Journal of Neurology (2023) 270:348–356
https://doi.org/10.1007/s00415-022-11364-9

ORIGINAL COMMUNICATION

A real-world study of interleukin-6 receptor blockade in patients with neuromyelitis optica spectrum disorder

Shu Yang[1] · Chen Zhang[1] · Tian-Xiang Zhang[1] · Bin Feng[1] · Dongmei Jia[1] · Shasha Han[1] · Ting Li[1] · Yi Shen[1] · Guangxun Yan[1] · Chao Zhang[1,2]

Received: 28 June 2022 / Revised: 22 August 2022 / Accepted: 31 August 2022 / Published online: 6 September 2022

Abstract

Neuromyelitis optica spectrum disorder (NMOSD) is a relapsing autoimmune disease that can cause permanent neurological disabilities. However, the interleukin-6 (IL-6) signaling pathway is a promising therapeutic target for relapse prevention. Therefore, this study evaluated the long-term effectiveness of tocilizumab, a humanized anti-IL-6 receptor antibody, for NMOSD. We enrolled 65 patients with NMOSD who received regular intravenous administration of tocilizumab (8 mg/kg) between October 2017 and January 2022. Then, we retrospectively collected data on the clinical characteristics and baseline glial fibrillary acidic protein (GFAP) and neurofilament light chain levels. The primary outcome was the annualized relapse rate (ARR). Risk factors were assessed using a multivariable logistic regression model. During the median follow-up of 34.1 (interquartile range: 25.5–39.3) months, 23% (15/65) of patients relapsed during tocilizumab treatment, but the median ARR decreased from 1.9 (range 0.12–6.29) to 0.1 (range 0–1.43, $p < 0.0001$). A prolonged infusion interval (> 4 weeks, odds ratio [OR]: 10.7, 95% confidence interval [CI]: 1.6–71.4, $p = 0.014$) and a baseline plasma GFAP level of > 220 pg/mL (OR: 20.6, 95% CI 3.3–129.4, $p = 0.001$) were risk factors for future relapses. During treatment, the median Expanded Disability Status Scale score significantly decreased in aquaporin-4 antibody-positive and -negative patients, but the pain did not considerably improve. There were no severe safety concerns. Tocilizumab treatment significantly reduced the relapse rate in patients with NMOSD. However, prolonged infusion intervals and high baseline plasma GFAP levels may increase the relapse risk during tocilizumab therapy.

Keywords Neuromyelitis optica spectrum disorder · Tocilizumab · Relapse · Risk · Glial fibrillary acidic protein

Introduction

Neuromyelitis optica spectrum disorder (NMOSD) is a chronic autoimmune disease of the central nervous system (CNS) predominantly characterized by recurrent attacks of optic neuritis (ONs), longitudinally extensive transverse myelitis, or area postrema syndrome [1]. Most patients with NMOSD receive antibody-mediating therapy specific for astrocytic water channel aquaporin-4 (AQP4) [2]. The AQP4 antibody (AQP4-ab) plays a critical role in the astrocytopathy immunopathogenesis in NMOSD, which typically induces chronic relapses in adults [3–6]. Furthermore, the high NMOSD recurrence rate correlates with severe neurological disability. Thus, relapse prevention is critical for cases where irreversible disability accumulates [7, 8]. From 2019 to 2020, five placebo-controlled, randomized trials for NMOSD have been completed, assessing highly effective disease-modifying strategies, including B cell depletion, complement C5 inhibition, and interleukin-6 (IL-6) receptor blockade [9–14]. However, few studies have investigated the relapse risk among patients receiving these treatments in a real-world setting.

NMOSD is hypothesized to be closely associated with humoral immunity involving abnormal B cell responses. IL-6 participates in B-cell differentiation into plasmablasts that produce AQP4-ab [15], and patients with NMOSD

✉ Chao Zhang
chaozhang@tmu.edu.cn

1 Department of Neurology and Institute of Neuroimmunology, Tianjin Medical University General Hospital, Tianjin, China

2 Centers of Neuroimmunology and Neurological Diseases, China National Clinical Research Center for Neurological Diseases, Beijing Tiantan Hospital, Capital Medical University, Beijing, China

have significant increases in serum and cerebral spinal fluid (CSF) IL-6 levels [16–18]. IL-6 also regulates glial fibrillary acidic protein (GFAP) CSF levels, which are related to astrocyte injury [19]. Thus, IL-6 signaling inhibition reduces the AQP4-ab level, blood–brain barrier permeability, and pro-inflammatory T helper 17 cell activity [15, 20, 21]. Three comparative trials (SAkuraSky, SAkuraStar with satralizumab, and TANGO with tocilizumab) have demonstrated the efficacy of an IL-6 receptor blockade in AQP4-ab (+) patients with NMOSD [12–14]. However, the follow-up periods in these trials were relatively short and the efficacy of long-term tocilizumab treatment remains unclear.

Therefore, this retrospective study investigated the relapse risk factors for patients with NMOSD undergoing tocilizumab treatment and explored potential biomarkers for the prediction of disease recurrence.

Methods

Patient cohort

This is a retrospective data collection study. Sixty-five patients were enrolled and followed up at the Department of Neurology of Tianjin Medical University General Hospital between October 2017 and January 2022. The inclusion criteria were: (1) adults (≥ 18 years) diagnosed with NMOSD based on the 2015 International Panel for Neuromyelitis Optica Diagnosis criteria; (2) patients who received tocilizumab treatment. The exclusion criteria were as follows: (1) patients who were treated with other immunosuppressants within expected pharmacodynamics effect window prior to tocilizumab initiation, such as mycophenolate mofetil for 3 months, azathioprine for 6 months, and rituximab for 6 months; (2) B-cell count < lower limit of normal if patients had a history of receiving a B-cell-targeted therapy; (3) patients who were diagnosed with myelin oligodendrocyte glycoprotein antibody-associated disease or anti-glial fibrillary acidic protein encephalomyelitis; (4) patients with a history of clinically significant infection (Herpes simplex virus, cytomegalovirus, human immunodeficiency virus, hepatitis viruses, syphilis, etc.); (5) patients with heart, liver, or kidney insufficiency; 6) patients with tumor disease currently or within last 5 years. Clinical data were collected, including sex, history of attacks, concomitant autoimmune diseases, the annual relapse rate, the Expanded Disability Status Scale (EDSS) score. NMOSD-related pain was assessed by the numerical rating scale (NRS) rated between 0, no pain, to 10, worst pain imaginable. Patients were requested to score the pain related to their myelitis rather than non-myelitis pain problems (e.g., migraine, joint pain, etc.). Patients were regularly administered intravenous 8 mg/kg of tocilizumab. The primary outcome was the annualized relapse rate (ARR). Relapse was defined as new neurologic symptoms or acute worsening of previous neurologic deficits with objective clinical signs lasting for at least 24 h and attributed to an inflammatory CNS event.

GFAP and neurofilament light chain (NfL) measurements

Blood samples were collected before tocilizumab treatment and stored in liquid argon. Briefly, a SIMOA HD-1 analyzer and a SIMOA Neurology 2-Plex assay reagent kit (103520; both from Quanterix, Billerica, MA, USA) were used to measure plasma GFAP and NfL levels.

Statistical analyses

Patient characteristics were described as frequency and percentages for categorical variables and means with 95% confidence intervals (CIs) or medians with interquartile ranges (IQRs) for continuous variables. Groups were compared using analysis of variance and independent samples *t*-test, Mann–Whitney *U* test, and Kruskal–Wallis analyses for continuous variables, and X^2 analysis (Fisher's exact test) for categorical variables.

We used a paired t-test and Wilcoxon signed-rank test to compare the ARR before and after tocilizumab treatment for patients who relapsed during the follow-up period. Finally, we assessed the risk factors using a multivariable logistic regression model, reported as adjusted odds ratios (ORs). Furthermore, we divided the patients into high and low-level groups based on the baseline plasma GFAP (pGFAP) and plasma NfL (pNfL) concentration using the geometric mean as a cut-off value. The geometric mean was calculated as:

$$\left[10^{(\text{mean}+\text{SD})_{\text{non-relapsed}}} \times 10^{(\text{mean}-\text{SD})_{\text{relapsed}}}\right].$$

The pGFAP cut-off value was 220 pg/mL, and the pNfL cut-off value was 20 pg/mL. All statistical analyses were performed using SPSS (version 26, IBM Corp., Armonk, NY, USA).

Standard protocol approval, registration, and patient consent

The Institutional Review Board of Tianjin Medical University General Hospital and each participating center's local institutional review board provided ethical approval based on the ICH-Good Clinical Practices guidelines. In addition, all patients provided written informed consent for retrospective chart review.

Results

Clinical characteristics

Between October 2017 and January 2022, 212 patients were diagnosed as NMOSD in our center and received preventive therapeutics. Among them, 65 (30.7%) patients met the criteria for this study. Table 1 presents the participants' characteristics ($n=65$). Sixty patients (92.3%) were female, and 54 (83.1%) were AQP4-ab (+). The mean age at disease onset was 44.2 (14.5) years, and the mean age at tocilizumab initiation was 48.3 (14.5) years. The median baseline EDSS score was 5.5 (IQR: 3.0–6.0).

Tocilizumab (8 mg/kg) was administered intravenously at a mean interval of 37.5 (range 27–61) days. However, 38 patients (58.5%) received tocilizumab every 4 weeks at a median interval of 29.5 (range 27–31) days. Twenty-five patients (38.5%) underwent prolonged infusions due to a patient-oriented economic burden, of which 18 patients (27.7%) underwent infusion every 6 weeks (median interval: 45 days; range 43–47 days) and 7 patients (10.8%) underwent infusion every 8 weeks (median interval: 58 days; range 56–61 days). Overall, 27 patients (41.5%) had concomitant systemic autoimmune diseases, including Sjogren's syndrome (12.3%), Graves' disease (7.7%), Hashimoto's thyroiditis (4.6%), rheumatoid arthritis (3.1%), and systemic lupus erythematosus (1.5%).

All patients discontinued prior immunosuppressants to start tocilizumab, except for oral corticosteroids. Patients switched to tocilizumab treatment mainly due to disease breakthrough or adverse events under prior immunosuppressants, including leukopenia, infections, or abnormal liver function. 59 (90.8%) patients were taking oral prednisone at the dose of a median 25 mg (range 15–40 mg) at the start of tocilizumab, and thus, tocilizumab was administered as an add-on treatment. The corticosteroids were tapered gradually and discontinued within a median of 4.2 (range 3–8) months, and then tocilizumab was used alone in the following months. Six patients (9.2%) discontinued all prior treatments and tocilizumab was used as monotherapy.

Table 1 Baseline demographic and clinical characteristics of patients with NMOSD

Patients ($n=65$)	
Sex	
Women	60 (92.3%)
Men	5 (7.7%)
Age at disease onset, years	44.2 (40.6,47.8)
Disease duration, years	4.1 (2.9, 5.3)
AQP4-IgG positivity	54 (83.1%)
Annualized relapse rate before tocilizumab	1.9 (0.1,6.3)
Infusion interval	
4 weeks	38 (58.4%)
6 weeks	18 (27.7%)
8 weeks	7 (10.8%)
Concomitant autoimmune disease	27 (41.5%)
Sjögren's syndrome	8 (12.3%)
Undifferentiated connective tissue diseases	8 (12.3%)
Grave's disease	5 (7.7)
Hashimoto's thyroiditis	3 (4.6%)
Rheumatoid arthritis	2 (3.1%)
Systemic lupus erythematosus	1 (1.5%)
Immunomodulatory agents before tocilizumab treatment	
Corticosteroids	65 (100%)
Intravenous immunoglobulin	35 (53.8%)
Mycophenolate mofetil	17 (26.1%)
Azathioprine	15 (23.1%)
Rituximab	12 (18.5%)
Cyclophosphamide	1 (1.5%)

Data are n, n (%), mean (95% CI), or median (IQR). AQP4, aquaporin 4

Tocilizumab treatment relapses

By the end of the follow-up period (median: 34.1 months, IQR 25.5–39.3), 50 (76.9%) patients were relapse-free, and 15 patients (23.1%) experienced 20 relapses. Ten patients (15.4%) suffered one attack, and five (7.7%) suffered two attacks.

Overall, the median ARR significantly decreased from 1.9 (range 0.1–6.3) before tocilizumab therapy to 0.1 (range 0–1.4, $p<0.0001$, 95% CI 1.4–2.1) after therapy. In addition, the median time to the first relapse was 15.5 (range 4–42) months.

Next, we compared the demographic characteristics of relapse and relapse-free patients. The median age (45.1 vs. 44.0 years, $p=0.8019$) and disease duration (61.5 vs. 45.1 months, $p=0.3364$) were similar in both groups. Furthermore, the median ARR (1.4 vs. 1.9, $p=0.2029$) and median EDSS score (4.0 vs. 3.3, $p=0.1928$) before tocilizumab treatment did not differ between the relapse and relapse-free groups. Thus, relapse and relapse-free patients had comparable ages, disease durations, disease activities, and disability severities.

Relapse risk factors during tocilizumab treatment

AQP4-ab serostatus

In total, 24.1% (13/54) of AQP4-ab (+) patients and 18.1% (2/11) of AQP4-ab (−) patients experienced relapses during the follow-up period. The median ARR decreased from 1.89 to 0.14 ($p<0.0001$, 95% CI 1.38–2.12) for AQP4-ab

(+) patients and from 1.75 to 0.06 ($p < 0.0001$, 95% CI 1.22–2.49) for AQP4-ab (−) patients. However, the ARR after treatment did not differ between the AQP4-ab (+) and AQP4-ab (−) groups (0.14 vs. 0.06, $p = 0.3618$; Fig. 1A) and the median times to the first relapse in each group were comparable (18.6 vs. 15.5 months, $p = 0.7210$). Finally, the logistic regression analysis indicated that AQP4-ab serostatus was not a significant relapse risk factor (OR: 6.2, 95% CI 0.4–92.0, $p = 0.19$).

Concomitant systematic autoimmune diseases

Seven of 36 patients (19.4%) with concomitant systematic autoimmune diseases and eight of 29 patients (27.6%) without concomitant diseases experienced relapse. After treatment, the ARR decreased from 1.73 to 0.17 ($p < 0.0001$, 95% CI 1.05–2.06) for patients with autoimmune diseases and from 2.05 to 0.09 ($p < 0.0001$, 95% CI 1.52–2.39) for patients without autoimmune diseases. However, the ARR after treatment did not differ between the groups (0.17 vs. 0.09, $p = 0.2586$; Fig. 1B) and the median times to the first relapse in each group were comparable (20.1 vs. 15.8 months, $p = 0.5028$). Concomitant autoimmune disease was not a relapse risk factor (OR: 1.8, 95% CI 0.4–9.2, $p = 0.47$).

Treatment infusion interval

Seven of eighteen patients (38.9%) who were infused every 6 weeks and 3 of 7 patients who were (42.9%) infused every 8 weeks experienced relapses, whereas 5 of 38 patients (13.2%) patients who were infused every 4 weeks relapsed during the follow-up period. ARR significantly decreased in all groups after treatment (4 weeks: from 2.00 to 0.09, $p < 0.0001$, 95% CI 1.59–2.23; 6 weeks: from 1.55 to 0.18, $p = 0.0004$, 95% CI 0.66–2.07; 8 weeks: from 2.69 to 0.24, $p = 0.0225$, 95% CI 0.42–4.47; Fig. 1C). The median times to the first relapse per group were also comparable (4 weeks: 17.3 months vs. 6 weeks: 18.8 months vs. 8 weeks: 14 months, $p = 0.8779$). However, logistic regression analysis indicated that an infusion interval > 4 weeks increased the relapse risk (OR: 10.7, 95% CI 1.6–71.4, $p = 0.014$).

Concomitant corticosteroids

Overall, 59 patients (90.7%) were taking oral corticosteroids when starting tocilizumab (i.e., the add-on group), and 11 (18.6%) relapsed; 4 of 6 patients (66.7%) receiving tocilizumab as monotherapy at initiation had relapsed. After treatment, the ARR decreased from 1.95 to 0.09

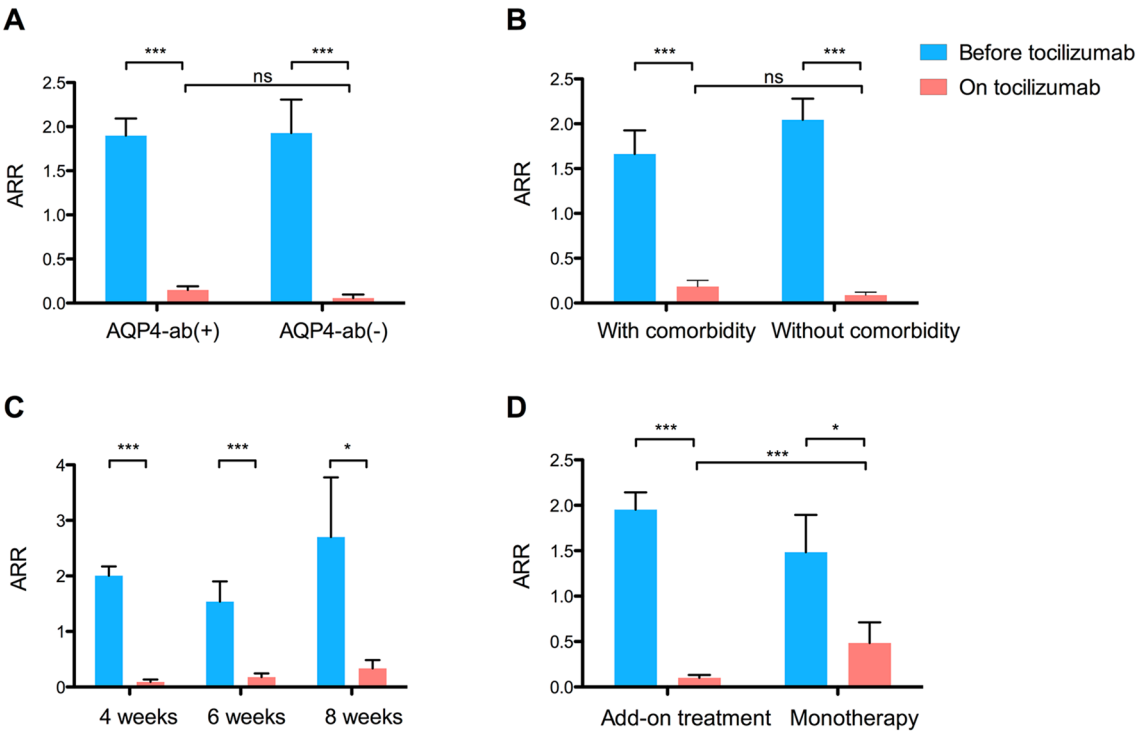

Fig. 1 The annualized relapse rate (ARR) of patients with NMOSD before and on tocilizumab treatment. **A** The comparison of ARR between patients with AQP4-ab (+) and patients with AQP4-ab (−). **B** The ARR in patients with and without concomitant autoimmune diseases. **C** The ARR in patients who received tocilizumab treatment at 4-week, 6-week, and 8-week intervals. **D** The comparison of ARR in patients receiving tocilizumab as add-on and monotherapy. *$p < 0.05$, ***$p < 0.001$, ns, $p > 0.05$

(p < 0.0001, 95% CI 1.51–2.21) in the add-on group and from 1.48 to 0.49 in the monotherapy group ($p = 0.0495$, 95% CI 0.05–2.02); the add-on group ARR was significantly lower than in the monotherapy group (0.09 vs. 0.49, $p = 0.0005$; Fig. 1D). Furthermore, a survival curve analysis determined that the add-on and monotherapy groups significantly differed in the early stages ($p = 0.0067$), and the logistic regression analysis determined that the add-on group had a decreased relapse risk than in the monotherapy group (OR: 11.4, 95% CI 0.8–166.4, $p = 0.08$).

Neuronal biomarkers

We also evaluated whether pGFAP and pNfL levels were associated with relapse in patients with NMOSD receiving tocilizumab. The pGFAP and pNfL levels were measured at baseline. For convenient calculations, we used the natural logarithm by reducing the absolute values. The log(pGFAP) and log(pNfL) values at baseline were significantly higher in relapsing patients than in relapse-free patients (log(pGFAP): 3.1 vs. 2.1, $p < 0.0001$; log(pNfL): 1.6 vs. 1.3, $p = 0.0178$; Fig. 2A, B).

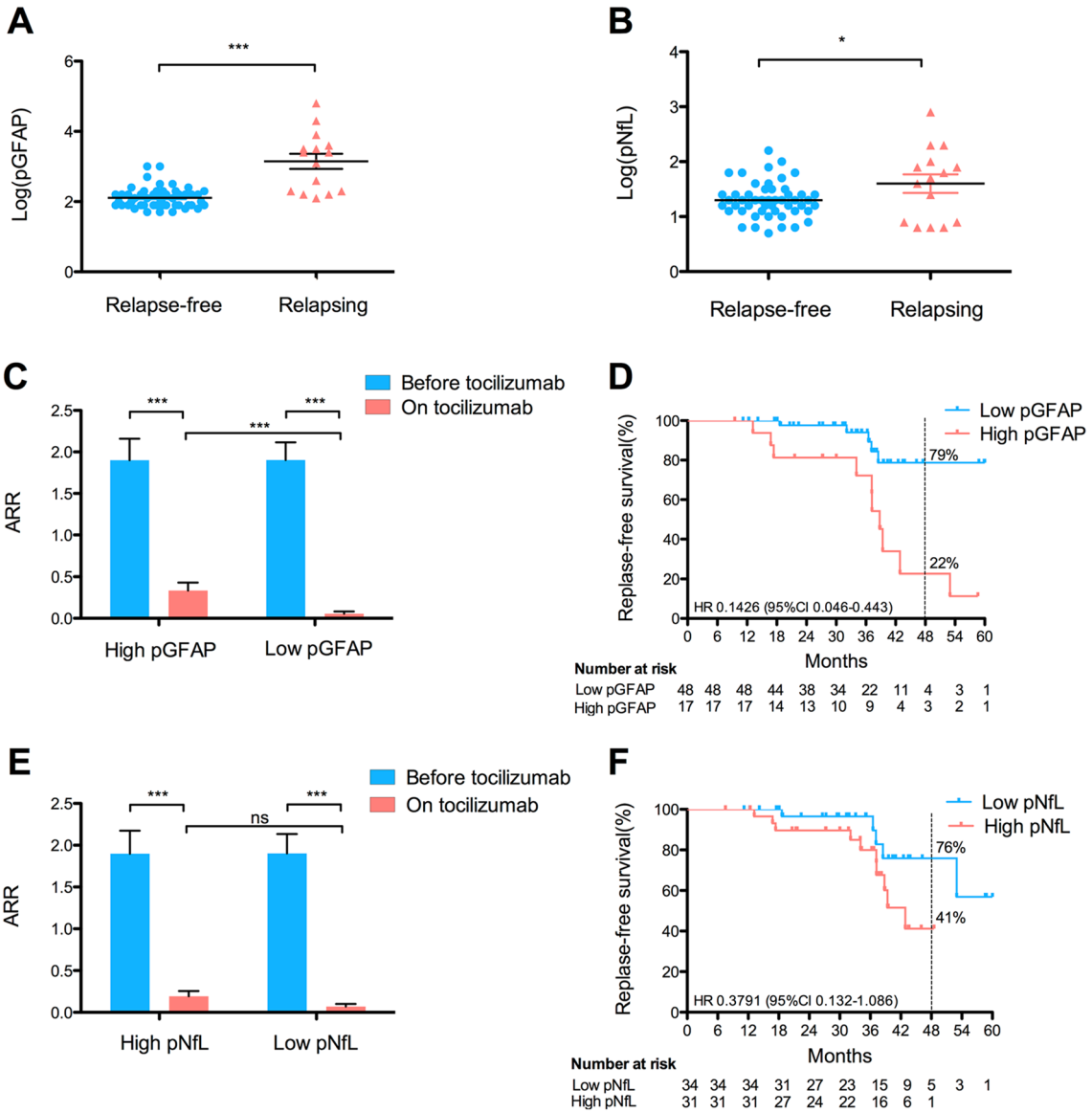

Fig. 2 Association of neuronal biomarker levels with the ARR in patients with NMOSD. **A**, **B** The comparisons of baseline log(pGFAP) and log(pNfL) in relapsing and relapse-free patients with NMOSD. **C** The ARR in patients with high pGFAP levels and low pGFAP levels. The cutoff of pGFAP was 220 pg/ml. **D** Time to first relapse stratified by high and low levels of pGFAP in the full analysis set. **E** The ARR in patients with high pNfL levels and low pNfL levels. The cutoff of pNfL was 20 pg/ml. **F** Time to first relapse stratified by high and low levels of pNfL in the full analysis set. *$p < 0.05$, ***$p < 0.001$, ns, $p > 0.05$

Next, we divided the patients into high and low groups based on the pGFAP (220 pg/mL) or pNfL (20 pg/mL) cut-off values. At the end of the follow-up period, 10 of 17 patients (59%) in the high pGFAP group relapsed, whereas only 5 of 48 patients (10.4%) in the low group relapsed. The proportion of patients with relapse in the high pGFAP group was significantly higher than the low group ($p = 0.0002$). After treatment, the ARR decreased from 1.90 to 0.33 ($p < 0.0001$, 95% CI 1.061–2.07) in the high group and from 1.90 to 0.06 in the low group ($p < 0.0001$, 95% CI 1.44–2.25); the ARR was significantly higher in the high than in the low group (0.33 vs. 0.05, $p = 0.0002$; Fig. 2C). The survival curve analysis also indicated a significant difference between the high and low groups ($p = 0.0015$; Fig. 2D) and a higher baseline pGFAP level was associated with an increased relapse risk (OR: 20.6, 95% CI 3.3–129.4, $p = 0.001$).

Moreover, 10 of 31 patients (32.2%) in the high pNfL group relapsed, whereas 5 of 34 patients (14.7%) in the low group relapsed. After treatment, the ARRs did not differ between the groups (0.17 vs. 0.07, $p = 0.07$; Fig. 2E), which was confirmed in the survival curve analysis ($p = 0.0708$; Fig. 2F).

Figure 3 presents the adjusted ORs for all potential risk factors. Overall, a higher baseline pGFAP level (> 220 pg/mL) and an infusion interval longer than 4 weeks may increase the relapse risk in patients with NMOSD.

Disability

After tocilizumab treatment, 14 myelitis cases and six ONs were recorded among all relapsing patients. The EDSS score worsened in 4 of 54 AQP4-ab (+) patients (7.4%) and 1 of 11 AQP4-ab (−) patients (9.1%). The EDSS score increased by less than 1 in acute attacks, indicating no severe relapse occurred during tocilizumab treatment. Overall, at the end of the follow-up period, the median EDSS score significantly decreased, from 5.75 (range 1–8.5) to 3.5 (range 0–8) for AQP4-ab (+) patients ($p < 0.001$; Fig. 4A) and from 5 (range 1.5–6.0) to 2.5(range 0–5.5) for AQP4-ab (−) patients ($p = 0.043$; Fig. 4B).

Based on the numerical rating scale, 34 of 65 patients (52.3%) with NMOSD experienced disease-related chronic pain before tocilizumab therapy; the median pain intensity was 2 (IQR: 1.5–3.5) according to the numerical rating scale. After treatment, the median pain score did not decrease (2.5, IQR: 1.5–4.0).

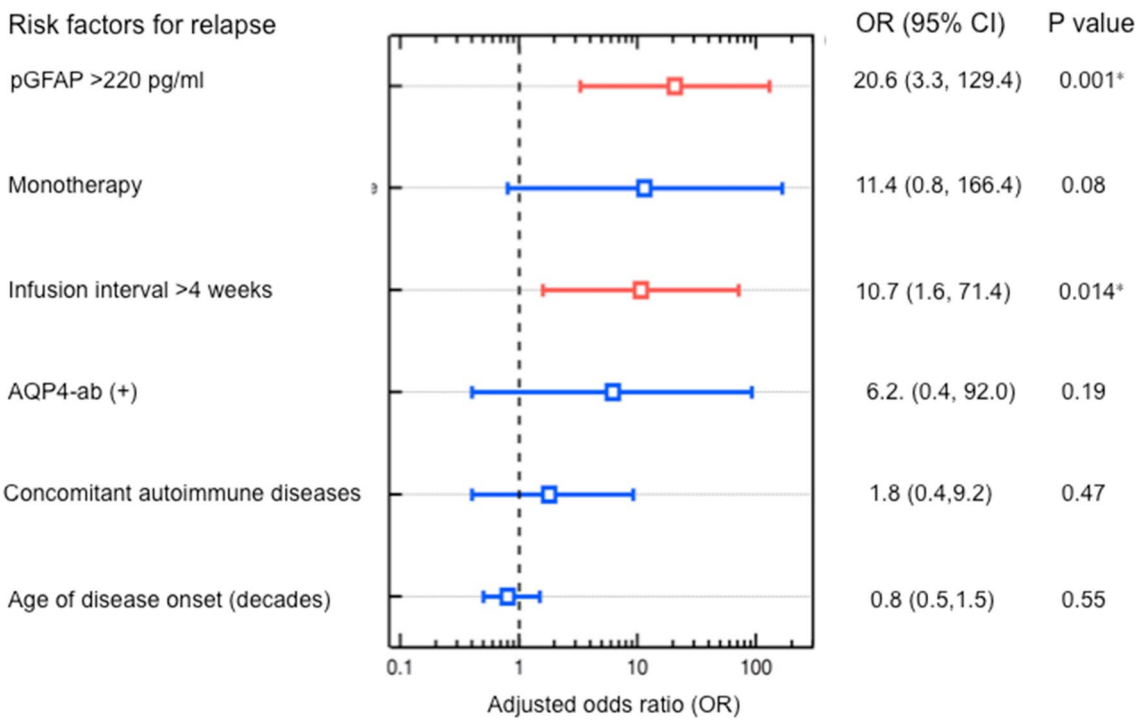

Fig. 3 Risk factors and their adjusted odds ratios (ORs) obtained from multivariable logistics regression analysis. The baseline pGFAP levels, add-on therapy, infusion intervals, AQP4-ab positivity, concomitant autoimmune diseases, and age of disease onset were included as possible risk factors. Data points of the risk factors with significant p values (< 0.05) in the model were shown with the red color and were marked (*) in the right side of the figure. Data points of the risk factors with non-significant p values (≥ 0.05) in the model are shown with the blue color

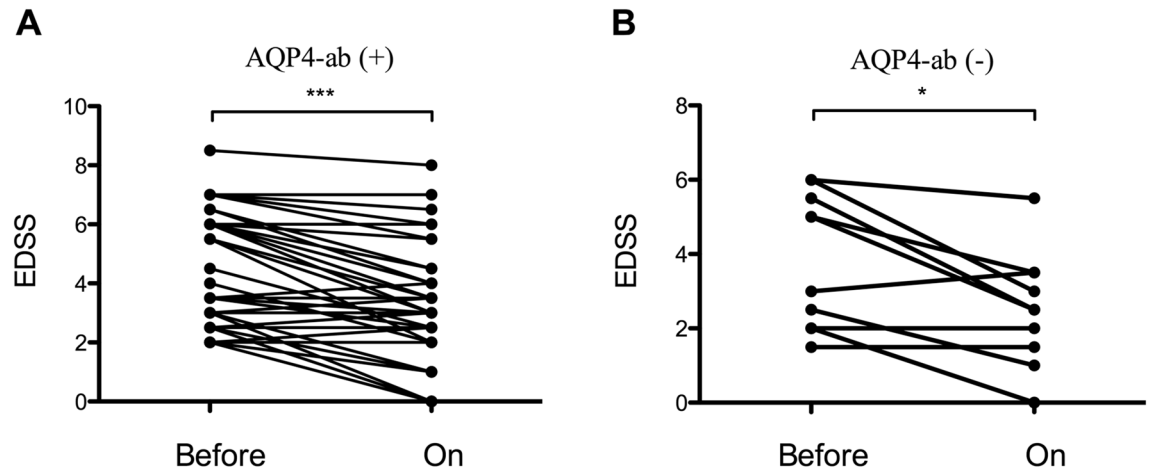

Fig. 4 The Expanded Disability Status Scale (EDSS) score of patients with NMOSD before and on tocilizumab treatment. The patients were grouped into **A** AQP4-ab (+) and **B** AQP4-ab (−) NMOSD**.** *$p < 0.05$, ***$p < 0.001$

Adverse events and safety

Overall, 28 patients (43%) had mild-to-moderate increases in the serum alanine transaminase (ALT) level, increasing to 78.4 U/L (mean; range 52–246 U/L) but decreasing to 49.8 U/L (mean; range 24–92 U/L) after symptomatic treatment with bicyclol and/or tiopronin.

Moreover, 15 of 65 patients (23.1%) reported transient fatigue lasting 3.4 days (mean; range 1–9 days). Eighteen patients (27.7%) developed infections, including urinary tract ($n = 11$), upper respiratory tract ($n = 8$), zoster virus ($n = 4$), and pneumonia ($n = 3$). Seven patients (10.7%) had hypercholesterolemia (mean: 6.4 mmol/L, range 5.9–7.4 mmol/L) that decreased within the normal range after rosuvastatin treatment. Infusion-related reactions occurred in 5 of 65 (7.7%) patients, including skin rash ($n = 2$), lower limb edema ($n = 2$), headache ($n = 1$), dizziness ($n = 1$), and hypotension ($n = 1$).

Discussion

This study confirmed the effectiveness of tocilizumab in patients with NMOSD in a real-world setting and explored the risk factors associated with NMOSD relapses during long-term tocilizumab therapy management.

We found that the median ARR significantly decreased with tocilizumab treatment for the entire cohort, demonstrating the beneficial effect of blocking the IL-6 receptor in NMOSD. Furthermore, tocilizumab reduced the relapse risk in AQP4-ab (+) and AQP4-ab (−) patients, and the ARR after treatment was similar in both groups. This result differs from the SAkuraSky and SAkuraStar trials, which provided insufficient evidence that satralizumab reduced the relapse risk in AQP4-ab (−) patients. However, their result was partly attributable to the small sample size for this subpopulation [12, 13]. Disease heterogeneity is greater among AQP4-ab (−) patients. However, recent studies have shown no major difference between AQP4-ab (+) and AQP4-ab (−) patients regarding clinical, imaging, and laboratory variables [22]. Our data supports this and implies that tocilizumab is similarly effective for reducing relapses in both subpopulations.

Conventional therapies for NMOSD relapse prevention usually begin with oral corticosteroids or immunomodulatory agents. However, monoclonal antibodies targeting cluster of differentiation (CD) 20, CD19, IL-6 receptor, and complement C5 are increasingly used in clinical practice [9–14, 23]. According to our previous study, follicular T helper (Tfh) cells, which were associated with IL-6 and plasmablast formation, was not significantly decreased until 3 months after tocilizumab treatment. B cell activation markers CD86, CD69, and HLA-DR were also downregulated only after 3 months. The findings mean that it may take at least 3 months for tocilizumab to maintain B- and T-cell homoeostasis by regulating B-cell differentiation and inhibiting lymphocyte activation in patients with NMOSD [24]. In this study, most patients were permitted to continue baseline steroids treatment when tocilizumab was commenced. Our study showed that the early administration of corticosteroids during tocilizumab treatment inhibited disease activity. Furthermore, the ARR with early add-on treatment was significantly lower than that with tocilizumab monotherapy. However, although both regimens reduced the relapse risk after tocilizumab treatment, the multivariable logistic regression analysis did not identify early conventional treatment add-ons as a significant risk-reducing factor for NMOSD. This result may

be attributed to the small number of patients not receiving add-on treatments ($n=6$), as it was too small for statistical analyses. Notably, corticosteroids alone may be enough to prevent relapses for some patients [25]. Regardless, early add-on treatments induce better therapeutic effects with tocilizumab by stably blocking the IL-6 signaling pathway.

Tocilizumab is recommended every 4 weeks for rheumatic diseases. Therefore, an extended infusion interval might not maintain a stable and effective serum pharmacological tocilizumab concentration, reducing the therapeutic effect. In our study, prolonging the infusion interval was a relapse risk factor during tocilizumab treatment. Overall, 38% of all patients received tocilizumab in intervals longer than 4 weeks, primarily due to a patient-oriented economic burden. A slightly higher proportion of patients experienced relapses with longer infusion intervals (> 4 weeks) but we had insufficient data to indicate an increased relapse risk. However, an infusion interval of > 12 weeks may increase the relapse risk for patients who discontinued tocilizumab treatment (data not shown). Therefore, our data suggest that long-term, regular maintenance of an IL-6 receptor blockade may be required to inhibit the disease activity.

Previous studies have reported an association between a higher baseline pGFAP level, but not pNfL level, with a shorter duration between attacks and a higher EDSS score in patients with AQP4-ab (+) NMOSD. These results suggest that pGFAP could be a disease severity and activity biomarker for patients with AQP4-IgG (+) NMOSD [26, 27]. Our findings are consistent with previous observations; a higher pGFAP level, but not pNfL level, was associated with a higher ARR and was regarded as a relapse risk factor. Thus, pGFAP is a potential biomarker for disease activity in patients with NMOSD receiving tocilizumab treatment. This result is also consistent with the pathophysiological characteristics of NMOSD, which is considered immune-mediated astrocytopathy [28, 29].

Tocilizumab is a steroid-sparing biological agent for various autoimmune diseases. Furthermore, the TANGO study suggested that tocilizumab provides evident clinical efficacy for patients with NMOSD regardless of their autoimmune disease status [14]. Our study supports this result, finding that concomitant systemic autoimmune disease was not a risk factor for disease activity during tocilizumab treatment. The therapeutic efficacy in patients with NMOSD and other autoimmune diseases may be attributed to the pleiotropic effects of the IL-6 signaling blockade.

Our study had several limitations. First, we recruited a relatively small number of patients owing to the rarity of NMOSD and the high cost of tocilizumab treatment. Second, our study was a real-world study without a randomized control. Thus, biases related to ARR comparisons before and after tocilizumab treatment, such as regression to the mean, are a study design limitation [30]. Third, the pGFAP and pNfL levels were not determined regularly during the follow-up period. However, monitoring the dynamic changes in these levels during tocilizumab treatment may provide valuable information, including relapse prediction, which should be investigated in future studies. Finally, we did not quantify the serum AQP4-ab titers among patients treated with tocilizumab at different infusion intervals. Thus, it remains unclear if and for how long tocilizumab induces lower AQP4-ab titers.

In conclusion, our study supports the theory that tocilizumab decreases the ARR for patients with NMOSD. However, prolonged infusion intervals and a high baseline pGFAP level increase the relapse risk during tocilizumab therapy. Finally, the pGFAP concentration could be a biomarker for predicting the therapeutic effect of tocilizumab.

Acknowledgements We thank the patients, doctors, and nurses from the Department of Neurology, Tianjin Medical University General Hospital, who participated in this study and all members of the Jing-Jin Center for Neuroinflammation for their support.

Author contributions CZ conceptualized and designed the study. SY, CZ, TXZ, BF, DJ, SH, TL, and GY performed the experiments. SY, TXZ, and DJ analyzed the results. LY, QL, and FDS supervised the study. SY and CZ wrote and edited the manuscript.

Declarations

Conflicts of interest The study was supported by the National Natural Science Foundation of China (grant no. 82171777 to C.Z.) and the Natural Science Foundation of Tianjin Province (grant no. 20JCJQJC00280 to C.Z.)

Standard protocol approval, registration, and patient consent The Institutional Review Board of Tianjin Medical University General Hospital provided ethical approval based on the ICH–Good Clinical Practice guidelines. All patients provided written informed consent.

References

1. Wingerchuk DM, Banwell B, Bennett JL et al (2015) International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology 85:177–189
2. Lennon VA, Wingerchuk DM, Kryzer TJ et al (2004) A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet 364(9451):2106–2112
3. Kinoshita M, Nakatsuji Y, Kimura T et al (2010) Anti- aquaporin-4 antibody induces astrocytic cytotoxicity in the absence of CNS antigen-specific T cells. Biochem Biophys Res Commun 394(1):205–210
4. Fujihara K (2019) Neuromyelitis optica spectrum disorders: still evolving and broadening. Curr Opin Neurol 32(3):385–394
5. Jarius S, Ruprecht K, Wildemann B et al (2012) Contrasting disease patterns in seropositive and seronegative neuromyelitis optica: a multicentre study of 175 patients. J Neuroinflammation 9:14

6. Kimbrough DJ, Mealy MA, Simpson A et al (2014) Predictors of recurrence following an initial episode of transverse myelitis. Neurol Neuroimmunol Neuroinflamm 1:e4
7. Papadopoulos MC, Bennett JL, Verkman AS (2014) Treatment of neuromyelitis optica: state-of-the-art and emerging therapies. Nat Rev Neurol 10:493–506
8. Trebst C, Jarius S, Berthele A et al (2014) Update on the diagnosis and treatment of neuromyelitis optica: recommendations of the Neuromyelitis Optica Study Group (NEMOS). J Neurol 261:1–16
9. Cree BAC, Bennett JL, Kim HJ et al (2019) Inebilizumab for the treatment of neuromyelitis optica spectrum disorder (N-MOmentum): a double-blind, randomised placebo- controlled phase 2/3 trial. Lancet 394(10206):1352–1363
10. Tahara M, Oeda T, Okada K et al (2020) Safety and efficacy of rituximab in neuromyelitis optica spectrum disorders (RIN-1 study): a multicentre, randomised, double-blind, placebo-controlled trial. Lancet Neurol 19(4):298–306
11. Pittock SJ, Berthele A, Fujihara K et al (2019) Eculizumab in aquaporin-4-positive neuro- myelitis optica spectrum disorder. N Engl J Med 381(7):614–625
12. Yamamura T, Kleiter I, Fujihara K et al (2019) Trial of satralizumab in neuromyelitis optica spectrum disorder. N Engl J Med 381(22):2114–2124
13. Traboulsee A, Greenberg BM, Bennett JL et al (2020) Safety and efficacy of satralizumab monotherapy in neuromyelitis optica spectrum disorder: a randomised, double-blind, multicentre, placebo-controlled phase 3 trial. Lancet Neurol 19(5):402–412
14. Zhang C, Zhang MN et al (2020) Safety and efficacy of tocilizumab versus azathioprine in highly relapsing neuromyelitis optica spectrum disorder (TANGO): an open-label, multicentre, randomised, phase 2 trial. Lancet Neurol 19(5):391–401
15. Chihara N, Aranami T, Sato W et al (2011) Interleukin 6 signaling promotes anti-aquaporin 4 autoantibody production from plasmablasts in neuromyelitis optica. Proc Natl Acad Sci U S A 108(9):3701–3706
16. Uzawa A, Mori M, Arai K et al (2010) Cytokine and chemokine profiles in neuromyelitis optica: significance of interleukin-6. Mult Scler 16(12):1443–1452
17. Sato DK, Callegaro D, de Haidar Jorge FM et al (2014) Cerebrospinal fluid aquaporin-4 antibody levels in neuromyelitis optica attacks. Ann Neurol 76(2):305–309
18. Uzawa A, Mori M, Kuwabara S (2014) Cytokines and chemokines in neuromyelitis optica: pathogenetic and therapeutic implications. Brain Pathol 24(1):67–73
19. Petković F, Campbell IL, Gonzalez B, Castellano B (2016) Astrocyte-targeted production of interleukin-6 reduces astroglial and microglial activation in the cuprizone demyelination model: Implications for myelin clearance and oligodendrocyte maturation. Glia 64(12):2104–2119
20. Agasing AM, Wu Q, Khatri B et al (2020) Transcriptomics and proteomics reveal a co- operation between interferon and T-helper 17 cells in neuromyelitis optica. Nat Commun 11(1):2856
21. Takeshita Y, Obermeier B, Cotleur AC et al (2017) Effects of neuromyelitis optica-IgG at the blood-brain barrier in vitro. Neurol Neuroimmunol Neuroinflamm 4(1):e311
22. Ortiz Salas PA, Gaviria Carrillo M, Cortés Bernal GA et al Neuromyelitis optica spectrum disorder: do patients positive and negative for anti-aquaporin-4 antibodies present distinct entities? A Colombian perspective. Neurologia (Engl Ed) S2173-5808(22)00052-9
23. Carreón Guarnizo E, Hernández Clares R, Castillo Triviño T et al (2022) Experience with tocilizumab in patients with neuromyelitis optica spectrum disorders. Neurologia (Engl Ed) 37(3):178–183
24. Liu Y, Zhang H, Zhang T-X et al (2021) Effects of tocilizumab therapy on circulating B cells and T helper cells in patients with neuromyelitis optica spectrum disorder. Front Immunol 12:703931
25. Takai Y, Kuroda H, Misu T et al (2021) Optimal management of neuromyelitis optica spectrum disorder with aquaporin-4 antibody by oral prednisolone maintenance therapy. Mult Scler Relat Disord 49:102750
26. Watanabe M, Nakamura Y, Michalak Z et al (2019) Serum GFAP and neurofilament light as biomarkers of disease activity and disability in NMOSD. Neurology 93(13):e1299–e1311
27. Kim H, Lee EJ, Kim S et al (2020) Serum biomarkers in myelin oligodendrocyte glycoprotein antibody-associated disease. Neurol Neuroimmunol Neuroinflamm 7(3):e708
28. Lucchinetti CF, Mandler RN, McGavern D et al (2002) A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. Brain 125(Pt 7):1450–1461
29. Fujihara K, Misu T, Nakashima I et al (2012) Neuromyelitis optica should be classified as an astrocytopathic disease rather than a demyelinating disease. Clin Exp Neuroimmunol 3(2):58–73
30. Cree BAC (2015) Placebo controlled trials in neuromyelitis optica are needed and ethical. Mult Scler Relat Disord 4(6):536–545