience, Università Cattolica del Sacro Cuore, L.go Gemelli, 1 00135 Rome, Italy.
Email: raffaele.iorio@policlinicogemelli.it

Abstract

Background and purpose: Serum levels of neurofilament light chain (sNfL) and glial fibrillary acidic protein (sGFAP) are promising neuro-axonal damage and astrocytic activation biomarkers. Susac syndrome (SS) is an increasingly recognized neurological condition and biomarkers that can help assess and monitor disease evolution are highly needed for the adequate management of these patients. sNfL and sGFAP levels were evaluated in patients with SS and their clinical relevance in the relapse and remission phase of the disease was assessed.

Methods: As part of a multicentre study that enrolled patients diagnosed with SS from six international centres, sNfL and sGFAP levels were assessed in 22 SS patients (nine during a relapse and 13 in remission) and 59 age- and sex-matched healthy controls using Simoa™ assay Neurology 2-Plex B Kit.

Authors Nicola De Stefano and Raffaele Iorio shared senior authorship.

See commentary by T. A. Hardy on page 2953.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2023 The Authors. *European Journal of Neurology* published by John Wiley & Sons Ltd on behalf of European Academy of Neurology.

Results: Serum NfL levels were higher than those of healthy controls ($p < 0.001$) in SS patients and in both subgroups of patients in relapse and in remission ($p < 0.001$ for both), with significantly higher levels in relapse than in remission ($p = 0.008$). sNfL levels showed a negative correlation with time from the last relapse ($r = -0.663$; $p = 0.001$). sGFAP levels were slightly higher in the whole group of patients than in healthy controls ($p = 0.046$) and were more pronounced in relapse than in remission ($p = 0.013$).

Conclusion: In SS patients, both sNfL and sGFAP levels increased compared with healthy controls. Both biomarkers had higher levels during clinical relapse and much lower levels in remission. sNfL was shown to be time sensitive to clinical changes and can be useful to monitor neuro-axonal damage in SS.

KEYWORDS

biomarkers, inflammation, neuro-ophthalmology, neuro-otology

INTRODUCTION

Susac syndrome (SS) is a neurological condition, characterized by encephalopathy, branch retinal artery occlusions and hearing loss [1]. Although the disease is considered rare, its real prevalence is unknown, most probably due to the fact that it is often underdiagnosed or misdiagnosed [1]. The clinical presentation of SS is variable and involves the central nervous system (CNS), with headache [1], progressive cognitive impairment [2], encephalopathy, unilateral or bilateral visual disturbances [3], sensorineural hearing loss [4] and other neurological symptoms including ataxia, aphasia, motor and sensory deficits [5]. The disease may have a severe course with serious long-term clinical outcomes which include bilateral visual and hearing loss and cognitive impairment associated with significant brain atrophy. The vast majority of SS patients experience relapses followed by periods of remission [6]. Unfortunately, the treatment guidance in SS is still expert-opinion based and mainly consists of immunosuppressive treatments due to its putative autoimmune aetiology [7]. In this context, biomarkers that can help assess and monitor disease evolution are highly needed for the adequate management of patients with this disorder.

Neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) represent two promising neuronal and glial degeneration biomarkers. Indeed, the recent development of ultrasensitive digital immunoassays has enabled reliable measurements of these CNS-relevant biomarkers in serum, where they were not previously detectable [8, 9]. Increased cerebrospinal fluid (CSF) and serum levels of NfL have been repeatedly associated with CNS damage in several different neurological conditions [9] and, due to its high specificity, it has been considered a distinctive marker of neuro-axonal damage. Thus, NfL serum levels have been used to assess and monitor neuronal damage in many neurological disorders such as multiple sclerosis, Alzheimer's disease and amyotrophic lateral sclerosis [10]. On the other hand, GFAP is an intermediate filament highly expressed in astrocytes [11] and its serum levels have been increasingly used as a reliable biomarker of astrocytic activation and damage, with an ever-growing body of evidence supporting its use to detect even subtle injuries to the CNS [12].

Despite their potential usefulness as biomarkers of CNS damage in a complex and severe disease such as SS, to the best of our knowledge no data are currently available regarding changes in NfL and GFAP levels in patients with this disease. The aim here is therefore to evaluate the serum NfL (sNfL) and serum GFAP (sGFAP) levels in patients with SS and assess their clinical relevance in the relapse and remission phase of this rare neurological condition.

MATERIALS AND METHODS

This cross-sectional international multicentre cohort study enrolled patients diagnosed with SS between May 2014 and July 2022 from six centres—Fondazione Policlinico Universitario A. Gemelli (Rome, Italy), Brugmann University Hospital (Brussels, Belgium), Helsinki University Hospital (Helsinki, Finland), Massachusetts General Hospital, Harvard Medical School (Boston, MA, USA), Uppsala University (Uppsala, Sweden), Fleni Institute (Buenos Aires, Argentina). The study was approved by the Ethics Committee of the Fondazione Policlinico A. Gemelli of Rome (ID601) and the local ethics committees of the participating centres.

The inclusion criteria of patients were (1) a definite diagnosis of SS; (2) being older than 18 years; and (3) the ability to give written informed consent. The diagnosis of SS was performed following the 2016 European Susac Consortium diagnostic criteria [13]. Thirteen patients were enrolled before the publication of the diagnostic criteria defined by the European Susac Consortium in 2016. All their medical records were carefully reviewed to confirm they all met the criteria [14]. The exclusion criteria were (1) other neurological disorders (e.g., previous stroke, multiple sclerosis, Parkinson's disease, neuropathies) and (2) severe comorbidities, including type 2 diabetes, cardiovascular disease, psychiatric, infectious, neoplastic and autoimmune diseases.

Individual clinical and paraclinical data were collected, including age, sex, age at onset, body mass index (BMI), disease duration at blood withdrawal, initial and subsequent clinical features, numbers of exacerbations, and treatment during the disease course. All patients had an ophthalmological workup including retinal fluorescein

angiography and inner ear examination. Vestibulocochlear involvement was defined as new tinnitus and/or sensorineural hearing loss, supported by an audiogram, and/or peripheral vertigo, supported by specific diagnostics. Brain involvement was documented by standardized neurological clinical examination and typical findings on intracranial magnetic resonance imaging, performed in 21/22 patients. CSF analysis was performed in 18/22 patients to exclude other diagnoses. SS patients were defined as relapsing if they presented with an acute worsening of existing symptoms, or new symptoms after 30 days of improvement or stable disease, and no evidence of any alternative explanation. The symptoms should have persisted for at least 24 h and should not be preceded or concurrent with infections or fever. Relapses were always confirmed by neurological examination and/or retinal fluorescein angiography. Patients were considered to be in remission after at least 3 months since the last relapse. Serum samples of SS patients were obtained at outpatient or inpatient clinical assessments. sNfL and sGFAP values of three patients, who had been previously evaluated during acute relapse, were then re-evaluated also in remission after a variable time interval.

Finally, serum samples from age- and sex-matched healthy controls (HCs) were collected by the University of Siena. They had no history of autoimmune, psychiatric or neurological diseases. Serum aliquots of SS patients and HCs were stored at -80°C until analysis.

Serum NfL and sGFAP single molecular array (SimoaTM) assay

Serum NfL and sGFAP concentrations were measured using SimoaTM assay Neurology 2-Plex B (GFAP, NfL) Assay Kit (Catalog #103520; Quanterix) run on the semi-automated ultrasensitive SR-X Biomarker Detection System (Quanterix). Samples were diluted at 1:4 and randomly distributed on 96-well plates. Quality control samples provided with the kit had concentrations within the predefined range and the coefficient of variance across the plates was $<10\%$. All samples were analysed blindly under alpha-numeric codes. The diagnostic codes were broken only after quality-control-verified NfL and GFAP concentrations were reported to the database manager.

Statistical analysis

Data were summarized as number of patients (percentage/frequency) and median (25th–75th percentiles). Group differences for normally distributed data were assessed using analysis of variance. Quantitative data were compared with the Fisher exact test. The Kolmogorov–Smirnov test was performed for the demonstration of normal distribution. sNfL and sGFAP values were skewed; therefore sGFAP and sNfL levels were log₁₀ transformed. Analysis of covariance was performed by analysing log₁₀sNfL and sGFAP levels as dependent variables, groups (SS patients and HCs; relapsing SS, SS patients in remission and HCs) as fixed variables, and age and BMI as covariates, to examine differences between sNfL and sGFAP levels between the groups. The importance of BMI and age, particularly when analysing sNfL concentrations, has been highlighted [14].

A value of $p < 0.05$ was considered statistically significant. Analysis results and graphs were generated with SPSS statistics (IBM SPSS V.26).

RESULTS

Blood samples of the 22 SS patients and the 59 HCs were collected and sNfL and sGFAP were assessed at the laboratory of the Centre of Precision and Translation Medicine, University of Siena, Italy. Demographic and clinical features of patients and HCs are summarized in Table 1.

The median age of HCs was 33 years (25th–75th percentiles, 28–52) and 37% were male. The median age of patients with SS was 35 years (25th–75th percentiles, 25.5–44; $p = 0.19$) and 27% were male. Age, gender and BMI did not significantly differ between relapsing patients and patients in remission with SS (relapsing SS, 30 years [25th–75th percentiles 19.5–37], 22% were male, BMI 23.5 kg/m^2 [25th–75th percentiles 20.25–25.5]; SS patients in remission, 37 years [25th–75th percentiles 32.5–47.5], 31% were male, BMI 25 kg/m^2 [25th–75th percentiles 22.5–26]) (Table 1). There was no statistical significance amongst relapsing SS patients, SS patients in remission and HCs in terms of sex and age. At the time of blood

TABLE 1 Demographic and clinical features of healthy controls (HCs), and patients with Susac syndrome (SS) in relapse and in remission included in the study.

	HCs	SS patients in relapse	SS patients in remission	p value
Number	59	9	13	
Sex (male %)	37%	25%	30%	NS
Age (years) (median, 25th–75th percentiles)	33, 28–52	30, 19.5–37	37, 32.5–47.5	NS
Percentage of patients with classic clinical triad at blood withdrawal	N/A	33%	31%	NS
Disease duration (median, 25th–75th percentiles)	N/A	2, 0.75–35.5	41, 30–67	NS
Number of relapses from the clinical onset (median, 25th–75th percentiles)	N/A	2, 1–4	3, 3–8.5	NS

Abbreviations: HCs, healthy controls; N/A, not applicable; NS, not significant; SS, Susac syndrome.

withdrawal, nine patients were experiencing a relapse of the disease, whilst 13 patients were in remission. Table 2 reports the detailed demographic features, sNFL and sGFAP values, and relapse/remission status of all SS patients. The detailed clinical features and drug treatments of all SS patients are summarized in Table 3.

Serum NFL and sGFAP levels in SS patients and HCs

Age- and BMI-corrected log₁₀sNFL levels were almost twice as high in SS patients (median log₁₀sNFL 1.46, 25th–75th percentiles 1.18–2.79) compared to HCs (median log₁₀sNFL 0.81, 25th–75th percentiles 0.69–0.91; $p < 0.001$). Age- and BMI-corrected sGFAP levels were slightly increased in patients with SS (median log₁₀sGFAP 1.85, 25th–75th percentiles 1.69–2.18) compared to HCs (median log₁₀sGFAP 1.76, 25th–75th percentiles 1.54–1.89; $p = 0.046$; Figure 1).

Serum NFL and sGFAP levels in patients with SS grouped for disease activity

When our cohort of SS patients was grouped for disease activity as relapsing patients or patients in remission, higher levels of both age- and

BMI-corrected sNFL and sGFAP were found in relapse than in remission (relapsing SS, median log₁₀sNFL 2.87, 25th–75th percentiles 2.03–3.22; SS patients in remission, median log₁₀sNFL 1.25, 25th–75th percentiles 1.03–1.46; $p = 0.008$; relapsing SS, median log₁₀sGFAP 2.16, 25th–75th percentiles 1.85–2.34; SS patients in remission, median log₁₀sGFAP 1.79, 25th–75th percentiles 1.65–1.89; $p = 0.013$).

Compared to HCs, SS patients showed increased age- and BMI-corrected sNFL levels in both relapse and remission ($p < 0.001$ for both). In contrast, age- and BMI-corrected sGFAP levels were higher than those of HCs only in relapsing SS patients ($p = 0.001$; Figure 2).

Interestingly, in three patients who were previously studied during relapse, sNFL and sGFAP levels were evaluated also in remission (see Table 2) and a clear reduction (range 38%–100%) was documented in all cases.

Association of sNFL and sGFAP levels in patients with clinical features of SS patients

Log₁₀sNFL levels showed a negative correlation with time from the last relapse ($r = -0.663$; $p = 0.001$; Figure 3). This correlation was not for sGFAP levels. Finally, no correlations were found between sNFL or sGFAP levels and disease duration.

TABLE 2 Detailed demographic features, sNFL and sGFAP values, and relapse/remission status of all Susac patients.

Patient	Origin	Sex	sGFAP (pg/mL)	sNFL (pg/mL)	Follow-up sGFAP (pg/mL, months)	Follow-up sNFL (pg/mL, months)	Relapse versus remission
Patient 1	Italy	F	186.09	2064.94			Relapse
Patient 2	Belgium	F	40.07	20.40			Remission
Patient 3	Belgium	F	18.89	14.53			Remission
Patient 4	Italy	M	49.03	10.69			Remission
Patient 5	Italy	F	82.53	125.07			Remission
Patient 6	USA	M	69.77	22.01			Remission
Patient 7	USA	F	98.34	28.97			Remission
Patient 8	Italy	F	245.04	742.61	71.68, 26	7.12, 26	Relapse
Patient 9	USA	M	73.05	15.38			Remission
Patient 10	Argentina	M	101.84	584.70			Relapse
Patient 11	Argentina	F	192.34	2018.20			Relapse
Patient 12	Argentina	F	86.93	17.09			Relapse
Patient 13	Argentina	F	143.45	993.60			Relapse
Patient 14	Argentina	F	57.26	208.01			Relapse
Patient 15	Finland	M	57.93	42.21			Remission
Patient 16	Finland	F	66.93	10.56			Remission
Patient 17	Finland	F	6.23	5.74			Remission
Patient 18	Finland	F	49.05	7.46			Remission
Patient 19	Italy	M	399.88	1416.20	34.8, 44	6.95, 44	Relapse
Patient 20	Italy	F	49.03	53.94	77.68, 6	33.54, 6	Relapse
Patient 21	Sweden	F	240.02	28.06			Remission
Patient 22	Sweden	F	61.33	17.80			Remission

Abbreviations: F, female; M, male; sGFAP, serum glial fibrillary acidic protein; sNFL, serum level of neurofilament light chain.

TABLE 3 Detailed clinical features and drug treatments of all Susac patients.

Patient	Disease duration at blood draw (months)	Time between blood draw and the last relapse (months)	Clinical triad at blood draw	Other neurological symptoms	Visual disturbances at onset	BRAO	Hearing loss at onset	Previous treatments	Immunosuppressive therapy at blood draw
Patient 1	9	0	No	Working memory deficit, language disturbance, ataxia	No	Yes	No	IV methylprednisolone; oral prednisone, IV cyclophosphamide, IVIg	Yes (PO steroids)
Patient 2	118	10	Yes	Headache, upper limb paresthesia, intermittent vertigo, memory loss, loss of concentration	Yes	Yes	Yes	IV methylprednisolone; PO methylprednisolone; azathioprine; cyclosporine; infliximab and AZA	Yes (PO steroids)
Patient 3	37	40	Yes	Headache, nausea, confusion, generalized hyposthenia, dysarthria, dyscalculia	Yes	Yes	Yes	IV methylprednisolone; PO methylprednisolone; mycophenolate mofetil; cyclophosphamide; plasmapheresis; infliximab; MTX	Yes (PO steroids)
Patient 4	20	20	No	Attentive disturbances; 4 limb paresthesia, ataxia	Yes	Yes	No	IV methylprednisolone; IV cyclophosphamide, AZA; aspirin; high dose vitamin D	Yes (AZA)
Patient 5	30	4	No	Language dysfunction; attentive disturbances, ataxia	Yes	Yes	No	IV methylprednisolone; prednisone; cyclosporine; aspirin	Yes (cyclosporine)
Patient 6	42	Not available	No	Confusion, walking difficulties, syncopal spell, nausea, anorexia, headache, ataxia	No	Yes	Yes	IVIg; rituximab; prednisone; mycophenolate mofetil	Yes (PO steroids, mycophenolate mofetil)
Patient 7	50	49	No	Headache, nausea, vomiting, 4 limb paresthesia, numbness of left cheek, chin and both hands, malaise, vertigo, attentive disturbances, perseveration, ataxia	Yes	Yes	Yes	Mycophenolate mofetil	Yes (mycophenolate mofetil)
Patient 8	65	0	Yes	Headache, ataxia	Yes	Yes	Yes	IV methylprednisolone; IVIg; PO steroids; aspirin; rituximab	Yes (PO steroids, rituximab)
Patient 9	38	14	No	Headache, inappropriate behaviour, memory loss, perseveration, nausea, ataxia	Yes	Yes	Yes	IV methylprednisolone; IV cyclophosphamide; IVIg; prednisone; mycophenolate mofetil	Yes (mycophenolate mofetil)

TABLE 3 (Continued)

Patient	Disease duration at blood draw (months)	Time between blood draw and the last relapse (months)	Clinical triad at blood draw	Other neurological symptoms	Visual disturbances at onset	BRAO	Hearing loss at onset	Previous treatments	Immunosuppressive therapy at blood draw
Patient 10	2	1	No	Confusion, headache, hemiparesis, aphasia, cognitive impairment	No	Yes	No	IVlg, IV cyclophosphamide; high dose steroids; rituximab	No
Patient 11	2	1.5	No	Vertigo, nausea, vomiting, confusion, headache, ataxia	No	Yes	No	High dose steroids, mophetil micophenolate	No
Patient 12	0.5	3	No	Headache, confusion	Yes	Yes	No	High dose steroids, mophetil micophenolate, cyclophosphamide, rituximab	No
Patient 13	0.5	1	No	Cognitive impairment, confusion, somnolence, tinnitus	No	Yes	No	High dose steroids, mophetil micophenolate	No
Patient 14	1	0	No	Vertigo, headache, confusion, cognitive impairment, ataxia	No	Yes	Yes	IVlg; cyclophosphamide; high dose steroids	No
Patient 15	144	21	Yes	Headache (migraine with aura)	No	Yes	Yes	LMWH, methotrexate	Yes (PO prednisolone, IVlg, mycofenolate mofetil)
Patient 16	72	43	No	No	Yes	Yes	No	Aspirin, IVlg	Yes (IVlg)
Patient 17	60	13	Yes	Headache (migraine with aura)	No	Yes	Yes	Aspirin, prednisolone, IVlg, mycofenolate mofetil	Yes (PO prednisolone, IVlg, mycofenolate mofetil)
Patient 18	24	6	No	No	Yes	Yes	No	0	No
Patient 19	59	0	Yes	No	No	Yes	Yes	Steroids, AZA	Yes (PO steroids)
Patient 20	12	0	Yes	Inappropriate behaviour	No	Yes	Yes	PO steroids, IV methylprednisolone, rituximab	Yes (PO steroids)
Patient 21	341	65	No	Headache (migraine with aura)	Yes	Yes	Yes	PO steroids	Yes (PO steroids)
Patient 22	41	41	No	Headache (migraine with aura)	Yes	Yes	Yes	PO steroids, AZA	Yes (PO steroids and AZA)

Note: Clinical triad has been defined according to the European Susac Consortium diagnostic criteria [23] when the patient presented with the unequivocal clinical and/or paraclinical involvement of all three main organs.
 Abbreviations: AZA, azathioprine; BRAO, branch retinal artery occlusion; F, female; IV, intravenous; IVlg, intravenous immunoglobulins; LMWH, low molecular weight heparin; M, male; MTX, methotrexate; N/A, not applicable; PO, per os.

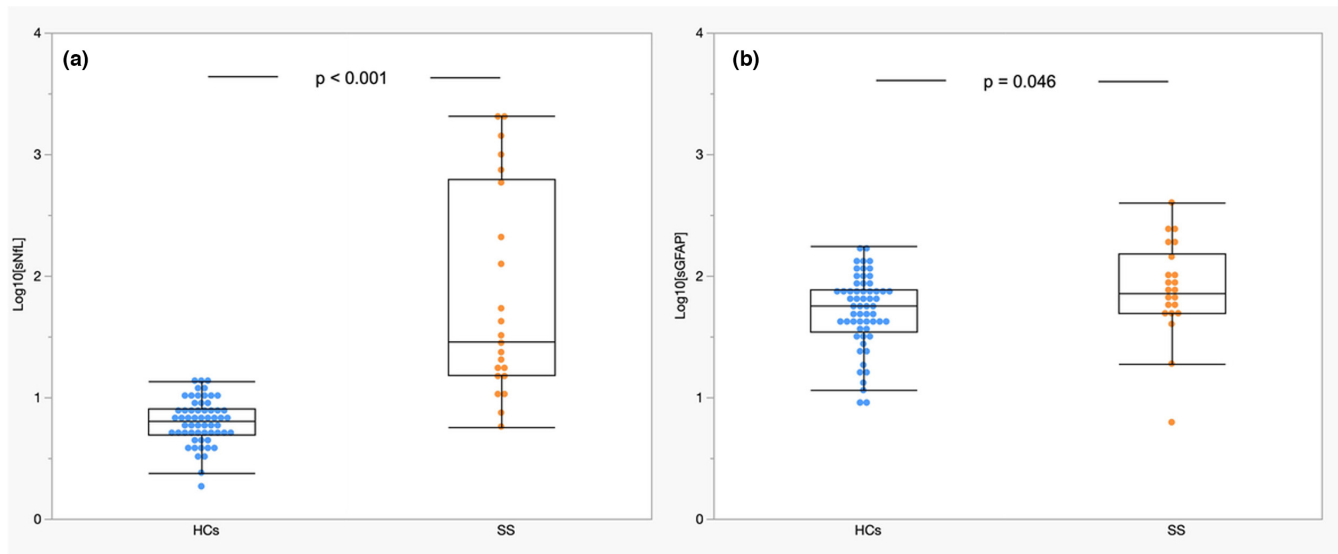


FIGURE 1 Log₁₀sNfL and log₁₀sGFAP values in healthy controls and patients with Susac syndrome. Log₁₀ serum levels of neurofilament light chain (sNfL) values (a), log₁₀ serum glial fibrillary acidic protein (sGFAP) values (b) in healthy controls (HCs) and patients with Susac syndrome (SS). Box plots express the first (Q1) and third (Q3) quartiles by the upper and lower horizontal lines in a rectangular box, in which there is a horizontal line showing the median. The whiskers extend upwards and downwards to the highest or lowest observation within the upper (Q3+1.5×IQR) and lower (Q1-1.5×IQR) limits. *p* values indicate statistical significance between the different groups. IQR, interquartile range.

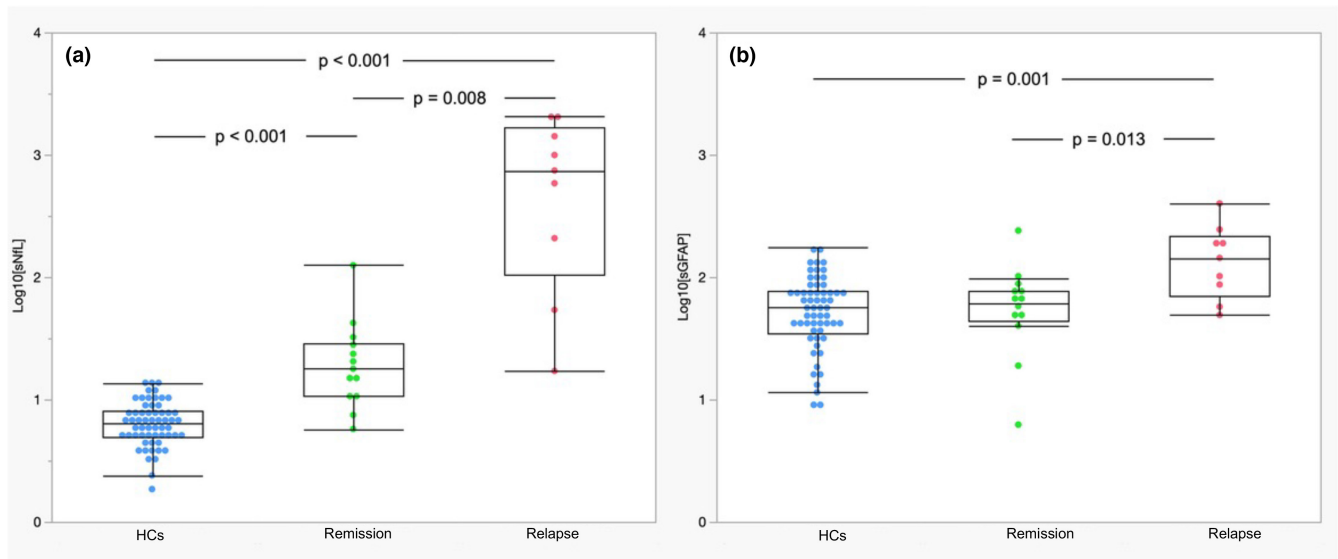


FIGURE 2 Log₁₀sNfL and log₁₀sGFAP values in healthy controls and in patients in remission and relapse with Susac syndrome. Log₁₀ serum levels of neurofilament light chain (sNfL) values (a), log₁₀ serum glial fibrillary acidic protein (sGFAP) values (b) in healthy controls (HCs) and in patients with Susac syndrome (SS) in relapse and in remission. Box plots express the first (Q1) and third (Q3) quartiles by the upper and lower horizontal lines in a rectangular box, in which there is a horizontal line showing the median. The whiskers extend upwards and downwards to the highest or lowest observation within the upper (Q3+1.5×IQR) and lower (Q1-1.5×IQR) limits. *p* values indicate statistical significance between the different groups. IQR, interquartile range.

DISCUSSION

In this cross-sectional cohort study, increased levels of sNfL and sGFAP were found in SS patients in comparison with HCs.

Interestingly, subgroup analyses showed that both sNfL and sGFAP levels were higher in relapsing patients than in SS patients in remission, suggesting the clinical relevance of these biomarkers of neuroaxonal damage and astrocytic activation in SS.

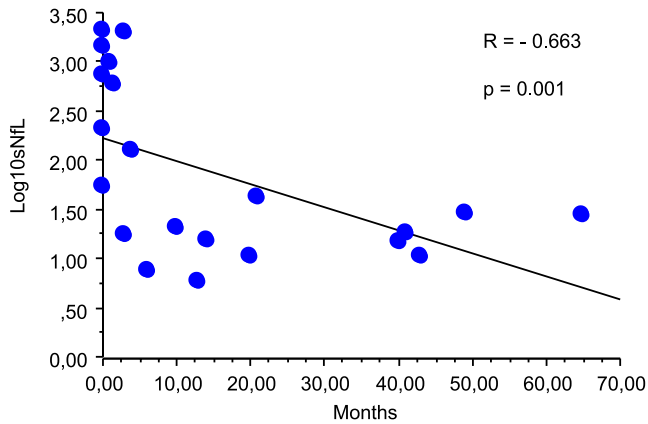


FIGURE 3 Correlation between log₁₀sNFL levels with the time between blood draw and the last relapse in patients with Susac syndrome. Correlation between log₁₀ serum levels of neurofilament light chain (sNFL) levels with the time between blood draw and the last relapse (in months) in patients with Susac syndrome. *p* and *r* values obtained by the Spearman correlation test are indicated.

Susac syndrome (SS) is considered an organ-specific immune-mediated endotheliopathy [1]. The limited available histopathological evidence demonstrates vascular changes characterized by endothelial necrosis, capillary wall thickening, endothelial swelling and collagen deposition [15–18]. The immune response seen in histopathological specimens of SS patients is usually limited and remains to be confirmed; therefore SS has been referred to as ‘pauci-inflammatory angiopathy’ [19]. Other studies support the role of cytotoxic CD8+ T cells in the pathogenesis of SS, adhering to the microvasculature within the CNS, promoting the apoptosis of the endothelial cells and the focal damage of the blood–brain barrier [18, 20]. The final result of this immune-mediated damage of the microvasculature of the brain, retina and inner ear is vessel occlusion with microinfarcts and atrophy diffusely distributed throughout the white and grey matter of the CNS [6, 15, 18, 20].

Overall, the patterns of the relative increase in sGFAP and sNFL levels in the acute stage found here in SS patients seem distinctive compared to other diseases. Specifically, in our cohort of relapsing SS patients, the levels of sNFL tended to be very high with a modest increase in sGFAP. This can be due to more prominent neuro-axonal damage and a minor astrocytic involvement occurring in SS and is very different, for example, to the pattern seen in neuromyelitis optica spectrum disease (NMOSD) patients [21] where the large elevation of sGFAP levels during relapses is consistent with the pathogenic mechanism of primary astrocytopathy [22]. Furthermore, our SS patients’ sNFL values are much higher than those reported for multiple sclerosis patients [14] and aquaporin-4 IgG+ NMOSD [23–25], which may represent an important clue towards the prominent neuro-axonal degeneration that occurs in SS. In contrast, levels of sGFAP were comparable between SS patients in remission and HCs. This is similar to what has already been shown both in aquaporin-4 IgG+ NMOSD [26] and in cerebral small vessel disease [27].

Finally, a negative correlation was found between sNFL levels and the time from the last relapse. These time-sensitive changes in sNFL levels are similar to those reported in multiple sclerosis, where sNFL levels appear to remain elevated for up to 3 months and then decrease [28], and to stroke where sNFL levels show a peak at the third week and then decrease [29].

A relatively large cohort was studied here for such a rare disease, which allowed for the first time an assessment of the clinical relevance of blood biomarkers in SS. However, our study is not without limitations. The main limitation lies in the cross-sectional and retrospective design of the study with limited clinical follow-up information. Moreover, it was not possible here to analyse NFL and GFAP levels in the CSF, which would have lent support to our serum findings.

In summary, it was found that sNFL and, to a lesser extent, sGFAP may be reliable and clinically relevant biomarkers of neuro-axonal damage and astrocytic activation. Larger and longitudinal studies are needed to validate our findings and establish whether sNFL and sGFAP are reliable biomarkers for monitoring patients with SS in the clinical setting.

AUTHOR CONTRIBUTIONS

Domenico Plantone: Conceptualization; investigation; writing—original draft; methodology; validation; writing—review and editing; formal analysis; project administration; resources; visualization; data curation. Eleonora Sabatelli: Conceptualization; methodology; investigation; writing—original draft; writing—review and editing; project administration; data curation; resources. Sara Locci: Investigation; methodology; data curation; visualization; validation. Mariano Marrodan: Investigation; writing—original draft; methodology; writing—review and editing; data curation. Sini M. Laakso: Conceptualization; writing—original draft; investigation; methodology; data curation. Farrah J. Mateen: Data curation; methodology; conceptualization; investigation; writing—original draft; writing—review and editing. Amalia Feresiadou: Conceptualization; writing—original draft; investigation; methodology; data curation; writing—review and editing. Tom Buelens: Writing—review and editing; writing—original draft; investigation; conceptualization; methodology; visualization; data curation. Assunta Bianco: Data curation; writing—review and editing; writing—original draft; investigation; conceptualization; methodology. Marcela P. Fiol: Conceptualization; investigation; writing—original draft; methodology; writing—review and editing; data curation. Jorge Correale: Conceptualization; investigation; methodology; data curation; writing—original draft. Pentti Tienari: Conceptualization; investigation; writing—original draft; methodology; data curation. Paolo Calabresi: Conceptualization; investigation; writing—original draft; methodology; data curation. Nicola De Stefano: Conceptualization; investigation; writing—original draft; methodology; data curation; supervision; project administration; visualization; validation; writing—review and editing; resources. Raffaele Iorio: Conceptualization; supervision; data curation; project administration; formal analysis; methodology; validation; visualization; writing—review and editing; writing—original draft; investigation; resources.

ACKNOWLEDGEMENTS

D.P. and N.D.S. are members of the European Reference Network for Rare Neurological Diseases.

FUNDING INFORMATION

This study received no funding.

CONFLICT OF INTEREST STATEMENT

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Domenico Plantone  <https://orcid.org/0000-0001-6666-7244>

Mariano Marrodan  <https://orcid.org/0000-0002-4142-8375>

Farrah J. Mateen  <https://orcid.org/0000-0002-4293-8115>

Assunta Bianco  <https://orcid.org/0000-0002-7101-0949>

Marcela P. Fiol  <https://orcid.org/0000-0003-0879-1831>

Jorge Correale  <https://orcid.org/0000-0003-4756-9889>

Pentti Tienari  <https://orcid.org/0000-0001-5686-2900>

Raffaele Iorio  <https://orcid.org/0000-0002-6270-0956>

REFERENCES

- Marrodan M, Fiol MP, Correale J. Susac syndrome: challenges in the diagnosis and treatment. *Brain*. 2022;145:858-871.
- Dörr J, Krautwald S, Wildemann B, et al. Characteristics of Susac syndrome: a review of all reported cases. *Nat Rev Neurol*. 2013;9(6):307-316.
- Redler Y, Chwalisz BK. Neuro-ophthalmic manifestations of Susac syndrome. *Curr Opin Ophthalmol*. 2020;31(6):495-502.
- Liu Y, Wang CW, Sung YF, Yang FC. Sudden onset hearing loss as initial presentation of Susac syndrome: a rare case report and brief review. *Neurol Sci*. 2022;43(1):683-686.
- Marrodan M, Correale J, Alessandro L, et al. Susac syndrome: a differential diagnosis of white matter lesions. *Mult Scler Relat Disord*. 2017;15:42-46.
- Greco A, De Virgilio A, Gallo A, et al. Susac's syndrome—pathogenesis, clinical variants and treatment approaches. *Autoimmun Rev*. 2014;13(8):814-821.
- Susac JO, Egan RA, Rennebohm RM, Lubow M. Susac's syndrome: 1975–2005 microangiopathy/autoimmune endotheliopathy. *J Neurol Sci*. 2007;257(1–2):270-272.
- Kuhle J, Barro C, Andreasson U, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. *Clin Chem Lab Med*. 2016;54:1655-1661.
- Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol*. 2018;14(10):577-589.
- Fyfe I. Neurofilament light chain—new potential for prediction and prognosis. *Nat Rev Neurol*. 2019;15(10):557.
- Abdelhak A, Foschi M, Abu-Rumeileh S, et al. Blood GFAP as an emerging biomarker in brain and spinal cord disorders. *Nat Rev Neurol*. 2022;18(3):158-172.
- Lumpkins KM, Bochicchio GV, Keledjian K, Simard JM, McCunn M, Scaea T. Glial fibrillary acidic protein is highly correlated with brain injury. *J Trauma*. 2008;65(4):778-782.
- Kleffner I, Dörr J, Ringelstein M, et al. Diagnostic criteria for Susac syndrome. *J Neurol Neurosurg Psychiatry*. 2016;87(12):1287-1295.
- Benkert P, Meier S, Schaedelin S, et al. Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. *Lancet Neurol*. 2022;21(3):246-257.
- Hardy TA, O'Brien B, Gerbis N, et al. Brain histopathology in three cases of Susac's syndrome: implications for lesion pathogenesis and treatment. *J Neurol Neurosurg Psychiatry*. 2015;86(5):582-584.
- Fox RJ, Costello F, Judkins AR, et al. Treatment of Susac syndrome with gamma globulin and corticosteroids. *J Neurol Sci*. 2006;251(1–2):17-22.
- Heiskala H, Somer H, Kovanen J, Poutiainen E, Karli H, Haltia M. Microangiopathy with encephalopathy, hearing loss and retinal arteriolar occlusions: two new cases. *J Neurol Sci*. 1988;86(2–3):239-250.
- Agamanolis DP, Prayson RA, Asdaghi N, Gultekin SH, Bigley K, Rennebohm RM. Brain microvascular pathology in Susac syndrome: an electron microscopic study of five cases. *Ultrastruct Pathol*. 2019;43(6):229-236.
- Magro CM, Poe JC, Lubow M, Susac JO. An organ-specific autoimmune endotheliopathy syndrome associated with anti-endothelial cell antibodies. *Am J Clin Pathol*. 2011;136(6):903-912.
- Gross CC, Meyer C, Bhatia U, et al. CD8+ T cell-mediated endotheliopathy is a targetable mechanism of neuro-inflammation in Susac syndrome. *Nat Commun*. 2019;10(1):5779.
- Watanabe M, Nakamura Y, Michalak Z, et al. Serum GFAP and neurofilament light as biomarkers of disease activity and disability in NMOSD. *Neurology*. 2019;93(13):e1299-e1311.
- Aktas O, Smith MA, Rees WA, et al. Serum glial fibrillary acidic protein: a neuromyelitis optica spectrum disorder biomarker. *Ann Neurol*. 2021;89(5):895-910.
- Zhang TX, Chen JS, Du C, et al. Longitudinal treatment responsiveness on plasma neurofilament light chain and glial fibrillary acidic protein levels in neuromyelitis optica spectrum disorder. *Ther Adv Neurol Disord*. 2021;14:17562864211054952.
- Chang X, Huang W, Wang L, et al. Serum neurofilament light and GFAP are associated with disease severity in inflammatory disorders with aquaporin-4 or myelin oligodendrocyte glycoprotein antibodies. *Front Immunol*. 2021;12:647618.
- Kim H, Lee EJ, Kim S, et al. Serum biomarkers in myelin oligodendrocyte glycoprotein antibody-associated disease. *Neurol Neuroimmunol Neuroinflamm*. 2020;7(3):e708.
- Hyun JW, Kim Y, Kim SY, Lee MY, Kim SH, Kim HJ. Investigating the presence of interattack astrocyte damage in neuromyelitis optica spectrum disorder: longitudinal analysis of serum glial fibrillary acidic protein. *Neurol Neuroimmunol Neuroinflamm*. 2021;8(3):e965.
- Gattringer T, Enzinger C, Pinter D, et al. Serum glial fibrillary acidic protein is sensitive to acute but not chronic tissue damage in cerebral small vessel disease. *J Neurol*. 2023;270(1):320-327.
- Rosso M, Gonzalez CT, Healy BC, et al. Temporal association of sNfL and Gad-enhancing lesions in multiple sclerosis. *Ann Clin Transl Neurol*. 2020;7(6):945-955.
- Pujol-Calderón F, Portelius E, Zetterberg H, Blennow K, Rosengren LE, Höglund K. Neurofilament changes in serum and cerebrospinal fluid after acute ischemic stroke. *Neurosci Lett*. 2019;698:58-63.

How to cite this article: Plantone D, Sabatelli E, Locci S, et al. Clinically relevant increases in serum neurofilament light chain and glial fibrillary acidic protein in patients with Susac syndrome. *Eur J Neurol*. 2023;30:3256-3264. doi:[10.1111/ene.15939](https://doi.org/10.1111/ene.15939)