Analysis of soluble interleukin-2 receptor as CSF biomarker for neurosarcoidosis

Carolin Otto, MD,* Oliver Wengert, MD,* Nadine Unterwalder, MD, Christian Meisel, MD,§ and Klemens Ruprecht, MD§

Neurol Neuroimmunol Neuroinflamm 2020;7:e725. doi:10.1212/NXI.0000000000000725

Abstract

Objective
To systematically analyze soluble interleukin-2 receptor (sIL-2R) in CSF as a diagnostic and disease activity biomarker in patients with sarcoidosis involving the CNS (neurosarcoidosis).

Methods
sIL-2R was determined by chemiluminescent immunoassays in CSF/serum samples from patients with neurosarcoidosis (n = 23), MS (n = 19), neurotuberculosis (n = 8), viral (n = 18) and bacterial (n = 9) meningitis, cerebral lymphoma (n = 8), Guillaumin-Barré syndrome (n = 8), and 115 patients with noninflammatory neurologic diseases (NINDs) as controls. The sIL-2R index was calculated by dividing the CSF/serum sIL-2R quotient (QsIL-2R) through the CSF/serum albumin quotient (QAlb). sIL-2R quotient diagrams were established by plotting QsIL-2R against QAlb. sIL-2R levels were correlated with clinical, MRI, and CSF disease activity markers of neurosarcoidosis.

Results
Patients with neurosarcoidosis had higher CSF sIL-2R, QsIL-2R, and sIL-2R index values than patients with NINDs (p < 0.0001 for all pairwise group comparisons). sIL-2R quotient diagrams demonstrated an intrathecal sIL-2R synthesis in >50% of neurosarcoidosis samples. Similar findings were observed in viral/bacterial meningitis, CNS lymphoma, and, most pronounced, in neurotuberculosis, but not in patients with MS. CSF sIL-2R parameters were associated with clinical disease activity, leptomeningeal gadolinium enhancement, and the CSF white cell count in patients with neurosarcoidosis.

Conclusions
CSF sIL-2R parameters are elevated in patients with neurosarcoidosis, but this finding is not specific for neurosarcoidosis. Nevertheless, CSF sIL-2R parameters may help distinguishing neurosarcoidosis from MS and are associated with clinical, radiologic, and CSF disease activity markers of neurosarcoidosis.

Classification of evidence
This study provides Class II evidence that CSF sIL-2R parameters distinguish neurosarcoidosis from NINDs and MS.

*These authors contributed equally to the manuscript.
§These authors contributed equally to the manuscript.

From the Department of Neurology (C.O., O.W., K.R.), Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health; Labor Berlin Charité-Vivantes GmbH (N.U., C.M.); and Institute for Medical Immunology (C.M.), Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany.

Go to Neurology.org/NN for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.
Sarcoidosis is a multisystem granulomatous disease of unknown etiology, whose histopathologic hallmark are noncaseating epitheloid granulomas, most often localized in the lung or mediastinal lymph nodes. Involvement of the CNS (herein referred to as neurosarcoidosis) occurs in at least 5%-15% of patients with systemic sarcoidosis. However, because of the heterogenous clinical and radiologic manifestations and the current absence of specific diagnostic markers, diagnosis of neurosarcoidosis can be challenging.

Soluble interleukin-2 receptor (sIL-2R) is produced by proteolytic cleavage of membrane-bound interleukin-2 receptor (IL-2R)α (CD25). IL-2Ra is part of the high-affinity IL-2R, which is a heterotrimeric receptor consisting of IL-2Ra, IL-2Rβ (CD122), and IL-2Rγ (CD132). Although resting T cells constitutively express the IL-2R β- and γ-chains, expression of IL-2Ra, which is required for formation of the high-affinity receptor, is low in resting T cells, but rapidly increases on activation of T cells, followed by shedding of IL2-Ra from the cell surface. Levels of sIL-2R in serum are therefore considered a marker of immune system activation. Specifically, serum sIL-2R has extensively been studied and, in some places, is already used in clinical routine as diagnostic and disease activity marker for systemic sarcoidosis. Nevertheless, data on the value of sIL-2R in CSF as a potential biomarker of neurosarcoidosis are scarce.

We systematically evaluated CSF sIL-2Rα as determined by semiautomated chemiluminescent immunoassays, as a diagnostic and disease activity biomarker for neurosarcoidosis.

**Methods**

**Standard protocol approvals, registrations, and patient consents**

All lumbar punctures were performed for diagnostic purposes only and with written informed consent of the patients or their guardians. The Institutional Review Board, Charité–Universitätsmedizin Berlin, Berlin, Germany, approved the usage of stored CSF and serum samples for the purposes of this study (EA1/126/10).

**Patients**

Patients were identified retrospectively from case files of the Department of Neurology, Charité–Universitätsmedizin Berlin, and the patients’ clinical and paraclinical data were retrieved from their medical records. A prerequisite for inclusion into the study was the availability of −80°C-stored CSF and serum samples, collected during routine lumbar punctures between 2004 and 2012.

Patients with neurosarcoidosis had to have a diagnosis of definite or probable neurosarcoidosis according to the Zajicek-criteria. Diagnosis of sarcoidosis was histologically proven in 21 of 23 patients, 17 of whom had a systemic biopsy and 4 a CNS biopsy. Clinical disease activity of patients with neurosarcoidosis at the time of CSF withdrawal was assessed by experienced neurologists and categorized as either clinically active disease or clinical remission. Clinically active disease was defined as new clinical symptoms or signs or worsening of preexisting symptoms or signs compared with a previous neurologic assessment. Clinical remission was defined as absence of clinical symptoms or signs or clinical disease stability or improvement compared with a previous neurologic assessment. Furthermore, based on cranial MRI findings at the time of CSF withdrawal, patients with neurosarcoidosis were grouped into those with and those without diffuse leptomeningeal gadolinium enhancement on cranial MRI, as described previously.

MS was diagnosed according to the McDonald 2017 criteria. Neurotuberculosis was diagnosed based on clinical, CSF, and MRI findings and PCR detection of *Mycobacterium tuberculosis* in CSF. Diagnosis of viral or acute bacterial meningitis was based on clinical and CSF findings and PCR detection of viral or bacterial DNA in CSF. Guillain-Barré syndrome (GBS) was diagnosed based on clinical, electrophysiologic, and CSF findings. In all patients with CNS lymphoma, the diagnosis was made by histopathologic examination of a brain biopsy. Patients with noninflammatory neurologic diseases (NINDs) had to have a normal CSF cell count (<4/μL) and no CSF-specific oligoclonal bands, but could have a wide range of normal to elevated CSF/serum albumin (Q\textsubscript{Ab}) quotient levels. Further detailed information on the diagnoses and clinical findings of the patients included in this work is provided in appendix e-1 (links.lww.com/NXI/A237).

**Routine CSF parameters**

Routine CSF parameters were determined as previously described. Total albumin concentrations in CSF and serum were measured nephelometrically (BN ProSpec, Siemens Healthcare GmbH, Erlangen, Germany). The age-adjusted upper limit of normal for Q\textsubscript{Ab} was calculated by the formula \((\text{age}/15) + 4\). \(^{12}\)

**Measurement of sIL-2R**

sIL-2R was determined in CSF and serum samples by IMMULITE\textsuperscript{™} semiautomated chemiluminescent immunoassays (Siemens Healthcare GmbH, Erlangen, Germany) according to the manufacturer’s instructions. sIL-2R measurements were performed between 2012 and 2013. Details of the calculation of sIL-2R values in U/mL are provided in appendix e-2 (links.lww.com/NXI/A238).
Analysis of sIL-2R data and generation of sIL-2R quotient diagrams

The CSF/serum sIL-2R quotient (Q_{sIL-2R}) was calculated by the formula: sIL-2R CSF/sIL-2R serum. The sIL-2R index was calculated by the formula: (sIL-2R CSF/sIL-2R serum)/(albumin CSF/albumin serum) = Q_{sIL-2R}/Q_{Alb}. sIL-2R quotient diagrams were generated by plotting Q_{sIL-2R} against Q_{Alb} values of patients with NINDs. A linear regression line was fitted into the diagram as well as the upper 99% prediction band, indicating the area in which 99% of all data points from patients with NINDs are expected to fall. The 99% prediction band was considered to represent the upper limit of the reference range (Q_{lim sIL-2R}), values above which were considered to indicate an intrathecal sIL-2R synthesis.

Statistical analyses

The primary research questions of this study were whether CSF/serum sIL-2R parameters distinguish patients with neurosarcoidosis, MS, GBS, viral meningitis, bacterial meningitis, neurotuberculosis, and CNS lymphoma from patients with NINDs and whether CSF sIL-2R parameters distinguish patients with neurosarcoidosis and MS. The classification of evidence assigned to these questions is Class II. For descriptive statistics, data are presented as median (interquartile range), absolute range (minimum–maximum), mean (SD), and absolute and relative frequencies in case of categorical data. Statistical significance of differences between 2 groups was assessed by Mann-Whitney U tests. A total of 32 group comparisons were associated with the primary research questions. Therefore, analyses were adjusted for multiple testing according to the Bonferroni method. The significance level of \( p < 0.05 \) was thus divided by 32, resulting in a new local significance level of \( p < 0.0016 \). The 2 group comparisons between patients with clinically active disease and in remission and between patients with and without diffuse leptomeningeal enhancement were considered as exploratory secondary end points and not corrected for multiple testing. Associations of sIL-2R with age and CSF cell counts were assessed by Spearman rank correlation coefficients. The correlation of Q_{Alb} and Q_{sIL-2R} in patients with NINDs was analyzed by linear regression. The capacity of CSF sIL-2R parameters to differentiate between different diseases was analyzed by receiver operating characteristic (ROC) curves. Analyses were performed with GraphPad Prism version 5.04.

Data availability

According to local data protection requirements, on requests from external researchers, approval for distribution of data will be obtained by the Institutional Review Board of Charité-Universitätsmedizin Berlin, and anonymized data will be shared with any qualified investigator.

Results

Patients

Demographics and CSF findings as well as sIL-2R in serum, sIL-2R in CSF, Q_{sIL-2R}, and the sIL-2R index in the different groups of patients analyzed in this study are summarized in table 1. Details of the patients’ diagnoses and clinical findings are included in appendix e-1 (links.lww.com/NXI/A237). In pairwise group comparisons, patients with neurosarcoidosis (\( p = 0.0002 \)) and MS (\( p = 0.0008 \)) were younger than patients with NINDs. The age of all other patient groups did not differ from that of patients with NINDs.

Reference values for sIL-2R in CSF and serum, Q_{sIL-2R}, and sIL-2R index

As measurements were performed in CSF and serum samples that had been stored frozen for prolonged periods of time, we analyzed the stability of sIL-2R in frozen CSF and serum samples by redetermining sIL-2R in 12 CSF and serum samples that had initially been measured in 2013 and were subsequently stored at −20°C for approximately 7 years. Bland-Altman plots (see appendix e-2, links.lww.com/NXI/A238) showed that sIL-2R values measured in CSF and serum in 2020 and 2013 did not substantially vary (difference between both measurements <15% with very few outliers). Likewise, there was no suggestion of a systematic bias between both measurements. These findings indicate that sIL-2R concentrations are stable in CSF/serum samples stored frozen for longer periods of time.

Reference values for sIL-2R in CSF and serum, Q_{sIL-2R}, and the sIL-2R index were established from sIL-2R values of the 115 patients with NINDs, who had no signs of CSF inflammation, but a wide range of Q_{Alb} values (table 2). The upper limit of the reference range was defined as the mean plus 3 SDs. Levels above this cutoff were regarded as elevated. Reference values for CSF sIL-2R and Q_{sIL-2R} are also provided separately for the subgroups of samples from patients with a normal blood-CSF barrier function, as indicated by normal age-adjusted Q_{Alb} values (\( n = 42 \)), and samples from patients with a disturbed blood-CSF barrier function, as indicated by elevated age-adjusted Q_{Alb} values (\( n = 74 \)). In the 115 patients with NINDs, sIL-2R in serum (\( p = 0.57 \)) and CSF (\( p = 0.13 \)) did not differ between women and men. In the 115 patients with NINDs, sIL-2R in serum was not associated with age (\( r = 0.17; p = 0.074 \)), but sIL-2R in CSF was found to be associated with age (\( r = 0.43; p < 0.001 \)).

CSF/serum quotient diagrams demonstrate intrathecal production of sIL-2R in neurosarcoidosis, viral/bacterial meningitis, neurotuberculosis, and CNS lymphoma

Plotting of Q_{sIL-2R} against Q_{Alb} values of the 115 patients with NINDs showed that Q_{sIL-2R} increases with increasing Q_{Alb} (\( r^2 = 0.715; p = 0.0001 \)), indicating that in patients without CNS inflammation, sIL-2R in CSF is derived from serum (figure 1). Next, we fitted the upper 99% prediction band into the sIL-2R CSF/serum quotient diagram, levels above which were considered to indicate an intrathecal production of sIL-2R in 52.6% of samples from neurosarcoidosis, 100% of samples from neurotuberculosis, 53.3% of samples from viral meningitis, 75% of samples from bacterial meningitis, and 76% of samples
Table 1 Demographics, CSF cell count, QAlb, as well as CSF and serum sIL-2R, QsIL-2R and sIL-2R index in the different groups of patients included in this study.

| Diagnosis             | No. of patients | Female/ | Age (y) | No. of samples | CSF cell count (cells/μL) QAlb (×10^3) sIL-2R CSF (U/mL) sIL-2R serum (U/mL) QsIL-2R (×10^3) sIL-2R index |
|-----------------------|-----------------|---------|---------|----------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| NINDs                 | 116             | 70/46  | 27(23–79) | 111/22         | 0.005 (0.001–0.017) 13 (8–20) 402 (201–2,401.3) 40 (20–1,605) 0.6 (0.3–1.2) |
| Neurosarcoidosis      | 23              | 19/4   | 37 (24–87) | 19/14          | 0.001 (0.001–0.003) 0.9 (0.1–8.6) 37 (2.6–1,740) 64 (8.5–254) 0.076 (0.01–0.75) |
| MS                    | 60              | 59/11  | 3 (2–83)  | 18/10          | 0.001 (0.001–0.003) 3.2 (0.1–17) 16 (2–22) 101 (0.01–100) 0.2 (0.01–1) |
| Neurotuberculosis     | 18              | 18     | 50 (8–70) | 18/10          | 0.001 (0.001–0.003) 0.7 (0.1–8.6) 37 (2.6–1,740) 64 (8.5–254) 0.076 (0.01–0.75) |
| Bacterial meningitis  | 8               | 8      | 5 (1–80)  | 8/10           | 0.001 (0.001–0.003) 1.3 (0.1–8.6) 37 (2.6–1,740) 64 (8.5–254) 0.076 (0.01–0.75) |
| GBS                   | 15              | 15     | 60 (61–78) | 15/10         | 0.001 (0.001–0.003) 1.3 (0.1–8.6) 37 (2.6–1,740) 64 (8.5–254) 0.076 (0.01–0.75) |

Abbreviations: GBS = Guillain-Barré syndrome; NINDs = noninflammatory neurologic diseases; QAlb = CSF/serum albumin quotient; QsIL-2R = CSF/serum soluble interleukin-2 receptor quotient; sIL-2R = soluble interleukin-2 receptor; sIL-2R index = soluble interleukin-2 receptor index (QsIL-2R/QAlb).

The highest CSF sIL-2R and QsIL-2R values were observed in patients with neurotuberculosis, and 13/14 samples of patients with neurotuberculosis showed CSF sIL-2R, QsIL-2R, and sIL-2R index values above the respective reference ranges.

**Association of sIL-2R with clinical, radiologic, and CSF parameters of disease activity in patients with neurosarcoidosis**

In a group comparison, the sIL-2R index was higher ($p = 0.0016$) in samples from patients with clinically active neurosarcoidosis ($n = 15$) than in samples from patients in clinical remission ($n = 13$).
The upper limit of normal was defined as the mean + 3 SDs; levels above this cutoff are considered pathologically elevated. Values for sIL-2R in CSF and QsIL-2R ELISA, this technology overall appears to be more standard-
terminated by IMMULITE laboratory diagnostics.

Abbreviations: QAlb = CSF/serum albumin quotient; sIL-2R = soluble interleukin-2 receptor.

|               | sIL-2R CSF (U/mL) | sIL-2R serum (U/mL) | QsIL-2R (×10³) | sIL-2R index | QAlb (U/mL) | sIL-2R CSF, normal | QAlb (U/mL) | sIL-2R CSF, elevated | QAlb (U/mL) | QsIL-2R, normal | QAlb (×10³) | QsIL-2R, elevated | QAlb (×10³) |
|---------------|-------------------|---------------------|----------------|--------------|--------------|-------------------|--------------|---------------------|--------------|----------------|---------------|----------------|---------------|
| Mean          | 17.7              | 498.4               | 40.9           | 4.4          | 11.2         | 13               | 20.4         | 30.4                | 46.8         | 57.6           | 1,819.3       | 124            | 11             |
| SD            | 13.3              | 440.3               | 27.7           | 2.2          | 9.6          | 5                | 15.6         | 11.7                | 32.2         | 5.6            | 65.5          | 67.2           | 65.5          |
| Upper limit of normal (mean + 3 SD) | 57.6 | 1,819.3 | 124 | 11 | 40 | 28 | 67.2 | 65.5 | 143.4 |

27) at the time of lumbar puncture (figure 3A). When analyzing sIL-2R index values intraindividually in patients (n = 7) who underwent 2 longitudinal lumbar punctures, sIL-2R index values declined from clinically active to clinical remission phases in 5/7 patients (figure 3B). From 2 of those patients, a third sample obtained during a clinically active disease phase was available, which in both patients demonstrated a reincrease of the sIL-2R index. sIL-2R indices were higher in samples from patients with neurosarcoidosis and diffuse leptomeningal enhancement on cranial MRI (n = 11) than in those from patients without diffuse leptomeningal enhancement (n = 4, p = 0.022; figure 3C). Finally, the CSF white cell count correlated strongly with the sIL-2R index in patients with neurosarcoidosis (r = 0.72, p < 0.0001; figure 3D). Similar results were obtained when clinical, MRI, and CSF parameters were correlated with CSF sIL-2R levels or QsIL-2R (appendix e-3, links.lww.com/NXI/A239).

## Discussion

The 3 key results of this systematic evaluation of sIL-2R in CSF as a biomarker for neurosarcoidosis are that CSF sIL-2R parameters are (1) elevated in more than 50% of samples from patients with neurosarcoidosis, (2) correlate with clinical, radiologic, and CSF disease activity markers of neurosarcoidosis, but (3) are also elevated in patients with viral/bacterial meningitis, neotuberculosis, and CNS lymphoma, although not in patients with MS and GBS.

In the present work, sIL-2R was measured in CSF by semi-automated chemiluminescent immunoassay technology (IMMULITE™), which has been used in routine laboratory diagnostics for measuring sIL-2R in serum for more than 15 years. Compared with determination of sIL-2R in CSF by ELISA, this technology overall appears to be more standardized and to have a higher potential for implementation in routine laboratory diagnostics.

As reference values for sIL-2R parameters in CSF, as determined by IMMULITE™, were hitherto not available, we herein provide reference values for CSF sIL-2R, Q_{sIL-2R}, and the sIL-2R index from CSF/serum samples of 115 patients with NINDs. Of note, because of ethical concerns regarding CSF withdrawal from healthy controls, our reference cohort did not include healthy controls. Nevertheless, similar to healthy controls, the 115 patients with NINDs had no evidence of inflammation in routine CSF examinations. The present reference values for individuals without inflammatory CNS conditions should thus prove valuable for future analyses of sIL-2R in CSF by IMMULITE™ technology. It needs to be mentioned that sIL-2R reference values were obtained with previously frozen CSF and serum samples. Although we thus cannot exclude that sIL-2R levels in fresh CSF and serum samples might be somewhat higher than those reported herein, we consider major differences to be rather unlikely.

Many proteins present in CSF are derived from blood, meaning that on disturbances of the blood-CSF barrier, the CSF concentrations of those proteins increase. To properly assess the CSF concentrations of such serum-derived proteins, their CSF concentrations need to be related to their serum concentrations by a CSF/serum quotient. Q_{Ab} is the most widely used indicator of blood-CSF barrier function. Thus, plotting the CSF/serum quotient of a given protein against Q_{Ab} in CSF/serum quotient diagrams (or Reiber diagrams) allows us to analyze the concentration of a given protein in CSF irrespective of blood-CSF barrier function. Similar to the approach pursued to establish CSF/serum quotient diagrams for immunoglobulin (Ig)G, IgA, and IgM, we here establish CSF/serum quotient diagrams for sIL-2R, leveraging sIL-2R serum and CSF data from 115 patients with NINDs, which were purposefully chosen to have a wide range of Q_{Ab} values. Plotting of the Q_{sIL-2R} against Q_{Ab} in CSF/serum quotient diagrams revealed an increase of Q_{sIL-2R} with increasing Q_{Ab}, demonstrating that under normal, i.e., noninflammatory conditions, sIL-2R in CSF is indeed derived from serum. Accordingly, the association of CSF sIL-2R levels with age in patients with NINDs is explained by the physiologic increase of Q_{Ab} across the lifespan, resulting from a more leaky blood-CSF barrier with increasing age.
Diffusion of serum proteins across the blood-CSF barrier depends on the size of the protein, with smaller molecules crossing the barrier more easily than larger ones. The fact that in patients with NINDs, QsIL-2R values were higher than QAlb values is thus explained by the lower molecular weight of sIL-2R (55 kDa) compared with albumin (69 kDa).

CSF/serum sIL-2R quotient diagrams permitted to detect an intrathecal synthesis of sIL-2R in neurosarcoidosis, viral/ bacterial meningitis, neurotuberculosis, and CNS lymphoma. The most likely explanation for this observation is that in these conditions, inflammatory or neoplastic cells that invade the CNS shed sIL-2R into the CSF, as also suggested by the strong...
A correlation of the CSF cell count and CSF sIL-2R parameters in patients with neurosarcoidosis.

An important conclusion of our findings is that determination of sIL-2R in CSF alone cannot define whether elevated CSF sIL-2R concentrations result from diffusion of sIL-2R across a disturbed blood-CSF barrier or from production of sIL-2R in CSF. Analysis of sIL-2R in CSF/serum quotient diagrams and calculation of the sIL-2R index, which both take into account blood-CSF barrier function, seem thus more informative than the isolated analysis of sIL-2R concentrations in CSF. This is clearly exemplified by patients with bacterial meningitis, who, compared with patients with NINDs, have markedly higher CSF sIL-2R levels but similar sIL-2R index levels, indicating that the elevated sIL-2R levels in CSF result from the strongly disturbed blood-CSF barrier function in this condition.

Importantly, elevated CSF sIL-2R parameters were not only seen in neurosarcoidosis but also in diseases that must be considered in the clinical differential diagnosis of neurosarcoidosis, such as neurotuberculosis and CNS lymphoma. These findings are consistent with previous reports on elevated CSF sIL-2R levels in neurotuberculosis and CNS lymphoma and show that elevated sIL-2R parameters in CSF are not specific for neurosarcoidosis. Nevertheless, in some situations, MS can be a relevant differential diagnosis of neurosarcoidosis. It is therefore noteworthy that none of the patients with MS studied in this work had elevated sIL-2R parameters in CSF, even if the majority of those patients had active disease at the time of lumbar puncture. The detection of elevated CSF sIL-2R parameters may therefore be useful for differentiation of patients with neurosarcoidosis from patients with active MS. Why CSF sIL-2R parameters are normal in MS, which is a prototypical inflammatory CNS disease, remains to be further explored. However, our findings suggest that in contrast to neurosarcoidosis, infectious CNS diseases, and CNS lymphoma, the cell types and pathways contributing to the shedding of sIL-2R into the CSF may not be activated in MS.

Figure 2: sIL-2R in serum and CSF, QsIL-2R, and sIL-2R index in the different groups of patients analyzed in this study. Data are presented as dot plots with median and interquartile ranges. The dotted lines indicate the cutoff value for the respective parameters (see table 2), levels above which are considered to be increased. The statistical significance (pairwise group comparisons by Mann-Whitney U tests) of values that were higher in the different groups of patients than in NINDs is indicated: ****p < 0.0001. GBS = Guillain-Barre syndrome; NINDs = noninflammatory neurologic diseases; ns = not significant; QAlb = CSF/serum albumin quotient; QsIL-2R = CSF/serum soluble interleukin-2 receptor quotient; sIL-2R index = soluble interleukin-2 receptor index (QsIL-2R/QAlb).
Diffuse leptomeningeal gadolinium enhancement on MRI is a typical MRI feature of active neurosarcoidosis that tends to revert under therapy. We have previously found that in patients with neurosarcoidosis, CSF cell count is associated with diffuse leptomeningeal gadolinium enhancement and clinical disease activity. The correlation of CSF sIL-2R parameters with clinical disease activity, leptomeningeal gadolinium enhancement, and the CSF cell count suggests that CSF sIL-2R parameters may represent disease activity biomarkers in neurosarcoidosis. Still, the added value of determining CSF sIL-2R parameters, compared with traditional disease activity markers, e.g., MRI, currently remains unclear and needs to be carefully weighted against the risk and burden of lumbar punctures.

The results of our present work are consistent with those of a previous smaller study that analyzed sIL-2R by ELISA in patients with neurosarcoidosis (n = 11), other inflammatory diseases, and healthy controls and found elevated CSF sIL-2R parameters in neurosarcoidosis compared with healthy controls and patients with MS, as well as an intraindividual correlation of CSF sIL-2R with disease activity. The current investigation extends the findings of this work by analyzing CSF sIL-2R parameters in a larger group of patients and additional relevant controls, including NINDs and CNS lymphoma, by a more detailed demonstration of a correlation of CSF sIL-2R parameters with disease activity in neurosarcoidosis and by establishing reference values for CSF sIL-2R parameters and sIL-2R CSF/serum quotient diagrams.

A limitation of the present study is its retrospective approach. Furthermore, inclusion of an even larger number of CSF/serum samples, in particular from patients with rarer diagnoses, e.g., neurotuberculosis, and inclusion of further inflammatory or infectious diseases, e.g., neuroborreliosis, might have been desirable. Still, to the best of our knowledge, the present study represents the largest and most comprehensive evaluation of CSF sIL-2R parameters as diagnostic and disease activity biomarkers for neurosarcoidosis compared with relevant control conditions conducted to date.
Altogether, the findings of this investigation should prove helpful in clinical practice for both informed decisions on when to determine sIL-2R in CSF and for the rational interpretation of CSF sIL-2R test results.

**Acknowledgment**
The authors thank Marina Schreiber, Liane Barnick, Ute Bergemann, and Rita Benz for excellent technical assistance.

**Study funding**
This work was supported by Stiftung Charité (BIH Clinical Fellow Program); kit reagents for measurements of soluble interleukin-2 receptor were partly provided by Siemens Healthcare Diagnostics GmbH.

**Disclosure**
C. Otto, O. Wengert, N. Unterwalder, and C. Meisel report no disclosures relevant to the manuscript. K. Ruprecht received research support from Novartis, Merck Serono, German Ministry of Education and Research, and Stiftung Charité and travel grants from Bayer, Biogen Idec, Merck Serono, Sanoﬁ-Aventis/Genzyme, Teva, Roche, Novartis, and the Guthy-Jackson Charitable Foundation. Go to Neurology.org/NN for full disclosures.

**Publication history**
Received by Neurology: Neuroimmunology & Neuroinflammation November 19, 2019. Accepted in final form March 26, 2020.

### Appendix (continued)

| Name               | Location                                    | Contribution                                                                                                   |
|--------------------|---------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| Nadine Unterwalder,  | Labor                                       | Analyzed and interpreted the data and revised the manuscript for intellectual content                           |
| MD                 | Berlin-Charité Vivantes GmbH, Germany       |                                                                                                               |
| Christian Meisel, MD | Labor                                       | Designed and conceptualized the study; analyzed and interpreted the data; and revised the manuscript for intellectual content |
|                    | Berlin-Charité Vivantes GmbH, Germany and Chari-té Universitätsmedizin Berlin, Germany |                                                                                                               |
| Klemens Ruprecht, MD | Charité-Universitätsmedizin Berlin, Germany | Designed and conceptualized the study; analyzed and interpreted the data; and drafted the manuscript for intellectual content |

### References
1. Grunewald J, Grutters JC, Arkema EV, Sakerkoo LA, Moller DR, Muller-Quernheim J. Sarcoidosis. Nat Rev Dis Primers 2019;5:45.
2. Hoitsma E, Faber CG, Drent M, Sharma OP. Neurosarcoidosis: a clinical dilemma. Lancet Neurol 2004;3:397–407.
3. Stern BJ, Royal W III, Gelfand JM, et al. Definition and consensus diagnostic criteria for neurosarcoidosis: from the neurosarcoidosis consortium consensus group. JAMA Neurol 2018;75:1546–1553.
4. Rubin LA, Nelson DL. The soluble interleukin-2 receptor: biology, function, and clinical application. Ann Intern Med 1990;113:619–627.
5. Abbas AK, Trotta E, Simeonov DR, Ma son A, B luestone JA. Revisiting IL-2: biology and therapeutic prospects. Sci Immunol 2018;3.
6. Morris JC, Waldmann TA. Advances in interleukin 2 receptor targeted treatment. Ann Rheum Dis 2000;59(suppl 1):i109–i114.
7. Ramos-Casals M, Retamozo S, Siso-Almirall A, Perez-Alvarez R, Pallares I, Brito-Zeron P. Clinically-useful serum biomarkers for diagnosis and prognosis of sarcoidosis. Expert Rev Clin Immunol 2019;15:391–405.
8. Peteret HF, Reske D, Tumani H, et al. Soluble CSF interleukin 2 receptor as indicator of neurosarcoidosis. J Neurol 2010;257:1855–1863.
9. Zajicek JP, Scolding NJ, Foster O, et al. Central nervous system sarcoidosis—diagnosis and management. QJM 1999;92:103–117.
10. Wengert O, Rothenfusser-Korber E, Voelrath B, et al. Neurosarcoidosis: correlation of cerebrospinal fluid findings with diffuse leptomeninged gadolinium enhancement on MRI and clinical disease activity. J Neurol Sci 2013;335:124–130.
11. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 2017;16:162–173.
12. Reiber H. Cerebrospinal ﬂuid—physiology, analysis and interpretation of protein patterns for diagnosis of neurological diseases. Mult Scler 1998;4:99–107.
13. Rothkrantz-Kos S, Drent M, Schmitz MP, Menheere PP, van Dieijen-Visser MP. Reference values of soluble interleukin-2 receptor on the IMMULITE. Clin Chem Lab Med 2004;42:976–977.
14. Scheibe F, Flick H, Wengert O, et al. Diagnostic pitfalls: a case of neurosarcoidosis mimicking tuberculous menigitis. J Neurol 2012;259:1736–1739.
15. Bhatla G, Soni N, Endo ze R, Ganeshsh B. Magnetic resonance texture analysis utility in differentiating intraparenchymal neurosarcoidosis from primary central nervous system lymphoma: a preliminary analysis. NeuroRadiol J 2019;32:203–209.
16. Ieguchi R, Shimizu Y, Shimizu S, Kitagawa K. CSF and clinical data are useful in differentiating CNS inflammatory demyelinating disease from CNS lymphoma. Mult Scler 2018;24:1212–1223.
17. Shah R, Roberson GH, Cure JK. Correlation of MR imaging findings and clinical manifestations in neurosarcoidosis. AJNR Am J Neuroradiol 2009;30:953–961.
