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Immunoglobulin levels may aid in the prediction of treatment response in anti-CD20 treatment of multiple sclerosis

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Abstract

Background: Anti-CD20 therapies are an emerging treatment strategy in multiple sclerosis (MS). **Objective:** Retrospective analysis of efficacy and safety in an MS cohort treated with rituximab (RTX) with identification of potential treatment response predictors. **Methods:** This retrospective study describes a monocentric cohort of 30 MS patients treated with RTX in a routine clinical setting. Patient characteristics, disease course, clinical and magnetic resonance imaging (MRI) treatment response markers, and laboratory assessments were analyzed. Logistic regression analysis corrected for demographic characteristics was used to identify treatment response predictors. **Results:** The RTX-treated cohort (mean age at RTX initiation 48 years (SD 14)) comprised patients with relapsing-remitting MS ($n = 9$), primary progressive MS ($n = 11$), and secondary progressive MS ($n = 10$). Two-thirds of patients had at least one MS medication prior to RTX; 27.6% of patients improved on the Expanded Disability Status Scale during RTX, whereas 72.4% of patients were stable or worsened. Based on this classification, we identified the presence of gadolinium enhancement in MRI before RTX as a predictor of response (odds ratio (OR) 12.2, confidence interval (CI) 1.02–144.55). After receiver operating characteristic curve definition of immunoglobulin (Ig) class cutoffs and creation of a sum score, the latter also predicted RTX response (OR 5.15, CI 1.21–21.88). Infectious complications were seen in three patients under RTX treatment. **Conclusion:** With the limitation of the retrospective approach and small sample size, this study confirms gadolinium enhancement before treatment initiation as a predictor of anti-CD20 response in MS. Lower Ig levels were associated with RTX response; however, these will have to be further investigated for a potential role for infectious complications.

Keywords

MS, rituximab, ocrelizumab, PPMS, SPMS, RRMS

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system with heterogeneous disease courses: a relapsing-remitting course (RRMS), a primary progressive course (PPMS), and a secondary progressive course (SPMS).[1] Treatment options for RRMS have broadly expanded over past decades with several substances approved or in the pipeline, whereas treatment options for progressive forms are sparse. Recently, ocrelizumab was the first agent ever approved for PPMS, a humanized monoclonal antibody against CD20.[2]

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CD20 is a cell surface protein expressed on circulating B cells, but not on bone marrow precursor cells or plasma cells.[3] A distinct pathophysiological antibody has never been identified in the context of MS as the inflammatory processes are pleiotropic.[4] Within these processes, the contribution of B cells is indisputable in MS, especially in progressive forms.[5–7] It has thus been identified as a potential target structure in MS immunotherapy even before ocrelizumab.

Rituximab (RTX) is a chimeric monoclonal antibody against CD20 first licensed by the FDA in 1997 and initially used in hematological indications, later followed by rheumatological disease. In a phase 2 trial in RRMS, RTX first demonstrated efficacy on both magnetic resonance imaging (MRI) parameters and the proportion of patients with relapses.[8] In PPMS, there was no difference between RTX and placebo. However, patients with MRI activity and younger age at RTX initiation profited from RTX treatment with a slight delay of time to confirmed disability progression.[9] Although RTX was never further developed to a formal licensing stage in MS, it is widely used as an off-label treatment option in both relapsing and progressive forms of MS. In the largest reported MS cohort from the Swedish MS registry comprising all disease courses, MRI parameters and disease progression were positively affected in an observational real-world study.[10]

Throughout the MS studies, safety data for both RTX and ocrelizumab are thus far generally favorable,[2,8–11] yet partly limited by short follow-up duration. The relationship of serological biomarkers with RTX efficacy and safety has not yet been investigated in MS.

Besides the evaluation of B cell counts under treatment, it is reasonable to assess immunoglobulin (Ig) levels. For RTX in the placebo-controlled trials, the frequency of decreases in IgG and IgA levels in the RTX group was comparable to the placebo group, whereas IgM was more frequently reduced in RTX; a relationship to increased infectious adverse events was not detected.[8,9] The larger Swedish registry reported on reduced IgG levels in 3% of the patients, again without an association with infectious complications.[10]

Data on the Ig level's association with infectious events are thus still limited for MS. There are no data on treatment efficacy in association with Ig levels. RTX-related IgG and IgA hypogammaglobulinemia has recently been associated with infections in vasculitis patients. Although this cohort was small ($n = 30$), it demonstrated significantly higher odds for severe infections (as defined by hospitalization) even after correction for age, race, and renal function.[12]

This study therefore set out to assess potential associations of clinical, MRI, and serological markers, especially Ig levels, with efficacy and infectious events in a cohort of MS patients treated with RTX.

Methods

Patients

This retrospective study was approved by the responsible cantonal ethics committee of Bern (registration no. KEK-BE 2017-01369) and carried out at Inselspital Bern, Switzerland.

Patients were identified by medical chart analysis. All patients diagnosed with MS according to the revised McDonald Criteria[13]—irrespective of disease course (RRMS, PPMS, SPMS)—and treated with RTX (MabThera®, Roche, Switzerland) at least once and followed up for at least 3 months were included. Available data on relapses, including calculation of an annualized relapse rate (ARR), evolution of disability as measured by the Expanded Disability Status Scale (EDSS),[14] MRI, laboratory parameters, and adverse events, were collected.

Change in disability before (latest available time point) and after RTX (latest available time point) as measured by the EDSS was used to categorize treatment response (improvement versus stable/worsening). Clinical and paraclinical parameters as well as serological markers were statistically analyzed for their potential of RTX treatment response prediction.

Statistical analysis

Descriptive statistics were used to summarize the assessed variables. Categorical variables are given in absolute and relative frequencies. Continuous variables are expressed as mean and standard deviation (SD).

The ARR was calculated for each patient over the whole available documented time interval before and since RTX initiation. Especially for patients with short follow-up after RTX initiation, this is a conservative approach as it would rather overestimate than underestimate the ARR.

Logistic regression analysis was used to identify clinical and paraclinical parameters for predicting RTX treatment response. These were parameters of disease activity/burden of disease obtained before RTX initiation: relapses, gadolinium enhancement in cerebral MRI, and disability level. The analysis was adjusted for gender and observation period since the initiation of RTX therapy.

To identify potential serological parameters obtained during RTX treatment serving as biomarkers of RTX treatment response, receiver operating characteristic (ROC) curves were used to define cutoffs for IgG, IgM, and IgA serum levels distinguishing between patients with and without RTX treatment response. These cutoffs were then implemented in an Ig score (range 0–3) to predict RTX treatment response in a logistic regression analysis. Prediction parameters are described as odds ratio (OR) with 95% confidence interval (CI). All analyses have been performed with the statistical software SPSS (IBM SPSS Statistics for Windows, Version 20.0. Released 2011. Armonk, NY: IBM Corp.).

Results

Description of the cohort

We identified 30 patients with RRMS (9/30), SPMS (11/30), and PPMS (10/30) who were treated with RTX in an off-label setting in the clinical routine of a tertiary university hospital between April 2015 and March 2017.

Patients had a mean age of 48 years (SD 14; Table 1), with a female-to-male ratio of 2:1. Mean EDSS at initiation of RTX was 4.7 (SD 1.8). In 10 of 30 patients, RTX was the first MS medication used; 6 of them were diagnosed with PPMS, 3 with SPMS, and 1 patient had an RRMS disease course. The mean observation period after first RTX treatment was 0.8 years (mean, SD 0.5), with a mean total RTX dosage of 1675 mg (SD 1135; Table 1).

Laboratory examinations before the initiation of RTX demonstrated B cell counts within the normal range (mean 239/µl, SD 188) and Ig class concentrations within the normal range: IgG 9.5 g/l (mean, SD 2.4), IgA 1.8 g/l (mean, SD 0.7), and IgM 1.2 g/l (mean, SD 0.6).

Table 1. Patient characteristics.

Variable	Mean	SD	Number of patients (%)	*n*
MS disease course				
RRMS			9/30 (30)	30
PPMS			11/30 (33)	30
SPMS			10/30 (37)	30
Gender (♀)			20/30 (67)	30
Number of preceding MS therapies				
0			10/30 (33)	30
1			11/30 (37)	30
2			2/30 (7)	30
3			6/30 (20)	30
4			1/30 (3)	30
Gd-enhancing lesion(s) on MRI before RTX			12/28 (40)	30
Age at start of RTX (years)	48	14		30
MS duration until start of RTX (years)[a]	9.3	9.1		30
Observation period before RTX initiation (years)	2.5	2.9		30
Observation period since RTX initiation (years)	0.8	0.5		30
ARR before RTX initiation	0.3	0.8		30
ARR since RTX initiation	0.28	0.73		30
EDSS at RTX initiation	4.7	1.8		30
Last EDSS score during RTX	4.6	1.8		29[b]
RTX dose in first cycle[c] (mg)	1375	625		30
Total RTX dose over all cycles (mg)	1675	1135		30
Number of RTX infusions in total	2.2	0.96		30
Laboratory results				
B cells/µl before RTX	239	188		30
Mean B cells/µl during RTX[d]	17	27		25
IgG before RTX (g/l)	9.5	2.4		27
IgA before RTX (g/l)	1.8	0.7		27
IgM before RTX (g/l)	1.2	0.6		26
IgG during RTX (g/l)[d]	8.7	2		29
IgA during RTX (g/l)[d]	1.6	0.6		29
IgM during RTX (g/l)[d]	0.9	0.5		29
Lymphocyte count/µl before RTX	1716	637		30
Lymphocyte count/µl during RTX[d]	1615	424		25

MS: multiple sclerosis; RRMS: relapsing-remitting MS; PPMS: primary progressive MS; SPMS: secondary progressive MS; MRI: magnetic resonance imaging; RTX: rituximab; ARR: annualized relapse rate; EDSS: Expanded Disability Status Scale; Gd: gadolinium; Ig: immunoglobulin; SD: standard deviation.

[a]MS disease duration is defined as interval between first diagnosis and initiation of RTX therapy.

[b]One patient had no documented EDSS after RTX initiation.

[c]One cycle comprises one to two single infusions within 4 weeks.

[d]Per patient, a minimum of one to a maximum of seven measurements were available 0.25–2.25 years after initiation of RTX.

Efficacy

In the whole patient group, the ARR remained unchanged after the initiation of RTX (Table 1; $p = 0.81$). However, in the subgroup of RRMS patients, the ARR decreased from 0.93 before (mean, SD 1.23) to 0.17 during RTX therapy (mean, SD 0.52; $p = 0.07$).

Due to the large proportion of progressive patients, the EDSS was used to classify treatment response resulting in 8/29 patients with EDSS improvement (responders) versus 21/29 patients with stable or worsened EDSS (nonresponders).

Of 30 patients, 28 had a cerebral MRI before the initiation of RTX (months prior RTX 4.6 (mean, SD 0.73) with gadolinium enhancement in 12 of 28 patients.

A follow-up scan 7.5 months (mean, SD 5.6) after the first RTX dose was available for 20 of these patients ($n = 9$ of the group with gadolinium enhancement; $n = 11$ of the group without gadolinium enhancement). These MRI data demonstrated gadolinium enhancement in 4 of 20 patients, in total. All of these four patients were patients who had gadolinium enhancement in the first scan. This results in an overall reduction of MRI activity of 55.6% (5/9).

Predictors of RTX treatment response

Logistic regression analysis determined the presence of gadolinium enhancement in the MRI scan before RTX as a predictor of RTX treatment response (OR 12.2, 95% CI 1.02–144.55), whereas EDSS progression and absence of relapses before treatment initiation were not identified as potential parameters for prediction (Tables 2 and 3).

Cutoffs for IgG, IgM, and IgA serum concentrations were calculated to distinguish between RTX responders versus nonresponders via ROC (see Online Supplementary Table S1). With these cutoffs, a score was created attributing 1 point per Ig class value below the defined cutoff (Ig score), therefore ranging from 0 (no value below cutoff) to 3 (IgG, IgM, and IgA value below cutoff). Each increase of

Table 2. Predictors of RTX response prior to RTX initiation.[a]

Variable	OR	95% CI	*p* Value
Relapse-free before RTX	0.57	0.06–5.79	0.63
Gd-enhancing lesion(s) on MRI before RTX	12.2	1.02–144.55	0.048
EDSS progression before RTX	4.16	0.45–38.81	0.21

OR: odds ratio; CI: confidence interval; EDSS: Expanded Disability Status Scale; Gd: gadolinium; MRI: magnetic resonance imaging; RTX: rituximab.
[a]Nagelkerke's R-Square 0.37; data available for $n = 27/30$. OR > 1 indicates higher likelihood for positive RTX response. Analysis was adjusted for gender and time of follow-up since RTX initiation.

Table 3. Predictors of RTX response during RTX therapy.[a]

Variable	OR	95% CI	*p* Value
Total RTX dose (mg)	1	0.99–1.002	0.87
Mean IgG, IgA, IgM sum score[b]	5.15	1.21–21.88	0.03

OR: odds ratio; EDSS: Expanded Disability Status Scale; Ig: immunoglobulin; MRI: magnetic resonance imaging; RTX: rituximab; CI: confidence interval.
[a]Nagelkerke's R-Square 0.42; data available for $n = 28/30$. OR > 1 indicates higher likelihood for positive RTX response. Analysis was adjusted for gender and time of follow-up since RTX initiation.
[b]Cutoffs for Ig levels were calculated using ROC analysis. Afterward, one point was given for each value below the cutoff (IgG 8.3 mg/l; IgA 2.0 mg/l; IgM 1.0 mg/l) resulting in a sum score ranging from 0 to 3.

Table 4. Adverse events and decrease of immunoglobulins during RTX therapy.

	Number of patients (%)
Any adverse event related to RTX	7/30 (23)
Infusion reaction	4/30 (13)
Infection	3/30 (10)
Ig class	
IgG 6–7 g/l	4/29 (14)
IgG <6 g/l	2/29 (7)
IgM 0.3–0.4 g/l	1/29 (3)
IgM <0.3g/l	3/29 (10)
IgA 0.6–0.7 g/l	1/29 (3)
IgA <0.6 g/l	0/29 (0)

RTX: rituximab; Ig: Immunoglobulin.

Table 5. Ig serum concentrations in patients with and without infectious complications.

	IgG (g/l) (mean, SD)	IgM (g/l) (mean, SD)	IgA (g/l) (mean, SD)
Patients with infectious complications ($n = 3$)	7.2 (0.7)	0.51 (0.05)	1.14 (0.46)
Patients without infectious complications ($n = 26$)[a]	8.8 (2.0)	0.91 (0.56)	1.71 (0.6)

Ig: immunoglobulin.
[a]One patient without infection had no Ig testing during RTX therapy.

1 point in the Ig score demonstrates a significant association with RTX response (OR 5.0, 95% CI 1.2–20.93) in the logistic regression analysis (Tables 2 and 3).

Adverse events

Adverse events occurred in 7 of 30 RTX-treated patients. Four patients experienced mild infusion reactions. Three patients had infections in mean 0.4 years (SD 0.5) after first dose of RTX leading to hospitalization in two patients ($n = 1$ pyelonephritis, $n = 1$ bladder infection). The third case reported to have had influenza in a follow-up visit, not further confirmed by additional data (Table 4). Table 5 outlines Ig serum concentrations of patients with infections compared to patients without infectious complications.

Discussion

This retrospective analysis of a small, monocentric cohort of RTX-treated MS patients describes an association of Ig levels with treatment response. Due to small patient numbers, a relation to infectious adverse events cannot be proven.

Anti-CD20 agents have recently proven high efficacy in both relapsing and primary progressive forms of MS.[2,10,11] Safety profiles seem generally favorable, for instance, in terms of progressive multifocal leukoencephalopathy (PML). However, infectious complications remain a risk for B cell–depleting therapies as well as for other disease-modifying drugs in MS.[15–18] This might become even more important with approved anti-CD20 treatment in clinical practice, as patient populations might be considerably older, suffer from comorbidities, or receive prolonged treatment in comparison to a trial setting.

In our mixed cohort of patients, the ARR did not serve as an adequate outcome parameter as only few patients were RRMS patients. For this reason, the EDSS was defined as the clinical outcome parameter. To be able to detect small effect sizes, we decided to set the following outcome groups: EDSS improvement ($n = 8$) versus stable/worsening EDSS ($n = 21$).

Whereas clinical baseline parameters did not serve as predictors for RTX treatment response in these groups, MRI activity 4.6 months (SD 0.73) before treatment initiation was associated with positive RTX response (OR 12.2 [1.02–144.55]). This is well in line with the phase 2 RTX study in PPMS[9] and underlines the fact that patients with active inflammation have better chances to respond to this mainly anti-inflammatory therapy. With our small cohort of different disease courses, it is well explained that the high MRI efficacy of more than 90% reduction of gadolinium-enhancing lesions compared to interferon beta[11] cannot be reproduced, here.

Although most of the studies on anti-CD20 treatments in MS provide some data on Ig levels, there are no distinct

analyses of their association with efficacy and only few information in association with safety. In ocrelizumab-treated RRMS patients, IgG and IgA levels were below the lower limit of normal at 1.5% and 2.4%, respectively, whereas the proportion of patients with IgM decrease was 16.5%. Unfortunately, the extent of decreases was not denoted and no association with outcome or infections was given (see supplementary material in the work of Hauser et al.[11]). These proportions were similar for the PPMS cohort. Here, no relationship to serious infections was detected (see supplementary material in the work of Montalban et al[2]).

Thus, consistently for RTX and ocrelizumab data, a small proportion of patients with lower IgG and IgA and a higher proportion of patients with lower IgM were reported.[2,8–11] Here, we created an Ig score combining cut-offs for all three Ig serum classes and detected a significant association with RTX response (OR 5.0 [1.2–20.93]). This finding needs confirmation in larger cohorts, but has thus far not been described elsewhere.

Consistent with other cohorts, infusion-related mild adverse events were reported for our cohort. Three patients with infectious complications ($n = 1$ mild, $n = 2$ with hospitalization) were seen in our cohort. Infectious complications and reactivation of latent infections, for example, hepatitis B, are a major concern in anti-CD20 treatment and will require further attention.[15]

With only three patients with infectious complications, a statistical analysis does not seem appropriate, here. Whether Ig levels in patients with compared to those without infectious complications are different needs to be assessed in larger MS cohorts. This has already been demonstrated in other conditions, for example, in vasculitis patients.[12] However, there are also data that cannot confirm the relationship between low Ig levels and infections in a population under multimodal immunosuppressive treatment.[19] The latter study focused only on IgG levels that might explain conflicting results. Hypogammaglobulinemia for Ig classes might serve as a predictor for infectious complications and could serve as a feasible tool in clinical practice, but needs further, putatively even disease-specific validation.

The major limitation of our study is of course the small sample size and retrospective approach leading to missing values in parts of the analyses and heterogeneity. However, we think this analysis is well suited to serve as a hypothesis-generating approach that should be further investigated in larger, prospective studies of anti-CD20 therapies in MS. As MRI activity as an established paraclinical parameter of treatment response was confirmed, here, our analysis may well be comparable to other, larger cohorts.

Especially for novel therapeutic interventions, the emersion of unanticipated adverse events that might not have been uncovered in phase 3 trials due to low incidence rates is feared. Surrogate markers to help in the identification of patients at risk for adverse events, but also at higher chance to respond to treatment are still an unmet need in MS treatment. If Ig serum levels were confirmed as serological markers for treatment response and maybe also for infectious adverse events, the longitudinal standardized assessment of Ig levels might help to identify patients at risk.

Authors' contribution

R Hoepner and A Miclea contributed equally to this work.

Declaration of conflicting interests

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Supplementary material

Supplementary material for this article is available online

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