https://doi.org/10.31925/farmacia.2022.4.8 **ORIGINAL ARTICLE**

MECHANISM OF RITUXIMAB IN THE TREATMENT OF NEUROMYELITIS OPTICA

CHUNHUA YUAN [1], YING LIU [2], YANKUN HAO [3], LEI YAN [4], JUN LIANG [5], HUIPENG JIN [2]*

[1]*Department of Neurology, Second Affiliated Hospital of Mudanjiang Medical University, Mudanjiang city, 157000, China*
[2]*Ophthalmic Function Room, Affiliated Hongqi Hospital of Mudanjiang Medical University, Mudanjiang city, 157000, China*
[3]*Department of Medical Function, Mudanjiang Medical University, Mudanjiang city, 157000, China*
[4]*Department of Histology and Embryology, Mudanjiang Medical University, Mudanjiang city, 157000, China*
[5]*Stem Cell Institute, Mudanjiang Medical University, Mudanjiang city, 157000, China*

**corresponding author: huipengjin@163.com*

Manuscript received: February 2022

Abstract

Rituximab (RTX) is increasingly used in neuromyelitis optica (NMO). Due to the lack of data on its safety and efficacy, we set out to determine the performance of rituximab (RTX) versus cyclophosphamide (CPM) as maintenance therapy for NMO. In this randomized, prospective clinical trial, the primary objective was to determine the efficacy of RTX *versus* CPM, as assessed by the mean Expanded Disability Status Scale (EDSS) score and overall efficacy rate. Secondary objectives included assessment of mean Activities of Daily Living (ADL) score, treatment safety profile, serum AQP4-Ab, IL-6, IL-27 and MMP-9 levels and recurrence rate after treatment. The results of our study showed that both RTX and CPM provided therapeutic benefits for the treatment of NMO. However, RTX was superior to CMP in terms of safety, efficacy and durability of remission. Further research is warranted to validate these results.

Rezumat

Rituximabul (RTX) este din ce în ce mai des utilizat în neuromielita optică (NMO). Datorită lipsei de date privind siguranța și eficacitatea sa, ne-am propus să determinăm performanța rituximabului (RTX) comparativ cu ciclofosfamida (CPM), ca terapie de întreținere pentru NMO. În acest studiu clinic randomizat, prospectiv, obiectivul principal a fost determinarea eficacității RTX *versus* CPM, evaluată prin scorul mediu al Scalei *Expanded Disability Status* (EDSS) și prin rata de eficacitate generală. Obiectivele secundare au inclus evaluarea scorului mediu al activităților de viață zilnică (ADL), profilul de siguranță al tratamentului, nivelurile serice ale AQP4-Ab, IL-6, IL-27 și MMP-9 și rata de recurență după tratament. Rezultatele studiului au arătat că atât RTX, cât și CPM au oferit beneficii terapeutice pentru tratamentul NMO. Cu toate acestea, RTX a fost superior CMP în ceea ce privește siguranța, eficacitatea și durabilitatea remisiunii. Sunt necesare cercetări suplimentare pentru validarea acestor rezultate.

Keywords: Devic's disease, neuromyelitis optica, demyelinating disease, aquaporin-4 autoantibody, rituximab

Introduction

Neuromyelitis optica (NMO), also known as Devic's disease, is a rare yet severe autoimmune central nervous system (CNS) disorder characterized by recurrent attacks primarily involving the optic nerves (optic neuritis) and the spinal cord (myelitis) [1, 2]. Similar to other autoimmune diseases, NMO is more prevalent in women than men [3]. Most patients have detectable serum IgG autoantibodies targeting the astrocyte water channel aquaporin 4 (AQP4-Ab), which are considered highly specific for the diagnosis of NMO [3].

Treatment of NMO focuses on both the management of acute symptoms and the prevention of future relapses. Acute attacks usually require intravenous corticosteroid pulse therapy to suppress CNS inflammation, with treatment escalation to plasma exchange if necessary [5, 6]. Preventive approaches are primarily immunosuppressive [7]. Azathioprine, mycophenolate mofetil and rituximab (RTX) are the most widely recommended agents for NMO prophylaxis, and prolonged (> 5 years) regimens may be required to reduce relapse risk and preserve neurological function [8, 9]. Symptomatic and supportive care, especially for immobility and neuropathic pain, should also be considered as adjunctive therapy [10, 11].

RTX, a human monoclonal antibody directed against the CD20 epitope on the surface of B cells, has been increasingly utilized in the preventive treatment of NMO. However, its safety and effectiveness have not yet been fully elucidated. We thus conducted a prospective, randomized clinical study to evaluate the performance of RTX *versus* cyclophosphamide (CPM) as maintenance therapy in NMO patients.

Materials and Methods

Study design

This was a single-centre, prospective, randomized clinical trial designed to evaluate the performance of

RTX *versus* CPM as maintenance therapy in NMO patients. The study included three groups, i.e., two case groups (RTX- and CPM-treated patients) and one control group (healthy subjects) and was divided into 3 parts, *i.e.*, treatment period 1 (acute phase - corticosteroid therapy), treatment period 2 (4-week RTX or CPM therapy) and treatment-free follow-up period (1 year).

The research protocol followed the ethical principles of the Declaration of Helsinki and was approved by the institutional ethics committee of Mudanjiang Medical University, Mudanjiang, China. Written informed consent was obtained from all participants prior to enrolment after a complete explanation of the study design and objectives, as well as possible adverse drug reactions.

Patients and setting

A total of 40 NMO patients (6 males, 34 females) with mean ± SD age at disease onset of 28.45 ± 17.08 years (range 16 - 58 years) and mean ± SD disease duration of 10.25 ± 6.38 years (range 2.25 - 22.18 years) were recruited as case groups at the Second Affiliated Hospital of Mudanjiang Medical University, Mudanjiang, China, from May 2018 to September 2019. The case population was randomly divided into an experimental group and control group (each group with 20 cases) assigned to receive weekly pulses of either RTX (RTX-group; n = 20) or CPM (CPM-group; n = 20) and followed up over 1 year.

Inclusion criteria were defined as: i) a diagnosis of NMO based on the revised 2015 diagnostic criteria for NMO Spectrum Disorder (NMOSD), ii) age ≥ 16 years; iii) disease duration > 1 year, and iv) AQP4-Ab seropositivity. Key exclusion criteria were: i) presence of uveitis, glaucoma, retinal disease, and/or any other ocular disorders; ii) presence of other autoimmune diseases; iii) cardiovascular, cerebrovascular, chronic kidney or liver comorbidities; iv) presence of hepatitis, tuberculosis, and/or other chronic infectious diseases; v) presence of neurological disorders; vi) pregnancy or lactation; vii) recent exposure to immunosuppressive or anti-inflammatory drugs before enrolment; viii) those whose contact was lost during treatment or follow-up; and ix) those with poor obedience.

In addition, a group of 20 healthy volunteers (2 males, 18 females) with mean ± SD age of 42.21 ± 19.84 years (age range 22 - 56 years) served as non-treated controls providing a reference standard to evaluate the case/control status of the selected serological biomarkers.

Treatment protocol

In the acute phase, all patients were given high-dose intravenous (IV) methylprednisolone (Tianjin Tianyao Pharmaceuticals, China) with gradual tapering from 1 g/day to 120 mg/day for 6 consecutive days and then switched to 60 mg/day oral prednisolone acetate (Shandong Octagon Chemicals Limited, China) gradually reducing the dose by 5 mg *per* week to maintenance 10 mg/day dose until remission. In parallel, potassium chloride sustained release tablets (Shenzhen Zhonglian Pharmaceutical Co., Ltd.) (1 g x 3 times a day) and omeprazole enteric-coated capsules (Shanghai Himed Pharmaceutical Co., Ltd, China) (20 mg twice daily) were orally administered.

Following remission, patients in the CPM group were treated with pulse doses of CPM (Shandong Octagon Chemicals Limited, China) 200 mg IV weekly in combination with 2-mercaptoethanesulfonate sodium (Mesna) (Shandong Octagon Chemicals Limited, China) for 4 weeks. In the RTX-group, patients received RTX (Genentech, Roche Group, Hoffmann-La Roche Ltd, USA) as a 375 mg/m^2 IV infusion once weekly for 4 weeks.

Study evaluations

The severity of disability in case groups was clinically assessed using the 10-point Expanded Disability Status Scale (EDSS; higher scores indicate greater levels of disability) and the Activities of daily living (ADL) scale (point scale of 0 - 100, with higher scores representing lower impact on daily activities). Clinical therapeutic outcome was classified as: i) markedly effective (complete disappearance of signs and symptoms); ii) effective (improved, but incomplete resolution of signs and symptoms), and iii) ineffective (no improvement in signs and symptoms). The total effective rate was defined as the proportion of patients that achieved markedly effective and effective curative outcomes.

Peripheral blood samples (4 mL) were obtained from all participants, centrifuged (3000 × g 10 min), and separated serum was stored at -80°C until further processing. All serum samples were analysed for AQP4-Ab (EH0967 Catalogue no., Wuhan Fine Biotech Co., Ltd., Wuhan, China), interleukin (IL)-6 (EH0201 Catalogue no., Wuhan Fine Biotech Co., Ltd., Wuhan, China), IL-27 (EH0193 Catalogue no., Wuhan Fine Biotech Co., Ltd., Wuhan, China), and Matrix metallo-proteinase (MMP)-9 (EH0238 Catalogue no., Wuhan Fine Biotech Co., Ltd., Wuhan, China) levels by Sandwich Enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions. Case and control serum levels were compared at baseline (prior to intervention), and pre- and post-treatment assays were compared in cases. The sensitivity for the AQP4-Ab ELISA kit was below 0.188 ng/mL, for IL-6 was 2.813 pg/mL, for IL-27 was 0.094 ng/mL and for MMP-9 was 0.188 ng/mL. After hospital discharge, patients were followed up for over 1 year, and relapses were evaluated every 6 months. The efficacy and specific incidence of adverse reactions were compared between the two groups. Adverse reactions included liver function damage (hormone shock therapy may cause liver function damage to a certain extent), fever, thrombocytopenia and vomiting.

Study objectives

The primary objective was to determine the effectiveness of RTX *versus* CPM concerning the mean EDSS score

and total effective rate. Secondary objectives included: i) mean Activities of daily living (ADL) score; ii) safety profile; iii) case/control status as well as pre- and post-treatment case status of serum AQP4-Ab, IL-6, IL-27 and MMP-9; and iv) recurrence rate.

Statistical analysis

Descriptive statistics are presented as means ± standard deviation (SD) for continuous variables and frequencies with percentages (%) for categorical variables. The independent samples t-test was used for comparisons between groups, while the paired t-test was applied for comparisons within each group. All statistical analyses were performed at a significance level of 0.05 using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, N.Y., USA).

Results and Discussion

Baseline statistics

According to the data in Table I, there was no significant difference in baseline data between the experimental group and the control group ($p > 0.05$).

Table I

Comparison of baseline indicators

Indicator	Experimental group	Control group
Age (years old)	43.08 ± 18.69	42.51 ± 19.22
Gender (male/female)	3/17	3/17
Mean age of onset (years old)	28.37 ± 17.16	28.59 ± 17.01
The course of disease (year)	10.19 ± 6.19	10.32 ± 6.41

EDSS status

Following completion of treatment, the mean values of EDSS score were significantly lower in the RTX group compared to the CPM group (2.57 ± 1.20 and 3.05 ± 1.18, respectively; $p = 0.028$). Improvement from mean baseline EDSS was also significantly greater with RTX (2.65 ± 1.08) than CPM (1.79 ± 1.23) ($p = 0.003$). No significant differences between the case groups were observed concerning mean ADL values before and after treatment (Table II).

Table II

Expanded Disability Status Scale and Activities of Daily Living Scale scores before and after rituximab and cyclophosphamide treatment

Group	RTX group	CPM group	p-value
EDSS score, mean ± SD			
- At treatment initiation	5.22 ± 1.14	4.84 ± 1.33	0.562
- After treatment completion	2.57 ± 1.20	3.05 ± 1.18	**0.028**
Mean ± SD EDSS reduction	2.65 ± 1.08	1.79 ± 1.23	**0.003**
ADL score, mean ± SD			
- At treatment initiation	67.68 ± 7.49	68.05 ± 8.34	0.869
- After treatment completion	89.19 ± 8.67	87.36 ± 9.05	0.058
Mean ± SD ADL reduction	21.51 ± 6.72	19.31 ± 8.30	0.216

ADL, Activities of daily living scale; CPM, cyclophosphamide; EDSS, Expanded Disability Status Scale; RTX, rituximab; SD, standard deviation

Clinical curative outcome

As shown in Table III, RTX was superior to CPM concerning the clinical curative effect, with 60% (n = 12) and 35% (n =7) of subjects in the RTX group and 50% (n = 10) and 20% (n = 4) in the CPM-group achieving markedly effective and effective therapeutic outcomes, respectively. Overall, RTX achieved a higher total effective rate compared to CPM throughout 4 weeks of treatment (95% *vs*. 70%; $p < 0.05$).

Table III

Clinical curative outcomes after rituximab and cyclophosphamide treatment

Group	RTX group	CPM group
The curative outcome, *n (%)*		
Markedly effective	12 (60%)	10 (50%)
Effective	7 (35%)	4 (20%)
Ineffective	1 (5%)	6 (30%)
Total effective rate, *n (%)*	19 (95%)*	14 (70%)

CPM, cyclophosphamide; RTX, rituximab, * $p < 0.05$ compared with CMP group

Safety profile

As shown in Table IV, the incidence of adverse events (AEs) was significantly lower in the RTX group compared with the CPM group (15% *vs*. 30%, respectively; $p < 0.05$). In the CPM group, six patients experienced AEs, including 2 hepatic events, 2 cases of nausea/ vomiting, 1 case of fever and 1 case of thrombocytopenia. In the RTX group, three patients experienced AEs, including 2 cases of nausea/vomiting and 1 case of fever.

Table IV

Treatment-related adverse events in rituximab- and cyclophosphamide-treated groups

Group	RTX-group	CPM-group
Adverse events, *n (%)*		
Nausea/vomiting	2 (10%)	2 (10%)
Fever	1 (5%)	1 (5%)
Hepatic events	0 (0%)	2 (10%)
Thrombocytopenia	0 (0%)	1 (5%)
Total, *n (%)*	3 (15%)*	6 (30%)

CPM, cyclophosphamide; RTX, rituximab, * $p < 0.05$ compared with CMP group

Serum biomarkers

Mean serum levels of AQP4-Ab, IL-6, MMP-9, and IL-27 in cases and controls are presented in Figure 1. No statistically significant difference in any of the studied biomarkers was found between cases at baseline ($p > 0.05$). However, baseline serum levels of AQP4-Ab, IL-6 and MMP-9 were markedly elevated, while serum IL-27 concentrations were significantly lower in both case groups compared to controls ($p < 0.05$ for both). Following treatment, serum AQP4-Ab, IL-6 and MMP-9 levels were considerably reduced, whereas serum IL-27 levels were significantly increased in both case groups ($p < 0.05$), but changes were greater in the RTX treated group ($p < 0.05$).

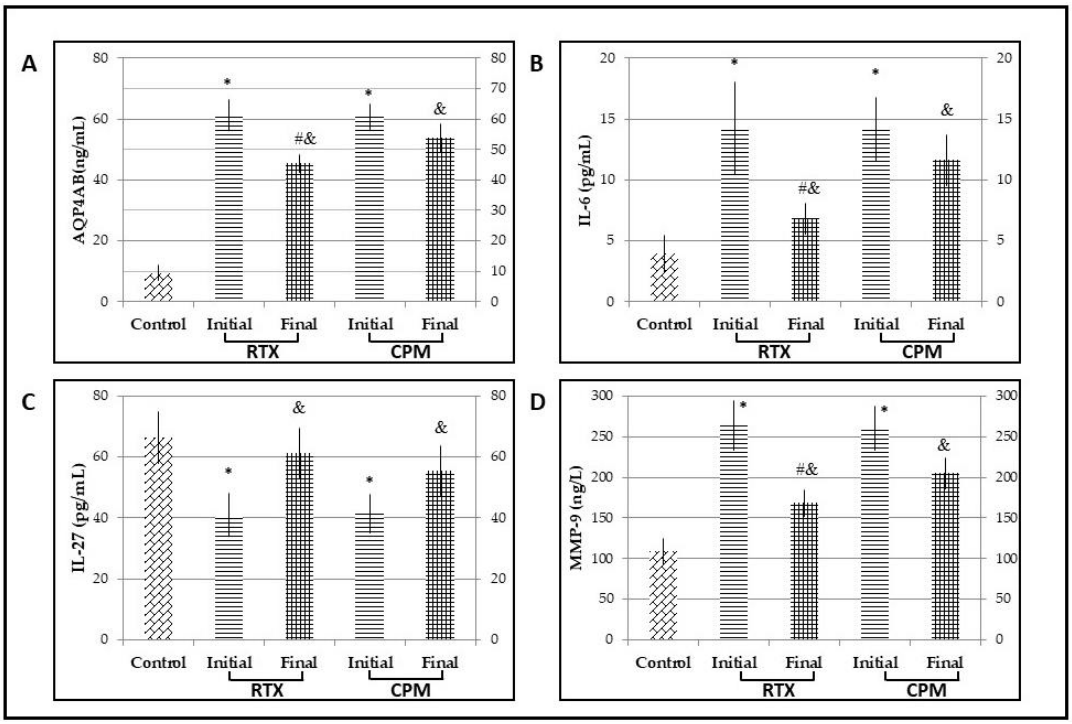

Figure 1.

Serum levels of anti-aquaporin 4 antibody, interleukin-6, interleukin-27, and Matrix metalloproteinase 9 among cases and controls. * $p < 0.05$ compared to control group; $p < 0.05$ compared to CPM group; $^{\&}$ $p < 0.05$ compared to initial levels within each group

Recurrence rate

Over the 1-year follow-up period, only 5 relapses were recorded in the RTX-treated patients, whereas 9 recurrent cases were observed in the CPM-treated subjects. No statistically significant difference was found regarding the frequency of relapses between the case groups within the first 6 months after treatment ($p = 0.681$). However, a significantly lower number of recurrent cases was recorded in the RTX-group ($n = 2$) compared with CPM-group ($n = 5$) during 7 to 12 months of follow-up ($p = 0.036$) (Table V).

Table V

Recurrence rate at 6- and 12-month follow-up in case groups

Relapse cases, *n*	**6-month follow-up**	**12-month follow-up**
RTX-group	3	2
CPM-group	4	5
p-value	0.681	0.036

CPM, cyclophosphamide; RTX, rituximab

NMO is an inflammatory demyelinating CNS disorder typically characterized by bilateral optic neuritis and

transverse myelitis. Several factors, such as infectious microorganisms and emotional stress, have been proposed as a trigger for NMO; however, many aspects of the disease pathogenesis remain unclear. Since visual impairment and neurologic dysfunction in NMO are commonly severe, prophylactic immunomodulatory therapy should be focused on reducing the frequency and severity of relapses and preventing disease progression [12]. Although RTX has been increasingly utilized in this setting, its safety and effectiveness in NMO patients have yet to be fully determined.

This study evaluated the effect of maintenance treatment with RTX or CPM on NMO patients. Based on our results, superior curative effects were achieved with RTX compared to CPM after 4 weeks of treatment. The proportion of subjects achieving markedly effective and effective therapeutic outcomes was significantly greater in the RTX group, yielding a higher total effective rate compared to the CPM group (95% in the RTX *vs*. 70% in the CPM group; $p < 0.05$). Improvement in EDSS score was also significantly greater with RTX (2.65 ± 1.08) than CPM (1.79 ± 1.23) ($p = 0.003$). Despite the relatively high rate of AEs (15%), RTX was better tolerated than CPM providing a more favourable safety profile. In addition, RTX was found to be superior to CPM for maintaining remission. Although recurrences were similar in both treated groups at the 6-month follow-up, RTX was associated with fewer relapses in the later follow-up phase (7 - 12 months) compared to CPM, indicating that RTX can more efficiently prolong relapse-free intervals and improve prognosis in NMO patients.

Apart from anti-AQP4 antibodies, a subgroup of NMO patients is seropositive for AQP1 or myelin oligodendrocyte glycoprotein (MOG) antibodies [13]. AQP4, a water channel transmembrane protein highly expressed in perivascular astrocyte end-feet, has been widely recognized as the major target antigen in NMO [14]. Anti-AQP4 antibodies not only serve as a sensitive and highly specific disease biomarker but also seem to be actively involved in the pathogenesis of NMO [15, 16]. Our findings showed that serum levels of AQP4-Ab in NMO subjects were significantly elevated compared to healthy controls, further supporting its pathogenic implications in the onset and evolution of the disease.

Although RTX has been shown to reduce relapse frequency and severity in NMO, previous studies have reported refractory cases [17-19]. A possible reason may be that RTX cannot suppress AQP4-IgG production because pre-B cells and plasmablasts can express CD19, but not CD20. However, RTX is able to deplete circulating CD20+ mature B cells and plasmablasts, generating anti-AQP4 antibodies [20, 21]. Given that AQP4-Ab titres may also correlate with NMO activity and progression, our study, demonstrating a post-treatment reduction in serum AQP4-Ab levels, has also indicated the neuroprotective effects of RTX, which could be utilized in the setting of NMO.

Recent progress in our understanding of NMO pathogenesis has provided evidence that various inflammatory cytokines and chemokines may also play important roles in NMO development by disrupting the blood-brain barrier (BBB) integrity, rendering AQP4 more easily accessible to immune attack [22, 23]. In this regard, the key role of IL-6 in the immunopathogenesis of NMO has already been suggested. Uchida *et al*. have recently reported that overexpression of MMP-2 in the cerebrospinal fluid (CSF) of NMO patients, possibly promoted by elevated IL-6 activation, can induce BBB disruption allowing serum anti-AQP4 antibodies to penetrate the CNS [24]. In this study, serum IL-6 levels were also found to be elevated in NMO patients compared to controls and decreased after treatment. In addition, IL-27 signalling has been increasingly implicated in the pathogenesis of several immune-mediated diseases. By inhibiting the differentiation of Th17 and Th2 cells, IL-27 has been described as an anti-inflammatory mediator [25]. Our results revealed that serum IL-27 levels were significantly decreased in NMO patients compared to controls and gradually increased following either RTX or CPM treatment. This finding is in line with previous studies indicating that IL-27 might play a protective role in NMO by mediating T-cell regulation [26, 27]. However, the reasons and mechanisms for reduced IL-27 in the course of NMO have yet to be determined.

As a main member of the MMP family, MMP-9 has been shown to play a central role in demyelinating diseases [28, 29]. MMPs can disturb the blood-CSF barrier by degrading type IV collagen, a major component of the endothelial basement membrane [30]. As a result, activated T cells can cross the BBB and gain access to the CNS, ultimately leading to inflammation and demyelination [31]. As suggested by Hosokawa *et al*., serum MMP-9 concentrations were significantly higher in NMO cases compared to healthy individuals. Similarly, we found that MMP-9 levels in the serum of NMO patients were significantly elevated compared to controls and decreased after treatment, more pronouncedly in the RTX-group.

Conclusions

In summary, the present study showed that RTX provided superior effectiveness and safety outcomes compared to CPM, enabling more NMO patients to reach a relapse-free state during a 1-year follow-up period. Moreover, RTX has proved superior to CPM in reducing AQP4-Ab, IL-6, and MMP-9 and increasing IL-27 levels in the serum of NMO patients. Further studies with larger sample sizes and extended follow-up periods are required to confirm these findings.

Acknowledgement

This work was supported by the Natural Science Foundation of Heilongjiang Province, China (No. LH2020H077).

Conflict of interest

The authors declare no conflict of interest.

References

1. Paul F, Murphy O, Pardo S, Levy M, Investigational drugs in development to prevent neuromyelitis optica relapses. *Expert Opin Investig Drugs*, 2018; 27: 265-271.
2. Akaishi T, Takahashi T, Nakashima I, Chloride imbalance between serum and CSF in the acute phase of neuromyelitis optica. *J Neuroimmunol*., 2018; 315: 45-49.
3. Kassa R, Raslau F, Smith C, Sudhakar P. Teaching NeuroImages: Leber hereditary optic neuropathy masquerading as neuromyelitis optica. *Neurology*., 2018; 90: e94-e95.
4. Khan TR, Wang C, Aquaporin-4 neuromyelitis optica spectrum disorder in a 2-year-old girl: Diagnostic and treatment considerations. *Mult Scler Relat Disord*., 2020; 41: 102030: 1-4.
5. Sarnat HB, Flores-Sarnat L, Boltshauser E, Area Postrema: Fetal Maturation, Tumors, Vomiting Center, Growth, Role in Neuromyelitis Optica. *Pediatr Neurol*., 2019; 94: 21-31.
6. Agasing AM, Wu Q, Khatri B, Borisow N, Ruprecht K, Brandt AU, Gawde S, Kumar G, Quinn JL, Ko RM, Mao-Draayer Y, Lessard CJ, Paul F, Axtell RC, Transcriptomics and proteomics reveal a cooperation between interferon and T-helper 17 cells in neuromyelitis optica. *Nat Commun.*, 2020; 11: 2856: 1-13.
7. Montcuquet A, Collongues N, Papeix C, Zephir H, Audoin B, Laplaud D, Bourre B, Brochet B, Camdessanche JP, Labauge P, Moreau T, Brassat D, Stankoff B, de Seze J, Vukusic S, Marignier R; NOMADMUS study group and the Observatoire Français de la Sclérose en Plaques (OFSEP), Effectiveness of mycophenolate mofetil as first-line therapy in AQP4-IgG, MOG-IgG, and seronegative neuromyelitis optica spectrum disorders. *Mult Scler.*, 2017; 23: 1377-1384.
8. Mealy MA, Levy M, A pilot safety study of ublituximab, a monoclonal antibody against CD20, in acute relapses of neuromyelitis optica spectrum disorder. *Medicine (Baltimore).*, 2019; 98(25): e15944: 1-4.
9. Nasralla S, Abboud H, Is neuromyelitis optica without AQP4-IgG a T-cell mediated disease? insights from checkpoint inhibitor immune-related adverse events. *Mult Scler Relat Disord.*, 2020; 46: 102451: 1-3.
10. Lu P, Yuan T, Liu X, Tian G, Zhang J, Sha Y, Role of Diffusional Kurtosis Imaging in Differentiating Neuromyelitis Optica-Related and Multiple Sclerosis-Related Acute Optic Neuritis: Comparison With Diffusion-Weighted Imaging. *J Comput Assist Tomogr.*, 2020; 44: 47-52.
11. Bilgener E, Gümüş B, Pregabalin consumption in Turkey: was it an abuse?. *Farmacia*, 2021; 69(6): 1189-1194.
12. Vodopivec I, Matiello M, Prasad S, Treatment of neuromyelitis optica. *Curr Opin Ophthalmol.*, 2015; 26: 476-483.
13. Cobo-Calvo A, Ruiz A, Richard C, Blondel S, Cavagna S, Strazielle N, Ghersi-Egea JF, Giraudon P, Marignier R, Purified IgG from aquaporin-4 neuromyelitis optica spectrum disorder patients alters blood-brain barrier permeability. *PLoS One*, 2020; 15(9): e0238301: 1-15.
14. Papadopoulos MC, Verkman AS, Aquaporin 4 and neuromyelitis optica. *Lancet Neurol.*, 2012; 11: 535-544.
15. Palace J, Leite MI, Nairne A, Vincent A, Interferon Beta treatment in neuromyelitis optica: increase in relapses and aquaporin 4 antibody titers. *Arch Neurol.*, 2010; 67: 1016-1017.
16. Tradtrantip L, Zhang H, Saadoun S, Phuan PW, Lam C, Papadopoulos MC, Bennett JL, Verkman AS, Anti-aquaporin-4 monoclonal antibody blocker therapy for neuromyelitis optica. *Ann Neurol.*, 2012; 71: 314-322.
17. Bourre B, Lefaucheur R, Girault C, Treatment of NMO relapse in the elderly: rituximab when plasma exchange fails?, *Acta Neurol Belg.*, 2013; 113: 335-336.
18. Radaelli M, Moiola L, Sangalli F, Esposito F, Barcella V, Ferrè L, Rodegher M, Colombo B, Fazio R, Martinelli V, Comi G, Neuromyelitis optica spectrum disorders: long-term safety and efficacy of rituximab in Caucasian patients. *Mult Scler.*, 2016; 22: 511-519.
19. Jade JD, Bansi S, Singhal B, Rituximab in Neuromyelitis Optica Spectrum Disorders: Our Experience. *Ann Indian Acad Neurol.*, 2017; 20: 229-232.
20. de Andrés C, Teijeiro R, Saiz A, Fernández P, Sánchez-Ramón S, Changes in B and T-cell subsets and NMO-IgG levels after immunoglobulins and rituximab treatment for an acute attack of neuromyelitis optica. *Neurologia*, 2015; 30: 276-282.
21. Kessler RA, Mealy MA, Levy M, Treatment of Neuromyelitis Optica Spectrum Disorder: Acute, Preventive, and Symptomatic. *Curr Treat Options Neurol.*, 2016; 18: 2: 1-15.
22. Içöz S, Tüzün E, Kürtüncü M, Durmuş H, Mutlu M, Eraksoy M, Akman-Demir G, Enhanced IL-6 production in aquaporin-4 antibody positive neuromyelitis optica patients. *Int J Neurosci.*, 2010; 120: 71-75.
23. Araki M, Matsuoka T, Miyamoto K, Kusunoki S, Okamoto T, Murata M, Miyake S, Aranami T, Yamamura T, Efficacy of the anti-IL-6 receptor antibody tocilizumab in neuromyelitis optica: a pilot study. *Neurology*, 2014;82(15): 1302-1306.
24. Uchida T, Mori M, Uzawa A, Masuda H, Muto M, Ohtani R, Kuwabara S, Increased cerebrospinal fluid metalloproteinase-2 and interleukin-6 are associated with albumin quotient in neuromyelitis optica: Their possible role on blood-brain barrier disruption. *Mult Scler.*, 2017; 23: 1072-1084.
25. Du HZ, Wang Q, Ji J, Shen BM, Wei SC, Liu LJ, Ding J, Ma DX, Wang W, Peng J, Hou M, Expression of IL-27, Th1 and Th17 in patients with aplastic anemia. *J Clin Immunol.*, 2013; 33: 436-445.
26. Kamiya S, Owaki T, Morishima N, Fukai F, Mizuguchi J, Yoshimoto T, An Indispensable Role for STAT1 in IL-27-Induced T-bet Expression but Not Proliferation of Naive CD4+ T Cells. *J Immunol.*, 2004; 173(6): 3871-3877.

27. Zhang DQ, Jia K, Wang R, Li T, Zhao N, Yang LN, Yang L, Decreased serum IL-27 and IL-35 levels are associated with disease severity in neuromyelitis optica spectrum disorders. J Neuroimmunol., 2016; 293: 100-104.
28. Tasaki A, Shimizu F, Sano Y, Fujisawa M, Takahashi T, Haruki H, Abe M, Koga M, Kanda T. *J Neurol Neurosurg Psychiatry*., 2014; 85: 419-430.
29. Opdenakker G, Nelissen I, Van Damme J, Functional roles and therapeutic targeting of gelatinase B and chemokines in multiple sclerosis. *Lancet Neurol.*, 2003; 2: 747-756.
30. Pană RD, Dehelean C, Pînzaru I, Marcovici I, Simu S, Crăiniceanu Z, Enache A, Challenges and limitations in developing an animal model of epidermolysis bullosa acquisita: a minireview. *Farmacia*, 2021; 69(4): 650-656.