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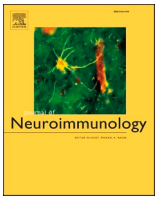

Inflammatory profiles in plasma and cerebrospinal fluid of patients with neurosarcoidosis

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ABSTRACT

Methods: Cerebrospinal fluid (CSF) and plasma levels of 38 biomarkers from 20 neurosarcoidosis (NS) patients were compared to healthy controls (HC).
Results: In CSF, 25 biomarkers were significantly elevated compared to HC: IFNγ, TNFα, TNFβ, IL-2, IL-6, IL-10, IL-12B, IL-15, IL-16, CCL2, CCL3, CCL4, CCL11, CCL13, CCL17, CCL22, CCL26, CXCL8, CXCL10, TNFR2, VEGF-A, PIGF, SAA, VCAM1, and ICAM1.
In plasma, 12 biomarkers were significantly elevated compared to HC: IFNγ, TNFα, CCL2, CCL3, CCL4, CCL17, CXCL10, VEGFR1, PIGF, SAA, VCAM1, and ICAM1.
Conclusion: NS patients have profoundly elevated cytokines, chemokines, vascular angiogenesis, and vascular injury biomarkers in CSF and plasma.

1. Introduction

The histological hallmark of sarcoidosis is the presence of highly organized, non-caseating epithelioid granulomas that consist of epithelioid cells and multinucleated giant cells, formed from macrophages. Also seen are clusters of differentiation 4^+ ($CD4^+$) T helper (Th) lymphocytes and a few $CD8^+$ T lymphocytes in the periphery (American Thoracic Society, 1999).

The pathogenesis of sarcoidosis is still unclear but is characterized by a dysregulated, multifaceted inflammatory response. On activation, $CD4^+$ Th cells differentiate into interferon (IFN)γ- and interleukin (IL)-2-producing Th1 cells, IL-17A-producing Th17 cells, and IFNγ-producing Th17.1 cells (Grunewald et al., 2019; Zhou and Arce, 2020). The increased IFNγ levels contribute to the activation of macrophages followed by an increase in the synthesis of multiple cytokines and chemokines, e.g., tumor necrosis factor α (TNFα), IL-12, IL-18, C-C motif chemokine ligand 2 (CCL2), CCL3, CCL4, C-X-C motif chemokine ligand 9 (CXCL9), CXCL10, and CXCL11 (Grunewald et al., 2019; Su et al., 2013; Zhou and Arce, 2020; Ziegenhagen and Muller-Quernheim, 2003). TNFα is involved in developing and maintaining granuloma (Fehrenbach et al., 2003; Kindler et al., 1989). IL-12 contributes to the differentiation of Th1 (Hsieh et al., 1993) and Th17.1 cells (Arger et al., 2020; Lexberg et al., 2010). The cytokines are part of the recruitment of new cells to the inflamed area (Deshmane et al., 2009; Nishioka et al., 2007; Taub et al., 1993). Furthermore, T-regulatory (Treg) cells known to downregulate inflammation are altered in their ability to reduce inflammation (Idali et al., 2008; Miyara et al., 2006; Taflin et al., 2009).

While the pathogenesis of extra-neurological sarcoidosis is often

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investigated, less is known about the inflammation of NS due to the rarity of the disease and the limited access to diagnostic material in the form of biopsy material and cerebrospinal fluid (CSF). However, as in extra-neurological sarcoidosis, non-caseating epithelioid granulomas are present in histopathological CNS biopsies in NS (Herring and Urich, 1969; Jachiet et al., 2018; Saleh et al., 2006), and NS patients have blood-brain barrier (BBB) disruption (McLean et al., 1995).

Since NS and ocular sarcoidosis exist as an independent phenotype in cluster analysis (Lhote et al., 2021; Rubio-Rivas and Corbella, 2020; Schupp et al., 2018), specific underlying inflammation or other CNS cells (Orihuela et al., 2016; Rothhammer and Quintana, 2015) may be involved.

This study aimed to investigate the underlying inflammatory biomarkers in NS patients by using multiplex analytical assays. Our objective was to compare the CSF and plasma levels of cytokines, chemokines, and other inflammatory markers between NS patients and healthy controls (HC).

2. Material and methods

2.1. Study population

In this cross-sectional study, we consecutively recruited patients from the neurology and rheumatology departments at Odense University Hospital between January 2016 and August 2020.

The inclusion criteria were adult patients (>18 years) who fulfilled the criteria for "highly probable" NS according to the World Association of Sarcoidosis and Other Granulomatous Diseases (Judson et al., 2014b). To focus on the CNS disease, NS patients with polyneuropathy and diseases that could cause similar symptoms to NS were excluded. NS patients with CNS disease and small fiber neuropathy were accepted (Byg et al., 2021). The neurology departments at Odense University Hospital recruited healthy controls (HC) among patients admitted with unspecific symptoms without any signs of neurological or systemic inflammatory diseases and with normal CSF and MRI scans. Some of the baseline data have been published previously (Byg et al., 2021).

2.2. Sample collection

CSF and plasma were collected at the time of inclusion. Whole blood was obtained using 4 mL BD Vacutainer EDTA tubes, centrifuged 10 min at 2000 xg, plasma aliquoted using 2 mL Sarstedt polypropylene tubes, and stored at -80 °C until further analyses. CSF was collected through lumbar puncture into 10 mL Sarstedt polypropylene tubes, centrifuged 20 min at 450 xg, aliquoted in 2 mL Sarstedt polypropylene tubes, and stored at -80 °C until further analyses.

2.3. Blood, plasma, and CSF examination

Leukocyte count, leukocyte differential counts, C-reactive protein (CRP), CSF cell counts, CSF protein, CSF/plasma albumin quotient, and CSF oligoclonal bands were routinely analyzed at Odense University Hospital.

A CSF/plasma albumin quotient at 8×10^{-3} or above was considered elevated.

For each biomarker a biomarker index is calculated by the biomarker CSF/plasma quotient divided by CSF/serum albumin quotient.

Flow-cytometric examination of the complete lymphocyte subset panel (TBNK analysis) subpopulations was performed using a BD FACSCanto™ II flow cytometer with BD FACSDiva software at Odense University Hospital. In brief, cells were stained using BD Multitest 6-color TBNK reagent with optional BD Trucount tubes. A total of 50,000 events were collected to identify and determine the percentages and absolute counts of $CD3^+$ T cells, $CD3^+CD4^+$ Th cells, $CD3^+CD8^+$ cytotoxic T cells, $CD3^+CD19^+$ B cells, and $CD3^-CD16/56^+$ NK cells. The lymphocyte gate was set to only include a well-defined population of $CD45^{high}$ cells with low side scatter expression.

2.4. MRI scans

The MRI was graded by the number of affected anatomical sites visualized by post-contrast enhanced lesions. Supratentorial meningeal enhancement, infratentorial meningeal enhancement, parenchymal enhancement, and enhancing lesions in the cervical spine, thoracic spine, lumbar spine, or cauda equina give one point each. A score of 0 points was graded no enhancement, 1–2 points were graded mild enhancement, and a score of 3–6 points was graded moderate/severe enhancement (Byg et al., 2021).

2.5. Electrochemiluminescence analysis

Plasma and CSF levels of 38 potential biomarkers (IFNγ, TNFα, TNFβ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL12B, IL13, IL-15, IL-16, IL-17A, tumor necrosis factor receptor 1 (TNFR1), TNFR2, chemokine C-C motif ligand 2 (CCL2) (MCP-1), CCL3 (MIP-1α), CCL4 (MIP-1β), CCL11 (eotaxin-1), CCL13 (MCP-4), CCL17 (TARC), CCL22 (MDC), CCL26 (eotaxin-3), CXCL8 (IL-8), CXCL10 (IP10), vascular endothelial growth factor (VEGF)-A, VEGF-C, VEGF-D, VEGF receptor 1 (VEGFR1), tyrosine kinase 2 (Tie-2), placental growth factor (PIGF), basic fibroblast growth factor (bFGF), serum amyloid A (SAA), cell adhesion molecules vascular 1 (VCAM1), and cell adhesion molecules intercellular 1 (ICAM1) were measured by electrochemiluminescence multiplex analysis (MesoScale Diagnostic, Rockville, MD, US) using a V-PLEX Human Biomarker Plex kit, a human TNF-RI Ultra-Sensitive kit, and a human TNF-RII Ultra-Sensitive kit, according to the manufacturer's instructions. The total coefficient of variation was <20%. Samples below the assay detection limit were set to 50% of the detection limit.

For each biomarker, the fold elevation is calculated by the median value in NS patients divided by the median value in healthy controls.

2.6. Statistical analysis

Categorical variables are reported as counts (n) and proportions (%). Continuous data are presented as the median and interquartile range (IQR) or mean and standard deviation (SD). For statistical analysis, the biomarkers were first grouped into their type of function in the inflammation processes; cytokines, chemokines, TNFR1 and TNFR2, vascular angiogenesis, and injury biomarkers. Second, the Wilcoxon rank-sum test with Bonferroni-Holms correction for multiple testing was used to test between NS patients and HC in each group. Spearman's rank correlation test was used to analyze possible associations between CSF and plasma levels, and the Kruskal-Wallis test was used to compare groups. All analyses were performed in Stata 16.1 (StataCorp LCC, College Station, TX, USA). A *P*-value below 0.05 was considered statistically significant.

2.7. Ethical approval

The study was approved by the Regional Committee of Health Research Ethics for Southern Denmark (ID: S-20120066, S-20140051) and was reported to the Danish Data Protection Agency (ID: 15/3744).

2.8. Data availability statement

The data underlying this article will be shared at a reasonable request to the corresponding author and with approval from the Committee of Health Research Ethics, Southern Denmark.

3. Results

3.1. Patient population

We included 20 NS patients and 11 HC (Table 1). The median age of 51.6 years in NS patients was significantly higher than 37.0 years in HC ($p = 0.03$). The percentage of females was significantly lower in NS patients than in HC ($p = 0.03$). The median duration of symptoms in NS patients was eight months before inclusion, and four patients were on immunosuppressive medication. None of the NS patients had severe comorbidity (Byg et al., 2021). Eighteen patients (90%) had systemic sarcoidosis, and 2 (10%) had isolated NS. The most frequent symptoms were headache (60%), vertigo (55%), and tinnitus (50%). Forty percent of patients had chest symptoms, ocular symptoms (30%), and skin rash (35%). On neurological examination, the findings were peripheral sensory signs (50%), peripheral motor signs (40%), and cranial nerve involvement (30%). Two patients (10%) had more than one cranial nerve involved.

3.2. Baseline MRI, blood, and CSF characteristics

The MRI scans of the CNS were abnormal in 12 NS patients, of which two displayed cerebral abnormalities, three spinal cord abnormalities, and seven revealed cerebral and spinal cord abnormalities. In the contrast-enhancing lesions on MRI, eight patients had no enhancement, five patients were grouped as mild enhancement, and seven patients were grouped as moderate/severe enhancement. (Byg et al., 2021).

We initially estimated total blood leukocyte count and blood leukocyte differential counts using flow cytometry (Table 2). The total leukocyte count in the blood was comparable between NS patients and healthy controls (HC) ($p = 0.8$), as was the total number of neutrophils ($p = 0.5$), monocytes ($p = 0.3$), and basophils ($n = 0.8$). In contrast, the total lymphocyte count was significantly lower in NS patients compared to HC ($p = 0.0007$), demonstrating an impaired peripheral lymphocyte response in NS patients.

On TBNK analysis (Table 2), the median number of $CD4^+$ T cells, $CD8^+$ T cells, B cells, and NK cells was lower than in the normal population (McNerlan et al., 1999): $CD4^+$ T cells 605 × 10^6/L in NS patients vs. 780 × 10^6/L in the normal population, $CD8^+$ T cells 290 × 10^6/L in NS patients vs. 400 × 10^6/L, B cells 185 × 10^6/L in NS patients vs. 210 × 10^6/L, and NK cells 155 × 10^6/L in NS patients vs. 200 × 10^6/L (McNerlan et al., 1999).

We observed no significant differences in CRP levels between NS patients and HC ($p = 0.4$) (Table 2).

The CSF was abnormal in 19 NS patients; 16 patients had pleocytosis (median 22.5 × 10^6 cells/L, IQR 18.0–40.4), and 17 patients had elevated protein levels (median 0.59 g/L, IQR 0.55–0.99) (Table 2) (Byg et al., 2021). Furthermore, 60% of NS patients had a CSF/plasma albumin quotient of 8 × 10^{-3} or over.

In the CSF of NS patients, the T cell population constituted median 88.5% (IQR 79.1–91.5%) of the white blood cells, with a CD4/CD8 ratio of median 3.3 (IQR 2.6–5.2). B cells constituted median 0.7% (IQR 0.2–2.0%) of the white blood cells, monocytes median 0.88% (IQR 0.3–3.6%), granulocytes median 0.2% (IQR 0.0–2.3%), and NK cells median 2.3% (IQR 1.3–5.5%).

Table 1
Clinical data at baseline for patients with neurosarcoidosis and healthy controls.

	Neurosarcoidosis	Healthy controls	P-value[a]
N	20	11	
Age, years	51.6 (43.0–56.4)	37.0 (25.8–44.5)	0.03
Female (%)	9 (45)	10 (91)	0.03
Duration of symptoms, months	8 (4–16)		
Immunosuppression			
Glucocorticosteroid	4 (20)	0	
Methotrexate/Azathioprine	2 (10)	0	

Values are median, interquartile (IQR), or count (percentage). [a] Wilcoxon rank-sum test.

Table 2
Baseline paraclinical characteristics in plasma and cerebrospinal fluid.

	Neurosarcoidosis	Healthy controls	P-value[c]
N	20	11	
Leukocytes[a]	7.5 (3.0)	7.7 (3.5)	0.8
Neutrophilocytes[a]	5.2 (2.9)	4.4 (3.0)	0.5
Lymphocytes[a]	1.4 (0.6)	2.6 (1.1)	0.0007
Monocytes[a]	0.6 (0.3)	0.5 (0.2)	0.3
Basophilocytes[a]	0.02 (0.03)	0.02 (0.03)	0.8
T-lymphocytes CD4 + [a]	605 (415, 905)	780[f]	
T-lymphocytes CD8 + [a]	290 (95, 455)	400[f]	
B-lymphocytes CD19 + [a]	185 (110, 205)	210[f]	
Neutral killer cells[a]	155 (90, 205)	200[f]	
C-reactive protein[b]	7.9 (14.5)	2.7 (2.5)	0.4[d]
Cerebrospinal fluid (CSF)			
Pleocytosis, N	16		
Cells x 10^6/L	22.5 (18.0, 40.4)		
Protein elevation, N	17		
g/L	0.59 (0.55, 0.99)		
CSF/plasma albumin quotient x 10^{-3}	8.2 (7.3, 15)		

Values are in mean and standard division (SD) or median and interquartile range (IQR). [a] 10^6 cells/L. [b] g/mL. [c] *t*-test. [d] Wilcoxon rank-sum test. [f] from ref. (McNerlan et al., 1999).

3.3. Cytokines in CSF and plasma

We analyzed CSF and plasma levels of 16 cytokines in NS patients and compared these to HC (Fig. 1 and Tables 3 & 4).

In CSF, the levels of nine cytokines (IFNγ, TNFα, TNFβ, IL-2, IL-6, IL-10, IL-12B, IL-15, and IL-16) were significantly increased in NS patients compared to HC (Fig. 1 and Table 3). Compared to HC, we found a significant increase in CSF levels in NS patients of IFNγ ($p = 0.001$), TNFα ($p = 0.03$), TNFβ ($p = 0.03$), IL-2 ($p = 0.006$), IL-6 ($p = 0.001$), IL-10 ($p = 0.04$), IL-12B ($p = 0.001$), IL-15 ($p = 0.001$), and IL-16 ($p = 0.001$).

In addition (Table 3), CSF IFNγ and IL12B had the highest fold elevation in NS patients compared to HC. All cytokines had a high biomarker index (Table 3), and none of the cytokines displayed any significant difference by the extent of contrast enhancement on MRI (Table 5).

In plasma, only IFNγ ($p = 0.03$) and TNFα ($p = 0.001$) were significantly increased in NS patients compared to HC. We observed no changes in the plasma levels of TNFβ, IL-2, IL-6, IL-10, IL-12B, IL-15, or IL-16 between NS patients and HC (Table 4).

CSF and plasma IL-1α, IL-1β, IL-4, IL-5, IL-7, IL-13, and IL-17A levels were below detection limits or did not show any differences between groups (Tables 3 & 4).

As IFNγ and TNFα levels significantly increased both in the CSF and plasma of NS patients, we used Spearman's rank correlation test to investigate a possible association. However, we did not observe any significant association between IFNγ levels ($r_s = 0.06$, $p = 0.8$) or TNFα levels ($r_s = -0.18$, $p = 0.45$) in CSF and plasma.

3.4. TNF receptors in CSF and plasma

While we found no significant difference in CSF (Table 3) or plasma (Table 4) TNFR1 levels, TNFR2 CSF levels were significantly higher in NS patients compared to HC ($p = 0.009$, Fig. 1j). Furthermore, the highest TNFR2 level (Table 5) was in NS patients with moderate/severe enhancement 7376 pg/mL compared to mild enhancement 1636 pg/mL and no enhancement 1660 pg/mL ($p = 0.047$). No significant change was observed in plasma TNFR2 levels ($p = 0.06$).

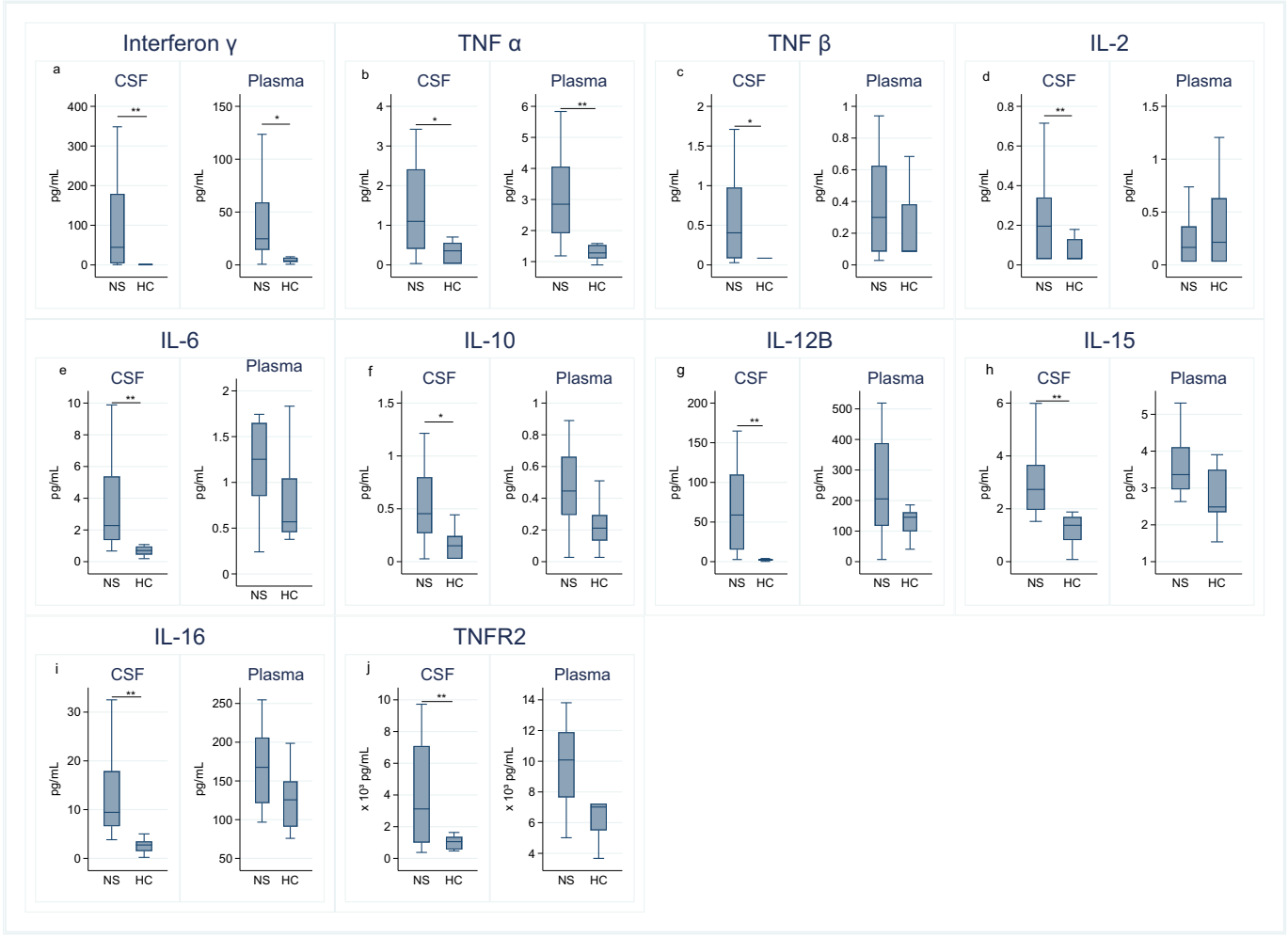

Fig. 1. Box plots of cytokine levels and TNFR2 levels in cerebrospinal fluid and plasma. CSF: cerebrospinal fluid. NS: neurosarcoidosis patients ($n = 20$). HC: Healthy controls ($n = 11$).

3.5. Chemokines in CSF and plasma

Next, we analyzed CSF and plasma levels of ten selected chemokines in NS patients and compared these to HC (Fig. 2 and Tables 3 & 4). In CSF, we found the levels of CCL2 ($p = 0.02$), CCL3 ($p = 0.0009$), CCL4 ($p = 0.003$), CCL11 (p = 0.0009), CCL13 (p = 0.0009), CCL17 (p = 0.0009), CCL22 (p = 0.0009), CCL26 ($p = 0.002$), CXCL8 (p = 0.0009), and CXCL10 (p = 0.0009) to be significantly increased in NS patients compared to HC (Table 3). In addition, CSF CCL3 and CXCL10 had the highest fold elevation in NS patients compared to HC.

In plasma, only CCL2 (p = 0.02), CCL3 (p = 0.02), CCL4 (p = 0.002), CCL17 ($p = 0.001$), and CXCL10 (p = 0.002) levels were significantly increased in NS patients as compared to HC. We observed no differences in plasma levels of the remaining chemokines (Table 4).

Because the levels of CCL2, CCL3, CCL4, CCL17, and CXCL10 all increased significantly in both plasma and CSF of NS patients, we analyzed possible associations between plasma and CSF levels using Spearman's rank correlation test. We did not observe any significant correlation between plasma and CSF levels of CCL2 ($r_s = -0.44$, $p = 0.06$), CCL3 ($r_s = 0.21$, $p = 0.4$), CCL4 ($r_s = -0.17$, $p = 0.49$), CCL17 ($r_s = 0.07$, $p = 0.79$), and CXCL10 ($r_s = 0.07$, $p = 0.78$).

Compared to plasma, the CSF CCL2 level was increased in all patients (Fig. 3a), CSF CCL3 in 13 patients (Fig. 3b), CSF CCL4 in 2 patients (Fig. 3c), CSF CCL17 in 7 patients (Fig. 3d), and CSF CXCL10 in 18 patients (Fig. 3e).

3.6. Vascular angiogenesis and injury biomarkers

Finally, we investigated possible changes in vascular angiogenesis markers and injury markers (Fig. 4 and Tables 3 & 4).

In CSF, we found that VEGF-A levels were significantly increased in NS patients compared to HC ($p = 0.008$) whereas no differences were observed in VEGFR1. Also, PIGF ($p = 0.002$), SAA ($p = 0.03$), VCAM1 ($p = 0.0003$), and ICAM1 (p = 0.0003) levels were significantly increased in NS patients compared to HC. Furthermore, the highest ICAM1 level (Table 5) was in NS patients with moderate/severe enhancement 57 ng/mL compared to mild enhancement 35 ng/mL and no enhancement 23 ng/mL (p = 0.03).

In plasma, we found that plasma levels of VEGFR1 ($p = 0.049$), PIGF ($p = 0.0006$), SAA ($p = 0.02$), VCAM1 ($p = 0.01$), and ICAM1 (p = 0.02) were significantly increased in NS patients compared to HC.

Because PIGF, VCAM1, ICAM1, and SAA levels all increased significantly in both plasma and CSF of NS patients, we analyzed possible associations between CSF and plasma levels using Spearman's rank correlation test. We did not observe any significant correlation between CSF and plasma levels of PIGF ($r_s = 0.39$, $p = 0.1$), VCAM1 ($r_s = 0.09$, $p = 0.72$), or ICAM1 ($r_s = -0.25$, $p = 0.32$) whereas SAA levels in CSF and plasma levels displayed a strong positive correlation ($r_s = 0.73$, p = 0.0006, Fig. 5). In addition, the SAA index was low 0.11 (Table 3).

Table 3
Cytokine, chemokine, soluble TNF-receptor, vascular angiogenesis, and injury biomarker in cerebrospinal fluid.

	Neurosarcoidosis	Healthy controls	P-value[c]	Fold elevation[d]	Index[e]
Cytokines[a]					
IFNγ	44.5 (4.0, 179.3)	*Below detc.*	0.001	68	120
TNFα	1.10 (0.41, 2.41)	0.36 (0.04, 0.56)	0.03	3.1	27
TNFβ	0.40 (0.09, 0.98)	*Below detc.*	0.03	4.8	120
IL-1α	*Below detc.*	0.63 (0.09, 8.34)	0.6		
IL-1β	*Below detc.*	*Below detc.*			
IL-2	0.20 (0.03, 0.34)	*Below detc.*	0.006	6.7	105
IL-4	*Below detc.*	*Below detc.*			
IL-5	*Below detc.*	*Below detc.*			
IL-6	2.28 (1.36, 5.37)	0.70 (0.45, 0.97)	0.001	3.3	204
IL-7	0.41 (0.31, 0.52)	0.46 (0.35, 0.48)	1		
IL-10	0.45 (0.27, 0.80)	0.15 (0.03, 0.24)	0.04	3.0	88
IL-12B	59 (15, 110)	2.0 (1.5, 3.3)	0.001	29	23
IL-13	1.22 (0.20, 2.50)	*Below detc.*	1		
IL-15	2.73 (1.96, 3.66)	1.38 (0.81, 1.69)	0.001	2.0	71
IL-16	9.4 (6.6, 17.9)	2.8 (1.5, 3.5)	0.001	3.4	7
IL-17A	1.59 (0.21, 2.51)	*Below detc.*	0.08		
Chemokines[a]					
CCL2	304 (232, 346)	218 (176, 259)	0.02	1.4	271
CCL3	49 (26, 64)	*Below detc.*	0.0009	32	150
CCL4	20 (16, 29)	9 (6, 13)	0.003	2.3	28
CCL11	37 (22, 50)	*Below detc.*	0.0009	4.0	33
CCL13	24 (5, 40)	*Below detc.*	0.0009	13	58
CCL17	56 (15, 369)	3.2 (0.4, 4.0)	0.0009	18	89
CCL22	132 (53, 258)	10 (7, 14)	0.0009	14	15
CCL26	6.0 (4.0, 10.7)	*Below detc.*	0.002	2.5	31
CXCL8	75 (42, 123)	22 (17, 30)	0.0009	3.4	2747
CXCL10	5373 (1906, 9786)	225 (144, 344)	0.0009	24	604
TNF receptors[a]					
TNFR1	725 (531, 2582)	1118 (748, 1397)	0.6		
TNFR2	3123 (999, 7093)	1055 (559, 1359)	0.009	3.0	33
Vascular angiogenesis[a]					
VEGF-A	3.2 (0.5, 3.9)	*Below detc.*	0.008	6.0	7
VEGF-C	*Below detc.*	*Below detc.*			
VEGF-D	25 (16, 37)	27 (13, 36)	1		
VEGFR1	39 (36, 81)	53 (38, 65)	1		
Tie-2	*Below detc.*	*Below detc.*			
PIGF	17 (10, 26)	8 (6, 10)	0.002	2.2	192
bFGF	*Below detc.*	*Below detc.*			
Vascular injury[b]					
SAA	11.5 (4.8, 55.6)	6.1 (1.9, 7.7)	0.03	1.9	0.11
VCAM1	50 (34, 74)	20 (16, 25)	0.0003	2.5	9
ICAM1	35 (20, 57)	13 (8, 18)	0.0003	2.6	6

Values are median and interquartile range (IQR). [a] Value unit is pg/mL. [b] Value unit is ng/mL.
[c]Wilcoxon rank-sum test with Bonferroni-Holm correction. [d] Represents the median value divided by the median in healthy controls. [e] Calculated by CSF/plasma quotient divided by CSF/serum albumin quotient. Below detc.: more than half of the samples are below the detections limit.

4. Discussion

In this observational cross-sectional study, we used electrochemiluminescence multiplex analytical assays to assess the immunological response of 38 different cytokines, chemokines, vascular angiogenesis, and injury biomarkers in NS. In NS patients, we demonstrated a strong systemic inflammation; 25 biomarkers in CSF and 12 biomarkers in plasma were significantly increased compared to healthy controls.

Most immunological studies are based on pulmonary sarcoidosis patients by investigating bronchoalveolar lavage (BAL) and blood (Grunewald et al., 2019; Zhou and Arce, 2020). The results are sometimes inconsistent, however, probably because of different patient populations, sample sizes, and analysis techniques for immunohistochemistry, Elisa assay, and cell culture.

4.1. Cells counts

Significant lymphopenia involving $CD4^+$, $CD8^+$, and B cells is common in sarcoidosis patients and correlates with disease severity (Sweiss et al., 2010). In NS patients, we found the total lymphocyte count, $CD4^+$ T cells, $CD8^+$ T cells, B cells, and NK cells to be reduced, demonstrating that NS patients also experience lymphopenia.

4.2. Cytokines

4.2.1. Th1 and Th17 cytokines

We found substantially increased IFNγ levels in the CSF and plasma, although they did not correlate with each other. IFNγ is an essential cytokine in the pathogenesis of sarcoidosis produced by Th1 and TH17.1 cells (Möllers et al., 2001; Prasse et al., 2000; Ramstein et al., 2016; Shigehara et al., 2003; Tøndell et al., 2014). Our data indicate that IFNγ is also important in NS inflammation, but the cytokine's production seems independent in CSF and plasma. However, IFNγ is not specific for sarcoidosis or NS and can be elevated in various inflammatory conditions of the CNS. (Lepennetier et al., 2019). Further supporting the role of Th1 cells in NS, we also found elevated concentrations of IL-2, albeit at low concentrations and only in the CSF. In sarcoidosis, elevated IL-2 concentration in serum and BAL has been reported (Thillai et al., 2012) as well as an increased number of lymphocytes expressing intracellular IL-2 in BAL and blood (Möllers et al., 2001; Prasse et al., 2000).

Although IL-17A is essential for granuloma inflammation in mice (Okamoto Yoshida et al., 2010), and an enhanced IL-17A expression is found in sarcoidosis granulomas (Ten Berge et al., 2012), we did not find

Table 4
Cytokines, chemokines, soluble TNF-receptors, vascular angiogenesis and injury biomarkers in plasma.

	Neurosarcoidosis	Healthy controls	P-value[d]	Fold elevation[e]
Cytokines[a]				
IFNγ	24.7 (14.3, 59.2)	3.7 (2.9, 7.0)	0.03	6.7
TNFα	2.85 (1.91, 4.06)	1.28 (1.10, 1.53)	0.001	2.2
TNFβ	0.30 (0.09, 0.63)	*Below detc.*	0.8	
IL-1α	1.46 (0.28, 2.93)	1.33 (0.38, 3.42)	1	
IL-1β	*Below detc.*	*Below detc.*		
IL-2	0.17 (0.03, 0.36)	0.21 (0.03, 0.63)	1	
IL-4	*Below detc.*	*Below detc.*		
IL-5	*Below detc.*	*Below detc.*		
IL-6	1.25 (0.85, 1.65)	0.60 (0.46, 1.04)	0.2	
IL-7	2.8 (1.9, 5.2)	2.3 (1.8, 2.9)	0.9	
IL-10	0.45 (0.29, 0.66)	0.21 (0.13, 0.29)	0.2	
IL-12B	205 (117, 388)	145 (99, 162)	0.4	
IL-13	*Below detc.*	*Below detc.*		
IL-15	3.4 (3.0, 4.1)	2.5 (2.3, 3.5)	0.2	
IL-16	168 (121, 206)	126 (91, 149)	0.2	
IL-17A	7.3 (3.8, 9.9)	4.2 (2.5, 4.6)	0.2	
Chemokines[a]				
CCL2	111 (98, 119)	82 (74, 90)	0.02	1.3
CCL3	31 (19, 40)	15 (2, 20)	0.02	2.0
CCL4	60 (45, 75)	33 (28, 42)	0.002	2.0
CCL11	125 (91, 143)	87 (68, 108)	0.08	
CCL13	53 (35, 72)	45 (41, 50)	0.4	
CCL17	70 (47, 148)	36 (29, 40)	0.001	1.9
CCL22	871 (575, 1138)	770 (542, 985)	0.5	
CCL26	18 (14, 36)	14 (10, 19)	0.3	
CXCL8	2.5 (1.8, 3.7)	1.6 (1.2, 2.2)	0.08	
CXCL10	660 (415, 992)	152 (128, 224)	0.002	4.3
TNF receptors[a]				
TNFR1	1223 (1042, 1720)	1734 (1378, 1842)	0.08	
TNFR2	10,083 (7651, 11,895)	7019 (5500, 7237)	0.06	
Vascular angiogenesis[a]				
VEGF-A	38 (29, 46)	36 (28, 36)	1	
VEGF-C	63 (46, 86)	48 (37, 62)	0.5	
VEGF-D	379 (320, 497)	379 (303, 461)	1	
VEGFR1	92 (83, 126)	70 (65, 87)	0.049	1.3
Tie-2	5417 (5002, 6931)	5248 (4745, 5564)	1	
PIGF	7.4 (6.9, 9.2)	5.6 (3.9, 6.5)	0.0006	1.3
bFGF	1.15 (0.50, 2.39)	2.29 (0.91, 3.56)	1	
Vascular injury				
SAA[b]	11.3 (6.1, 35.0)	4.7 (3.0, 9.5)	0.02	2.4
VCAM1[c]	573 (493, 746)	454 (351, 477)	0.01	1.3
ICAM1[c]	590 (478, 663)	357 (295, 467)	0.02	1.7

Values are median and interquartile range (IQR). [a] Value unit is pg/mL. [b] Values unit is μg/mL. [c] Values unit is ng/mL. [d] Wilcoxon rank-sum test with Bonferroni-Holm correction. [e] Represents the median value divided by the median in healthy controls. Below detc.: more than half of the samples are below the detection limit.

a significant increase in IL-17A level in NS patients similar to others (Beirne et al., 2009; Minasyan et al., 2021).

4.2.2. Macrophage cytokines

Our data support considerable inflammatory response in the CNS as TNFα, IL-6, IL-10, IL-12B, IL-15, and IL-16 were all elevated in NS patients in CSF, and TNFα was also increased in the plasma. In sarcoidosis, activated macrophages are contributors to these cytokines, although CNS microglia may also contribute (Orihuela et al., 2016).

TNFα is an essential cytokine in sarcoidosis (Fehrenbach et al., 2003; Thillai et al., 2012) and develops and maintains granuloma (Kindler et al., 1989). Its release can be compartmentalized to the affected organ (Müller-Quernheim et al., 1992), and the TNFα level correlated to the activity in sarcoidosis (Geyer et al., 2013; Loza et al., 2011). TNFα inhibition is used in the management of patients with refractory sarcoidosis (Baughman et al., 2021; Drent et al., 2014) and refractory NS (Baughman et al., 2021; Cohen Aubart et al., 2017; Gelfand et al., 2017; Voortman et al., 2019). We found significantly higher TNFα levels in NS patients in both the CSF and the plasma, but without correlation between the two. This may suggest that both systemic and CNS inflammatory events are important in the production of TNFα in NS and contribute to granuloma formations in both compartments. However, our finding of elevation of TNFα levels supports the rationale of TNFα inhibition in NS.

In the CSF, we found significantly elevated IL-6 in NS patients compared to HC. A previous study reported that CSF IL6 is the highest in patients with active NS and that a high level was associated with greater risk of relapse or progression-free survival (Chazal et al., 2019).

The high CSF IL-12B levels may support the role of both Th1 and Th17 cells in NS as IL-12B is a subunit of IL-12 and IL-23. Previous studies found elevated IL-12B in the serum in patients with systemic sarcoidosis and correlation with the number of organ involvement (Beirne et al., 2009; Hata et al., 2007; Shigehara et al., 2003). However, in contrast to other autoimmune diseases, ustekinumab (Feagan et al., 2016; Kimball et al., 2012), which binds to IL-12B, failed to demonstrate efficacy in pulmonary sarcoidosis (Judson et al., 2014a).

We found a significantly higher level of IL-15 in both CSF and plasma of NS patients compared to HC. IL-15 is a Th1-related cytokine that shares many biologic activities with IL-2 (Waldmann, 2013). Earlier studies reported elevated IL-15 levels in BAL (Minasyan et al., 2021) and serum (Beirne et al., 2009).

IL-10 and IL-16 are also produced by other cells than macrophages. We found that IL-10 concentration was significantly higher in the CSF of NS patients than in HC, but the concentration was low. IL-10 is an anti-inflammatory cytokine (Saraiva and O'Garra, 2010). However, IL-10 from alveolar macrophages is upregulated in patients with acute sarcoidosis (Oltmanns et al., 2003) and in BAL (Thillai et al., 2012) although not always in plasma and serum (Bansal et al., 1997; Geyer et al., 2013; Thillai et al., 2012). In CSF, a previous study did not find any difference in IL-10 concentration in CSF between NS patients and those with various CNS inflammatory disorders (Chazal et al., 2019).

4.2.3. Th2 cytokines

It has been proposed that a transition from Th1 to Th2 cytokine profile may be involved in chronic sarcoidosis and tissue fibrosis (Möllers et al., 2001; Patterson et al., 2012). We did not find any elevation of Th2 cytokines IL-4 or IL-5 and IL-13 in either CSF or plasma, although the elevated level of IL-10 may indicate a shift to Th2 responses.

4.2.4. TNF receptors

We found a significant elevation of TNFR2 in the CSF, particularly in NS patients with extended inflammation on MRI. TNFR1 did not show any difference compared to controls. TNFR2 is expressed mainly on immune cells, oligodendrocytes, and endothelial cells (Gough and Myles, 2020; Madsen et al., 2016). There are only a few reports of

Table 5
Cytokine, chemokine, soluble TNF-receptor, vascular angiogenesis, and injury biomarker levels in cerebrospinal fluid related to contrast-enhancing lesions on MRI.

	No Enhancement	Mild Enhancement	Moderate/Severe Enhancement	P-value[c]
N	8	5	7	
Cytokines[a]				
IFNγ	21 (2.6, 147)	22 (6, 93)	82 (57, 179)	0.4
TNFα	0.81 (0.25, 2.20)	0.99 (0.41, 2.07)	1.91 (1.10, 2.63)	0.2
TNFβ	0.29 (0.09, 1.24)	*Below detc.*	0.69 (0.40, 1.14)	0.4
IL-1α	0.26 (0.09, 1.23)	*Below detc.*	0.28 (0.09, 0.28)	0.6
IL-1β	0.06 (0.12, 0.18)	*Below detc.*	0.14 (0.02, 0.18)	0.9
IL-2	0.09 (0.03, 0.30)	0.21 (0.20, 0.22)	0.25 (0.03, 0.34)	0.8
IL-4	*Below detc.*	*Below detc.*	*Below detc.*	
IL-5	*Below detc.*	*Below detc.*	0.49 (0.39, 0.63)	
IL-6	1.82 (1.03, 3.45)	2.06 (1.59, 9.88)	3.09 (2.74, 55.6)	0.2
IL-7	0.45 (0.36, 0.51)	0.36 (0.32, 1.09)	0.36 (0.31, 0.41)	0.7
IL-10	0.45 (0.25, 0.72)	0.34 (0.03, 0.54)	0.69 (0.38, 2.75)	0.3
IL-12B	53 (14, 140)	49 (6, 59)	80 (31, 110)	0.5
IL-13	0.65 (0.20, 2.19)	1.25 (0.20, 1.25)	3.36 (0.88, 7.44)	0.4
IL-15	2.36 (1.92, 3.79)	2.83 (2.46, 3.21)	2.74 (1.96, 5.99)	0.7
IL-16	9 (5, 13)	9 (7, 17)	25 (9, 36)	0.2
IL-17A	1.67 (0.21, 3.35)	*Below detc.*	1.92 (1.16, 2.79)	0.4
Chemokines[a]				
CCL2	295 (244, 492)	227 (190, 243)	333 (304, 356)	0.06
CCL3	46 (23,57)	49 (27, 64)	47 (26, 67)	0.9
CCL4	20 (16, 27)	14 (8, 20)	29 (24, 34)	0.03
CCL11	34 (23, 52)	25 (22, 44)	44 (37, 50)	0.4
CCL13	15 (4, 34)	25 (23, 27)	32 (20, 57)	0.4
CCL17	77 (20, 311)	52 (13, 65)	212 (38, 1113)	0.5
CCL22	137 (45, 326)	114 (73, 128)	193 (123, 352)	0.4
CCL26	9.2 (5.4, 12.1)	4.0 (3.6, 6.0)	5.1 (2.7, 8.6)	0.09
CXCL8	63 (37, 92)	75 (34, 99)	126 (63, 365)	0.3
CXCL10	4486 (2286, 12,093)	1906 (1687, 5373)	6938 (5502, 11,285)	0.2
TNF receptors[a]				
TNFR1	692 (594, 2072)	531 (387, 596)	2652 (991, 3294)	0.07
TNFR2	1660 (988, 4554)	1636 (916, 3123)	7376 (4956, 7946)	0.047
Vascular angiogenesis[a]				
VEGF-A	1.85 (0.54, 3.74)	2.85 (0.54, 3.72)	5.48 (0.54, 13.4)	0.3
VEGF-C	*Below detc.*	*Below detc.*	*Below detc.*	
VEGF-D	30 (21, 37)	16 (16, 25)	25 (3, 50)	0.4
VEGFR1	47 (39, 72)	35 (29, 64)	38 (36, 118)	0.4
Tie-2	*Below detc.*	*Below detc.*	42 (29, 59)	
PIGF	11 (10, 18)	19 (10, 20)	29 (12, 46)	0.2
bFGF	*Below detc.*	*Below detc.*	*Below detc.*	
Vascular injury[b]				
SAA				0.2

(*continued on next page*)

Table 5 (*continued*)

	No Enhancement	Mild Enhancement	Moderate/Severe Enhancement	P-value[c]
	5	8	44	
	(4, 25)	(4, 111)	(40, 87)	
VCAM1	44	46	66	0.06
	(32, 50)	(32, 74)	(66, 110)	
ICAM1	23	35	57	0.03
	(19, 34)	(20, 45)	(54, 92)	

Values are median and interquantile range (IQR). [a] Value unit is pg/mL. [b] Values unit is ng/mL. Below detc.: more than half of the samples being below the detections limit. No Enhancement: no contrast enhancing lesions. Mild Enhancement: contrast enhancing in one or two anatomical sites. Moderate/Severe Enhancement: contrast enhancement in three to six anatomical sites. [c] Kruskal-Wallis test.

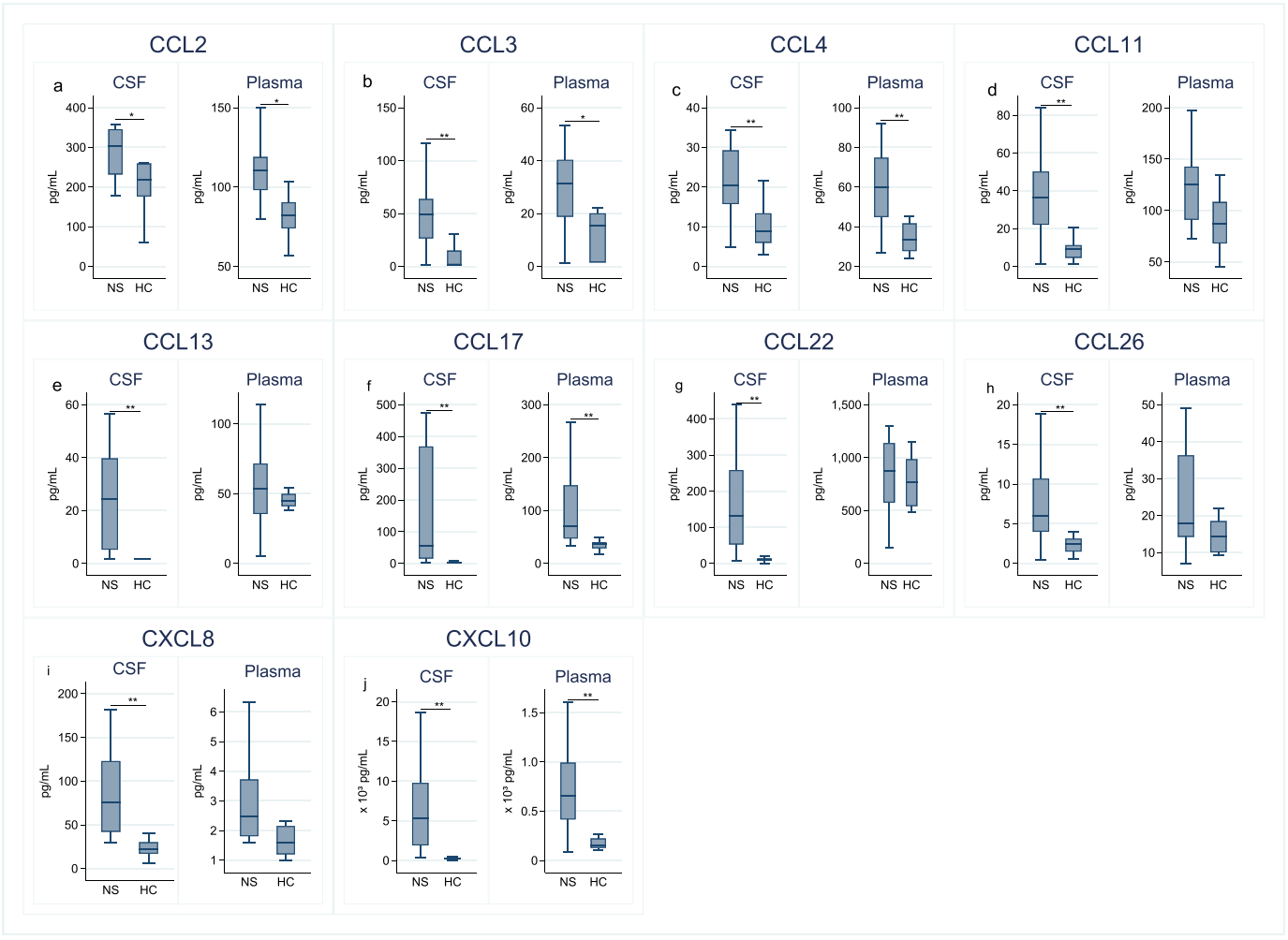

Fig. 2. Box plots of chemokine levels in cerebrospinal fluid and plasma. CSF: cerebrospinal fluid. NS: neurosarcoidosis patients (n = 20). HC: Healthy controls (n = 11).

TNFR1 and TNFR2 expression in sarcoidosis patients. One study reported increased TNFR2 but not TNFR1 in the serum of sarcoidosis patients, and the percentage of $CD4^+TNFR2^+$ cells in blood was especially increased in patients in remission or with stable disease (Kieszko et al., 2007). Another study reported higher serum TNFR1 and TNFR2 concentrations in sarcoidosis patients compared to controls and higher levels in active sarcoidosis. Furthermore, serum TNFR2 levels were significantly higher in sarcoidosis patients with advanced radiological stages II and III (Ziegenhagen et al., 2000). Our data support the role of TNFR2 in NS.

4.3. Chemokines

In this study, levels of chemokines were profoundly changed, and all chemokines in the panel were significantly elevated in CSF compared to HC.

In sarcoidosis, there can be conflicting results concerning chemokines and their association with sarcoidosis.

4.3.1. Recruitment of macrophages, $CD4^+$ cells, and $CD8^+$ cells

In both the CSF and plasma of NS patients, we found significant elevation of CCL2, CCL3, and CCL4. CCL2 is a key chemokine regulating the migration and infiltration of monocytes and macrophages (Deshmane et al., 2009). Increased CCL2 has been reported in plasma, serum,

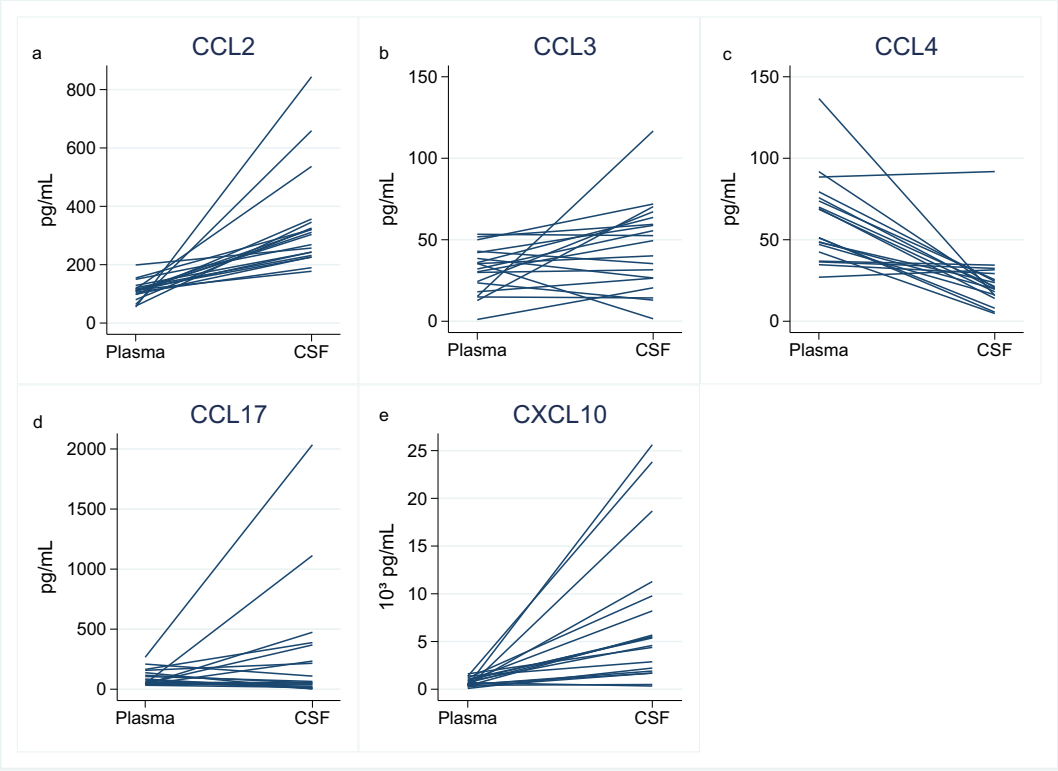

Fig. 3. Spaghetti plot of chemokine levels in plasma and cerebrospinal fluid in neurosarcoidosis patients.

and BAL (Car et al., 1994; Hashimoto et al., 1998; Loza et al., 2011; Palchevskiy et al., 2011) and was correlated with the activity and course of sarcoidosis (Hashimoto et al., 1998). In CNS, CCL2 is a commonly expressed chemokine during inflammation from various cells and increases the BBB permeability (Cédile et al., 2017; Stamatovic et al., 2005). In addition, CCL2 recruits $CD4^+$ cells in a chemokine receptor 2 (CCR2)-independent manner (Cédile et al., 2017). In NS patients, we found a higher CSF CCL2 level compared to HC. In addition, the CSF CLL2 level was higher than the plasma CCL2 level in all NS patients, indicating that CCL2 is important in NS inflammation.

The chemokine CCL3 preferentially mediates chemotaxis of $CD8^+$ cells, and CCL4 mediates chemotaxis for $CD4^+$ cells (Taub et al., 1993). Plasma CCL3 (Hashimoto et al., 1998) and serum CCL4 (Loza et al., 2011) are significantly elevated in patients with active sarcoidosis. Also in BAL, CCL3 was significantly increased in Stage II and III sarcoidosis, and the concentration of CCL4 was significantly increased at all stages (Capelli et al., 2002), but the data are not entirely consistent (Palchevskiy et al., 2011). Despite a significant elevation of CCL3 and CCL4 in NS patients compared to HC, there was no clear tendency regarding which compartment had the highest CLL3, and most NS patients had a lower CSF CCL4 level than plasma CCL4 level. This may indicate that CCL4 is not the most important $CD4^+$ cytokine in cell traffic regulation in NS.

4.3.2. *Th2 inflammation*

In CSF, we found elevated CCL11, CCL13, CCL17, CCL22, and CCL26 levels in NS patients, and for CCL17 also in plasma. In principle, they are all part of Th2 inflammation through CCR3 and CCR4.

Previous studies report elevated CCL11 and CCL26 levels in aqueous humor from patients with active sarcoidosis uveitis (Abu El-Asrar et al., 2019) and serum in sarcoidosis (Loza et al., 2011). The present study indicates CCL11, CCL13, and CCL26 are implicated in NS inflammation in CSF.

CCL17 and CCL22 bind to CCR4, which is highly expressed by activated Th2 cells and Treg cells and has been reported to be elevated in CNS diseases (Scheu et al., 2017). In serum but not BAL, CCL17 levels were elevated and correlated with disease severity in sarcoidosis (Nguyen et al., 2018; Nureki et al., 2008). As CCL17 was significantly elevated in CSF and plasma compared to HC, CCL17 seems imported in NS inflammation. However, we did not find any tendency for either CSF or plasma levels to be highest.

4.3.3. *CXCL chemokines*

The CSF CXCL10 had a high elevation in NS patients compared to HC. Furthermore, most NS patients had a higher CSF CXCL10 level than plasma CXCL10 level. This indicates that CXCL10 is important in NS. CXCL10 is categorized functionally as a Th1-chemokine and is induced by IFNγ. In sarcoidosis, CXCL10 levels are elevated in the plasma, serum, and BAL (Antoniou et al., 2006; Beirne et al., 2009; Geyer et al., 2013; Nureki et al., 2008; Su et al., 2013). The plasma and serum levels corresponded to the clinical course (Geyer et al., 2013; Su et al., 2013). Further studies are needed to clarify CXCL10 as a prognostic factor in NS.

CXCL8 level was higher in the CSF of NS patients compared to HC. In addition, we found a very high CXCL8 index indicating considerable CSF production. CXCL8 induces chemotaxis mainly of neutrophils granulocytes. In sarcoidosis, some studies reported elevated CXCL8 levels in serum (Mortaz et al., 2015) and BAL (Car et al., 1994). In contrast, other studies failed to demonstrate such an increase (Antoniou et al., 2006; Loza et al., 2011; Ziora et al., 2015).

4.4. *Blood-brain barrier disruption and vascular biomarkers*

We found an elevated CSF/plasma quotient as an indication of disruption of the BBB in 60% of NS patients, in agreement with previous studies (McLean et al., 1995).

In the CSF, we found significantly elevated VEGF-A levels in NS patients, and the plasma VEGFR1 level was higher in NS patients. The elevation of PIGF in the CSF is a novel finding in the context of sarcoidosis. VEGF-A is neuroprotective, and is essential in maintaining the BBB. (Lange et al., 2016). In sarcoidosis patients, single nucleotide

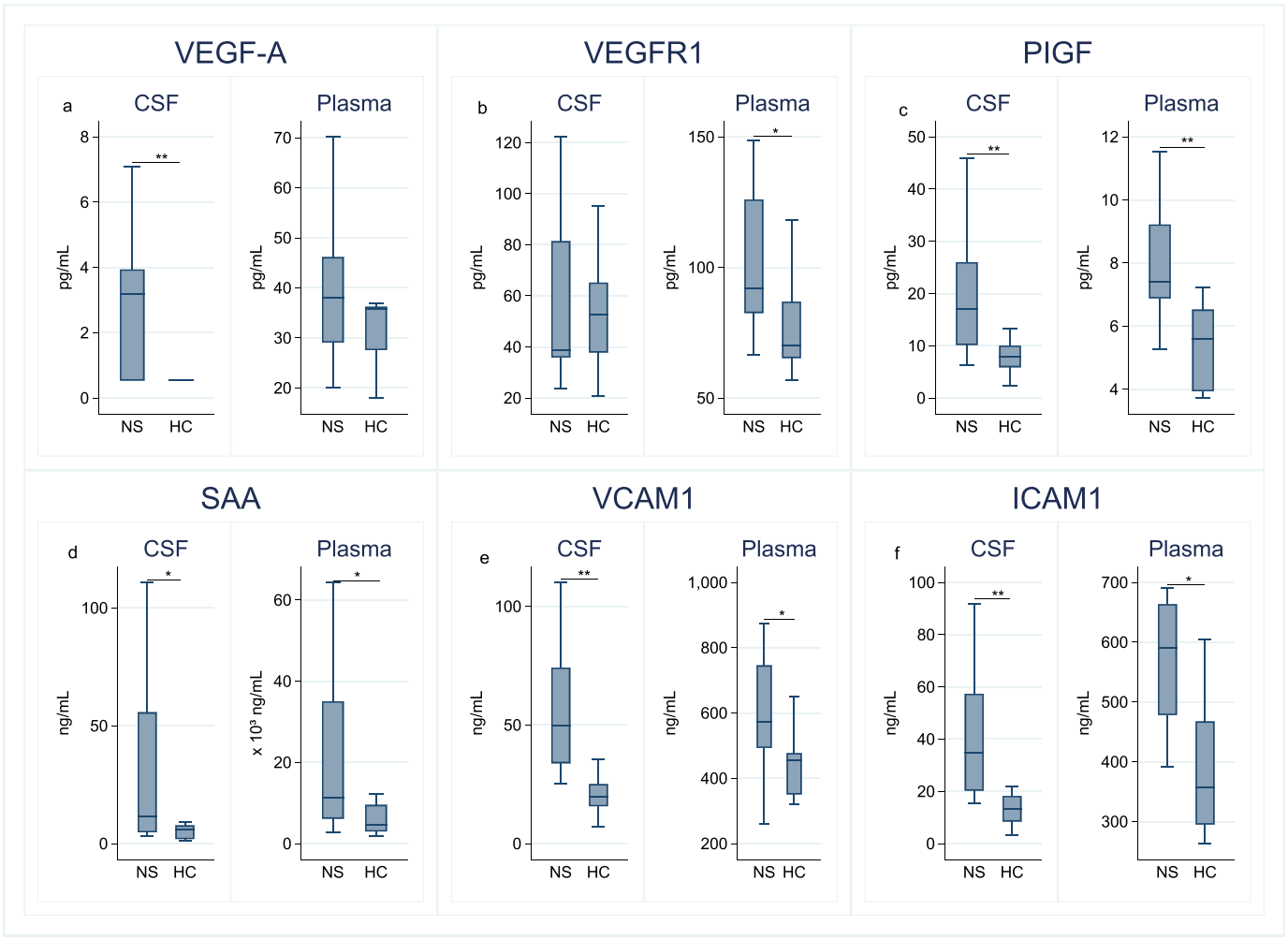

Fig. 4. Box plots of vascular angiogenesis and vascular injury biomarker levels in cerebrospinal fluid and plasma. CSF: cerebrospinal fluid. NS: neurosarcoidosis patients (n = 20). HC: Healthy controls (n = 11).

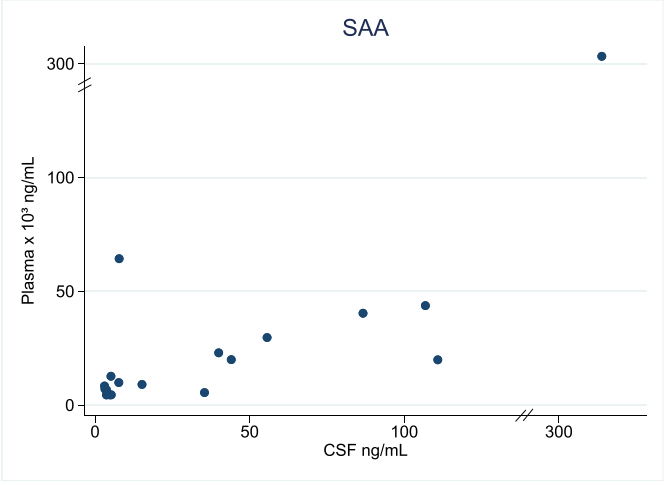

Fig. 5. Correlation between serum amyloid A (SAA) levels in cerebrospinal fluid and plasma. CSF: cerebrospinal fluid. Spearman's rho = 0.73, $p = 0.0006$.

polymorphisms in the VEGF, VEGFR1, and VEGFR2 genes are associated with the onset and course of sarcoidosis (Pabst et al., 2010). In addition, increased serum VEGF-A levels have been reported in sarcoidosis and correlated to disease activity and severity (Loza et al., 2011; Sekiya et al., 2003). The data are inconsistent, however (Ziora et al., 2015).

We also found significantly elevated VCAM1 and ICAM1 levels in both the CSF and plasma in NS patients. TNFα can induce cell adhesion molecules like VCAM-1 and ICAM-1 on the surface of the endothelial cells in the brain and facilitates the adhesion and transmigration of leukocytes (O'Carroll et al., 2015; Steiner et al., 2010). In sarcoidosis, VCAM1 and ICAM1 are present on vessel endothelium and ICAM1 on alveolar macrophages and epithelioid cells in granuloma (Kim et al., 1999; van Dinther-Janssen et al., 1993). The VCAM1 and ICAM1 levels correlated with the clinical stage and the disease activity (Berlin et al., 1998; Ishii and Kitamura, 1995; Kim et al., 1999). Our data revealed that VCAM1 and ICAM1 are important in NS, and they may be involved in the adhesion and transmigration of leukocytes.

We found a significant increase in CSF and plasma SAA in NS patients and a strong positive correlation between CSF and plasma, indicating a pathogenetic role for SAA in NS. However, the SAA index was low, indicating the primary SAA production outside the CNS. A previous study reported SAA was expressed much more intensely and with a characteristic pattern in tissues involved with sarcoidosis compared to non-sarcoidosis granulomatous tissues (Chen et al., 2010). In agreement with our study, other studies have reported elevated serum SAA levels in sarcoidosis patients, particularly with subacute onset requiring prolonged and multiple steroid treatments (Bargagli et al., 2011). In addition, fibrotic sarcoidosis patients showed higher SAA levels than sarcoidosis patients without fibrosis (Beijer et al., 2021). Further studies are needed to clarify the potential of SAA as a prognostic factor in NS.

However, the strong positive correlation between CSF SAA and plasma SAA indicates plasma measurements might be sufficient.

4.5. Strength and limitations

The strength of this study is that all patients were enrolled prospectively when NS diagnosis was established. We evaluated NS inflammation in both the CSF and the plasma using multiplex analytical assays.

However, the study is not without limitations. First, the number of patients was low due to the rarity of NS. Second, NS patients and HC had different age and sex distributions. Although cytokine levels are influenced by age (Michaud et al., 2013), none of the NS patients had severe comorbidity; thus, the age difference might not have a significant impact. Finally, extensive multianalyte profiling is typically not as sensitive as a standard single-analyte assay, which could explain why several biomarkers were below detection limits.

5. Conclusion

In summary, we provide evidence that NS is an inflammatory disease with strong involvement of multiple cytokines, chemokines, and vascular biomarkers. The cytokine profile showed a typical sarcoidosis Th1 inflammation with macrophage activity. The chemokines display the potential recruitment of monocytes, macrophages, $CD4^+$, $CD8^+$ cells, but also Th2 cells. In addition, elevation of both VCAM1 and ICAM1 indicates an upregulation and active facilitating of leukocyte endothelial transmigration over the blood-brain barrier. Interestingly, CSF CLL2 levels were higher than plasma CCL2 levels in all patients. CSF CXCL10 had a high elevation, and SAA levels in CSF and plasma were strongly correlated with each other, indicating that CCL2, CXCL10, and SAA are important in NS inflammation.

Further longitudinal studies are needed to clarify which biomarker has potential as an indicator of disease activity and prognosis.

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Declaration of Competing Interest

For all authors, none.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2022.577849.

References

Abu El-Asrar, A.M., Berghmans, N., Al-Obeidan, S.A., Gikandi, P.W., Opdenakker, G., Van Damme, J., Struyf, S., 2019. The CC chemokines CCL8, CCL13 and CCL20 are local inflammatory biomarkers of HLA-B27-associated uveitis. Acta Ophthalmol. 97 e122-e8.

American Thoracic Society, 1999. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. Am. J. Respir. Crit. Care Med. 160, 736–55.

Antoniou, K.M., Tzouvelekis, A., Alexandrakis, M.G., Sfiridaki, K., Tsiligianni, I., Rachiotis, G., Tzanakis, N., Bouros, D., Milic-Emili, J., Siafakas, N.M., 2006. Different angiogenic activity in pulmonary sarcoidosis and idiopathic pulmonary fibrosis. Chest. 130, 982–988.

Arger, N.K., Machiraju, S., Allen, I.E., Woodruff, P.G., Koth, L.L., 2020. T-bet expression in peripheral Th17.0 cells is associated with pulmonary function changes in sarcoidosis. Front. Immunol. 11, 1129.

Bansal, A.S., Bruce, J., Hogan, P.G., Allen, R.K., 1997. An assessment of peripheral immunity in patients with sarcoidosis using measurements of serum vitamin D3, cytokines and soluble CD23. Clin. Exp. Immunol. 110, 92–97.

Bargagli, E., Magi, B., Olivieri, C., Bianchi, N., Landi, C., Rottoli, P., 2011. Analysis of serum amyloid A in sarcoidosis patients. Respir. Med. 105, 775–780.

Baughman, R.P., Valeyre, D., Korsten, P., Mathioudakis, A.G., Wuyts, W.A., Wells, A., Rottoli, P., Nunes, H., Lower, E.E., Judson, M.A., Israel-Biet, D., Grutters, J.C., Drent, M., Culver, D.A., Bonella, F., Antoniou, K., Martone, F., Quadder, B., Spitzer, G., Nagavci, B., Tonia, T., Rigau, D., Ouellette, D.R., 2021. ERS clinical practice guidelines on treatment of sarcoidosis. Eur. Respir. J. 2004079.

Beijer, E., Roodenburg-Benschop, C., Schimmelpennink, M.C., Grutters, J.C., Meek, B., Veltkamp, M., 2021. Elevated serum amyloid A levels are not specific for sarcoidosis but associate with a fibrotic pulmonary phenotype. Cells. 10.

Beirne, P., Pantelidis, P., Charles, P., Wells, A.U., Abraham, D.J., Denton, C.P., Welsh, K. I., Shah, P.L., du Bois, R.M., Kelleher, P., 2009. Multiplex immune serum biomarker profiling in sarcoidosis and systemic sclerosis. Eur. Respir. J. 34, 1376–1382.

Berlin, M., Lundahl, J., Skold, C.M., Grunewald, J., Eklund, A., 1998. The lymphocytic alveolitis in sarcoidosis is associated with increased amounts of soluble and cell-bound adhesion molecules in bronchoalveolar lavage fluid and serum. J. Intern. Med. 244, 333–340.

Byg, K.E., Nielsen, H.H., Sejbaek, T., Madsen, J.S., Olsen, D.A., Nguyen, N., Kindt, A., Grauslund, J., Illes, Z., Ellingsen, T., 2021. Elevated neurofilament light chain in cerebrospinal fluid and plasma reflect inflammatory MRI activity in neurosarcoidosis. Brain Sci. 11.

Capelli, A., Di Stefano, A., Lusuardi, M., Gnemmi, I., Donner, C.F., 2002. Increased macrophage inflammatory protein-1alpha and macrophage inflammatory protein-1beta levels in bronchoalveolar lavage fluid of patients affected by different stages of pulmonary sarcoidosis. Am. J. Respir. Crit. Care Med. 165, 236–241.

Car, B.D., Meloni, F., Luisetti, M., Semenzato, G., Gialdroni-Grassi, G., Walz, A., 1994. Elevated IL-8 and MCP-1 in the bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis and pulmonary sarcoidosis. Am. J. Respir. Crit. Care Med. 149, 655–659.

Cédile, O., Wlodarczyk, A., Owens, T., 2017. CCL2 recruits T cells into the brain in a CCR2-independent manner. APMIS. 125, 945–956.

Chazal, T., Costopoulos, M., Maillart, E., Fleury, C., Psimaras, D., Legendre, P., Pineton de Chambrun, M., Haroche, J., Lubetzki, C., Amoura, Z., Legarff-Tavernier, M., Cohen, Aubart F., 2019. The cerebrospinal fluid CD4/CD8 ratio and interleukin-6 and -10 levels in neurosarcoidosis: a multicenter, pragmatic, comparative study. Eur. J. Neurol. 26, 1274–1280.

Chen, E.S., Song, Z., Willett, M.H., Heine, S., Yung, R.C., Liu, M.C., Groshong, S.D., Zhang, Y., Tuder, R.M., Moller, D.R., 2010. Serum amyloid A regulates granulomatous inflammation in sarcoidosis through Toll-like receptor-2. Am. J. Respir. Crit. Care Med. 181, 360–373.

Cohen Aubart, F., Bouvry, D., Galanaud, D., Dehais, C., Mathey, G., Psimaras, D., Haroche, J., Pottier, C., Hie, M., Mathian, A., Devilliers, H., Nunes, H., Valeyre, D., Amoura, Z., 2017. Long-term outcomes of refractory neurosarcoidosis treated with infliximab. J. Neurol. 264, 891–897.

Deshmane, S.L., Kremlev, S., Amini, S., Sawaya, B.E., 2009. Monocyte chemoattractant protein-1 (MCP-1): an overview. J. Interf. Cytokine Res. 29, 313–326.

Drent, M., Cremers, J.P., Jansen, T.L., Baughman, R.P., 2014. Practical eminence and experience-based recommendations for use of TNF-α inhibitors in sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 31, 91–107.

Feagan, B.G., Sandborn, W.J., Gasink, C., Jacobstein, D., Lang, Y., Friedman, J.R., Blank, M.A., Johanns, J., Gao, L.L., Miao, Y., Adedokun, O.J., Sands, B.E., Hanauer, S.B., Vermeire, S., Targan, S., Ghosh, S., de Villiers, W.J., Colombel, J.F., Tulassay, Z., Seidler, U., Salzberg, B.A., Desreumaux, P., Lee, S.D., Loftus Jr., E.V., Dieleman, L.A., Katz, S., Rutgeerts, P., 2016. Ustekinumab as induction and maintenance therapy for Crohn's disease. N. Engl. J. Med. 375, 1946–1960.

Fehrenbach, H., Zissel, G., Goldmann, T., Tschernig, T., Vollmer, E., Pabst, R., Müller-Quernheim, J., 2003. Alveolar macrophages are the main source for tumour necrosis factor-alpha in patients with sarcoidosis. Eur. Respir. J. 21, 421–428.

Gelfand, J.M., Bradshaw, M.J., Stern, B.J., Clifford, D.B., Wang, Y., Cho, T.A., Koth, L.L., Hauser, S.L., Dierkhising, J., Vu, N., Sriram, S., Moses, H., Bagnato, F., Kaufmann, J. A., Ammah, D.J., Yohannes, T.H., Hamblin, M.J., Venna, N., Green, A.J., Pawate, S., 2017. Infliximab for the treatment of CNS sarcoidosis: a multi-institutional series. Neurology. 89, 2092–2100.

Geyer, A.I., Kraus, T., Roberts, M., Wisnivesky, J., Eber, C.D., Hiensch, R., Moran, T.M., 2013. Plasma level of interferon γ induced protein 10 is a marker of sarcoidosis disease activity. Cytokine. 64, 152–157.

Gough, P., Myles, I.A., 2020. Tumor necrosis factor receptors: pleiotropic signaling complexes and their differential effects. Front. Immunol. 11, 585880.

Grunewald, J., Grutters, J.C., Arkema, E.V., Saketkoo, L.A., Moller, D.R., Muller-Quernheim, J., 2019. Sarcoidosis. Nat. Rev. Dis. Primers 5, 45.

Hashimoto, S., Nakayama, T., Gon, Y., Hata, N., Koura, T., Maruoka, S., Matsumoto, K., Hayashi, S., Abe, Y., Horie, T., 1998. Correlation of plasma monocyte chemoattractant protein-1 (MCP-1) and monocyte inflammatory protein-1alpha (MIP-1alpha) levels with disease activity and clinical course of sarcoidosis. Clin. Exp. Immunol. 111, 604–610.

Hata, M., Sugisaki, K., Miyazaki, E., Kumamoto, T., Tsuda, T., 2007. Circulating IL-12 p40 is increased in the patients with sarcoidosis, correlation with clinical markers. Intern. Med. 46, 1387–1393.
Herring, A.B., Urich, H., 1969. Sarcoidosis of the central nervous system. J. Neurol. Sci. 9, 405–422.
Hsieh, C.S., Macatonia, S.E., Tripp, C.S., Wolf, S.F., O'Garra, A., Murphy, K.M., 1993. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. Science. 260, 547–549.
Idali, F., Wahlström, J., Müller-Suur, C., Eklund, A., Grunewald, J., 2008. Analysis of regulatory T cell associated forkhead box P3 expression in the lungs of patients with sarcoidosis. Clin. Exp. Immunol. 152, 127–137.
Ishii, Y., Kitamura, S., 1995. Elevated levels of soluble ICAM-1 in serum and BAL fluid in patients with active sarcoidosis. Chest. 107, 1636–1640.
Jachiet, V., Lhote, R., Rufat, P., Pha, M., Haroche, J., Crozier, S., Dupel-Potier, C., Psimaras, D., Amoura, Z., Cohen, Aubart F., 2018. Clinical, imaging, and histological presentations and outcomes of stroke related to sarcoidosis. J. Neurol. 265, 2333–2341.
Judson, M.A., Baughman, R.P., Costabel, U., Drent, M., Gibson, K.F., Raghu, G., Shigemitsu, H., Barney, J.B., Culver, D.A., Hamzeh, N.Y., Wijsenbeek, M.S., Albera, C., Huizar, I., Agarwal, P., Brodmerkel, C., Watt, R., Barnathan, E.S., 2014a. Safety and efficacy of ustekinumab or golimumab in patients with chronic sarcoidosis. Eur. Respir. J. 44, 1296–1307.
Judson, M.A., Costabel, U., Drent, M., Wells, A., Maier, L., Koth, L., Shigemitsu, H., Culver, D.A., Gelfand, J., Valeyre, D., Sweiss, N., Crouser, E., Morgenthau, A.S., Lower, E.E., Azuma, A., Ishihara, M., Morimoto, S., Tetsuo Yamaguchi, T., Shijubo, N., Grutters, J.C., Rosenbach, M., Li, H.P., Rottoli, P., Inoue, Y., Prasse, A., Baughman, R.P., Organ Assessment Instrument Investigators TW, 2014b. The WASOG Sarcoidosis Organ Assessment Instrument: an update of a previous clinical tool. Sarcoidosis Vasc Diffuse Lung Dis. 31, 19–27.
Kieszko, R., Krawczyk, P., Chocholska, S., Bojarska-Junak, A., Jankowska, O., Król, A., Roliński, J., Milanowski, J., 2007. Tumor necrosis factor receptors (TNFRs) on T lymphocytes and soluble TNFRs in different clinical courses of sarcoidosis. Respir. Med. 101, 645–654.
Kim, D.S., Paik, S.H., Lim, C.M., Lee, S.D., Koh, Y., Kim, W.S., Kim, W.D., 1999. Value of ICAM-1 expression and soluble ICAM-1 level as a marker of activity in sarcoidosis. Chest. 115, 1059–1065.
Kimball, A.B., Gordon, K.B., Fakharzadeh, S., Yeilding, N., Szapary, P.O., Schenkel, B., Guzzo, C., Li, S., Papp, K.A., 2012. Long-term efficacy of ustekinumab in patients with moderate-to-severe psoriasis: results from the PHOENIX 1 trial through up to 3 years. Br. J. Dermatol. 166, 861–872.
Kindler, V., Sappino, A.P., Grau, G.E., Piguet, P.F., Vassalli, P., 1989. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell. 56, 731–740.
Lange, C., Storkebaum, E., de Almodovar, C.R., Dewerchin, M., Carmeliet, P., 2016. Vascular endothelial growth factor: a neurovascular target in neurological diseases. Nat. Rev. Neurol. 12, 439–454.
Lepennetier, G., Hracsko, Z., Unger, M., Van Griensven, M., Grummel, V., Krumbholz, M., Berthele, A., Hemmer, B., Kowarik, M.C., 2019. Cytokine and immune cell profiling in the cerebrospinal fluid of patients with neuro-inflammatory diseases. J. Neuroinflammation 16, 219.
Lexberg, M.H., Taubner, A., Albrecht, I., Lepenies, I., Richter, A., Kamradt, T., Radbruch, A., Chang, H.D., 2010. IFN-γ and IL-12 synergize to convert in vivo generated Th17 into Th1/Th17 cells. Eur. J. Immunol. 40, 3017–3027.
Lhote, R., Annesi-Maesano, I., Nunes, H., Launay, D., Borie, R., Sacre, K., Schleinitz, N., Hamidou, M., Mahevas, M., Devilliers, H., Bonniaud, P., Lhote, F., Haroche, J., Rufat, P., Amoura, Z., Valeyre, D., Cohen, Aubart F., 2021. Clinical phenotypes of extrapulmonary sarcoidosis: an analysis of a French, multi-ethnic, multicentre cohort. Eur. Respir. J. 57.
Loza, M.J., Brodmerkel, C., Du Bois, R.M., Judson, M.A., Costabel, U., Drent, M., Kavuru, M., Flavin, S., Lo, K.H., Barnathan, E.S., Baughman, R.P., 2011. Inflammatory profile and response to anti-tumor necrosis factor therapy in patients with chronic pulmonary sarcoidosis. Clin. Vaccine Immunol. 18, 931–939.
Madsen, P.M., Motti, D., Karmally, S., Szymkowski, D.E., Lambertsen, K.L., Bethea, J.R., Brambilla, R., 2016. Oligodendroglial TNFR2 mediates membrane TNF-dependent repair in experimental autoimmune encephalomyelitis by promoting oligodendrocyte differentiation and remyelination. J. Neurosci. 36, 5128–5143.
McLean, B.N., Miller, D., Thompson, E.J., 1995. Oligoclonal banding of IgG in CSF, blood-brain barrier function, and MRI findings in patients with sarcoidosis, systemic lupus erythematosus, and Behçet's disease involving the nervous system. J. Neurol. Neurosurg. Psychiatry 58, 548–554.
McNerlan, S.E., Alexander, H.D., Rea, I.M., 1999. Age-related reference intervals for lymphocyte subsets in whole blood of healthy individuals. Scand. J. Clin. Lab. Invest. 59, 89–92.
Michaud, M., Balardy, L., Moulis, G., Gaudin, C., Peyrot, C., Vellas, B., Cesari, M., Nourhashemi, F., 2013. Proinflammatory cytokines, aging, and age-related diseases. J. Am. Med. Dir. Assoc. 14, 877–882.
Minasyan, M., Sharma, L., Pivarnik, T., Liu, W., Adams, T., Bermejo, S., Peng, X., Liu, A., Ishikawa, G., Perry, C., Kaminski, N., Gulati, M., Herzog, E.L., Dela Cruz, C.S., Ryu, C., 2021. Elevated IL-15 concentrations in the sarcoidosis lung is independent of granuloma burden and disease phenotypes. Am. J. Phys. Lung Cell. Mol. Phys. 320, L1137–L1146.
Miyara, M., Amoura, Z., Parizot, C., Badoual, C., Dorgham, K., Trad, S., Kambouchner, M., Valeyre, D., Chapelon-Abric, C., Debré, P., Piette, J.C., Gorochov, G., 2006. The immune paradox of sarcoidosis and regulatory T cells. J. Exp. Med. 203, 359–370.
Möllers, M., Aries, S.P., Drömann, D., Mascher, B., Braun, J., Dalhoff, K., 2001. Intracellular cytokine repertoire in different T cell subsets from patients with sarcoidosis. Thorax. 56, 487–493.
Mortaz, E., Sereshki, H.A., Abedini, A., Kiani, A., Mirsaeidi, M., Soroush, D., Garssen, J., Velayati, A., Redegeld, F.A., Adcock, I.M., 2015. Association of serum TNF-α, IL-8 and free light chain with HLA-DR B alleles expression in pulmonary and extra-pulmonary sarcoidosis. J. Inflamm. (Lond). 12, 21.
Müller-Quernheim, J., Pfeifer, S., Männel, D., Strausz, J., Ferlinz, R., 1992. Lung-restricted activation of the alveolar macrophage/monocyte system in pulmonary sarcoidosis. Am. Rev. Respir. Dis. 145, 187–192.
Nguyen, C.T.H., Kambe, N., Ueda-Hayakawa, I., Kishimoto, I., Ly, N.T.M., Mizuno, K., Okamoto, H., 2018. TARC expression in the circulation and cutaneous granulomas correlates with disease severity and indicates Th2-mediated progression in patients with sarcoidosis. Allergol. Int. 67, 487–495.
Nishioka, Y., Manabe, K., Kishi, J., Wang, W., Inayama, M., Azuma, M., Sone, S., 2007. CXCL9 and 11 in patients with pulmonary sarcoidosis: a role of alveolar macrophages. Clin. Exp. Immunol. 149, 317–326.
Nureki, S., Miyazaki, E., Ando, M., Ueno, T., Fukami, T., Kumamoto, T., Sugisaki, K., Tsuda, T., 2008. Circulating levels of both Th1 and Th2 chemokines are elevated in patients with sarcoidosis. Respir. Med. 102, 239–247.
O'Carroll, S.J., Kho, D.T., Wiltshire, R., Nelson, V., Rotimi, O., Johnson, R., Angel, C.E., Graham, E.S., 2015. Pro-inflammatory TNFα and IL-1β differentially regulate the inflammatory phenotype of brain microvascular endothelial cells. J. Neuroinflammation 12, 131.
Okamoto Yoshida, Y., Umemura, M., Yahagi, A., O'Brien, R.L., Ikuta, K., Kishihara, K., Hara, H., Nakae, S., Iwakura, Y., Matsuzaki, G., 2010. Essential role of IL-17A in the formation of a mycobacterial infection-induced granuloma in the lung. J. Immunol. 184, 4414–4422.
Oltmanns, U., Schmidt, B., Hoernig, S., Witt, C., John, M., 2003. Increased spontaneous interleukin-10 release from alveolar macrophages in active pulmonary sarcoidosis. Exp. Lung Res. 29, 315–328.
Orihuela, R., McPherson, C.A., Harry, G.J., 2016. Microglial M1/M2 polarization and metabolic states. Br. J. Pharmacol. 173, 649–665.
Pabst, S., Karpushova, A., Diaz-Lacava, A., Herms, S., Walier, M., Zimmer, S., Cichon, S., Nickenig, G., Nothen, M.M., Wienker, T.F., Grohe, C., 2010. VEGF gene haplotypes are associated with sarcoidosis. Chest. 137, 156–163.
Palchevskiy, V., Hashemi, N., Weigt, S.S., Xue, Y.Y., Derhovanessian, A., Keane, M.P., Strieter, R.M., Fishbein, M.C., Deng, J.C., Lynch 3rd, J.P., Elashoff, R., Belperio, J.A., 2011. Immune response CC chemokines CCL2 and CCL5 are associated with pulmonary sarcoidosis. Fibrogenesis Tissue Repair 4, 10.
Patterson, K.C., Hogarth, K., Husain, A.N., Sperling, A.I., Niewold, T.B., 2012. The clinical and immunologic features of pulmonary fibrosis in sarcoidosis. Transl. Res. 160, 321–331.
Prasse, A., Georges, C.G., Biller, H., Hamm, H., Matthys, H., Luttmann, W., Virchow Jr., J.C., 2000. Th1 cytokine pattern in sarcoidosis is expressed by bronchoalveolar CD4+ and CD8+ T cells. Clin. Exp. Immunol. 122, 241–248.
Ramstein, J., Broos, C.E., Simpson, L.J., Ansel, K.M., Sun, S.A., Ho, M.E., Woodruff, P.G., Bhakta, N.R., Christian, L., Nguyen, C.P., Antalek, B.J., Benn, B.S., Hendriks, R.W., van den Blink, B., Kool, M., Koth, L.L., 2016. IFN-γ-producing T-helper 17.1 cells are increased in sarcoidosis and are more prevalent than T-helper type 1 cells. Am. J. Respir. Crit. Care Med. 193, 1281–1291.
Rothhammer, V., Quintana, F.J., 2015. Control of autoimmune CNS inflammation by astrocytes. Semin. Immunopathol. 37, 625–638.
Rubio-Rivas, M., Corbella, X., 2020. Clinical phenotypes and prediction of chronicity in sarcoidosis using cluster analysis in a prospective cohort of 694 patients. Eur. J. Intern. Med. 77, 59–65.
Saleh, S., Saw, C., Marzouk, K., Sharma, O., 2006. Sarcoidosis of the spinal cord: literature review and report of eight cases. J. Natl. Med. Assoc. 98, 965–976.
Saraiva, M., O'Garra, A., 2010. The regulation of IL-10 production by immune cells. Nat. Rev. Immunol. 10, 170–181.
Scheu, S., Ali, S., Ruland, C., Arolt, V., Alferink, J., 2017. The C-C chemokines CCL17 and CCL22 and their receptor CCR4 in CNS autoimmunity. Int. J. Mol. Sci. 18.
Schupp, J.C., Freitag-Wolf, S., Bargagli, E., Mihailovic-Vucinic, V., Rottoli, P., Grubanovic, A., Muller, A., Jochens, A., Tittmann, L., Schnerch, J., Olivieri, C., Fischer, A., Jovanovic, D., Filipovic, S., Videnovic-Ivanovic, J., Bresser, P., Jonkers, R., O'Reilly, K., Ho, L.P., Gaede, K.I., Zabel, P., Dubaniewicz, A., Marshall, B., Kieszko, R., Milanowski, J., Gunther, A., Weihrich, A., Petrek, M., Kolek, V., Keane, M.P., O'Beirne, S., Donnelly, S., Haraldsdottir, S.O., Jorundsdottir, K.B., Costabel, U., Bonella, F., Wallaert, B., Grah, C., Peros-Golubicic, T., Luisetti, M., Kadija, Z., Pabst, S., Grohe, C., Strausz, J., Vasakova, M., Sterclova, M., Millar, A., Homolka, J., Slovakova, A., Kendrick, Y., Crawshaw, A., Wuyts, W., Spencer, L., Pfeifer, M., Valeyre, D., Poletti, V., Wirtz, H., Prasse, A., Schreiber, S., Krawczak, M., Muller-Quernheim, J., 2018. Phenotypes of organ involvement in sarcoidosis. Eur. Respir. J. 51.
Sekiya, M., Ohwada, A., Miura, K., Takahashi, S., Fukuchi, Y., 2003. Serum vascular endothelial growth factor as a possible prognostic indicator in sarcoidosis. Lung. 181, 259–265.
Shigehara, K., Shijubo, N., Ohmichi, M., Kamiguchi, K., Takahashi, R., Morita-Ichimura, S., Ohchi, T., Tatsuno, T., Hiraga, Y., Abe, S., Sato, N., 2003. Increased circulating interleukin-12 (IL-12) p40 in pulmonary sarcoidosis. Clin. Exp. Immunol. 132, 152–157.
Stamatovic, S.M., Shakui, P., Keep, R.F., Moore, B.B., Kunkel, S.L., Van Rooijen, N., Andjelkovic, A.V., 2005. Monocyte chemoattractant protein-1 regulation of blood-brain barrier permeability. J. Cereb. Blood Flow Metab. 25, 593–606.
Steiner, O., Coisne, C., Cecchelli, R., Boscacci, R., Deutsch, U., Engelhardt, B., Lyck, R., 2010. Differential roles for endothelial ICAM-1, ICAM-2, and VCAM-1 in shear-

resistant T cell arrest, polarization, and directed crawling on blood-brain barrier endothelium. J. Immunol. 185, 4846–4855.
Su, R., Nguyen, M.L., Agarwal, M.R., Kirby, C., Nguyen, C.P., Ramstein, J., Darnell, E.P., Gomez, A.D., Ho, M., Woodruff, P.G., Koth, L.L., 2013. Interferon-inducible chemokines reflect severity and progression in sarcoidosis. Respir. Res. 14, 121.
Sweiss, N.J., Salloum, R., Gandhi, S., Alegre, M.L., Sawaqed, R., Badaracco, M., Pursell, K., Pitrak, D., Baughman, R.P., Moller, D.R., Garcia, J.G., Niewold, T.B., 2010. Significant CD4, CD8, and CD19 lymphopenia in peripheral blood of sarcoidosis patients correlates with severe disease manifestations. PLoS One 5, e9088.
Taflin, C., Miyara, M., Nochy, D., Valeyre, D., Naccache, J.M., Altare, F., Salek-Peyron, P., Badoual, C., Bruneval, P., Haroche, J., Mathian, A., Amoura, Z., Hill, G., Gorochov, G., 2009. FoxP3+ regulatory T cells suppress early stages of granuloma formation but have little impact on sarcoidosis lesions. Am. J. Pathol. 174, 497–508.
Taub, D.D., Conlon, K., Lloyd, A.R., Oppenheim, J.J., Kelvin, D.J., 1993. Preferential migration of activated CD4+ and CD8+ T cells in response to MIP-1 alpha and MIP-1 beta. Science. 260, 355–358.
Ten Berge, B., Paats, M.S., Bergen, I.M., van den Blink, B., Hoogsteden, H.C., Lambrecht, B.N., Hendriks, R.W., Kleinjan, A., 2012. Increased IL-17A expression in granulomas and in circulating memory T cells in sarcoidosis. Rheumatology. 51, 37–46.
Thillai, M., Eberhardt, C., Lewin, A.M., Potiphar, L., Hingley-Wilson, S., Sridhar, S., Macintyre, J., Kon, O.M., Wickremasinghe, M., Wells, A., Weeks, M.E., Mitchell, D., Lalvani, A., 2012. Sarcoidosis and tuberculosis cytokine profiles: indistinguishable in bronchoalveolar lavage but different in blood. PLoS One 7, e38083.
Tøndell, A., Moen, T., Børset, M., Salvesen, Ø., Rø, A.D., Sue-Chu, M., 2014. Bronchoalveolar lavage fluid IFN-γ+ Th17 cells and regulatory T cells in pulmonary sarcoidosis. Mediat. Inflamm. 2014, 438070.
van Dinther-Janssen, A.C., van Maarsseveen, T.C., Eckert, H., Newman, W., Meijer, C.J., 1993. Identical expression of ELAM-1, VCAM-1, and ICAM-1 in sarcoidosis and usual interstitial pneumonitis. J. Pathol. 170, 157–164.
Voortman, M., Drent, M., Baughman, R.P., 2019. Management of neurosarcoidosis: a clinical challenge. Curr. Opin. Neurol. 32, 475–483.
Waldmann, T.A., 2013. The biology of IL-15: implications for cancer therapy and the treatment of autoimmune disorders. J. Investig. Dermatol. Symp. Proc. 16, S28–S30.
Zhou, E.R., Arce, S., 2020. Key players and biomarkers of the adaptive immune system in the pathogenesis of sarcoidosis. Int. J. Mol. Sci. 21.
Ziegenhagen, M.W., Muller-Quernheim, J., 2003. The cytokine network in sarcoidosis and its clinical relevance. J. Intern. Med. 253, 18–30.
Ziegenhagen, M.W., Fitschen, J., Martinet, N., Schlaak, M., Müller-Quernheim, J., 2000. Serum level of soluble tumour necrosis factor receptor II (75 kDa) indicates inflammatory activity of sarcoidosis. J. Intern. Med. 248, 33–41.
Ziora, D., Jastrzębski, D., Adamek, M., Czuba, Z., Kozielski, J.J., Grzanka, A., Kasperska-Zajac, A., 2015. Circulating concentration of markers of angiogenic activity in patients with sarcoidosis and idiopathic pulmonary fibrosis. BMC Pulm Med. 15, 113.