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Tesi di Dottorato:

Interleukin-6 Receptor Blockade in Treatment- Refractory MOG-Ab–Associated Disease and Neuromyelitis Optica Spectrum Disorders

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Introduction:

Neuromyelitis optica spectrum disorder (NMOSD) is an autoimmune disease mostly characterized by recurrent episodes of optic neuritis and myelitis, alone or in combination(1). NMOSD is characterized, in the vast majority of patients, by the presence of auto-antibodies (Ab) against aquaporin 4 (AQP4), a serological diagnostic marker with direct pathological role (1). Recently, anti-myelin oligodendrocyte glycoprotein (MOG) Ab, a serological marker with putative pathophysiological role, have also been found in a small proportion of anti-AQ4 Ab negative NMOSD patients (2). Thus, MOG Ab–associated disease (MOGAD) and NMOSD with or without AQP4 Ab are both considered antibody-mediated, relapsing, chronic inflammatory disease of the central nervous system (CNS)(3,4). Although the clinical presentation with unilateral or bilateral optic neuritis (ON), longitudinally extensive transverse myelitis, or brain stem syndromes may be similar in MOGAD and NMOSD, demographic, clinical, imaging, and pathophysiologic findings strongly suggest the presence of 2 distinct disease entities (5–7). Both MOGAD, excluding acute disseminated encephalomyelitis (ADEM), and NMOSD typically follow a relapsing course in adults (3,8). Before introduction of disease modifying treatments, NMOSD (and MOGAD) prognosis was almost invariably poor with clinical attacks leading to permanent neurological disability or, especially for NMOSD, even death in few years after onset, due to involvement of brainstem structures and complications of neurological disability (3,9,10). Since disability accumulation is mainly driven by attacks, the aim of maintenance therapy in NMOSD and MOGAD is to prevent any additional attacks (3,8,10).

Recently, a variety of therapeutic strategies such as anti-CD19 or anti-CD20-targeted B-cell depletion, complement inhibition, and interleukin-6 (IL-6) receptor blockade were successfully investigated in pivotal NMOSD trials, particularly in AQP4-Ab+ patients (11–15). Yet, insights concerning the effectiveness and safety of such agents in MOGAD are scarce.

IL-6 plays an important role in the pathophysiology of NMOSD (16,17). Increased levels were detected in the serum and CSF, particularly during attacks (16,17). IL-6 promotes the differentiation of inflammatory Th17 cells and the production of AQP4-Ab by B cell–derived plasmablasts in NMOSD and increases the permeability of the blood-brain barrier, facilitating CNS inflammation (18–20). The efficacy of IL-6 receptor blockade in AQP4-Ab+ NMOSD was suggested by studies using tocilizumab (TCZ) in adults and children (21–24) and demonstrated by 2 pivotal trials of satralizumab (14,15), whereas the effect in AQP4-Ab–seronegative patients was less evident. Since AQP4-Ab+ NMOSD and MOGAD both display antibody and complement-mediated CNS injury and similar inflammatory CSF profiles (with elevated IL-6), IL6-blockade may also be beneficial in MOGAD(25,26). However, due to the low frequency of the disease, no large studies dissecting IL-6 blockade effect in MOGAD have been performed yet.

Aim of the study:

We designed a retrospective, international, multicenter study to explore safety and efficacy of tocilizumab (TCZ) administration in patients with MOGAD and to compare TCZ effect in MOGAD patients to the effect of TCZ in classical NMOSD (*i.e.:* AQP4-Ab+) or double-seronegative NMOSD patients.

Methods

All clinical and paraclinical data were analyzed retrospectively by chart review. Patients were continuously treated at the contributing centers, specialized in clinical neuroimmunology, with regular assessment of clinical (attacks, EDSS score, and pain levels) and paraclinical (MRI, AQP4-Ab and MOG-Ab, and other laboratory tests) data. AQP4-Ab and MOG-Ab antibodies were exclusively measured by cell-based assays. The primary outcome was the annualized relapse rate (ARR). An attack was defined as a new neurologic symptom or clear acute worsening of previous neurologic deficits with objective clinical signs, lasting for at least 24 hours and attributed to an inflammatory CNS event, confirmed by the treating physician. Safety aspects comprised infusion-related reactions, infections, tumors, cardiovascular events, standard laboratory tests, AQP4-Ab titers, EDSS score, and chronic pain (occurrence and intensity, classified as mild = 1, moderate = 2, or severe = 3) were assessed at TCZ start and, if available, at last follow-up during TCZ. MRI of the cervicothoracic spinal cord and the brain, evaluated at TCZ onset and last available follow-up, was classified as nonactive or active, indicated by the presence of new T2 or contrast-enhancing lesions.

Patients and treatment

Demographical and clinical characteristics are shown in Table 1. Fifty-seven patients with relapsing MOGAD (n = 14), excluding ADEM, classical AQP4-Ab+ NMOSD (n = 36), or double-seronegative NMOSD (n = 7), mainly of Caucasian descent (n = 50), from neurologic departments of 23 tertiary referral centers in Germany (n = 13), France (n = 5), Austria (1), Italy (1),

Switzerland (1), United Kingdom (1), and United States of America (1) were retrospectively analyzed. The evaluated TCZ treatment period ranged from December 2010 until November 2019. Regarding demographic parameters (Table 1), the mean age at disease manifestation was similar for patients with MOGAD or AQP4-Ab+ NMOSD (35.5 or 36.1 years, respectively; $p = 0.89$), as well as the age when TCZ was started (38.4 or 42.8 years, $p = 0.35$). Five patients were younger than 18 years at disease onset, and 3 of them younger than 18 years at TCZ initiation. Of note, patients with AQP4-Ab+ NMOSD were predominantly female, in contrast to patients with MOGAD (91% vs 35% female, respectively). Patients with AQP4-Ab+ NMOSD tended to have a longer history of disease (median 5.5 years) and were more severely affected at TCZ start (median Expanded Disability Status Scale [EDSS] score 6.25) than patients with MOGAD (median disease duration 2.2 years, $p = 0.13$; median EDSS score 2.75, $p < 0.01$). In the MOGAD group, 7 patients (50%) fulfilled the 2015 revised international consensus diagnostic criteria for NMOSD (27). Before TCZ therapy, patients with MOGAD had had a median of 6 attacks (range 1–12 attacks) with 4.5 ON (median; range 1–10 ON) and 2.0 myelitis events (median; range 1–5 myelitis events). In the NMOSD group, 5/7 double-seronegative (71%) and 27/36 AQP4-Ab+ (75%) patients fulfilled the 2006 NMO diagnostic criteria(28), whereas all AQP4-Ab+ and double-seronegative patients fulfilled the 2015 NMOSD diagnostic criteria(27). Of note, 47/57 (83%) patients were tested for both antibodies, and none was double positive. Ten AQP4-Ab+ patients were not tested for MOG-Ab. Before TCZ, all patients had been treated with different immunotherapies, as shown in Table 2, following established recommendations, and, remarkably, all had received rituximab (RTX).

Within the last 24, 12, and 6 months before TCZ switch, 53/57 (93%), 44/57 (77%), and 31/57 (54%) of the patients were treated with RTX, respectively. B-cell counts, collected briefly before the start of TCZ (median interval 0.9 months; interquartile range [IQR] 0.4–1.9 months), were available for 33/57 (58%) patients (25/36 [69%] AQP4-Ab+ NMOSD, 6/14 [43%] MOGAD, and 2/7 [29%] double-seronegative patients). Of these 33 patients, 28 (85%) patients (21/25 [84%] AQP4-Ab+ NMOSD, 5/6 [83%] MOGAD, and 2/2 [100%] double-seronegative patients) showed markedly reduced or depleted B cells. During the total pre-TCZ treatment phase (median duration of 2.9 years), patients had 6.0 attacks (median; range 1–30 attacks). Considering the last 2 years before TCZ start, 3.0 attacks (median; range 0–10 at- tacks) were recorded (Table 1).

Cohort	MOGAD (n = 14)	AQP4-IgG+ NMOSD (n = 36)	Double seronegatives (n = 7)	Total (N = 57)
Ethnicity, n (%)				
Caucasian	13 (93)	30 (83)	7 (100)	50 (88)
African	—	3 (8)	—	3 (5)
Arabian	1 (7)	2 (6)	—	3 (5)
Latin American	—	1 (3)	—	1 (2)
Sex, n: female/male (% female)	5/9 (35)	33/3 (91)	6/1 (85)	44/13 (77)
AQP4 serostatus, n: pos/neg (% positive)	0/14 (0)	36/36 (100)	0/7 (0)	36/21 (63)
MOG serostatus, n: pos/neg/NA[a] (% positive)	14/0/0 (100)	0/26/10 (0)	0/7/0 (0)	14/33/10 (25)
NMO based on 2006 criteria, n: yes/no (% yes)	4/10 (28)	27/9 (75)	5/2 (71)	36/21 (63)
NMO based on 2015 criteria, n: yes/no (% yes)	7/7 (50)	36/0 (100)	7/0 (100)	50/7 (87)
Age at disease manifestation, y: mean (SD)	35.5 (14.7)	36.1 (15.2)	42.7 (11.5)	36.8 (14.6)
Disease duration before TCZ, y: median (IQR)	2.2 (1.2–3.4)	5.5 (1.2–9.0)	2.4 (2.3–5.1)	2.9 (1.3–8.2)
Relapses under last immunotherapy, n: median (IQR)	1.5 (1.0–2.0)	1.0 (1.0–2.0)	3.0 (1.5–3)	1.0 (1.0–2.0)
Relapses during last 2 y before TCZ, n: median (IQR)	3.5 (2.2–5.0)	3.0 (2.0–5.0)	6.0 (2.5–6.0)	3.0 (2.0–5.0)
Age at TCZ start, y: mean (SD)	38.4 (15.0)	42.8 (14.6)	46.5 (10.8)	42.2 (14.3)
Number of TCZ infusions, n: mean (SD)	26.9 (21.7)	37.6 (31.1)	28.4 (21.9)	34.0 (28.2)
TCZ intervals, d: mean (SD)	30.8 (4.6)	32.1 (4.6)	30.4 (0.8)	31.6 (4.3)
TCZ treatment duration, mo: median (IQR)	16.3 (14.2–44.6)	27.9 (12.9–53.2)	30.4 (10.3–38.1)	23.8 (13.0–51.1)
Relapses before TCZ, n: median (IQR)	6.0 (4.2–8.0)	5.0 (3.0–10.2)	6.0 (5.5–8.5)	6.0 (3.0–9.0)
Relapses under TCZ, n: median (IQR)	0 (0–0)	0 (0–1.0)	1.0 (0–2.0)	0 (0–1.0)
EDSS score before TCZ: median (IQR)	2.75 (2.0–3.5)	6.25 (3.0–7.6)	5.0 (4.5–5.8)	4.5 (3.0–7.0)
EDSS score under TCZ: median (IQR)	2.0 (1.2–2.9)	4.25 (2.5–7.0)	5.0 (3.5–6.8)	3.5 (2.0–6.5)

Table 1: Demographic and clinical characteristics of enrolled patients.

Abbreviations: AQP4 = aquaporin-4; EDSS = Expanded Disability Status Scale; MOG = myelin oligodendrocyte glycoprotein; MOGAD = MOG-IgG–

associated disorder; NMOSD = neuromyelitis optica spectrum disorder; TCZ = tocilizumab. (a) MOG-Ab not tested.

Cohort	MOGAD (n = 14)	AQP4-IgG+ NMOSD (n = 36)	Double seronegatives (n = 7)	Total (N = 57)
Rituximab	14 (100)	36 (100)	7 (100)	57 (100)
Azathioprine	3 (21)	13 (36)	2 (29)	18 (32)
Mycophenolate mofetil	3 (21)	7 (19)	1 (14)	11 (19)
Low-dose steroid monotherapy	4 (29)	7 (19)	0 (0)	11 (19)
Methotrexate	1 (7)	7 (19)	3 (43)	11 (19)
Cyclophosphamide	2 (14)	8 (22)	1 (14)	11 (19)
IVIG	3 (21)	4 (11)	0 (0)	7 (12)
Interferon-beta	0 (0)	5 (14)	1 (14)	6 (11)
Mitoxantrone	0 (0)	5 (14)	0 (0)	5 (9)
Glatiramer acetate	0 (0)	2 (6)	1 (14)	3 (5)
Natalizumab	0 (0)	1 (3)	1 (14)	2 (4)
Long-term plasma exchange	1 (7)	1 (3)	0 (0)	2 (4)
Alemtuzumab	0 (0)	1 (3)	0 (0)	1 (2)
Fingolimod	0 (0)	1 (3)	0 (0)	1 (2)
Cyclosporin A	0 (0)	1 (3)	0 (0)	1 (2)
Belimumab	1 (7)	0 (0)	0 (0)	1 (2)
Etanercept	0 (0)	1 (3)	0 (0)	1 (2)

Table 2: Treatments of enrolled patients before TCZ start

Statistical Analysis

In general, the ARR was calculated by dividing the number of attacks within the last 2 years before TCZ switch or during TCZ treatment time by 2. However, for 19 patients, who had a TCZ pretreatment phase of <2 years (median 1.1 years), we categorically divided the total number of attacks by 2, and for 13 patients with a follow-up period of <1 year (median 0.5 years) during TCZ treatment, we divided the number of attacks by the concrete treatment duration and thus extrapolated this measure to 1 year. To avoid possible overestimation of the relapse-free proportion in the latter group, we excluded those 13 patients with TCZ treatment durations of <12 months for subgroup analyses. In the descriptive analysis, values are given as mean or median, with the appropriate measures of dispersion (i.e., range, SD, or IQR). In all cases, the assumption of normal distribution could not be affirmed.

Therefore, only nonparametrical tests were used. To test for statistically significant differences between 2 related samples like ARR before TCZ switch and ARR under TCZ therapy, the Wilcoxon signed-rank test was used. In case of paired categorical data with a dichotomous trait, the exact binomial test was used. For count data–like relapses, we also applied an unconditional Poisson regression. Statistical results are presented as p values and 95% confidence intervals. p values <0.05 were considered to indicate statistically significant results. Because of the exploratory nature of the study, no adjustment for multiple comparisons was made. Version 3.6.3 of the R statistics package was used for statistical analysis.

Results:

Annualized relapse rate

Forty-five of 57 (79%) patients switched to TCZ due to ongoing disease activity, 5/57 (9%) due side effects of prior immunotherapies (including allergic reactions on RTX in 3 patients), and 6/57 (10%) because of concomitant disease activity and adverse events. In 1 patient, the detection of neutralizing antibodies against RTX was the reason for treatment switch. TCZ was administered IV (mean 34 infusions, range 3–109) in 56 patients (98%) at a mean interval of 31.6 days (range 26.1–44.2 days) and with a median dose of 8.0 mg/kg body weight (range 6.0–12.0 mg/kg body weight; Table 1) and subcutaneously in 1 patient (2%) with weekly injections of 162 mg. The interval from last relapse to initiation of TCZ was similar for all groups, i.e., 2.2 months (median, IQR 1.1–5.1 months) for patients with AQP4-Ab+ NMOSD, 3.2 months (1.5–4.8 months) for the MOGAD subgroup, and 2.4 months (1.7–6.2 months) for double seronegatives.

The median treatment duration was 23.8 months (IQR 13.0–51.1 months), with patients with AQP4-Ab+ NMOSD showing the longest TCZ exposure (27.1 months), compared with MOGAD (16.3 months) and double-seronegative (30.4 months) patients. In one-third of patients (20/57), TCZ was given as an add-on treatment; in 2 of them due to comorbidities (psoriasis cotreated with methotrexate [MTX]; chronic poly-arthritis with oral low-dose steroids [LDSs]). Additional medications included LDS (n = 10), MTX (n = 4), mycophenolate mofetil (MMF; n = 2), azathioprine (AZA; n = 1), IV immunoglobulins (IVIG; n = 1), RTX (n = 1), and monthly high-dose steroids (HDS; n = 1), administered for <6 months in 3 patients and >6 months in 17 patients during TCZ treatment.

Initiation of TCZ was followed by a decrease of the median ARR in patients with AQP4-Ab+ NMOSD from 1.5 to 0 ($p < 0.001$, 95% CI 0–0.2) compared with the last 2 years before TCZ start. Of note, patients with MOGAD showed a similar median ARR reduction from 1.75 to 0 ($p = 0.0011$, 95% CI 1.3–2.6). For patients with double-seronegative NMOSD, median ARR reduction was less prominent but still significant (from 3.0 to 0.2 [$p < 0.032$, 95% CI 0.3–2.8]). For the total cohort, the median ARR decreased from 1.5 to 0 ($p < 0.001$, 95% CI 1.1–1.8; Figure 1). Of note, ARR reductions were also detectable when analysis was confined to those patients treated with TCZ for at least 12 months, including MOGAD and AQP4-Ab+ NMOSD, but not double-seronegative patients.

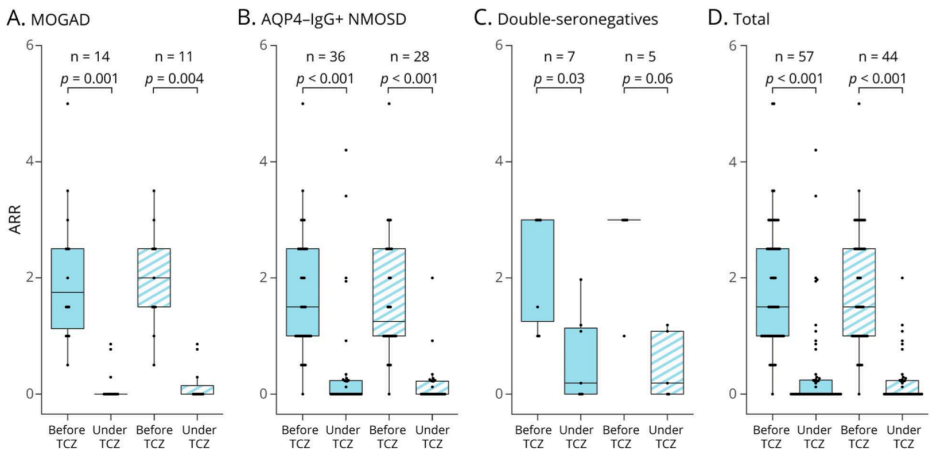

Figure 1: annualized relapse rate during TCZ treatment.

Box-and-whisker plots showing the median, IQR, and range of the annualized relapse rate 2 years before and during TCZ treatment for the MOGAD (A), the AQP4-Ab+ NMOSD (B) and the double seronegative (C) subgroups of patients, as well as for the total cohort (D). Each dot indicates 1 single patient. Hatched bars represent those patients who had been treated with TCZ for at least 12 months. AQP4 = aquaporin-4; ARR = annualized relapse rate; IQR

= interquartile range; MOGAD = MOG-IgG–associated disease; NMOSD = neuromyelitis optica spectrum disorder; TCZ = tocilizumab.

Regarding individual patients, 3/14 (21.4%) patients with MOGAD (Figure 2) and 14/36 (39%) patients with AQP4-Ab+ NMOSD (Figure 3) had at least 1 attack during TCZ treatment, and 2/14 (14.3%) patients with MOGAD and 2/ 36 (5.6%) patients with AQP4-Ab+ NMOSD showed 2 or more attacks. Sixty percent of all patients were relapse free (79% for MOGAD, 56% for AQP4-Ab+ NMOSD, and 43% for double-seronegative NMOSD). When analyzing only patients treated with TCZ for at least 12 months, 26/44 (59%) of all patients, 8/11 (73%) MOGAD, 16/28 (57%) AQP4-Ab+, and 2/5 (40%) double-seronegative patients, remained relapse free.

The median time to first relapse was 9 months (range 0.5–47 months) for the whole group, 9.4 months for MOGAD (range 9–15 months), 4.4 months for AQP4-Ab+ NMOSD (range 0.5–47 months), and 12.2 months for double-seronegative NMOSD (range 2.6–18.9 months). An unconditional Poisson regression analysis showed an average increase in relapses by 16% per year under TCZ therapy, indicating that a relapse is not expected until after 5 years under TCZ in the total cohort ($p < 0.03$). Moreover, double-seronegative patients had average 2.6 times the relapse counts compared with patients with AQP4-Ab+ NMOSD ($p < 0.03$), and in the MOGAD subgroup, relapses occurred 8% less than in AQP4-Ab+ NMOSD, which was not significant ($p = 0.86$).

When comparing patients treated with TCZ plus add-on treatment (20/57) with those on TCZ monotherapy (37/ 57), the ARR in the add-on group was higher in the 2 years before TCZ initiation (median 2.0 [IQR 1–3] vs 1.5 [IQR

1–2.5]) as well as during TCZ treatment (0.2 [IQR 0–0.8] vs 0 [IQR 0-0]). In line, freedom from relapses was achieved in 40% of patients in the add-on group and in 78% in the monotherapy group. By comparing the 2 groups of patients who switched to TCZ due to ongoing disease activity or side effects, the median ARR in the first group was 2.0 (IQR 1.0–2.5) during the 2 years prior TCZ and was 0 (IQR 0–0.2) during TCZ treatment, whereas the median ARRs were 1.0 (IQR 0.5–1.0) and 0 (IQR 0-0), respectively, for both intervals in the second subgroup.

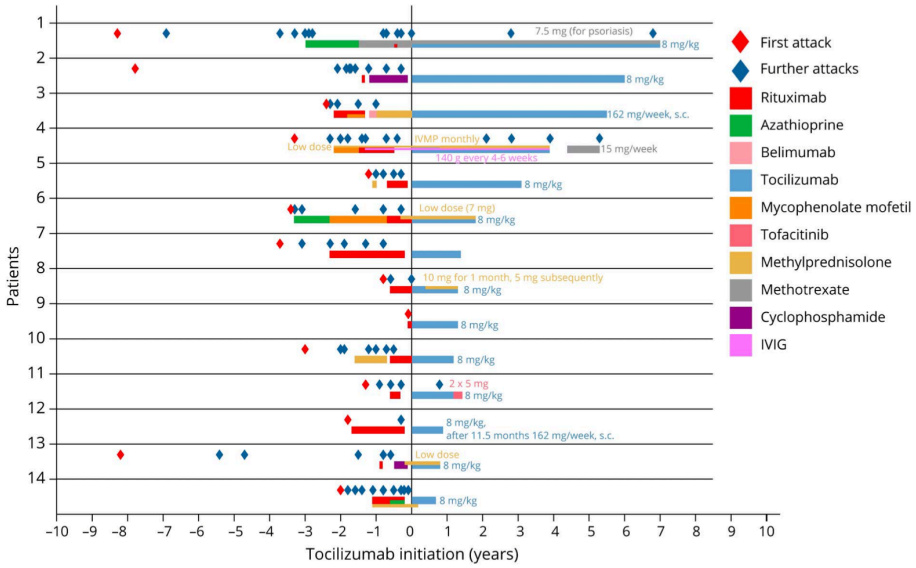

Figure 2: Disease courses and individual treatments for MOGAD patients

First attacks are indicated as red diamonds and further attacks as blue diamonds. IVIG = IV immunoglobulin; IVMP = IV methylprednisolone.

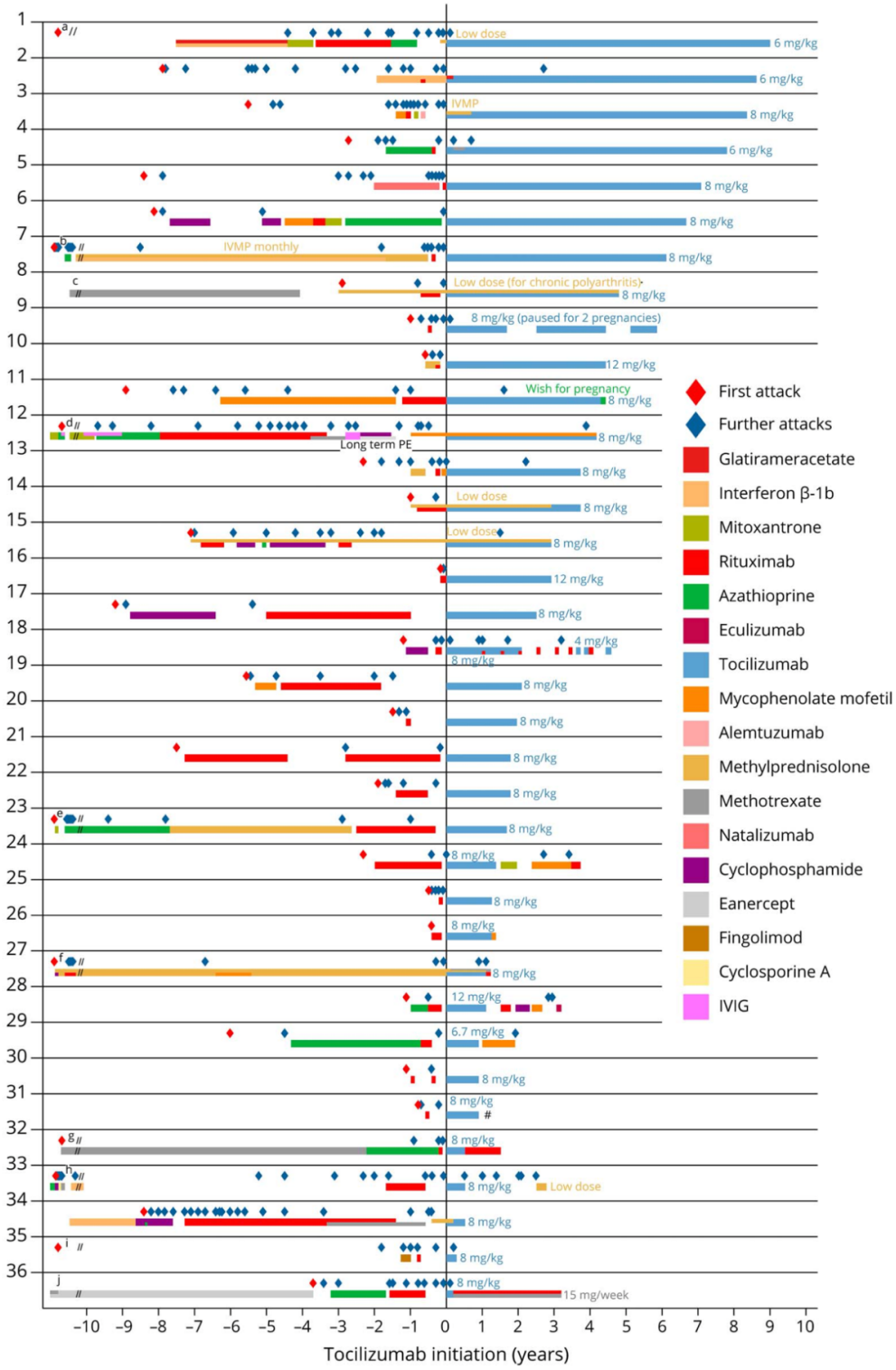

Figure 3: Disease courses and individual treatments for AQP4-Ab+ patients First attacks are indicated as red diamonds and further attacks as blue diamonds. IVMP = IV methylprednisolone; PE = plasma exchange. (a) Twelve years before tocilizumab (TCZ) initiation. (b) Twenty-four years before TCZ initiation. (c) Therapy of chronic polyarthritis 2 and a half years

before TCZ initiation. (d) Fifteen years before TCZ initiation. (e and f) Sixteen years before TCZ initiation. (g) Ten and a half years before TCZ initiation. (h) Twenty-two years before TCZ initiation. (i) Eleven and a half years before TCZ initiation. (j) Psoriasis therapy; psoriasis flare-up finally remitted completely under rituximab; #loss to follow-up.

When comparing patients who relapsed vs those who did not relapse during TCZ treatment (across the different sub-groups), relapsing patients with AQP4-Ab+ NMOSD were younger at disease manifestation than nonrelapsing patients (years, median, relapsing vs nonrelapsing, 31.4 vs 36.4, re- spectively). At TCZ start, MOGAD and double-seronegative patients who later relapsed were older than nonrelapsing patients, whereas relapsing and nonrelapsing AQP4-Ab+ patients had comparable age (years, median, relapsing vs nonrelapsing, AQP4-Ab+ 43.7 vs 43.6, MOGAD 48.5 vs 41.2, double seronegatives 50.7 vs 37.8, respectively). Relapsing patients had a longer disease duration than non-relapsing in the AQP4-Ab+ and MOGAD groups (years, median, AQP4-Ab+ NMOSD 8.76 vs 2.93, MOGAD 3.33 vs 2.11, respectively). Sex had no effect on relapses in all sub-groups. Under TCZ therapy, most of the myelitis and ON attacks occurred in AQP4-Ab+ NMOSD and double-seronegative patients (AQP4-Ab+ NMOSD: myelitis [14], ON [4]; MOGAD: myelitis [2], ON + myelitis [1]; double seronegatives: myelitis [4]).

Disability:

The median EDSS score significantly decreased in both seropositive groups, in MOGAD from 2.75 to 2.0 ($p < 0.031$) and in AQP-Ab+ NMOSD from 6.25

to 4.25 ($p < 0.003$). The median EDSS score remained stable on 5.0 in 7/7 double-seronegative patients ($p < 0.77$; Table 1; Figure 4).

When including patients with TCZ treatment duration >12 months only, the EDSS score improvement was still significant for AQP4-Ab+ NMOSD and the whole cohort (Figure 4). The EDSS score worsened in only 5/57 (9%) patients of the entire cohort, i.e., none of patients with MOGAD, 3/36 (8%) patients with AQP4-Ab+ NMOSD, and 2/7 (29%) double-seronegative patients.

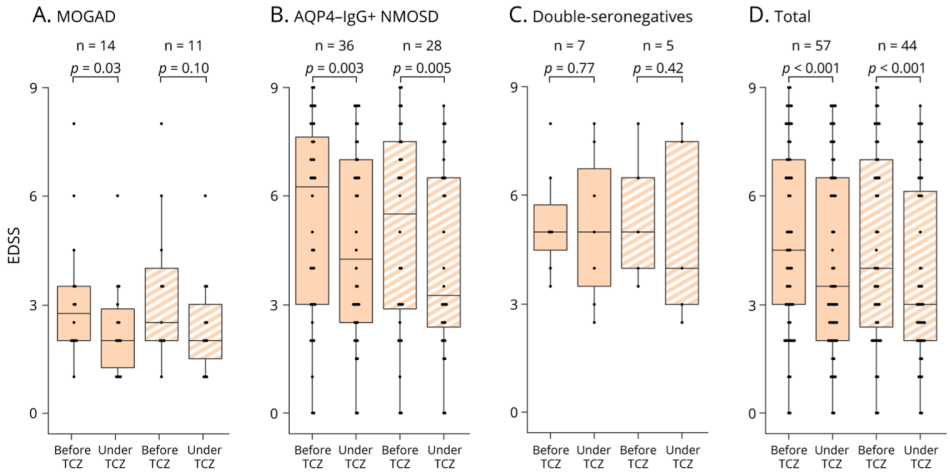

Figure 4: EDSS score before and during TCZ treatment.

Box-and-whisker plots showing the median, IQR, and range of the EDSS score 2 years before and during TCZ treatment for the MOGAD (A), the AQP4-Ab+ NMOSD (B) and the double seronegative (C) subgroups of patients, as well as for the total cohort (D). Each dot indicates 1 single patient. Hatched bars represent those patients who had been treated with TCZ for at least 12 months. AQP4 = aquaporin-4; EDSS = Expanded Disability Status Scale; IQR = interquartile range; MOGAD = MOG-Ab–associated disease; NMOSD = neuromyelitis optica spectrum disorder; TCZ = tocilizumab.

Pain

Initial disease-related chronic pain was reported in 28/51 patients (55%), with a median pain intensity of 2.0 (IQR 1–3; data from 27 patients). Presence and intensity of pain were not modulated during TCZ treatment, as 25/52 patients (48%) still had ongoing chronic pain with a median intensity of 2.0 (IQR 1–3; data available from 24 patients) at last follow-up, regardless of the AQP4-Ab/MOG-Ab serostatus.

Antibody Titers

Regarding AQP4-Ab immunoreactivity, most patients (12/16) showed decreased or stable titers after initiation of TCZ. Longitudinally assessed MOG-Ab anti- body titers were available in only 2 of 14 patients and showed a similar pattern as seen in AQP4-Ab+ NMOSD, i.e., a decrease from 1:320 to 1:32 and from 1:1,280 to 1:10, respectively.

Magnetic Resonance Imaging

For brain MRI, the proportion of patients with active scans (presence of new T2 or contrast-enhancing lesions) significantly decreased from 43.5% at TCZ baseline (20/46 patients with available longitudinal data at TCZ onset and follow-up) to 15.2% (7/46 patients; $p = 0.007$) at last available scan, within 31.6 months (mean; range 4.2–95.8 months). This reduction was detectable for MOGAD (change from 7/13 [53.8%] to 1/13 patients [7.7%] with active scans; $p = 0.031$), but not for AQP4-Ab+ (9/26 [34.6%] to 3/26 [11.5%]; $p = 0.146$) or seronegative (4/7 [57.1%] to 3/7 [42.9%]; $p = 1$) subgroups.

For spinal cord MRI, the proportion of patients with active scans decreased from 71.4% (25/35 patients) to 28.6% (10/ 35; $p = 0.00006$) during TCZ

(mean interval 40.5 months; range 3.7–111.3). This effect was mainly driven by the AQP4-Ab+ group with a decrease from 74.1% (20/27) to 25.9% (7/27) of patients during TCZ (p = 0.0002). For double-seronegative NMOSD and MOGAD, the proportion of patients with active scans was low and stable during TCZ.

Safety Data

Clinical Events

Infusion-related reactions occurred in 7/57 (12.3%) patients and included headache, abdominal pain, vertigo, nausea, fatigue, leg edema, rash, mild bruising, and bloating (Table 3). Infections comprised recurrent urinary tract infections (UTI, in 16% of patients), upper respiratory tract infections, common cold, bronchitis and pneumonia (in 16%), oral or lip infections (in 7%; including herpes simplex virus, ulcers, and candidiasis), erysipelas and skin lesions compatible with SLE (in 5%), and pyelonephritis (in 3.5%). In 19/57 (33%) patients, 23 chronic underlying inflammatory diseases were reported, including Hashimoto thyroiditis (N = 7), SLE (5), psoriasis (4), Sjogren syndrome (2), and vitiligo, polyarthritis, immune thrombocytopenic purpura, myasthenia gravis, and Crohn disease (1 each). Exacerbation of SLE and psoriasis during TCZ occurred in 4 patients (2 from each) and led to TCZ discontinuation in 2 of these 19 (11%) patients (both AQP-Ab+ NMOSD). No new cancer occurred. One case of type 1 focal nodular hyperplasia of the liver was diagnosed during TCZ. Cardiovascular events occurred in 3 patients, including a non-ST elevation myocardial infarction after the initial infusion, a deep vein thrombosis, and a slight increase in blood pressure. One death due to recurrent pneumonia occurred 2 months after discontinuation of

a 6-month TCZ treatment period, but this was not regarded as treatment related by the treating physician, as the 58-year-old patient had a history of severe AQP4-Ab+ NMOSD with concomitant SLE and uterus carcinoma, including surgery and radiation.

TCZ-treated patients with additional immunotherapies suffered more frequently from pneumonia compared with the monotherapy group (18% vs 6%); other side effects like reactivation of chronic latent infections (5% vs 6%) were equally distributed in both groups.

Laboratory Changes

Neutropenia during TCZ treatment, with a maximum cell count reduction of 61% below the lower reference level, occurred in 10/57 (18%) patients, with 3 patients on a concomitant immunotherapy (MTX, RTX, and LDS; Table 3). However, these 10 patients had no higher frequency of common neutropenia-related conditions such as UTI, pneumonia, and other (unspecific) infections. Transient and mild to moderate increases of liver enzymes and lipase (2- to 3-fold above the upper reference level) were reported in 20/57 (35.1%) patients. In particular, alanine aminotransferase was elevated at least once in 17/57 (29.8%) patients during TCZ and increased from 28.2 U/L (mean; range 8–90 U/L; at TCZ start) to 75.6 U/L (range 21–179 U/L; $p < 0.001$). Mean total cholesterol levels increased slightly during TCZ treatment from 195.3 mg/dL (n = 37/57; range 59–311 mg/dL) to 203.8 mg/dL (n = 44/57; range 85–372 mg/dL; $p = 0.5554$), with no changes within the subgroups as well. Similarly, low- and high-density lipo- protein (LDL/HDL) cholesterol levels were stable during TCZ (Table 2).

Cohort	MOGAD (n = 14)	AQP4-IgG+ NMOSD (n = 36)	Double seronegatives (n = 7)	Total (N = 57)
Infusion-related reactions, n (%)	1 (7%)	6 (17%)	0 (0%)	7 (12%)
Infections				
Recurrent urinary tract infections	1 (7%)	7 (19%)	1 (14%)	9 (16%)
Viral upper respiratory tract infections/common cold/bronchitis/ pneumonia	2 (14%)	5 (14%)	2 (29%)	9 (16%)
Oral or lip infections	0 (0%)	4 (11%)	0 (0%)	4 (7%)
Erysipelas and skin lesions compatible with SLE	0 (0%)	3 (8%)	0 (0%)	3 (5%)
(Pyelo)nephritis	1 (/%)	1 (3%)	0 (0%)	2 (4%)
Reactivation of chronic latent infection, n (%)	0 (0%)	3 (8%)	0 (0%)	3 (5%)
Tumor, n (%)	0 (0%)	1[a] (3%)	0 (0%)	1[a] (2%)
Cardiovascular events, n (%)	0 (0%)	3 (8%)	1 (14%)	4 (7%)
Neutropenia, n (%)	2 (14%)	8 (22%)	0 (0%)	10 (17%)
Any liver enzyme changes, n (%)	2 (14%)	12 (33%)	6 (86%)	20 (35%)
Cholesterol before TCZ, mg/dL: mean (SD)	195.8 (42.2)	190.5 (65.0)	220.3 (66.8)	195.3 (58.5)
Cholesterol under TCZ, mg/dL: mean (SD)	199.6 (66.3)	199.9 (46.2)	235.2 (80.4)	203.8 (56.6)
LDL before TCZ, mg/dL: mean (SD)	126.9 (50.0)	114.0 (47.4)	140.7 (54.5)	121.0 (48.0)
LDL under TCZ, mg/dL: mean (SD)	129.8 (44.9)	119.2 (43.3)	166.3 (49.5)	126.4 (44.9)
HDL before TCZ, mg/dL: mean (SD)	60.6 (21.4)	59.4 (22.7)	66.3 (35.7)	60.5 (22.8)
HDL under TCZ, mg/dL: mean (SD)	70.7 (40.3)	69.8 (41.9)	57.8 (38.5)	68.9 (40.2)

Table 3: Safety profile of TCZ treatment

Abbreviations: AQP4 = aquaporin-4; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; MOG = myelin oligodendrocyte glycoprotein; MOGAD = MOG-IgG–associated disorder; NMOSD = neuromyelitis optica spectrum disorder; SLE = systemic lupus erythematosus; TCZ = tocilizumab. (a) FNH (focal nodular hyperplasia).

TCZ discontinuation

TCZ therapy was discontinued in 20/57 patients (35.1%; 15/36 [41.7%] AQP4-Ab+, 2/14 [14%] MOGAD, and 3/7 [42.9%] double-seronegative patients) after 14.5 treatment months (median; range 2.9–53.9 treatment months). Of note, 45% (9/20) of them stopped TCZ for general reasons such as pregnancy, plans for pregnancy, and patient's preference (e.g., for oral medications), and 2 patients were lost to follow-up. However, 6 of the 20 patients (2 AQP4-Ab+, 3 double se- ronegative, and 1 MOGAD) presented ongoing MRI activity or attacks, and 5/20 patients (all AQP4-Ab+)

discontinued due to suspected side effects such as ileus (n = 1), nephritis and urticaria in the context of systemic lupus erythematosus (SLE; n = 1), psoriasis exacerbation (n = 1), and upper respiratory tract infections (n = 3). Two patients who stopped TCZ restarted it after completion of pregnancy and ileus treatment. Of the 11 patients with disease activity or suspected side effects, 6 patients (55%) received TCZ as add-on therapy, and 5 patients (45%) showed relapse activity, which occurred 256 days (median, IQR 73–329 days) after TCZ initiation, indicating that delayed onset of efficacy may have contributed to early discontinuation.

Discussion:

Our real-word, retrospective, multicenter, international study confirms existing evidence that, in MOG-Ab or AQP4-Ab–mediated inflammatory demyelinating syndromes, IL-6 blockade offers a therapeutic perspective, even in highly treated patients that were previously exposed immunotherapies, including B-cell depleting therapies.

Additionally, our data provide safety and efficacy insight into therapeutic long-term management of these diseases, with a follow-up far beyond the observation periods in existing pivotal trials.

Previous retrospective studies and case series in NMOSD suggested that IL-6 blockade with TCZ might be beneficial for relapse prevention(29,30). Additionally, a recent randomized clinical trial (TANGO trial(31)) showed that patients treated with TCZ performed better than those that were treated with azathioprine. Moreover, two randomized clinical trials with satralizumab confirmed that IL-6 blockage if effective in relapse prevention in AQP4-Ab+ NMOSD patients(14,15).

However, clinical trials observation/treatment period is limited and did not investigate the effect of IL-6 blockade in MOGAD patients.

We enrolled in our study 14 individuals with MOGAD and 36 individuals with AQP4-Ab+ NMOSD, providing real-world data with a mean and maximum treatment duration of nearly 3 and 9 years, respectively.

We show that the primary outcome measure (ARR) is significantly reduced by TCZ by 80% in the total cohort and by 76% in the AQP4-Ab+ subgroup. Additionally, ARR reduction was consistently reduced in both groups of TCZ treated patients (*i.e.,* whole cohort and those treated for at least 12 months).

Our clinical findings are also supported by MRI data showing a reduce spinal cord MRI activity together with AQP4-Ab titer stabilization.

Of note, however, MRI activity reduction, AQP4-Ab titer stabilization and even EDSS reduction could also be partially explained by a regression to the mean effect due to enrollment of active/relapsing patients in the study.

While some early reports showed a beneficial effect of TCZ on pain, such effect was not shown in our study, with similar results also reported satralizumab randomized clinical trials(14,15).

Regarding the patients with double-seronegative NMOSD, we observed a significant ARR reduction independently of the treatment duration. However, such evidence was not reported in the pivotal trials for satralizumab and could be explained by the heterogeneity of this less-defined patient group, hampering direct comparisons.

For MOGAD, treatment recommendations are scarce, and approaches well established for AQP4-Ab+ NMOSD such as CD20-mediated B-cell depletion have shown limited efficacy in MOGAD(32–34). Several reports showed that IVIG maintenance therapy might outperform other immunosuppressants (AZA, MMF and RTX) in MOGAD(34). Additionally, only few cases of MOGAD patients treated with TCZ were reported(35–37). Here, in our series of 14 patients with MOGAD, the ARR decreased by 93%, the median EDSS score was reduced from 2.75 to 2.0 over a mean TCZ treatment duration of 31 months. Notably, the ARR reduction persisted when considering only those patients who were treated for more than 12 months. Again, the effect on EDSS score and MRI activity was mainly driven by the fact of high disease activity at baseline and remission phase at follow-up assessment. Most patients with MOGAD (79% and 73% for the patients treated for >12 months,

respectively) remained relapse free, and in 57% of them, TCZ was used as monotherapy.

Overall, when considering disease activity, including ARR, as well as suspected side effects during TCZ therapy, we did not observe a clear advantage of add-on treatments, supporting the use of TCZ as monotherapy.

Considering safety, adverse events occurred within the expected range based on the established use of TCZ in clinical practice. Infusion-related reactions were the most common adverse events, followed by infections of the urinary or respiratory tract. Of note, (re)activation or worsening of chronic latent inflammatory diseases was observed in patients with already established SLE (n = 2) and psoriasis (n = 2), indicating that these patients should be particularly monitored. As expected, few patients developed mild to moderate neutropenia (18%) and liver enzyme changes (35%). Total cholesterol, as well as HDL and LDL cholesterol levels, did not change during TCZ treatment

An obvious limitation of this study is the retrospective multicenter design resulting in heterogeneity of TCZ treatment regimens and MRI protocols, as well as missing data. Another limitation is the relatively small sample size, which is justifiable by the rarity of NMOSD and MOGAD on a concomitant rare and off-label treatment with TCZ. Moreover, because of the lack of a control cohort and the timing of the switch to TCZ (i.e., during a phase of active disease), we have to consider regression to the mean as an important limitation of our study design, as mean disease activity could decrease spontaneously even without treatment.

Despite these limitations, this largest real-world study supports the long-term safety and therapeutic relevance of the IL-6 blockade in RTX-refractory AQP4-Ab+ NMOSD for up to 9 years. Moreover, our findings suggest a similar role for MOGAD, pointing toward the need for randomized controlled trials to evaluate the efficacy of IL-6 blockade in patients with MOGAD.

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