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Analysis of Soluble Interleukin-2 Receptor as a Prognostic Biomarker in NMOSD and MOGAD

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ABSTRACT

Objective: Soluble interleukin-2 receptor (sIL-2R) is a biomarker for T cell activity. T cells are involved in neuromyelitis optica spectrum disorders (NMOSD) and myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) pathogenesis. However, sIL-2R has so far not been evaluated in these conditions. Here, we compared sIL-2R levels in serum and cerebrospinal fluid (CSF) of patients with aquaporin-4-IgG-seropositive and seronegative (AQP4-IgG+/−) NMOSD, MOGAD, and noninflammatory neurologic disorders (NINDs), and assessed the prognostic value of sIL-2R for future attacks.

Methods: Retrospective analysis of real-world data of patients treated at Charité—Universitätsmedizin Berlin was conducted (45 MOGAD, 14 AQP4-IgG+NMOSD, 10 AQP4-IgG−NMOSD, 69 NINDs) between 2010 and 2024. Mean (SD) follow-up time was 40 (35) months. sIL-2R differences were assessed by linear mixed models. Cox regression analysis was performed to investigate the predictive value for subsequent attacks.

Results: Serum sIL-2R was higher in AQP4-IgG+NMOSD (estimated marginal mean [EMM] 802 IU/mL) and MOGAD (569 IU/mL) compared to NINDs (404 IU/mL). In patients with a first manifestation of MOGAD, but not NMOSD, serum sIL-2R (HR = 9.07 [95% CI 1.37–60.01]) and CSF sIL-2R (HR = 3.27 [95% CI 0.61–17.45]) levels were predictive for subsequent attacks.

Interpretation: Serum sIL-2R is elevated in AQP4-IgG+NMOSD and MOGAD and may be a prognostic biomarker for a relapsing disease course in MOGAD.

1 | Introduction

Neuromyelitis optica spectrum disorders (NMOSD) and myelin oligodendrocyte glycoprotein antibody (MOG-IgG)-associated disease (MOGAD) are autoimmune inflammatory diseases of the central nervous system (CNS) [1, 2]. In the majority of patients, NMOSD is associated with IgG autoantibodies against aquaporin-4 (AQP4-IgG+NMOSD), a water channel expressed on astrocytes. A minority of patients with NMOSD do not exhibit AQP4-IgG (AQP-IgG−NMOSD) [3]. While the mechanisms in AQP4-IgG−NMOSD remain unclear, in AQP4-IgG+NMOSD antibodies against AQP4 induce complement activation leading to destruction of astrocytes [4]. In contrast, in MOGAD, the exact role of MOG-IgG in lesion formation is still controversial. Experimental studies suggest an important role for MOG-specific T cells in combination

with MOG-IgG affecting the blood–brain barrier (BBB) as well as causing oligodendrocyte damage with consecutive demyelination [5].

While AQP4-IgG+NMOSD exhibits a relapsing course, leading necessarily to a long-term immune immunotherapy to prevent relapses and accumulation of disability, MOGAD represents a monophasic disease in up to 50% [6, 7]. Early discrimination between mono- or polyphasic disease courses is crucial to either begin relapse-preventing therapies early in the disease course or to avoid potentially harmful overtreatment. Therefore, reliable prognostic biomarkers for the disease course of MOGAD are an important, yet currently unmet, clinical need.

Interleukin-2 (IL-2) has pleiotropic effects on T cells. While IL-2 plays an essential role in regulatory T-cell (Treg) differentiation and therefore in establishing immune tolerance, it also induces proliferation of effector T cells, consequently enhancing immune reactions [8]. The central effect of IL-2 is by interacting with the IL-2 receptor (IL-2R) on T cells, and to some extent on other immune cells such as dendritic cells and B-cells [9]. Elevated concentrations of soluble IL-2R (sIL-2R) have been described in a wide range of diseases such as neoplasms, infections, and autoimmune diseases [10–12]. In sarcoidosis, sIL-2R levels are being used as a biomarker for diagnosis and to monitor inflammatory activity [13]. So far, no systemic analyses of sIL-2R in MOGAD or NMOSD have been conducted.

Here, we compared levels of sIL-2R in serum and cerebrospinal fluid (CSF) between patients with MOGAD, with AQP-IgG+/AQP-IgG−NMOSD, and with noninflammatory neurologic diseases (NINDs). Furthermore, (2) we assessed sIL-2R concentrations during acute attacks and clinical remission as well as (3) the utility of sIL-2R as a prognostic biomarker for subsequent attacks.

2 | Methods

2.1 | Study Population

Data for this retrospective trial was collected from medical records of patients treated at Charité—Universitätsmedizin Berlin from January 1 2010 to December 31 2024. In summary, we filtered the hospital information system to identify all patients above 18 years for the following ICD-10 codes: G04.8 (other encephalitis, myelitis and encephalomyelitis), G04.9 (encephalitis, myelitis and encephalomyelitis, unspecified), G05.8 (encephalitis, myelitis and encephalomyelitis in other diseases classified elsewhere), G35.9 (multiple sclerosis, unspecified), G36.8 (other specified acute disseminated demyelination), G36.9 (acute disseminated demyelination, unspecified), G37.3 (acute transverse myelitis in demyelinating disease of CNS), G37.8 (other specified demyelinating diseases of CNS), G37.9 (demyelinating disease of CNS, unspecified), and H46 (optic neuritis). Subsequently, we filtered patients that were MOG-/AQP4-IgG positive or diagnosed as G36.0 (neuromyelitis optica) according to ICD-10. Based on the resulting record list, we proceeded with the patients who had at least one measurement of sIL-2R in serum or CSF performed. Lastly, we evaluated the remaining patients regarding the correct diagnosis using existing medical reports. Diagnosis of AQP4-IgG+/AQP4-IgG−NMOSD was based on the International Panel for NMO Diagnosis (IPND) 2015 criteria [14] and diagnosis of MOGAD was based on the International MOGAD Panel proposed criteria [15]. Data of participants with NINDs were taken from a previous study by our group [12] and 1:1 age and sex matched to the rest of the cohort. Information regarding these patients can be found in the supplement (Table S1).

2.2 | Data Acquisition and Laboratory Procedures

Demographic data, date of disease onset, number and dates of previous attacks, number and dates of future attacks, attack characteristics, comorbidities, medication, routine CSF findings, AQP4-/MOG-IgG titers, and serum and CSF sIL-2R levels were obtained from medical records. We included serum samples of sIL-2R measurements that were considered to be withdrawn in remission (> 90 days after symptom onset of previous attack) and during the attack (≤ 90 days after symptom onset). All CSF samples of sIL-2R measurements were withdrawn during theattack.

Patients were considered on therapy if they were receiving ongoing immunosuppressive treatment at standard dosing intervals at the time of sample collection. The last documented visit at a neurologic outpatient clinic or neurology ward at Charité—Universitätsmedizin was considered the date of last follow-up.

All abovementioned laboratory parameters were obtained at the same clinical laboratory (Labor Berlin) applying the following procedures: CSF white cells, CSF albumin, and CSF/serum albumin quotients (Q_{Alb}) were determined as described previously [16]. AQP4-IgG and MOG-IgG were determined using serum fixed cell-based assays (CBAs, Euroimmun, Lübeck, Germany). sIL-2R levels in CSF and serum (in U/mL) were determined using IMMULITE chemiluminescent immunoassays (Siemens Healthcare GmbH, Erlangen, Germany). The upper limit of normal serum sIL-2R is 710 IU/mL and for CSF sIL-2R is 50 IU/mL; levels above these thresholds were considered elevated. The upper limits of normal for sIL-2R levels in serum (≤ 710 IU/mL) and CSF (≤ 50 IU/mL) were based on the manufacturer's specification. We dichotomized CSF sIL-2R values into “normal” and “elevated” CSF sIL-2R concentrations below the assay's lower limit of quantification, as stated in the manufacturer's instructions for use (≤ 50 IU/mL) were reported by the laboratory as “normal” without indication of exact values. This accounted for the majority of CSF samples analyzed in this study (97/103). We calculated the sIL-2R index in all patients with available absolute concentrations of CSF and serum sIL-2R using the following formula: (sIL-2R CSF/sIL-2R serum)/(albumin CSF/albumin serum) = $Q_{sIL\text{-}2R}/Q_{Alb}$. The reference value for the sIL-2R index was applied as described previously [12].

2.3 | Statistical Analysis

In this retrospective, observational study the date of the first sIL-2R measurement was set as baseline. For baseline characteristics, categoric variables are expressed as absolute

numbers and relative percentages, ordinal variables are reported as medians and interquartile ranges (IQR), and continuous variables are described as means and standard deviations (SD). We refrained from formal hypothesis testing between disease groups of demographic characteristics. Non-normally distributed variables were log-transformed (sIL-2R serum, AQP4-/MOG-IgG titers, CSF cell count, CSF protein). No negative values of the aforementioned variables were present. Thirteen patients (seven MOGAD, three AQP4-IgG+NMOSD, and three AQP4-IgG−NMOSD) had more than one available sIL-2R measurement. Group differences and associations were assessed using linear mixed models to account for multiple measurements with sIL-2R as the dependent variable, (1|Patient) as the random intercept (to account for multiple measurements of individual patients) and the independent variable based on the respective analysis (e.g., diagnosis, age). Estimated marginal means (EMM, when comparing continuous and categorical/ordinal scaled variables) or β (when comparing only continuous variables) and 95% confidence intervals are reported. For easier understanding, original values instead of log-transformed values are shown. No correlation analysis was performed between current relapse activity and sIL-2R CSF as either all measurements were done during relapse (patients with MOGAD) or all measurements were normal (patients with AQP4-IgG+NMOSD and AQP4-IgG−NMOSD). Cox proportional hazards regression analyses were employed to evaluate sIL-2R in serum, CSF, and the combination of both as a marker for prognosis for first subsequent relapse. For this analysis, serum sIL-2R was transformed into a binary variable (elevated >710 IU/mL, normal ≤ 710 IU/mL). The observation period was calculated between the date of initial sIL-2R measurement and the date of last visit. Cox regression models were adjusted for age and sex. We refrained from including further potential predictors of relapse risk into the model to avoid overfitting due to the relatively small number of patients. Additional exploratory adjustments for time from symptom onset to start of attack treatment, presence of CSF-specific supernumerary oligoclonal bands (OCB), and current attack at CSF/serum withdrawal in patients with a first manifestation of MOGAD did not have a relevant influence on the results.

No Cox regression analysis was performed for CSF sIL-2R in patients with AQP-IgG+NMOSD and AQP-IgG−NMOSD due to only normal (≤ 50 IU/mL) results in these patients. A post hoc power analysis yielded a power of 71% for the Cox regression analysis in patients with a first manifestation of MOGAD. All statistical analyses were performed using R (version 4.2.2). p-values < 0.05 were considered statistically significant. Due to the exploratory nature of this trial, no correction for multiple testing was done, and all p-values should be interpreted cautiously. Interpretations are based on effect sizes.

2.4 | Ethics Approval

The local ethics committee at Charité—Universitätsmedizin Berlin approved the current study (EA4/171/24). The study was conducted in accordance with the Declaration of Helsinki in its currently applicable version and the applicable European and German laws. The requirement for written consent was waived by the ethics committee due to the use of retrospective clinical routine data.

3 | Results

3.1 | Patients

The process of data collection is summarized in Figure 1. In total, 69 patients with a total of 115 sIL-2R measurements in serum or CSF were included in the final analysis.

The patients' demographic and clinical data are provided in Table 1. Most patients were diagnosed with MOGAD (45, 65%), followed by AQP4-IgG+NMOSD (14, 20%) and AQP4-IgG−NMOSD (10, 15%). Patients with AQP4+NMOSD (mean age 57, SD 19 years) were on average older than patients with AQP4-IgG−NMOSD (46, SD 11 years) and patients with MOGAD (37, SD 15 years). While most patients with AQP4-IgG+NMOSD (11, 79%) and AQP4-IgG−NMOSD (7, 70%) were female, the majority of patients with MOGAD were male (26, 58%). The mean observation time was similar in AQP4-IgG+NMOSD (36 months, SD 36 months) and MOGAD (36 months, SD 34 months), but was longer in AQP4-IgG−NMOSD (62 months, SD 37 months).

3.2 | Patients With AQP4-IgG+NMOSD and MOGAD Have Higher sIL-2R Serum Levels Than NINDs

Serum sIL-2R levels in patients with AQP4-IgG+NMOSD (802 IU/mL) were higher than in patients with MOGAD (569 IU/mL, $p = 0.001$), patients with AQP4-IgG−NMOSD (472 IU/mL, $p = 0.004$), and NINDs (404 IU/mL, $p < 0.001$, Figure 2). Patients with MOGAD had significantly higher serum sIL-2R than NINDs ($p = 0.001$), but not than patients with AQP4-IgG−NMOSD ($p = 0.661$). No significant difference was seen between NINDs and AQP4-IgG−NMOSD ($p = 0.123$). As current disease activity potentially influences sIL-2R levels, a subgroup analysis including measurements taken during remission was performed. Here, patients with AQP4-IgG+NMOSD (738 IU/mL, $p < 0.001$) and MOGAD (476 IU/mL, $p = 0.045$) still had significantly higher serum sIL-2R levels than NINDs. Patients with AQP4-IgG+NMOSD in remission also had higher levels of serum sIL-2R than patients in remission with AQP4-IgG−NMOSD (442 IU/mL, $p = 0.024$) and MOGAD ($p = 0.027$).

No significant association was found between use of immunosuppressive medications at the time of obtainment of serum samples and levels of serum sIL-2R (on therapy vs. not on therapy samples: 587 IU/mL vs. 545 IU/mL, $p = 0.972$). When analyzing only samples from patients considered not on therapy, patients with AQP4-IgG+NMOSD still had significantly higher levels of serum sIL-2R than patients with AQP4-IgG−NMOSD (869 IU/mL vs. 476 IU/mL, $p = 0.021$) and non-significantly higher levels than patients with MOGAD (869 IU/mL vs. 589 IU/mL, $p = 0.052$).

Thirty-four sIL-2R measurements in CSF were performed (21 in MOGAD, five in AQP4-IgG+NMOSD, eight in

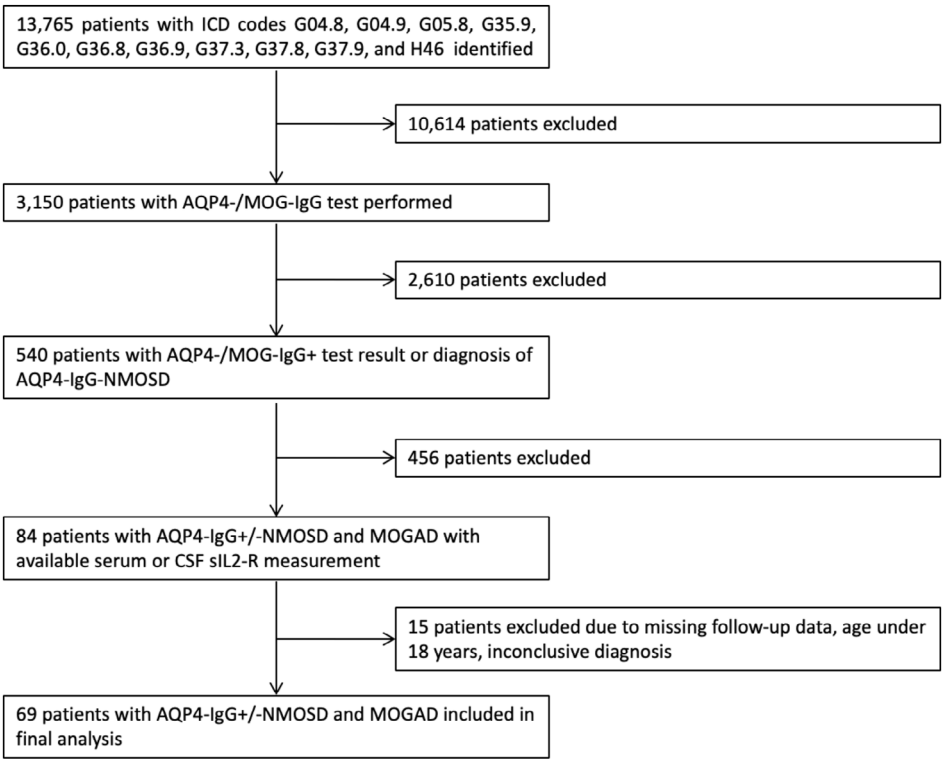

FIGURE 1 | Flowchart of data acquisition.

AQP4-IgG−NMOSD). One patient with AQP4-IgG+NMOSD had two CSF sIL-2R tests available (both normal). Elevated sIL-2R CSF levels (> 50 IU/mL) were only recorded in patients with MOGAD (6/21, 29%). Serum sIL-2R levels did not differ between MOGAD patients with elevated or normal CSF sIL-2R (EMM of serum sIL-2R in normal vs. elevated CSF sIL-2R: 577 IU/mL vs. 602 IU/mL, $p = 0.990$).

3.3 | Associations of sIL-2R Levels With Age, Sex, and CSF Parameters

In the total study cohort, serum sIL-2R levels were not associated with sex (EMM female vs. male: 497 IU/mL vs. 521 IU/mL, $p = 0.889$) but non-significantly with age ($\beta = 0.004$ [95% CI −0.00–0.01], $p = 0.055$). Male patients (1233 IU/mL, $n = 3$) with AQP4-IgG+NMOSD had higher levels of serum sIL-2R than female patients (615 IU/mL, $p = 0.005$), although the analysis was limited by the small sample size. Further, in patients with AQP4-IgG+NMOSD, higher serum sIL-2R levels were significantly associated with higher age ($\beta = 0.01$ [95% CI 0.00–0.02], $p = 0.016$). These associations were not seen in patients with NINDs, AQP4-IgG−NMOSD, and MOGAD (Table S2).

Elevated sIL-2R results in CSF were associated with higher CSF cell counts (normal vs. elevated: 14/μL vs. 250/μL, $p = 0.006$). Patients with elevated CSF sIL-2R had no significantly different Q_{Alb} compared to patients with normal CSF sIL-2R ($Q_{Alb} \times 10^3$ normal vs. elevated CSF sIL-2R: 8.1 vs. 10.2, $p = 0.560$). Five patients with MOGAD with elevated CSF sIL-2R had absolute CSF and serum sIL-2R concentrations available. The mean sIL-2R index in these patients was 38 (SD 37). Four of these patients had a sIL-2R index above 11, indicating an intrathecal synthesis of sIL-2R, although interpretation is limited by the small sample size.

3.4 | Acute Myelitis but Not Current Attack Is Associated to Higher sIL-2R Serum Levels

Fifty-three measurements of serum sIL-2R (35 in MOGAD, eight in AQP4-IgG+NMOSD, 10 in AQP4-IgG−NMOSD) were performed during acute attacks. No sIL-2R CSF test was done during remission. There was no statistically significant difference between serum sIL-2R levels during an attack and in remission in patients with AQP4-IgG+NMOSD (EMM attack vs. remission: 808 IU/mL vs. 738 IU/mL, $p = 0.862$), AQP4-IgG−NMOSD (490 IU/mL vs. 491 IU/mL, $p = 0.568$) or MOGAD (607 IU/mL vs. 476 IU/mL, $p = 0.703$).

When comparing sIL-2R serum levels measured during an acute attack between different types of attacks, patients with MOGAD and acute myelitis ($n = 14$) had a higher sIL-2R serum than other attack types ($n = 26$; myelitis against all other attack types: 848 IU/mL vs. 536 IU/mL, $p = 0.014$). No statistically significant differences between any attack types in AQP4-IgG+NMOSD or AQP4-IgG−NMOSD were observed (Table S3).

3.5 | sIL-2R in Serum and CSF at First Disease Manifestation Predict a Recurrent Disease Course in Patients With MOGAD

Mean follow-up time was 40 months (SD 35 months). Of all included MOGAD patients, 28 (62%) patients with MOGAD had a sIL-2R measurement at the time of first attack and follow-up

TABLE 1 | Demographic and clinical findings.

	Overall, *N* = 138	**AQP4-IgG+NMOSD, *N* = 14**	**AQP4-IgG−NMOSD, *N* = 10**	**MOGAD, *N* = 45**	**NINDs, *N* = 69**
Serum samples analyzed for sIL-2R, *n*	150	16	16	49	69
CSF samples analyzed for sIL-2R, *n*	103	5	8	21	69
Serum sIL-2R tests during attack/all serum samples, *n*/*N* (%)	53/79 (67)	8/14 (57)	10/16 (63)	35/49 (71)	.
CSF sIL-2R tests during attack/all CSF samples, *n*/*N* (%)	29/34 (85)	*3/5 (60)*	5/8 (63)	21/21 (100)	.
Sex, female, *n*/*N* (%)	72/138 (52)	11/14 (79)	7/10 (70)	19/45 (42)	35/69 (51)
Age in years, mean (SD)	43 (17)	57 (19)	46 (11)	37 (15)	44 (17)
Antibody titer at diagnosis, median (IQR)	1:100 (1:32, 1:200)	1:320 (1:32, 1:3200)	.	1:80 (1:10, 1:200)	.
Immunotherapy at baseline, *n*/*N* (%)					.
None	60/69 (87)	9/14 (64)	9/10 (90)	42/45 (93)	.
Azathioprin	*2/69 (3)*	*1/14 (7)*	*0/10 (0)*	*1/45 (2)*	.
Rituximab	*4/69 (6)*	*3/14 (22)*	*1/10 (10)*	*0/45 (0)*	.
Satralizumab	*1/69 (1)*	*1/14 (7)*	*0/10 (0)*	*0/45 (0)*	.
Tocilizumab	*2/69 (3)*	0/14 (0)	*0/10 (0)*	*2/45 (5)*	.
Number of serum sIL-2R tests considered on treatment/all serum samples, *n*/*N* (%)[a]	18/81 (22)	6/16 (38)	5/16 (31)	7/49 (14)	.
Number of CSF sIL-2R tests considered on treatment/all CSF samples, *n*/*N* (%)[a]	4/34 (12)	*2/5 (40)*	*2/8 (25)*	*0/21 (0)*	.
Time between last application of immunosuppressive treatment and sIL-2R test in days, mean (SD)	51 (70)	75 (88)	35 (77)	4 (15)	.
Observation time in months, mean (SD)	40 (35)	36 (36)	62 (37)	36 (34)	.
Number of previous attacks, median (IQR)	0 (0, 1)	1 (0, 3)	2 (0, 2)	0 (0, 1)	.
Type of previous attack before baseline, *n*/*N* (%)					.
None	42/69 (61)	6/14 (43)	5/10 (50)	31/45 (69)	.
ADEM	*2/69 (3)*	*0/14 (0)*	*0/10 (0)*	*2/45 (5)*	.
Brainstem	*1/69 (1)*	*1/14 (7)*	*0/10 (0)*	*0/45 (0)*	.
Myelitis	17/69 (25)	7/14 (50)	*4/10 (40)*	6/45 (13)	.

(Continues)

TABLE 1 | (Continued)

	Overall, $N=138$	AQP4-IgG+NMOSD, $N=14$	AQP4-IgG−NMOSD, $N=10$	MOGAD, $N=45$	NINDs, $N=69$
ON	7/69 (10)	*0/14 (0)*	*1/10 (10)*	6/45 (13)	.
CSF cell count/μL, median (IQR)	6 (3, 17)	11 (9, 12)	2 (1, 4)	8 (3, 102)	.
CSF protein in mg/dL, median (IQR)	428 (330, 627)	399 (334, 528)	501 (286, 625)	440 (337, 665)	.

Note: Cells with a sample count of $n<5$ are marked italicized. "." indicates data not available or not applicable.
Abbreviations: ADEM, acute disseminated encephalomyelitis; AQP4-IgG+NMOSD, aquaporin 4-IgG seropositive neuromyelitis optica spectrum disorders; AQP4-IgG−NMOSD, aquaporin 4-IgG seronegative neuromyelitis optica spectrum disorders; CSF, cerebrospinal fluid; IQR, interquartile range; MOGAD, myelin oligodendrocyte glycoprotein antibody-associated disease; ON, optic neuritis; SD, standard deviation; sIL-2R, soluble interleukin-2 receptor.
[a]On therapy was defined as receiving immunosuppressive treatment at standard dosing intervals depending on the respective drug at the time of sample collection.

data available (Figure 3). In these patients, elevated serum sIL-2R levels at first attack were associated with the risk for a subsequent relapse (HR = 9.07 [95% CI 1.37–60.01], $p=0.022$, $n=23$). When including serum sIL-2R as a log-transformed continuous variable into the Cox regression analysis, patients with higher serum sIL-2R had a shorter time to relapse, although this result was not statistically significant (HR 3.26 [95% CI 0.79–17.53], $p=0.100$, $n=23$).

Patients with elevated CSF sIL-2R also had a non-significantly greater risk for a following relapse (HR = 3.27 [95% CI 0.61–17.45], $p=0.165$, $n=20$). The association between the risk for a subsequent relapse after the first attack in patients with MOGAD and elevated sIL-2R levels persisted in the combined analysis of serum and CSF sIL-2R (HR = 8.24 [95% CI 1.67–40.62], $p=0.009$, $n=30$). When including all MOGAD patients in the analysis, neither serum sIL-2R (HR = 2.91 [95% CI 0.57–14.96], $p=0.200$, $n=36$) nor CSF sIL-2R (HR = 4.42 [95% CI 0.88–22.22], $p=0.071$, $n=21$) was significantly associated with the risk of a subsequent attack following the respective test.

In patients with AQP4-IgG+NMOSD and AQP4-IgG−NMOSD, no association exists between baseline serum sIL-2R and the risk of subsequent attacks (AQP4-IgG+NMOSD: HR = 0.91 [95% CI 0.01–60.90], $p=0.963$, $n=11$; AQP4-IgG−NMOSD: HR = 1.00 [95% CI 0.99–1.01], $p=0.775$, $n=10$).

4 | Discussion

In the current study, we showed that (1) sIL-2R serum concentrations are elevated in AQP4-IgG+NMOSD and MOGAD compared to NINDs, with the highest values in patients with AQP4-IgG+NMOSD. (2) Further, sIL-2R levels were not affected by current disease activity at the time of measurement. (3) Last, we demonstrated that sIL-2R concentrations in serum and potentially CSF at the time of a first attack are predictive of a relapsing disease course in patients with MOGAD.

This is the first study to systematically analyze sIL-2R in serum and CSF in patients with MOGAD, AQP4-IgG+NMOSD, and AQP4-IgG−NMOSD. To the best of our knowledge, only one prior study assessed levels of CSF sIL-2R in patients with NMOSD in comparison to MS and CNS lymphoma [17]. However, the focus of the study was on finding CSF biomarkers to differentiate CNS lymphoma from the other diseases.

Previous studies have shown that T cells play a central role in the pathophysiology of both NMOSD and MOGAD. Post-mortem histopathological studies showed complement deposition and T cell infiltration in AQP4-IgG+NMOSD lesions [4]. Further, peripheral blood cell profiles demonstrated a significant increase of Th17 T cells during attacks in AQP4-Ig+NMOSD and MOGAD while still being elevated in remission [18, 19]. Since sIL-2R is considered a marker of T cell activation [20], elevated sIL-2R levels in AQP4-IgG+NMOSD and MOGAD, compared to NINDs, align with these underlying immunopathological mechanisms. However, while sIL-2R levels showed promising results as a marker of disease activity in neurosarcoidosis [12], it did not show any association in discriminating between relapse or remission in AQP4-Ig+NMOSD, AQP4-IgG−NMOSD,

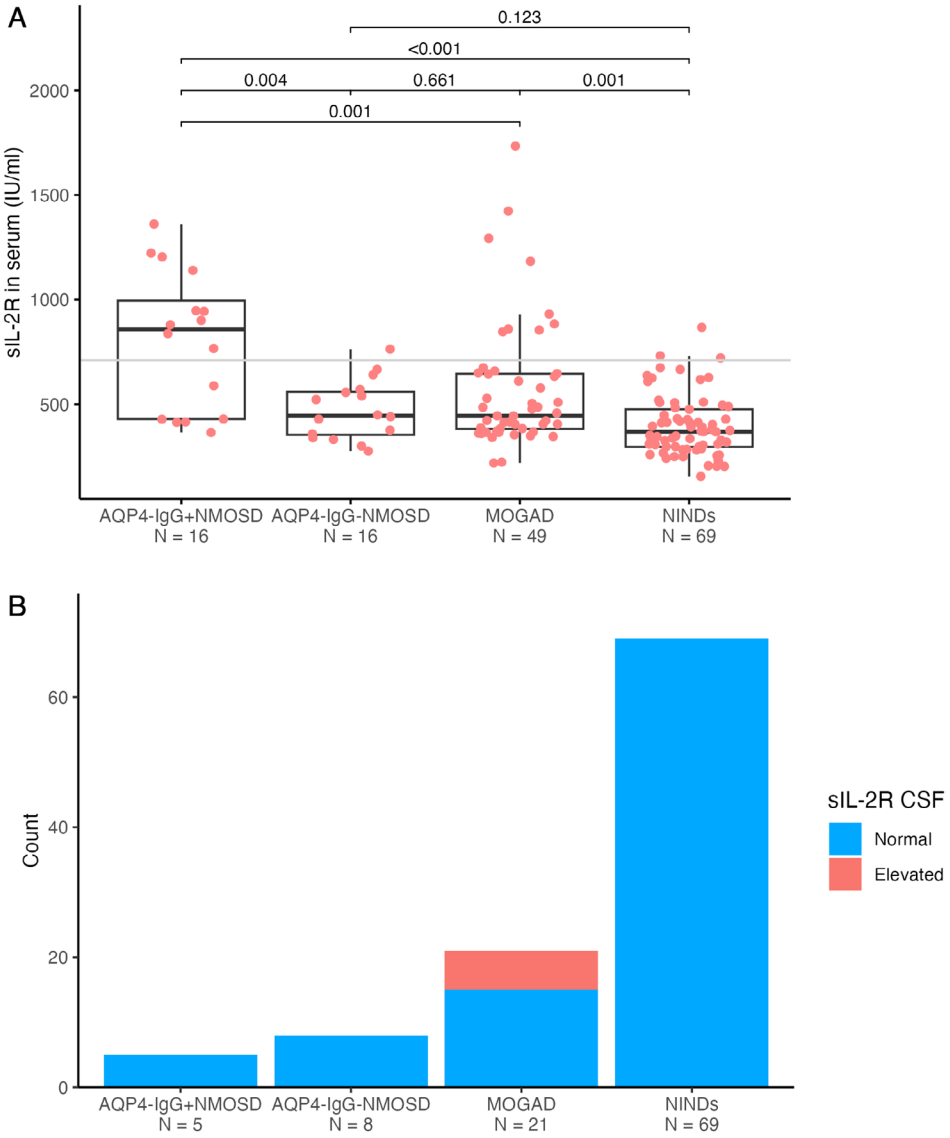

FIGURE 2 | Comparisons of baseline sIL-2R values at baseline. (A) Boxplot with overlayed scatterplot depicting absolute sIL-2R measurements. Gray line indicates threshold of 710 IU/mL between normal and elevated serum sIL-2R levels. (B) Bar chart comparing counts of elevated (> 50 IU/mL)/normal (≤ 50 IU/mL) measurements of CSF sIL-2R. *N* represents the number of serum and CSF samples for each group. Abbreviations: AQP4-IgG+NMOSD, aquaporin 4-IgG seropositive neuromyelitis optica spectrum disorders; AQP4-IgG−NMOSD, aquaporin 4-IgG seronegative neuromyelitis optica spectrum disorders; MOGAD, myelin oligodendrocyte glycoprotein antibody-associated disease; NINDs, noninflammatory neurologic diseases; SIL-2R, soluble interleukin-2 receptor.

or MOGAD. Thus, our data suggest that the intensity of T cell activation during attack might not be enough to notably raise sIL-2R levels. Therefore, sIL-2R could act as a supplementary biomarker to CNS-specific markers such as neurofilament light, which reflect tissue damages [21].

The most important finding of the current study is the potential prognostic value of sIL-2R in serum and to some extent in CSF for prediction of a polyphasic versus monophasic disease course in patients with MOGAD. Previous studies showed that Hispanic ethnicity, female sex, persistent detection of MOG-IgG after a first attack, and specific MOG-IgG epitopes were associated with recurrent attacks in MOGAD [22–24]. sIL-2R measurement could help guide clinicians in the future in deciding which patients with MOGAD should receive immunosuppressive medications already after the first attack. However, the association of serum sIL-2R and the risk for relapse was statistically significant only when dichotomizing serum sIL-2R concentrations, but not when including continuous values. This is potentially explained by a threshold, non-linear effect of serum sIL-2R whereby risk remains low until levels of sIL-2R exceed 710 IU/mL. Larger, follow-up studies are needed to further investigate the potential value of serum sIL-2R for assessment of the relapse risk in MOGAD.

Beyond clinical practice, the lack of biomarkers for early identification of MOGAD patients with a high relapse risk poses an obstacle for clinical trials: in ongoing phase 3 trials investigating the effect of satralizumab (clinicaltrials.gov ID NCT05271409) and rozanolixizumab (clinicaltrials.gov ID NCT05063162) the presence of at least two attacks is a requirement for patient inclusions.

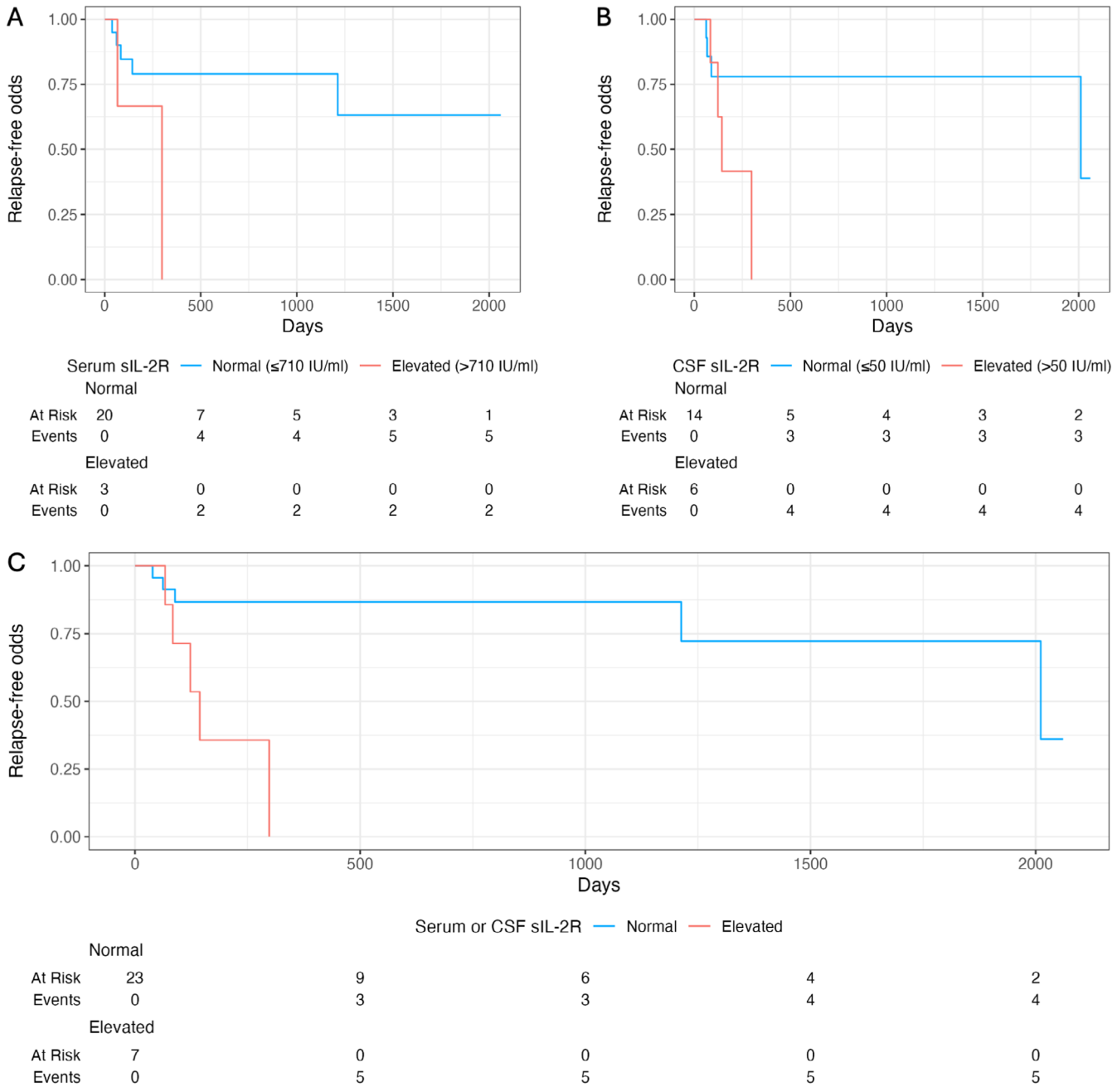

FIGURE 3 | Relapse-free survival in patients with first onset MOGAD with elevated or normal sIL-2R. Kaplan–Meier curves for serum (A), CSF (B) and combined (C) sIL-2R measurements. Serum and CSF sIL-2R concentrations measured during an attack at disease onset were dichotomized in "elevated" (serum: > 710 IU/mL; CSF: > 50 IU/mL) or "normal" (serum: ≤ 710 IU/mL; CSF: ≤ 50 IU/mL) according to the routine laboratory cut-off concentration. Risk tables are shown for serum sIL-2R, CSF sIL-2R, and combined sIL-2R variable (i.e., elevated result in either serum or CSF). Hazard ratios with 95% confidence intervals were determined by Cox proportional hazard regression adjusted for age and sex. Abbreviations: CI, confidence intervals; CSF, cerebrospinal fluid; HR, hazard ratio; sIL-2R, soluble interleukin-2 receptor.

The strength of the current study is the systematic inclusion of real-world data from a tertiary university hospital. All included patients were thoroughly assessed including medical history, MRI parameters, and laboratory measurement according to the current diagnostic guidelines. Further, appropriate control groups were chosen from an identical clinical setting.

However, this study also has several limitations: First, follow-up data on subsequent relapses could potentially be incomplete due to the study design. Yet, most patients who are being diagnosed at Charité—Universitätsmedizin Berlin with MOGAD, AQP4-IgG+NMOSD, or AQP4-IgG−NMOSD are being monitored at the Charité—Universitätsmedizin Berlin outpatient clinics and admitted to our neurology wards in case of a relapse. Second, in NMOSD patients almost all measurements were performed during attacks. Third, we dichotomized CSF sIL-2R measurements into "elevated" and "normal" categories, since continuous values ≤ 50 IU/mL were not available. The sIL-2R index could only be calculated in a small subset of patients ($n = 5$). Follow-up investigations using the sIL-2R index might provide additional

value for disease prediction and offer further insights into pathophysiological mechanisms. Fourth, the current hypothesis-generating study was formally underpowered leading to wide confidence intervals and reflecting the limited precision of the reported estimates. Larger prospective trials with sufficient numbers of patients are necessary to verify our results. Fifth, as our control group consisted only of patients with NINDs, a spectrum bias cannot be excluded. Future studies should additionally include patients with inflammatory diseases such as MS to elucidate the potential discriminatory value of sIL-2R.

In summary, we show that sIL-2R in serum is elevated in patients with MOGAD, AQP4-IgG+NMOSD, and AQP4-IgG−NMOSD. While sIL-2R levels did not correlate with current attacks, sIL-2R in serum and potentially in CSF predicted recurrent disease courses in patients with MOGAD. Therefore, sIL-2R might be a useful prognostic biomarker in MOGAD to support the decision on early initiation of long-term immunotherapy after the first disease manifestation.

Author Contributions

P.K. and P.S. conceptualized the study, wrote the first draft, and were responsible for data acquisition, analysis, methodology, literature review, and project administration. All other authors were responsible for data collection, gave critical input to the manuscript, supported the literature research, and helped edit the paper.

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Conflicts of Interest

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Data Availability Statement

The data supporting the findings in the present study will be made available upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Description of patients with NINDs. **Table S2:** Comparison of serum sIL-2R levels with sex and age. **Table S3:** Differences of serum sIL2-R levels between attack types in patients with AQP4-IgG+NMOSD, AQP4-IgG−NMOSD, and MOGAD.