Journal of the Neurological Sciences 377 (2017) 127–132

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Lower dosages of rituximab used successfully in the treatment of anti-NMDA receptor encephalitis without tumour

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ARTICLE INFO

Article history:
Received 30 November 2016
Received in revised form 10 March 2017
Accepted 6 April 2017
Available online 8 April 2017

Keywords:
Rituximab
Anti-NMDA receptor encephalitis
Immunotherapy
Relapse

ABSTRACT

Objective: The aim of this study was to evaluate the use and efficacy of lower dosages of rituximab for treating anti *N*-methyl-D-aspartate receptor (NMDAR) encephalitis without tumour.
Methods: We performed a prospective study of 10 patients with anti-NMDAR encephalitis who did not respond to 10 to 14 days first-line immunotherapy and received rituximab administered intravenously (IV) at a dosage of 100 mg once per week for 4 consecutive weeks. Reinfusion of rituximab was given when $CD19^+$ B-cell counts of total lymphocytes in peripheral blood >1%. The annualized relapse rate (ARR), modified Rankin scale (mRS) and $CD19^+$ B-cell counts were measured every 4 to 10 weeks after initial rituximab treatment in order to assess the clinical outcome and efficacy of rituximab.
Results: Lower dosages of rituximab led to a significant reduction of mRS and $CD19^+$ B-cells when compared with before the rituximab infusion ($P < 0.05$) and allowed 9 (90%) patients to maintain a stabilised neurological status. One patient experienced a relapse at 19 weeks after initial rituximab infusion. Although ARR reduction of all 10 patients did not achieve statistical significance ($P > 0.05$), in the 4 patients who had relapses before rituximab treatment there was an apparent reduction in ARR over 56 weeks. At the last follow up, 9 patients (90%) had a good outcome (mRS ≤ 2) including 3 patients (30%) who recovered completely (mRS = 0). Transient infusion adverse events occurred in 2 patients. We observed no serious delayed adverse events during the 56 weeks follow-up.
Conclusions: In patients with anti-NMDAR encephalitis who did not respond to first-line immunotherapy, early application of lower dosages of rituximab could efficiently reduce $CD19^+$ B-cell counts of peripheral blood and improve the prognosis of anti-NMDAR encephalitis.

1. Introduction

In 2005, Dalmau reported 4 young women with ovarian teratoma who developed acute psychiatric symptoms, seizures, memory deficits, decreased levels of consciousness, and central hypoventilation [1]. Two years later Dalmau found the pathogenic antibodies against NR1/NR2 heteromers of the NMDAR [2]. Management of anti-NMDAR encephalitis is focused on immunotherapy and the investigation and removal of the neoplasm. But nearly 50% of patients did not show obvious improvement after first-line treatment. Therefore, experts suggested an early application of second-line immunotherapy that includes rituximab, cyclophosphamide or mycophenolate mofetil [3].

Rituximab is a human chimeric monoclonal antibody against $CD20^+$ B cells that leads to B-cell depletion and is primarily used to treat non-Hodgkin's B-cell lymphoma. Accumulating evidence has supported the application of rituximab in autoimmune and inflammatory CNS disease to improve clinical outcome and decrease the occurrence of relapses. Multiple therapeutic dosages have been used in previous studies [4,5]. Taking into consideration the high price of rituximab for patients in China, and that no data exist on the efficacy and safety of lower dosages rituximab, we present 10 patients with anti-NMDAR encephalitis who were not associated with a tumour, and who had limited clinical improvement after a combination of corticosteroids and IVIG, but responded well to lower dosages of rituximab.

2. Methods

2.1. Patients

The study was approved by the Committee of Clinical Investigation at Shandong Provincial Hospital affiliated to Shandong University of Science and Technology and conformed to the principles of the Declaration of Helsinki. Because of the off-label use of rituximab and for permission to conduct clinical evaluation and follow-up prior to their inclusion in this study, informed consents were signed by the patient's parents or spouse due to these patients lacking the capacity to consent.

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http://dx.doi.org/10.1016/j.jns.2017.04.007
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Patients who were identified as having anti-NMDAR encephalitis fulfilled the following criteria: 1) the presence of IgG antibodies against the GluN1 subunit of the NMDA receptor in CSF 2) the presence of one or more of the following 6 major groups of symptoms, abnormal psychiatric/behaviour or cognitive dysfunction, speech dysfunction (pressured speech, verbal reduction, mutism), seizures, movement disorder, dyskinesias, or rigidity/abnormal postures, decreased level of consciousness, autonomic dysfunction or central hypoventilation 3) the exclusion of other disorders [6]. All patients underwent a CT-scan of thorax/abdomen/pelvic, ultrasound of abdomen and pelvic region and transvaginal ultrasound in married women. No evidence of systemic tumour was identified after primary and repeated screening every 3 months for 56 weeks in the 10 patients.

2.2. Laboratory assessments and collection of clinical data

NMDAR antibodies were determined by indirect immunofluorescence, using transfected human embryonic kidney cells (HEK293) expressing NR1 subunits of the NMDA receptor. Lymphocyte immunophenotyping was performed by flow cytometry in freshly acquired blood samples. Serum immunoglobulin levels containing IgG, IgM, IgE and IgA were performed by routine turbimetry. Clinical data including clinical presentations, ancillary examinations and treatments at the acute course of the disorder were collected by the study investigators. Only the first cerebrospinal fluid (CSF), electroencephalogram (EEG) and magnetic resonance imaging (MRI) were considered.

2.3. Rituximab treatment

First-line immunotherapy was defined as the application of corticosteroids and intravenous immunoglobulins (IVIG). All patients received at least 10 days first-line immunotherapy before rituximab infusion [7]. The therapeutic regimen of rituximab was 100 mg IV infusion, once per week for 4 consecutive weeks. Before the infusion of rituximab, 10 mg dexamethasone and 25 mg promethazine were given to prevent allergic reaction. An ECG monitor was used for 3 to 4 h to observe vital signs. Infusion adverse events were symptoms that occurred during the infusion or during the follow-up period and were recorded by the study investigator. Second-line immunotherapy had not been used in the 10 patients previously. The time between the first neurological symptom and the anti-NMDAR encephalitis diagnosis, between the first symptom and the rituximab treatment, and between the end of 10 days first-line treatment and initial rituximab treatment were all calculated.

2.4. Evaluation of efficacy and safety of rituximab

The ARR (annualized relapse rate), mRS (modified Rankin scale), $CD19^+$ B cells and immunoglobulin levels in peripheral blood were measured before and after rituximab therapy to evaluate the efficacy and safety of rituximab. Follow-up information was obtained every 4 to 10 weeks after initial infusion of rituximab for 56 weeks. Relapse was defined as the appearance of new onset symptoms or the worsening of pre-existing symptoms after improvement or stabilization of the disorder for at least 2 months, not explained by other causes [3]. Good outcome was defined as mRS 0 to 2 and complete recovery was defined as mRS 0 [8]. The depletion of $CD19^+$ B cells was defined as <1% of total lymphocytes [5]. Reinfusion of rituximab was given when $CD19^+$ B-cell counts exceeded 1% during the follow-up period.

2.5. Statistical analysis

The ARR, mRS score, and $CD19^+$ B-cell counts were compared pre-rituximab and post-rituximab treatment using the Wilcoxon signed rank test and the 2-sided sign test. Differences were considered significant when P values were <0.05. All statistical analyses were performed using GraphPad Prism software, version 5.0.

3. Results

3.1. Demographics and clinical characteristics

The 10 patients who met the inclusion criteria were hospitalised between May 1, 2014 and April 30, 2015 at the Department of Neurology, Shandong Provincial Hospital affiliated to Shandong University and received first-line immunotherapy (corticosteroids or combined with IVIG), but improvement was not observed after at least 10 days of first-line treatment. The age of patients at first neurologic manifestation was a median of 34.50 years (range 19.75 to 39.50 years). Initial symptoms could be divided into 3 groups: seizure (4 cases, 40%), cognitive dysfunction (1 case, 10%) and abnormal psychiatric/behaviour (5 cases, 50%). During the course of the disease, 8 patients (80%) presented with abnormal psychiatric/behaviour or cognitive dysfunction, 5 patients (50%) experienced seizure, 5 patients (50%) showed a decreased level of consciousness, 3 patients (30%) presented with speech dysfunction (repetitive speech, verbal reduction), 4 patients (40%) experienced autonomic dysfunction (sinus bradycardia) or central hypoventilation and 7 patients (70%) showed movement disorder (2 limb dyskinesia, 3 limb involuntary movements, 2 oro-lingual-facial dyskinesias). The median mRS at the peak of the disease was 4.5 (range 4 to 5) and 5 patients (50%) had a maximum mRS score of 5. Six patients (60%) were admitted into the intensive care unit due to a critical illness such as status epilepticus and central hypoventilation. The median disease duration before diagnosis was 50 days (range 24 to 95.75 days) and 4 patients experienced relapses (one patient had 2 relapses, three had 1 relapse) before rituximab infusion.

3.2. Ancillary examinations

Initial MRI results were abnormal in 4 (40%) patients with fluid-attenuated inversion recovery (FLAIR) or T_2 weighted imaging (T_2WI) hyperintensity signals located in the hippocampi, occipital lobes, temporal lobe, and cortex. Findings from CSF samples were abnormal in 7 (70%) cases, with increased white blood cell counts (>5, range 8 to 27) in 7 (70%) and elevated protein levels (>0.45 g/L, range 0.56 to 0.69) in 4 (40%) cases. The EEG showed abnormal results in 5 (50%) cases presenting with focal or diffuse slowing waves or epileptic discharge. The median value for $CD19^+$ B cells in peripheral blood before rituximab treatment was 15.60% (range 11.71% to 21.14%). Detailed demographic profiles and clinical features are outlined in Table 1.

3.3. Efficacy evaluation of rituximab

All 10 patients were treated with first-line immunotherapy for at least 10 days. Seven (70%) patients received corticosteroids (1000 mg/d × 5d) combined with IVIG (400 mg/kg/d × 5d) and 3 (30%) received a single corticosteroid (1000 mg/d × 5d). We observed a median time of 5 days (range 2.75 to 14.50 days) to assess the efficiency of first-line treatment but no improvement occurred. Hence, we started rituximab infusion. Rituximab was given after a median duration of disease of 76 days (range 42.50 to 115.25 days). For all 10 patients $CD19^+$ B-cell depletion occurred rapidly within 4 weeks after initial administration of rituximab (Fig. 1). Of the 10 patients, 7 (70%) showed an increase in $CD19^+$ B cells exceeding 1% at a median time of 25 weeks (range 19 to 39 weeks) (Table 2). A repeated infusion of rituximab was given at a median time of 32 weeks (range 23–56 weeks) to 6 patients (Fig. 2). Patient 4 refused reinfusion because of a stable neurological status. During the 56-week follow-up, $CD19^+$ B cells remained at <1% in 3 patients (30%). At the last follow-up, $CD19^+$ B-cell counts of total lymphocytes in peripheral blood had a median value of 0.93% (range 0.74% to 1.91%) as compared with 15.60% (range 11.71% to 21.14%) before rituximab treatment ($P < 0.05$) (Fig. 3).

In the 10 patients treated with rituximab, the median time between initiation of rituximab treatment and the first sign of clinical

Table 1
Description of the 10 patients with anti-NMDAR antibodies encephalitis at the acute stage of disease.

Patient	Onset age, y	First symptom	Main subsequent symptoms	CSF: ICP WBC Prot	MRI	EEG	Disease duration before diagnosis, (d)	Previous treatment	ICU
1	F/15	GTCS	Aggressiveness, unconsciousness, limb dyskinesia, speech disorder	110 mm H_2O 16/mm^3 0.18 g/L	Bihippocampa FH	Diffuse slow waves	153	Corticosteroids IVIG	Yes
2	M/35	Anterograde amnesia	GTCS, hallucinations, central hypoventilation, limb involuntary movements	125 mm H_2O 8/mm^3 0.25 g/L	–	–	86	Corticosteroids	Yes
3	F/20	Talk nonsense behavioral changes	Cognitive impairment, seizure, loss of consciousness	160 mm H_2O 180/mm^3 0.59 g/L	Corona radiata FH	–	25	Corticosteroids IVIG	No
4	F/33	Abnormal behaviour	Limb dystonia, decreased level of consciousness	–	–	–	125	Corticosteroids	Yes
5	M/37	Abnormal behaviour	GTCS, disorientation, repetitive speech	140 mm H_2O 8/mm^3 0.29 g/L	–	–	75	Corticosteroids IVIG	No
6	M/55	Abnormal behaviour	Status epilepticus, memory dysfunction, oro-lingual-facial dyskinesias, decreased level of consciousness	–	Bihippocampa temporal lobes FH	Epileptic discharge	32	Corticosteroids	Yes
7	F/34	GTCS	Autonomic dysfunction, oro-lingual-facial dyskinesias, central hypoventilation	145 mm H_2O 23/mm^3 0.66 g/L	–	Diffuse slow waves	58	Corticosteroids IVIG	Yes
8	F/19	Abnormal psychiatric	GTCS, memory dysfunction, chorea	120 mm H_2O 19/mm^3 0.46 g/L	Multiple abnormal signals	Focal slow waves	21	Corticosteroids IVIG	Yes
9	F/37	GTCS	Abnormal psychiatric, verbal reduction, chorea	110 mm H_2O 27/mm^3 0.76 g/L	–	–	20	Corticosteroids IVIG	No
10	F/47	GTCS	Abnormal behaviour, sinus bradycardia, decreased level of consciousness	–	–	Diffuse slow waves	42	Corticosteroids IVIG	No

GTCS: generalized tonic-clonic seizure, CSF: cerebrospinal fluid, EEG: electroencephalogram, RTX: rituximab, ARR: annualized relapse rate, mRS: modified Rankin scale, ICP: intracranial pressure, WBC: white blood count, Prot: protein, FH: MRI fluid-attenuated inversion recovery hyperintensity, IVIG: intravenous immunoglobulins, — negative.

improvement was 15 days (range 5 to 38 days). Dramatic clinical remission occurred in patient 3, the level of consciousness notably improved, seizures were significantly controlled but anterograde amnesia and disorientation still existed. The median mRS at the peak of the disease was 4.5 (range 4 to 5), at the last follow-up, the median mRS was 1 (range 0 to 1.25) ($P < 0.05$) (Fig. 3). The median time between initial administration of rituximab and good outcome (mRS ≤ 2) was 8 weeks (range 4 to 40 weeks). At the last follow up, 9 patients (90%) had a good outcome (mRS ≤ 2) including 3 patients (30%) who recovered completely (mRS = 0) (Fig. 4). The median pre-rituximab ARR was 0 (range 0 to 1), and the median post-rituximab ARR over 56 weeks was 0 (0) ($P = 0.16$).

3.4. Relapse during treatment

Only patient 3 experienced relapse, she presented with abnormal behaviour and seizure at 19 weeks after initial rituximab infusion (mRS = 3). The $CD19^+$ B-cells in peripheral blood rapidly increased to 9.56%. Though ARR reduction of all 10 patients did not achieve statistical significance ($P = 0.16$), the 4 patients who had relapses before rituximab treatment showed an apparent reduction in ARR over 56 weeks.

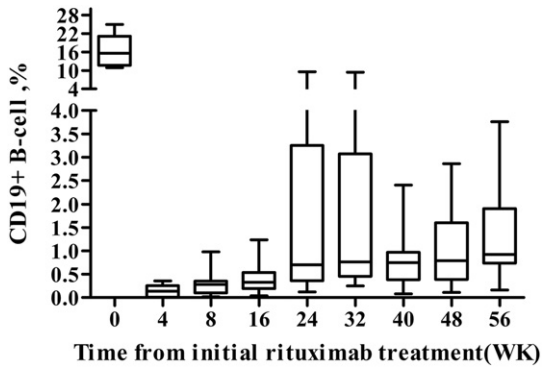

Fig. 1. Evolution of $CD19^+$ B-cell counts during the 56 week follow-up. 10 patients had a depletion of $CD19^+$ B-cells within 4 weeks after initial infusion of rituximab.

3.5. Infusion adverse events

Patient 3 had transient infusion-related symptoms which presented as hyperpyrexia during the administration of rituximab, but we immediately stopped the infusion and 10 mg dexamethasone was given. Symptoms showed gradual improvement and any adverse events did not reappear after restored use of rituximab. Patient 7 had a reduction in immunoglobulin G after repeated rituximab infusion. During the 56-week follow-up period, severe infusion adverse events did not occur.

4. Discussion

Rituximab is considered an optional therapeutic agent for patients with anti-NMDAR encephalitis at present, especially for those with refractory clinical pictures or who do not respond to first-line immunotherapy. In this current study, we report on a 56-week follow-up of 10 patients with anti-NMDAR encephalitis without tumour who were treated with lower dosages of rituximab. Obvious improvement of neurological status and $CD19^+$ B-cell depletion occurred within 4 weeks after initial infusion in all 10 patients. In a multi-institutional observational study, aggressive application of immunotherapy and tumour removal was an independent predictor of good outcome. However, nearly 50% of patients did not show obvious improvement after first-line treatment [3]. Moreover, >40% of adult patients and 75% of paediatric patients with anti-NMDAR encephalitis have no detectable neoplasm [9,10]. Hence, for these patients second-line immunotherapy is needed.

The intrathecal synthesis of NMDAR IgG antibody can lead to internalization of NMDAR in hippocampal neurons, rapidly reducing the

Table 2
Comparison between pre- and post-rituximab treatment.

Patient	Disease duration before RTX, (d)	The interval between the end of 10 days first-line treatment and initial RTX treatment, (d)[a]	mRS before RTX	mRS at 4 weeks after initial infusion	mRS at the last follow-up	$CD19^+$ B cell before RTX, (%)	$CD19^+$ B cell at 4 weeks after initial infusion, (%)	$CD19^+$ B cell at the last follow-up, (%)	Reappearance of $CD19^+$ B cell (wk)	ARR before RTX treatment[b]	ARR after RTX treatment
1	185	22	5	3	1	20.63	0.24	0.83	24	2	0
2	107	7	5	2	1	24.94	0	0.46	–	1	0
3	46	11	5	3	0	16.98	0.12	0.92	19	0	1
4	140	3	4	2	2	11.73	0.15	2.94	25	1	0
5	101	12	4	1	1	16.44	0.21	0.98	–	1	0
6	45	3	5	1	0	22.68	0.02	0.93	–	0	0
7	97	25	4	2	0	13.25	0	0.89	17	0	0
8	35	2	5	2	1	10.88	0.30	0.16	32	0	0
9	35	1	3	2	3	11.64	0.36	3.76	39	0	0
10	55	3	4	2	1	14.75	0.01	1.56	47	0	0

RTX: rituximab, ARR: annualized relapse rate, mRS: modified Rankin scale, [a]Observation on the efficiency of first-line immunotherapy, [b]These 4 patients (patients 1, 2, 4 and 5) who had relapses received first-line treatment once or twice with corticosteroids/IVIG during the initial episode of encephalitis and they showed clinical improvement after first-line treatment. But 2 months after the disorder had improved, pre-existing symptoms became worse and they showed almost no improvement after first-line treatment when they were re-admitted to hospital.

level of synaptic NMDAR and subsequently leading to a reversible NMDAR hypofunction [9,11]. Though a recent study demonstrated that only 6% of antibody-secreting cells or memory B cells in the CSF reacted against NR1, these are sufficient for encephalitis pathogenesis [12]. In an examination of lymphocyte immunophenotyping in the CSF of patients with NMDAR encephalitis, significant B-cell expansion appeared which supports the role of CSF humoral autoimmunity in the course of the disorder and was strong evidence of support for the use of rituximab [13].

Rituximab is a monoclonal antibody directed against the CD20 surface antigen expressed on pre-B lymphocytes. Its mechanism includes cell-mediated and complement dependent cytotoxic effects and the induction of apoptosis to prevent the maturation of pre-B cells into antibody-secreting cells. Rituximab has been used for non-Hodgkin lymphoma and rheumatoid arthritis for several years [14–16]. In recent years, it has been widely used in CNS autoimmune and inflammatory disease, such as relapsing-remitting multiple sclerosis (RRMS) [17], neuromyelitis optica spectrum disorders (NMOSD) [18], opsoclonus myoclonus ataxia syndrome (OMAS) and anti-NMDAR encephalitis [19–21].

Two rituximab regimens have been widely used in the past: 375 mg/m^2 infused once per week for 4 weeks and 1000 mg infused twice, 2 weeks apart. But in some small cohort studies [5,22,23], lower dosages of rituximab at 100 mg weekly for 3 or 4 consecutive weeks were also effective in achieving good outcomes. In this current study, 100 mg rituximab was given as a second-line immunosuppressant to

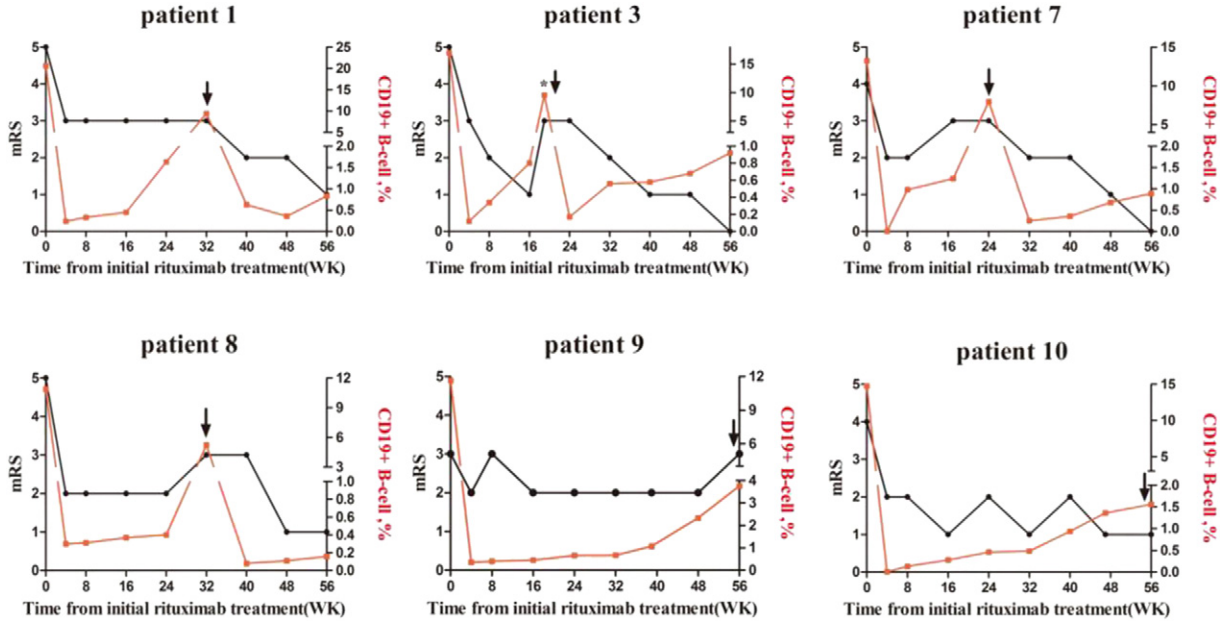

Fig. 2. The reappearance of $CD19^+$ B cells and fluctuation of mRS in patients 1, 3, 7, 8, 9 and 10, who accepted reinfusion of rituximab after initial administration during the 56 week follow up. The black line and red line indicate the fluctuation of mRS and $CD19^+$ B cells as a proportion of total lymphocytes in peripheral blood, respectively. A "downward arrow" indicates rituximab reinfusion; an "asterisk" indicates relapse. Patients 1, 7, 8, 9 and 10 received one additional reinfusion (100 mg) when they had $CD19^+$ B-cell counts which exceeded 1%. Patient 3 received 4 additional weekly reinfusions (4 × 100 mg) when she experienced relapse. Patient 8, who had stable clinical status for at least 2 months, when she came back for regular lymphocyte subsets and mRS measurement at 32 weeks after initial rituximab she told us that due to anterior communicating artery aneurysm she experienced subarachnoid hemorrhage (SAH) at 26 weeks, even though some of the symptoms (especially in physical activity) have improved, in daily life she still need help from her family. Therefore, the increased mRS was influenced by the co-morbid conditions instead of anti-NMDAR encephalitis, so we do not consider this is a relapse. Patient 10, the fourth timepoint (24 weeks) when she came back to our hospital, her mother told us she presented with temper tantrums and irritability occasionally (once or twice per month) (mRS = 2), almost according with the presentation at the third timepoint (16 weeks) (mRS = 1). Due to the mRS score does not involve specific parameter of daily life, the assessment is relatively subjective. Variation in grading between physicians cannot be excluded theoretically. If two grades are equally applicable to the patient, we choose the more severe grade. So, even though patient10 had an increase in mRS, her symptom showed almost no change.

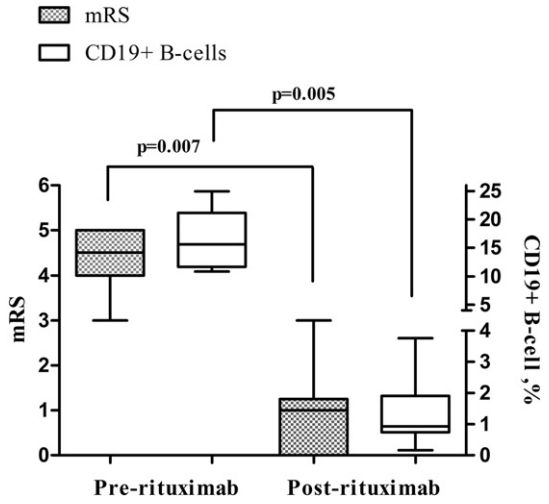

Fig. 3. The median and interquartile range are shown. The median mRS significantly decreased from 4.5 (interquartile range, 4 to 5) before rituximab treatment to 1 (interquartile range, 0 to 1.25) at the last follow-up ($P = 0.007$). The median value of $CD19^+$ B cells significantly decreased from 15.60% (interquartile range, 11.71% to 21.14%) before rituximab treatment to 0.93% (interquartile range, 0.74% to 1.91%) at the last follow up ($P = 0.005$).

the 10 patients who did not respond to corticosteroids or combined with IVIG. The $CD19^+$ B-cell counts were ≤1% in all 10 patients within 4 weeks after the first administration. The time at which $CD19^+$ B cells started to increase >1% was a median of 25 weeks (range 19 to 39 weeks) in 7 cases. In a prospective long-term cohort study of 10 patients with NMOSD treated with rituximab, B cells became undetectable in 90% of patients within 14 days after the first administration. The time of reappearance of B cells varied between 6 and 11 months [24]. Data from another report showed that $CD19^+$ B cells started to increase in 80% of patients 20 weeks after initial infusion [5]. In order to maintain a complete B-cell depletion and to prevent clinical relapse while achieving a stabilised neurological status, rituximab infusion was repeated at a median time of 32 weeks (range 23 to 56 weeks) for 6 patients in our study. Experts have suggested that reinfusion of rituximab should be administered at a regular interval of 6 to 9 months or after B-cell re-emergence and this therapeutic regimen is considered safe and tolerable [24,25]. In a 5-year follow-up in patients with neuromyelitis optica spectrum disorder (NMOSD) who were treated with repeated rituximab, there was an apparent decline in ARR and improvement or stabilization of disability appeared. Furthermore, experts also showed that less frequent applications of repeated rituximab after the disease activity stabilised could lead to a longer time before subsequent relapse [26].

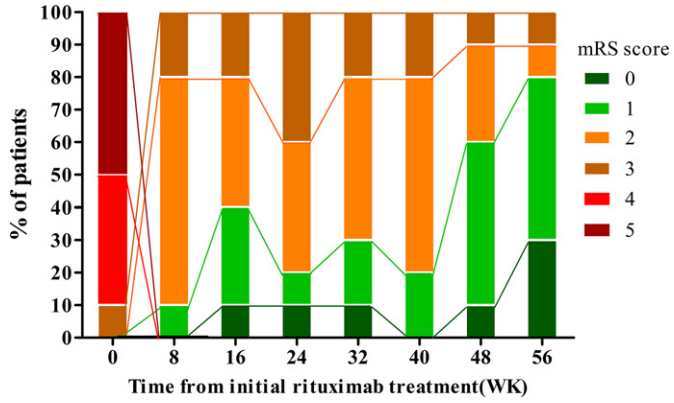

Fig. 4. Evolution of modified Rankin score (mRS) during 56-week follow-up. At the last follow up, 9 patients (90%) had a good outcome (mRS ≤ 2) including 3 patients (30%) who recovered completely (mRS = 0).

In this current study, the median time between initiation of rituximab treatment and the first sign of clinical improvement and between first administration of rituximab and good outcome (mRS ≤ 2) were 15 days (range 5 to 38 days) and 8 weeks (range 4 to 40 weeks) respectively which was shorter compared to previous reports [3,8]. This may be explained by the earlier use of rituximab in our study and the fact that rituximab can expedite the recovery of patients with anti-NMDAR encephalitis [21,27]. As the average half-life of immunoglobulins after completion of an infusion is 16 to 24 days, we can not exclude a late effect of immunoglobulins at the acute stage of the disorder. In general, the time to start rituximab infusion must be influenced by the patients' clinical status, $CD19^+$ B-cell counts and the possible side effects of the drug.

The relapse rate in our study was 10% which was not high compared with the 8% to 28% relapse rate reported in previous studies [3,4,8,10,28]. Titulaer and colleagues have demonstrated that second-line immunotherapy was associated with fewer relapses in patients without tumour [3]. In our patient the $CD19^+$ B-cell count was 9.56% during the relapse. In previous reports, patients with NMOSD and patients with multiple sclerosis, all had relapses which occurred after reappearance of B cells [24,29], indicating that disease activity or clinical remission has a close relationship with B-cell count. Moreover, a previous observational analysis has demonstrated that high antibody titres are associated with bad outcome and the fluctuation of CSF titres correlated better with the worsening or improvement of pre-existing symptoms [30]. Titulaer and colleagues found that immunosuppressive treatment after the initial episode and second-line immunosuppressive treatment in patients without a tumour were associated with lower frequency of relapses. Furthermore, the application of second-line immunotherapy during relapses was associated with a reduced occurrence of subsequent relapses [3]. One recent study showed the best outcomes occurred in patients who were treated early (≤40 days), 90% of relapsing patients had received suboptimal or no immunotherapy during their previous episode [31]. In this current study, the time between the end of 10 days first-line therapy and rituximab infusion was 5 days (range 2.75 to 14.50 days). Although the ARR reduction of all 10 patients did not achieve statistical significance ($P = 0.16$), the 4 patients who had relapses before rituximab treatment showed an obvious reduction in ARR over 56 weeks. This may be explained by the limited number of relapsed patients before rituximab infusion in our study.

Rituximab was well tolerated in our study. Patient 3 had a transient infusion-related symptom and presented with hyperpyrexia during the infusion and patient 7 had a reduction in immunoglobulin G after repeated rituximab infusion. Prevalent infusion-related reactions include flu-like symptoms, hypertension, bronchospasm, pruritus and rash, often occurring at the first rituximab therapy or within the first 30 days following therapy [32,33]. In a long term follow-up of rituximab treatment in patients with NMOSD, long-term B-cell depletion after repeated rituximab treatment did not lead to any increased safety risks [26]. Even though severe adverse events include opportunistic infections, leukocytopenia, posterior reversible encephalopathy syndrome (PRES), hypogammaglobulinemia, or progressive multifocal leukoencephalopathy (PML), these did not appear during the 56-week follow-up period. We must increase our vigilance to prevent the occurrence of serious adverse effect.

Our study has some limitations including the small number of patients, relatively short follow-up period and absence of NMDAR antibody titre assessment, which might have been a crucial indicator of response to rituximab. With the extension of disease duration, spontaneous improvement cannot be excluded. Therefore, more multicentre studies must be conducted to confirm the long-term outcome of patients treated with rituximab.

This study found that lower rituximab doses are sufficient to improve mRS score and to decrease CD19 B-cell counts to <1% of the lymphocyte population in peripheral blood. In addition, there was a low incidence of infusion adverse events in patients who had an incomplete

response to first-line treatment. Therefore, we recommend that measurement of lymphocyte subsets should be performed regularly as these results can be used to decide the timing of rituximab reinfusion. Patients who had $CD19^+$ B-cell counts that exceeded 1% after the initial 4-week cycles of rituximab therapy should receive one additional reinfusion to prevent the increase of disease activity and the risk of relapse.

Disclosure of conflict of interest

The authors declare no financial or other conflicts of interest.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 81671183).

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