

Investigating the Role of Serum IL-6 in Predicting Outcomes of B- Cell Depleting Therapy in Multiple Sclerosis: A Retrospective Cohort Study

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Genzyme, Gesellschaft von Freunden und Förderern der Heinrich-Heine-Universität Düsseldorf e.V., HERZ Burgdorf, Hexal, Janssen, Merck Serono, Novartis, Novo Nordisk Pharma, ONO Pharma, Roche and Teva. TR: received honoraria and/or research support from Alexion, argenx, Biogen, Merck, Novartis, Sanofi, UCB, J&J, and Roche. MP: received honoraria for lecturing and travel expenses for attending meetings from Alexion, ArgenX, Bayer Health Care, Biogen, Hexal, Merck Serono, Novartis, Roche, Sanofi-Aventis, Takeda and Teva. His research is funded by ArgenX, Biogen, Demecan, Hexal, Horizon Merck Serono, Novartis, Roche, Viartis, Takeda and Teva, all outside the scope of this work. His research is funded by the by the German Multiple Sclerosis Foundation (DMSG), the B. Braun Foundation and the German Alzheimer Society.

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Abstract

Background: Interleukin-6 (IL-6) plays a pivotal role in autoimmune inflammation through its effects on B-cell differentiation, Th17 expansion, and regulatory T-cell suppression. Given ocrelizumab's (OCR) mechanism of selective CD20⁺ B-cell depletion, baseline IL-6 levels have been hypothesized to predict disease activity and long-term outcomes in multiple sclerosis (MS). However, the prognostic value of serum IL-6 in OCR-treated patients remains unclear.

Methods: This retrospective study included 73 patients with relapsing–remitting MS (RRMS, n = 30) or primary progressive MS (PPMS, n = 43) who initiated OCR at University Hospital Düsseldorf between 2018 and 2023. Baseline serum IL-6 was compared with 87 healthy controls (HC) and correlated with clinical, radiological, and biomarker outcomes over 24 months. Clinical endpoints included confirmed progression independent of relapse activity (PIRA), and relapse-associated worsening (RAW), alongside MRI activity and Serum Neurofilament Light Chain (sNfL) and Serum Glial Fibrillary Acidic Protein (sGFAP) levels. Between-group comparisons used Welch's t-test or Wilcoxon rank-sum test, paired longitudinal comparisons used paired t-tests or Wilcoxon signed-rank tests, and associations between baseline biomarkers and outcomes were evaluated using Cox regression, multivariable linear or logistic regression, and rank-based linear models for IL-6.

Results: Baseline serum IL-6 levels showed no significant differences between the overall MS cohort and HC, nor between RRMS and PPMS subgroups. No baseline biomarker, including IL-6, sNfL, and sGFAP predicted disease activity. Longitudinal analysis under OCR revealed largely stable IL-6 concentrations but patients maintaining No Evidence of Disease Activity-3 (NEDA-3) showed 50% reduction in IL-6 at 12 months, whereas those with loss of NEDA-3 remained stable.

Conclusion: Baseline serum IL-6 alone is insufficient to predict clinical or radiological outcomes in OCR-treated MS patients. However, the significant longitudinal decline specifically in stable patients suggests that IL-6 dynamics, rather than static baseline measures, may better reflect sustained therapeutic response. These findings underscore the limited utility of serum IL-6 alone as a biomarker and support further exploration of longitudinal, multiparametric approaches.

Introduction

Multiple sclerosis (MS) is a chronic demyelinating and inflammatory disease of the central nervous system in which both T-cells and B-cells play critical roles in driving tissue damage (1). Among pro-inflammatory mediators, interleukin-6 (IL-6) has emerged as a particularly interesting cytokine due to its multifaceted effects on immune cell differentiation and function. IL-6 promotes B-cell differentiation and immunoglobulin production, potentially contributing to MS pathology. It also facilitates Th17 cell expansion while inhibiting regulatory T-cell development, fostering a pro-inflammatory environment that may exacerbate neuroinflammation and demyelination (2). Consequently, IL-6 is considered a key driver of autoimmune pathology and a potential therapeutic target in MS (3).

Beyond MS, IL-6 has been implicated in other autoimmune and inflammatory conditions, including systemic lupus erythematosus (SLE) and neuromyelitis optica spectrum disorders (NMOSD), where IL-6 receptor blockade reduces relapse rates in the latter disease (4,5). Emerging data also associates peripheral blood IL-6 alterations with various neuropsychiatric conditions, underscoring its broader potential as a biomarker across central nervous system disorders (4). Previous investigations into serum IL-6 in MS cohorts have yielded diverse results, highlighting the complexity of peripheral cytokine monitoring. While IL-6 is frequently elevated in the cerebrospinal fluid (CSF) during clinical relapses (6,7), its presence in the serum is often lower or undetectable compared to central compartments (6). Furthermore, recent studies in diverse populations have shown that while serum IL-6 can be elevated during relapses, it does not consistently correlate with all clinical subtypes (8).

Ocrelizumab (OCR) is a humanized monoclonal antibody that selectively depletes CD20⁺ B-cells and is effective in both relapsing-remitting (RRMS) and primary progressive MS (PPMS) (9). Given IL-6's role in B-cell function, it has been hypothesized that baseline IL-6 levels may predict disease activity or long-term outcomes in OCR-treated patients. However, existing data remains limited and inconclusive (10).

Building on this hypothesis, we investigated whether baseline serum IL-6 levels correlate with established markers of disease activity in MS patients initiating OCR therapy, and we compared these levels to healthy controls (HC).

Methods

Study Population

This retrospective study used data from the MS database of the Department of Neurology, Heinrich-Heine-University Düsseldorf, Germany (2018–2023). Clinical data was recorded during routine visits every six months and made available for research after written informed consent. Only patients diagnosed with RRMS or PPMS according to the 2017 McDonald criteria were included. Additional inclusion criteria comprised: (1) initiation of ocrelizumab (OCR) therapy and (2) availability of baseline serum samples collected prior to the first OCR infusion. Patients who experienced a relapse within three months prior to OCR initiation or were unable to provide consent were excluded. For comparison, baseline IL-6 levels from 87 healthy individuals without any history of autoimmune or chronic inflammatory disease were included as HC. This study was conducted in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for cohort studies.

To minimize confounding from acute inflammation, baseline C-reactive protein (CRP) levels and leukocyte counts were obtained. Patients with evidence of active infection- defined as CRP above the reference range, leukocytosis, or clinical signs of infection at the time of sample collection were excluded from the analysis.

Disability status and disease severity of MS patients were assessed using the Expanded Disability Status Scale (EDSS), a clinician-rated scale ranging from 0 (normal neurological examination) to 10 (death due

to MS)(11) EDSS was recorded at baseline (within one month prior to treatment initiation) and every six months thereafter, with changes from baseline analyzed to evaluate disability progression.

Outcome Definitions

Disability progression and disease activity were defined using established clinical and radiological criteria. Confirmed Disability Accumulation (CDA) was defined as an increase in EDSS of ≥ 1.0 points for patients with baseline EDSS ≤ 5.5 , or ≥ 0.5 points for baseline EDSS > 5.5 , confirmed at a subsequent visit ≥ 12 weeks later. Progression Independent of Relapse Activity (PIRA) was defined as CDA occurring without a preceding relapse between the two relevant EDSS assessments or within the 12 weeks prior to baseline(12) Relapse-Associated Worsening (RAW) was defined as CDA occurring during a relapse, with sustained disability progression persisting for ≥ 12 weeks; transient EDSS changes related to relapses without confirmation were excluded from both PIRA and RAW(13) Relapses were defined as neurological deterioration lasting more than 24 hours, unrelated to infection, and verified within 7 days. Absence of EDSS progression was defined as no confirmed disability increase over six months, with thresholds depending on baseline EDSS (0: ≥ 1.5 points; 1–5: ≥ 1 point; > 5 : ≥ 0.5 points). MRI activity was defined by new T1 gadolinium-enhancing lesions or new/enlarging T2 lesions in clinical routine measurements. No Evidence of Disease Activity-3 (NEDA-3) was defined as the absence of: (1) new or enlarging T2 lesions or T1 gadolinium-enhancing lesions on MRI, (2) clinical relapses, and (3) confirmed disability accumulation (CDA) over the observation period. Conversely, loss of NEDA-3 (loss of NEDA-3) was defined as the occurrence of any of these events. This composite outcome was assessed according to the standardized criteria previously described(14).

Laboratory Measures

Blood samples were obtained from patients with MS at baseline and every six months for up to two years, scheduled within two weeks prior to each subsequent OCR infusion; HCs were sampled at baseline only. Blood was drawn via a short catheter from an antecubital vein into Monovettes (Sarstedt, Nümbrecht, Germany). Serum tubes were allowed to clot for 30–60 minutes, centrifuged at 3000 rpm for 10 minutes, aliquoted, and initially frozen at -20°C . Within four weeks, samples were transferred to a -80°C freezer for long-term storage until final analysis. EDTA tubes were used for leukocyte counts, which were analyzed directly without centrifugation.

Serum was used for C-reactive protein (CRP), immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), serum neurofilament light chain (sNfL), serum glial fibrillary acidic protein (sGFAP), and IL-6. All biomarkers were assessed at baseline, whereas IL-6 was additionally measured at six-month intervals throughout the study period.

Quantification of sNfL and sGFAP was performed at the Central Institute for Clinical Chemistry and Laboratory Diagnostics, Medical Faculty, University Hospital Düsseldorf, Germany. Both biomarkers were analyzed using research-use-only electrochemiluminescence immunoassays (ECLIA) in a two-step sandwich format on the Roche Cobas 8000 analyzer (Elecsys® module), according to the manufacturer's

instructions. Laboratory personnel were blinded to clinical data. Z-scores for sNfL and sGFAP were calculated based on previously published methods (15,16)

Statistical Analysis

All analyses were conducted in RStudio. Demographic, clinical, and biomarker data were summarized using descriptive statistics. Normal distribution was tested using the Shapiro–Wilk test. Non-normally distributed data were presented as median and interquartile range (IQR), whereas normally distributed data were reported as mean $\pm$ standard deviation (SD). Between-group comparisons of continuous variables were performed using Welch's t-test for normally distributed data or the Wilcoxon rank-sum test for non-normal data. Paired longitudinal comparisons within groups were conducted using paired t-tests or Wilcoxon signed-rank tests, as appropriate. Associations between baseline biomarkers and clinical outcomes, including CDA, PIRA, RAW, NEDA-3, loss of NEDA-3, and MRI activity, were assessed using Cox proportional hazards models, multivariable linear regression, or logistic regression, adjusting for age and sex where relevant. Rank-based linear models were applied for comparisons of IL-6 levels across MS subtypes and over time. A p-value <0.05 was considered statistically significant, with correction for multiple comparisons applied where relevant.

Results

A total of 73 MS patients were included, comprising 43 with PPMS (58.9%) and 30 with RRMS (41.1%). Baseline characteristics are presented in Table 1.1 and 1.2. The mean (SD) age of the entire MS cohort was 52.2 ± 12.7 years, and 54.8% were female, with PPMS patients being older than those with RRMS (58.3 ± 9.3 vs. 43.5 ± 12 years, $p < 0.001$) and showing a slight male predominance (58.1% vs. 50%, $p = 0.654$). HC ($n = 86$) had a mean age of 46.1 ± 11.8 years and were 72.1% female. Compared with MS patients HC were significantly younger ($p < 0.001$). Median disease duration was significantly longer in PPMS (115 months, IQR 78) compared with RRMS (73 months, IQR 48; $p < 0.001$). Baseline EDSS was also higher in PPMS (median 4.0, IQR 3.375) than in RRMS (median 2.0, IQR 2.5; $p < 0.001$) (Table 1.2). Baseline IL-6 levels were similar between the overall MS cohort and HC (MS: 1.90 [IQR 1.8] pg/mL vs. HC: 1.96 [IQR 1.1] pg/mL; rank-based linear model adjusted for age and sex; $p = 0.178$) (Fig. 1A). Subgroup analysis by MS type revealed that baseline mean IL-6 level was higher in PPMS (2.30 [IQR 2.7] pg/mL), compared to RRMS patients (1.75 [IQR 0.6] pg/mL; $p = 0.024$). A significant overall group effect was observed (ANOVA; $p = 0.011$); however, post-hoc pairwise comparisons adjusted for multiple testing did not reveal statistically significant differences between individual groups, although a trend toward lower IL-6 levels in RRMS compared with HC was evident (Fig. 1B).

While baseline sGFAP z-scores were similar between MS subtypes, median baseline sNfL z-scores were elevated in RRMS (2.12 [IQR 1.9]) compared with PPMS (-0.41 [IQR 1.6]; $p < 0.001$) (Suppl. Figure 1). CRP levels were slightly higher in PPMS compared to healthy controls, though all retained samples were below the clinical threshold for acute infection (Suppl. Figure 2).

During the 24-month follow-up, 22 MS patients (30.1%) experienced CDA, 20 experienced PIRA (27.4%), and 2 experienced RAW (2.7%), with loss of NEDA-3 observed in 31 of 73 patients (42.5%).

Cox proportional hazard models revealed no significant associations between baseline IL-6 and occurrence of CDA (Suppl. Table 1.3). Also, after dichotomization of IL-6 at the median, there was no statistically significant association with the risk of CDA (HR 1.83; 95% CI 0.73–4.61; $p_{\text{adj}} = 0.989$) (Fig. 2).

In RRMS, baseline IL-6 was not associated with relapse occurrence over two years (median 1.75 vs. 1.8 pg/mL; rank-based linear model adjusted for age and sex; $p = 0.913$). Multivariable Cox regression for RAW was not performed due to the limited number of events ($n = 3$).

Multivariable linear regression identified baseline EDSS as the sole predictor of EDSS at 24 months ($\beta = 0.74$; 95% CI 0.41–1.08; $p = 0.005$), while no other biomarkers, including sNfL and sGFAP z-scores and baseline IL-6, were associated with final disability accrual or PIRA. Similarly, logistic regression demonstrated no relationship between baseline IL-6 and new T2 lesions on follow-up MRI (OR 0.86; 95% CI 0.53–1.39; $p = 0.53$), with subgroup analyses in RRMS (OR 0.27; 95% CI 0.01–5.21; $p = 0.38$) and PPMS (OR 1.04; 95% CI 0.61–1.77; $p = 0.88$) showing consistent findings.

Longitudinal analysis of serum IL-6 levels of 25 patients over 12 months during B-cell depleting therapy revealed largely stable concentrations, with no significant differences observed at any follow-up compared to baseline. This stability persisted across MS subtypes (RRMS and PPMS). Upon stratification by clinical response, early IL-6 dynamics between baseline and 6 months did not differ significantly between patients maintaining NEDA-3 and those with loss of NEDA-3 ($p = 0.126$). However, by 12 months post-ocrelizumab initiation, patients maintaining NEDA-3 exhibited a mean decrease in IL-6 from baseline to 12 months (-1.30 pg/mL), whereas patients with disease activity showed no significant change (0.09 pg/mL). The between-group difference in IL-6 change over 12 months was statistically significant ($p = 0.0318$) (Fig. 3). The between-group difference in IL-6 changes from baseline to 12 months indicated a trend toward significance after adjustment for multiple comparisons ($p = 0.064$). No immediate temporal correlation was found between the exact timing of a relapse and a spike in IL-6, though the limited number of RAW events ($n = 3$) restricted this analysis.

Discussion

In this retrospective cohort of 73 MS patients, we found that baseline serum IL-6 levels do not predict clinical or radiological outcomes over 24 months of ocrelizumab therapy. However, we observed that patients who pertained NEDA-3 status experienced a significant 50% reduction in longitudinal IL-6 levels, a change not seen in those with disease activity.

Ocrelizumab therapy was intentionally incorporated in our cohort to investigate biomarkers predictive of disease activity in both PPMS and RRMS patients. The rationale for this investigation lies in the complex interplay between B cells and IL-6. IL-6-producing B-cells drive proinflammatory Th17 responses integral

to MS pathogenesis, and ocrelizumab effectively targets these cells, thereby reducing inflammation and relapses (17).

Hypothetically, the depletion of these CD20⁺ B-cells should result in a concurrent reduction of serum IL-6, serving as a marker for therapeutic efficacy. However, despite effective B-cell depletion, a subset of patients continued to experience disease activity, indicating that compensatory or IL-6-independent pathways underpin progression in these cases(18). Within this context, our findings indicate that serum IL-6 lacks sufficient sensitivity to predict clinical disease activity or associated neurodegenerative markers.

Although serum IL-6 did not differentiate MS patients from controls, mean baseline levels were highest in PPMS, suggesting a possible association with progressive disease. This pattern aligns with the concept that PPMS is characterized by chronic, low-grade inflammation and neurodegeneration, processes known to drive increased systemic IL-6 production, particularly with advancing age. Notably, previous transcriptomic studies have reported reduced IL-6 receptor expression in PPMS compared with RRMS, a finding that may reflect receptor downregulation or shedding in the setting of chronically elevated IL-6 levels (19).

The higher baseline IL-6 in PPMS patients might also reflect age-dependent immune signatures, as our PPMS cohort trended towards an older age. This chronic low-grade systemic inflammation may contribute to the relatively high rate of lo-NEDA-3 (42.5%) and CDA observed in this real-world cohort(20).

Taken together, higher IL-6 concentrations in PPMS combined with lower IL-6R expression may represent a compensatory or exhaustion-related immune signature rather than an acute inflammatory response. Furthermore, this may indicate that elevated IL-6 is more characteristic of PPMS and could help distinguish disease courses, though the small sample size limits firm conclusions.

Notably, sNfL and GFAP z-scores in this cohort did not predict outcomes, in contrast to other studies (15,21), which may be explained by the higher age of our patients and the relatively low relapse activity.

In our subgroup with available longitudinal serum samples, IL-6 levels remained largely stable over 24 months of B-cell depleting therapy. When patients were stratified by NEDA-3 status at 12 months, those who maintained NEDA-3 showed significant reductions in IL-6, whereas patients experiencing loss of NEDA-3 exhibited minimal change. The between-group difference at 12 months suggested a trend toward significance after correction for multiple testing, although sample sizes were limited. These observations raise the possibility that longitudinal IL-6 dynamics may reflect clinical stability under anti-CD20 therapy, warranting further investigation in larger cohorts to determine its potential utility as a biomarker.

Notably, prior research consistently links cerebrospinal fluid (CSF) IL-6 - not serum IL-6 - with MS disease activity and disability. Elevated CSF IL-6 correlates with severity and is higher in PPMS compared with

controls. This divergence likely reflects cytokine compartmentalization and blood-brain barrier selectivity limiting IL-6 spill-over into serum, except possibly in progressive MS with chronic blood-brain barrier disruption (22). Our lack of CSF IL-6 data notwithstanding, this mechanistic insight contextualizes our negative serum IL-6 findings, underscoring CSF IL-6 as a more sensitive CNS inflammation biomarker.

Thus, serum IL-6 alone cannot capture the full spectrum of inflammatory and neurodegenerative processes in MS progression under B-cell therapy. Research should emphasize the multifaceted B-cell - IL-6 axis and develop composite biomarkers covering diverse pathophysiological routes, improving patient stratification and refining therapeutic targeting beyond IL-6.

Comparatively, IL-6 is a robust biomarker in neuromyelitis optica spectrum disorder (NMOSD), where it closely correlates with disease activity, relapse severity, and disability both in serum and CSF. IL-6 drives NMOSD pathogenesis by promoting plasmablast survival, aquaporin-4 antibody production, blood-brain barrier disruption, and enhanced proinflammatory T-cell activation. IL-6 receptor blockade significantly reduces NMOSD relapses, underscoring IL-6's role as both biomarker and therapeutic target(23). In contrast, MS exhibits heterogeneous immunopathology with multiple cytokines and mechanisms fluctuating by stage and subtype, limiting IL-6's predictive value. This disease-specific distinction emphasizes the importance of appropriate biomarker validation tailored to distinct CNS autoimmune disorders.

Several methodological and biological factors may also explain our lack of serum IL-6 predictive value in MS: moderate sample size and incomplete longitudinal sampling limit statistical power; IL-6 variability from external factors obscures specific signals; single baseline measures miss post-treatment cytokine dynamics; and retrospective real-world design entails sampling heterogeneity reducing biomarker sensitivity.

In conclusion, our data reinforce IL-6's limited utility as a serum biomarker for disease monitoring in B-cell-treated MS. Future studies should integrate longitudinal, multiparametric approaches combining cytokine profiles, neuroaxonal injury markers, glial activation, and advanced imaging across larger, balanced cohorts to clarify IL-6's combined biomarker potential within the broader inflammatory milieu governing MS progression and therapy response. This will facilitate individualized disease monitoring and precision therapeutic strategies.

Abbreviations

- **CDA:** Confirmed Disability Accumulation
- **CRP:** C-reactive Protein
- **CSF:** Cerebrospinal Fluid
- **ECLIA:** Electrochemiluminescence Immunoassay
- **EDSS:** Expanded Disability Status Scale
- **HC:** Healthy Controls

- **IgA:** Immunoglobulin A
- **IgG:** Immunoglobulin G
- **IgM:** Immunoglobulin M
- **IL-6:** Interleukin-6
- **IQR:** Interquartile Range
- **MRI:** Magnetic Resonance Imaging
- **MS:** Multiple Sclerosis
- **NEDA-3:** No Evidence of Disease Activity-3
- **NMOSD:** Neuromyelitis Optica Spectrum Disorders
- **OCR:** Ocrelizumab
- **PIRA:** Progression Independent of Relapse Activity
- **PPMS:** Primary Progressive Multiple Sclerosis
- **RAW:** Relapse-Associated Worsening
- **RRMS:** Relapsing–Remitting Multiple Sclerosis
- **SD:** Standard Deviation
- **sGFAP:** Serum Glial Fibrillary Acidic Protein
- **SLE:** Systemic Lupus Erythematosus
- **sNfL:** Serum Neurofilament Light Chain
- **NA:** Not Applicable

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the local ethics committee of Heinrich-Heine-University Düsseldorf (registry number 5951R, approval date 28.06.2018, and registry number 2021–1775, approval date 12.07.2022). Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials:

The dataset used is available from the corresponding author on reasonable request.

Competing interests

TK: reports no conflicts of interest in connection to this study.

RH: reports no conflicts of interest in connection to this study.

NH: reports no conflicts of interest in connection to this study.

LM: reports no conflicts of interest related to this study. He reports honoraria for lecturing, consulting and travel expenses for attending meetings from Biogen, Merck, argenX, Bial, Roche, Hexal, Neuraxpharm, Sanofi, Alexion and Novartis, all outside the scope of this work. His research is funded by the by the German Multiple Sclerosis Foundation (DMSG), the B.Braun Foundation and the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – 493659010.

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Authors' contributions

TK: Conceptualization (equal); Investigation (lead); Formal analysis (equal); Writing – original draft preparation (lead); Visualization (equal); Validation (equal); Writing – review & editing (equal).

RH: Conceptualization (equal); Investigation (lead); Formal analysis (equal); Writing – original draft preparation (lead); Visualization (equal); Validation (equal); Writing – review & editing (equal).

NH: Data curation (supporting); Investigation (supporting); Writing – review & editing (equal).

LM: Data curation (supporting); Methodology (supporting); Writing – review & editing (equal).

BJK: Data curation (supporting); Investigation (supporting); Writing – review & editing (equal).

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PK: Investigation (supporting); Writing – review & editing (equal).

KSB: Resources (lead); Investigation (supporting); Methodology (supporting); Writing – review & editing (equal).

SGM: Supervision (supporting); Resources (lead); Writing – review & editing (equal).

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MP: Conceptualization (lead); Methodology (lead); Project administration (lead); Supervision (lead); Writing – review & editing (lead); Validation (supporting).

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Tables

Table 1.1: Baseline characteristics of the MS cohort and healthy controls

Variable	Total MS cohort (n=73)	HC (n=86)	p-value
Age (years, mean $\pm$ SD)	52.2 $\pm$ 12.7	46.1 $\pm$ 11.8	<0.001
Female sex (n, %)	40 (54.8%)	62 (72.1%)	0.036
Months since disease manifestation (median; range; IQR)	91 (16–427; IQR 71)	NA	NA
EDSS (median; range; IQR)	3 (0–7; IQR 3.3)	NA	NA
Relapse within 24M (n, %)	6 (7.8%)	NA	NA
CDA within 24M (n, %)	22 (30.1%)	NA	NA
PIRA within 24M (n, %)	20 (27.4%)	NA	NA
RAW within 24M (n, %)	2 (2.7%)	NA	NA
Lo-NEDA-3 within 24M (n, %)	31 (42.5%)	NA	NA
IL-6 [pg/ml] (median; IQR)	1.90 (IQR 1.8)	1.96 (IQR 1.1)	0.334
sNfL z-score (median; IQR)	-0.10 (IQR 2.6)	NA	NA
sGFAP z-score (median; IQR)	0.28 (IQR 1.5)	NA	NA
CRP [mg/L] (median; IQR)	0.09 (IQR 0.2)	0.07 (IQR 0.1)	<0.001
IgG [mg/dl] (median; IQR)	952 (IQR 348.5)	885 (IQR 250)	0.761
IgM [mg/dl] (median; IQR)	86 (IQR 58.5)	NA	NA
Vitamin D [ng/ml] (median; IQR)	23.0 (IQR 19)	NA	NA
Continuous variables are presented as mean $\pm$ SD or median (range; IQR). Categorical variables are presented as n (%). P-values represent comparisons between the total MS cohort and HC using Welch's t-test or Wilcoxon rank-sum test. <i>Abbreviations:</i> CDA: Confirmed Disability Accumulation; CRP: C-reactive Protein; EDSS: Expanded Disability Status Scale; HC: Healthy Controls; IQR: Interquartile Range; loss of NEDA-3: Loss of No Evidence of Disease Activity-3; MS: Multiple Sclerosis; NA: Not Applicable; PIRA: Progression Independent of Relapse Activity; RAW: Relapse-Associated Worsening; SD: Standard Deviation; sGFAP: Serum Glial Fibrillary Acidic Protein; sNfL: Serum Neurofilament Light Chain.			

Table 1.2: Baseline characteristics of PPMS and RRMS patients

Variable	PPMS (n=43)	RRMS (n=30)	p-value
Age (years, mean $\pm$ SD)	58.3 $\pm$ 9.3	43.5 $\pm$ 12	<0.001
Female sex (n, %)	25 (58.1%)	15 (50%)	0.654
Months since disease manifestation (median; range; IQR)	115 (16–427; IQR 78)	73 (37–415; IQR 48)	<0.001
EDSS (median; range; IQR)	4 (1.5–7; IQR 3.4)	2 (0.0–6.5; IQR 2.5)	<0.001
Relapse within 24M (n, %)	NA	6 (20%)	NA
CDA within 24M (n, %)	18 (41.9%)	4 (13.3%)	0.019
PIRA within 24M (n, %)	18 (41.9%)	2 (6.7%)	0.002
RAW within 24M (n, %)	NA	2 (8%)	NA
Lo-NEDA-3 within 24M (n, %)	20 (46.5%)	11 (36.7%)	0.551
IL-6 [pg/ml] (median; IQR)	2.30 (IQR 2.7)	1.75 (IQR 0.6)	0.024
sNfL z-score (median; IQR)	-0.41 (IQR 1.6)	2.12 (IQR 1.9)	0.005
sGFAP z-score (median; IQR)	0.28 (IQR 1.1)	0.62 (IQR 2.6)	0.806
CRP [mg/L] (median; IQR)	0.10 (IQR 0.3)	0.09 (IQR 0.11)	0.341
IgG [mg/dl] (median; IQR)	969 (IQR 225)	829 (IQR 459)	0.018
IgM [mg/dl] (median; IQR)	87 (IQR 62)	82 (IQR 68)	0.153
Vitamin D [ng/ml] (median; IQR)	22.0 (IQR 20)	23.5 (IQR 16.5)	0.742
Characteristics are stratified by Multiple Sclerosis subtype. P-values represent comparisons between PPMS and RRMS groups using Welch's t-test or Wilcoxon rank-sum test.. <i>Abbreviations:</i> CDA: Confirmed Disability Accumulation; CRP: C-reactive Protein; EDSS: Expanded Disability Status Scale; IQR: Interquartile Range; loss of NEDA-3: Loss of No Evidence of Disease Activity-3; NA: Not Applicable; PIRA: Progression Independent of Relapse Activity; PPMS: Primary Progressive Multiple Sclerosis; RAW: Relapse-Associated Worsening; RRMS: Relapsing-Remitting Multiple Sclerosis; SD: Standard Deviation; sGFAP: Serum Glial Fibrillary Acidic Protein; sNfL: Serum Neurofilament Light Chain.			

Figures

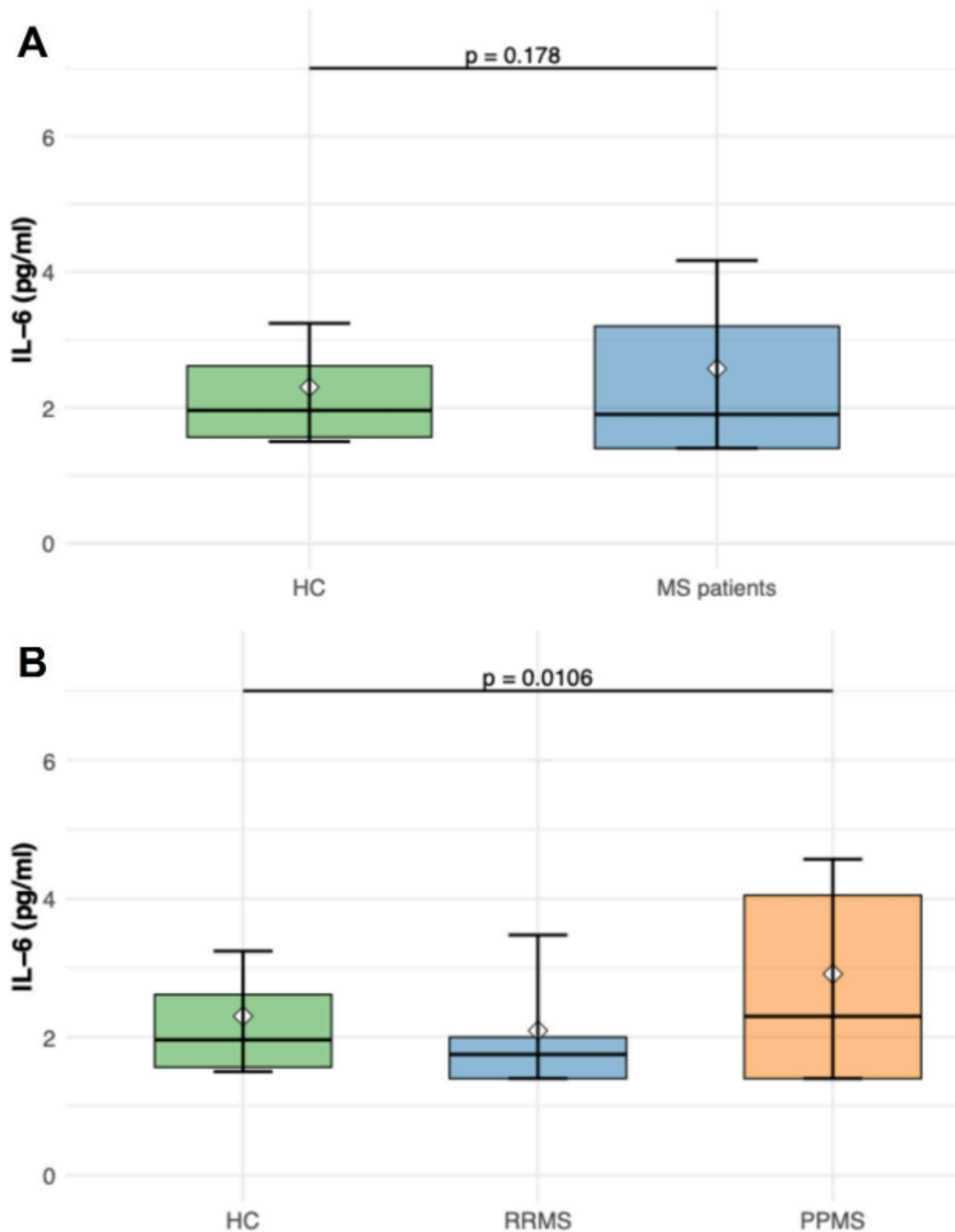


Figure 1

Comparison of baseline serum interleukin-6 (IL-6) levels between groups. (A) Comparison between the total MS cohort and HC. No significant difference was observed ($p = 0.178$, rank-based linear model adjusted for age and sex). (B) Subgroup analysis of IL-6 levels across RRMS, PPMS, and HC. While an overall group effect was noted ($p = 0.0106$, ANOVA), post-hoc pairwise comparisons did not reveal significant differences between individual groups.

Abbreviations: HC: Healthy Controls; IL-6: Interleukin-6; MS: Multiple Sclerosis; PPMS: Primary Progressive Multiple Sclerosis; RRMS: Relapsing-Remitting Multiple Sclerosis.

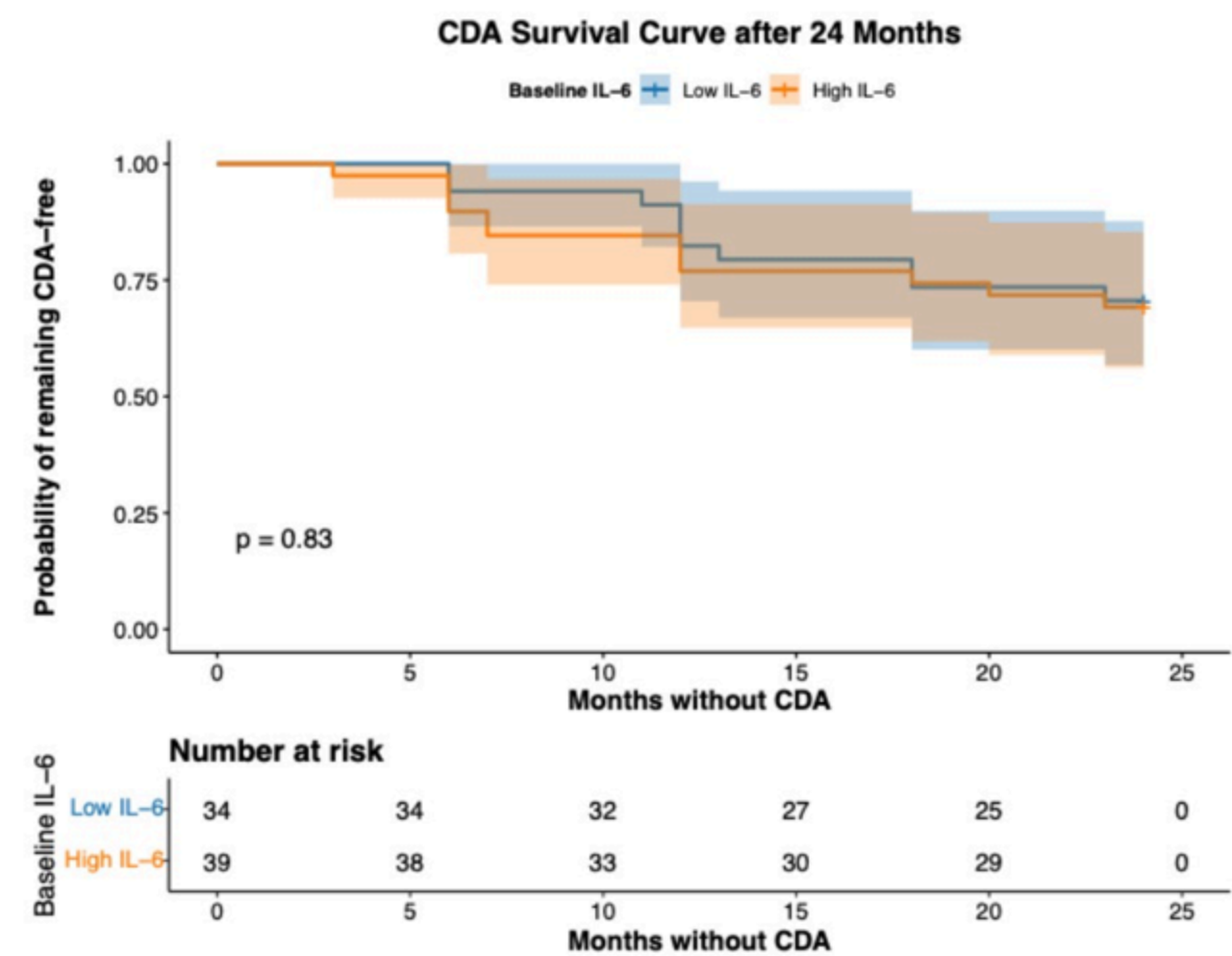


Figure 2

Time to confirmed disability accumulation (CDA) over 24 months stratified by baseline IL-6. Kaplan-Meier survival analysis showing the probability of remaining free from CDA. Patients were dichotomized into "High" and "Low" groups based on the median baseline IL-6 level. No significant difference in CDA risk was observed between the two groups (HR = 1.83; 95% CI: 0.73–4.61; p-value = 0.989).

Abbreviations: CDA: Confirmed Disability Accumulation; CI: Confidence Interval; HR: Hazard Ratio; IL-6: Interleukin-6.

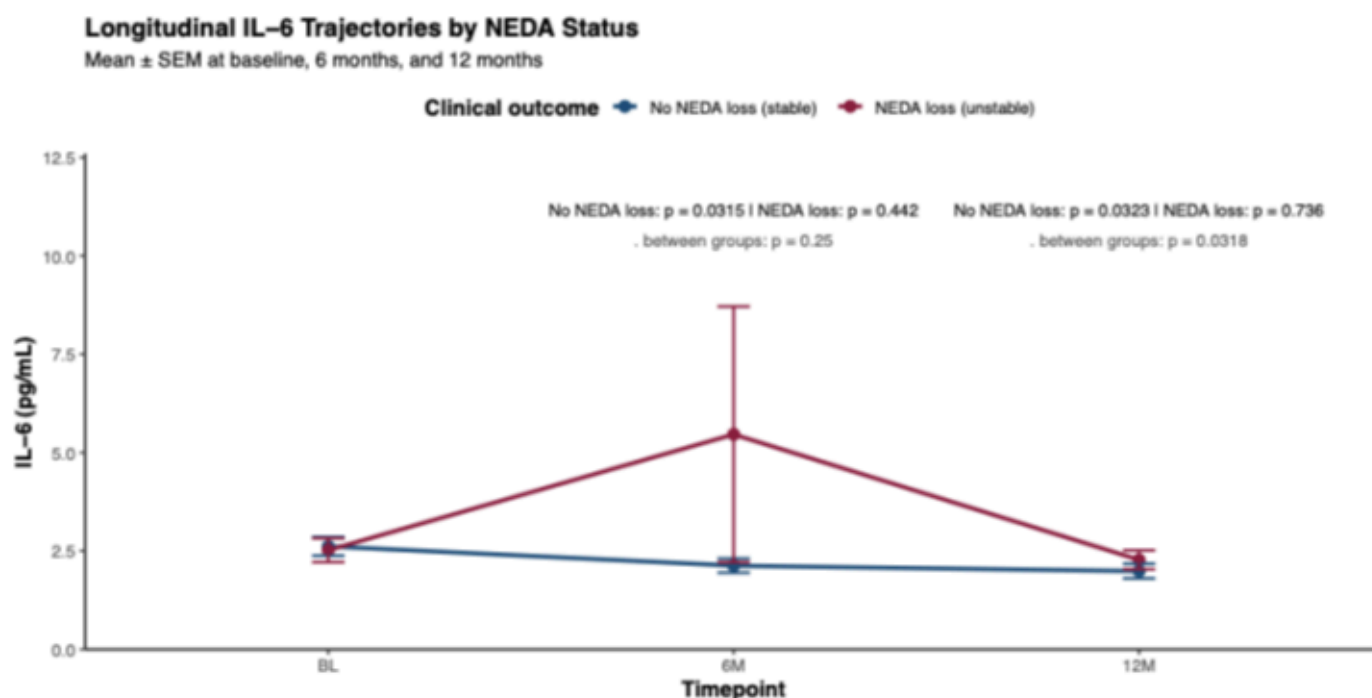


Figure 3

Longitudinal change in serum IL-6 levels at 12 months by loss of NEDA-3 status. Boxplots represent the mean change in IL-6 concentrations from baseline to 12 months post-ocrelizumab initiation. Patients maintaining NEDA-3 status exhibited a significant reduction in IL-6 (mean -1.30 pg/mL), whereas those with loss of NEDA-3 (loss of NEDA-3) remained stable (mean 0.09 pg/mL). The between-group difference was statistically significant ($p = 0.0318$), suggesting a potential association between IL-6 reduction and sustained clinical stability.

Abbreviations: IL-6: Interleukin-6; loss of NEDA-3: Loss of No Evidence of Disease Activity-3; NEDA-3: No Evidence of Disease Activity-3.

Supplementary Files

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