

Rituximab in multiple sclerosis: Frequency and clinical relevance of anti-drug antibodies

Nicky Dunn, Alexander Juto, Malin Ryner, Ali Manouchehrinia, Luca Piccoli, Katharina Fink, Fredrik Piehl and Anna Fogdell-Hahn

Multiple Sclerosis Journal

1–10

DOI: 10.1177/

1352458517720044

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Abstract

Background: Rituximab is a chimeric monoclonal anti-CD20 B-cell-depleting antibody increasingly used off-label in multiple sclerosis (MS). The clinical relevance of anti-drug antibodies (ADAs) against rituximab in MS is unknown.

Objective: To determine frequency of ADA in relation to B-cell counts, allergic reactions and clinical efficacy in a large cohort of MS-treated patients.

Methods: Cross-sectional study with collection of serum samples from 339 MS patients immediately before a scheduled rituximab infusion. ADAs were detected using an in-house-validated electrochemiluminescent immunoassay and a commercial enzyme-linked immunosorbent assay (ELISA) to compare methods. Data on patient demographics and clinical outcomes were retrieved from the Swedish MS Registry and patient records.

Results: ADAs were detected in 37% of relapsing–remitting MS and 26% in progressive forms of MS. Presence of ADAs decreased with increasing number of rituximab infusions. There was a significant association between both presence and titres of ADAs and incomplete B-cell depletion, but not with infusion/adverse reactions or clinical outcomes at the group level. Only five patients terminated rituximab during follow-up, four of which were ADA positive.

Conclusion: Rituximab treatment is associated with a high degree of ADAs, which correlates with efficacy of B-cell depletion; however, the clinical relevance of ADAs remains uncertain.

Correspondence to:

A Fogdell-Hahn

Center for Molecular
Medicine, Karolinska
University Hospital Solna,
171 76 Stockholm, Sweden.
anna.fogdell-hahn@ki.se

Nicky Dunn

Alexander Juto

Malin Ryner

Ali Manouchehrinia

Katharina Fink

Fredrik Piehl

Anna Fogdell-Hahn

Department of Clinical
Neuroscience, Karolinska
Institute, Stockholm, Sweden
/ Center for Molecular
Medicine, Karolinska
University Hospital Solna,
Stockholm, Sweden

Luca Piccoli

Immune Regulation
Laboratory, Institute for
Research in Biomedicine,
Bellinzona, Switzerland

Keywords: Antidrug antibodies, rituximab, multiple sclerosis, anti-CD20, MAb, immunogenicity, B-cell depletion

Date received: 17 February 2017; revised: 14 June 2017; accepted: 19 June 2017

Introduction

Rituximab (Mabthera; Roche, Basel, Switzerland) is a chimeric monoclonal anti-CD20 antibody approved for B-cell lymphoma, rheumatoid arthritis and systemic vasculitis. Rituximab has undergone clinical testing for multiple sclerosis (MS) in a phase II randomized placebo-controlled trial (RCT) for relapsing–remitting multiple sclerosis (RRMS) and a phase II/III RCT for primary-progressive multiple sclerosis (PPMS).^{1,2} However, the manufacturer has interrupted further clinical development for these indications. Nevertheless, data from several observational studies support the notion that rituximab is an effective and safe treatment option in both relapsing and progressive forms of MS.^{3,4} In addition, in two recent

studies, a superior efficacy of rituximab was shown compared to fingolimod in MS patients switching from natalizumab, as well as a beneficial safety profile and a high clinical efficacy in the largest rituximab cohort so far described.^{5,6} Still, it is highly motivated to further investigate aspects of the drug that may impact on safety and efficacy, such as anti-drug antibodies (ADAs).

The immunogenicity of rituximab has been described in other disease cohorts but with highly variable reported frequencies of ADAs. In patients with non-Hodgkin lymphoma (NHL), the reported frequency is 1%;⁷ in rheumatoid arthritis (RA), between 4% and 11%; and in systemic lupus erythematosus (SLE),

26%–65%.^{8,9} A correlation between ADA and incomplete B-cell depletion (BCD), serum sickness and hypersensitivity type 3 reactions has been described in SLE, but not in other diseases.^{9,10} In MS, the frequency of ADAs was reported to be 25% and 29% in two RRMS cohorts, respectively, and 7% in PPMS. However, no apparent correlation with clinical outcomes was found, and thus, the biological significance of ADAs remains unclear.^{1–3}

With a growing body of evidence suggesting a high clinical efficacy of rituximab in MS and increasing off-label use, there is a strong need to explore to what degree immunogenicity may affect treatment outcomes. Furthermore, there is ample evidence that the immunogenicity of other biological therapies such as interferon and natalizumab is associated with loss of clinical efficacy and risk of infusion reactions (IR) and adverse events (AE).¹¹ To this end, we here first compared two different assays regarding sensitivity to detect rituximab ADA in a large cross-sectional cohort of MS patients treated off-label with rituximab and then correlated the presence of ADAs with laboratory and clinical outcomes using the most sensitive assay.

Methods

Patients and clinical data

Serum samples ($n=453$ from $n=355$ patients) were collected immediately prior to a scheduled infusion from patients receiving rituximab off-label for MS at the Department of Neurology at the Karolinska University Hospital over a 12-month period (November 2015–November 2016). At 1 January 2016, the Department had 584 ongoing treatments with rituximab for patients with MS. There was no active patient selection; however, samples were not collected daily due to logistical reasons. No patient declined to participate; however, patients were excluded if a diagnosis of MS could not be confirmed (Figure 1). In the majority of cases (>95%), rituximab had been administered at a 6-month interval as a single infusion of 500 mg ($n=322$) or 1000 mg ($n=33$). Pre-treatment samples were used for validation of the electrochemiluminescent (ECL) method and were excluded from further statistical analyses.

Clinical information was retrieved from electronic medical charts and the Swedish Multiple Sclerosis Register (SMSreg).¹² Information about IR/AE related to the previous infusion and the infusion given immediately after ADA sampling was actively collected by the MS nurses using a structured format (graded according to Common Terminology Criteria

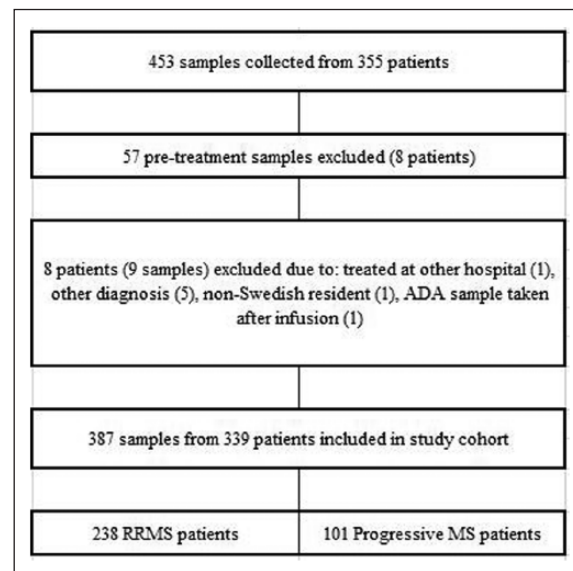


Figure 1. Flow chart of patient inclusion and exclusion.

for Adverse Events (CTCAE) v. 4.03). All magnetic resonance imaging (MRI) readings were performed by trained neuroradiologists reflecting routine practice in a real-world setting.

Disease severity at baseline and progression over the follow-up period was assessed using MRI gadolinium-enhancing (Gd+) lesions, the Expanded Disability Status Scale (EDSS) and relapses. Global multiple sclerosis severity score (MSSS) and age-related multiple sclerosis severity (ARMSS) score^{13,14} were calculated using the EDSS to further analyse disease severity and progression throughout the study. Relapses were defined as new onset of neurological symptoms lasting >24 hours which could not be explained by a concurrent condition. Relapse adjudication was based on clinical chart data by a single evaluator (A.J.) blinded to patients' ADA status. MRI for Gd enhancement was performed on an annual basis, with a mean of 1 year (standard deviation (SD): ± 0.7). All patients had given informed consent, and the study was approved by the Stockholm Regional Ethical Committee (2009/2107–31/12).

B-cell count

B-cell count (BCC) was determined by a certified clinical laboratory at the Department of Clinical Immunology, Karolinska University Hospital using flow cytometry (Navios; Beckman Coulter, Indianapolis, IN, USA) with the following antibodies: Mouse anti-human CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 (clones: B3821F4A/N901(NKH-1)/

J4.119/UCHT1; B3821F4A/N901/UCHT1; Beckman Coulter) and mouse anti-human CD16-PE (clone: B73.1; Becton Dickinson, New Jersey, USA). BCCs were taken within a 30-day period prior to serum sampling and rituximab infusion. For absolute quantification of BCC, 100- μ L Flow-Count fluorophores (Beckman Coulter) were added to a patient sample of 100 μ L, with a detection limit set at $<0.01 \times 10^9$ cells/L. Complete BCD was defined as BCC $<0.01 \times 10^9$ cells/L.

Detection of ADAs

Presence of anti-rituximab antibodies was determined using a slightly modified protocol of an ECL immunoassay developed by GlaxoSmithKline (GSK), using the Meso Scale Discovery® (MSD) technique. The ECL assay was validated in-house prior to analysis of study samples. ADAs were detected through a three-tiered approach. Samples detected as positive for ADAs in the screening assay were confirmed positive in a competitive assay to ascertain specificity of ADAs against rituximab. MS-specific cut-points were used, with a 1% false-positive rate after confirmation. In the third tier, the sample was titrated and the titre was determined as the last dilution step above the assay cut-point. The final titre was expressed as arbitrary units per millilitre (AU/mL), which corresponded to the inverse dilution at which the sample still gave positive signal in the assay. Drug interference was considered as a minor issue as the half-life of the drug is approximately 20 days and all samples were collected >5 months post-infusion.^{3,15} Nevertheless, in this ECL assay, the risk of drug interference was minimized by dissociating immune complexes with acid treatment prior to ADA detection. On validation, the assay was shown to detect a positive rat anti-rituximab control of 500 ng/mL in the presence of a drug concentration of 80 μ g/mL. The sensitivity of the ECL assay was 0.8 ng/mL for the rat anti-rituximab. Serum samples from 40 healthy donors, who gave written informed consent, were collected (Stockholm Blood Center, Stockholm, Sweden) to prepare a pool of normal healthy sera (NHS) used as negative control in the ECL assay. Low- and high-positive controls were prepared by spiking NHS with a rat anti-rituximab monoclonal antibody (MCA2260, clone MB2A4, Bio-Rad/AbD Serotec, Oxford, UK) to a concentration of 3 and 500 ng/mL, respectively.

Assay comparison

The ECL was selected after a pilot study comparing the frequency of ADAs in a subgroup of MS patients ($n=321$) showed a higher detection of ADAs with the ECL assay compared to the commercially available

anti-rituximab enzyme-linked immunosorbent assay (ELISA) kit (Promonitor, Bizkaia, Spain). The sensitivity was evaluated using three newly generated human anti-rituximab clones with different affinity as well as the rat anti-rituximab used as positive control in ECL.

Isolation and selection of human monoclonal anti-rituximab antibodies

A peripheral blood sample was obtained from a pemphigus patient who developed ADAs after treatment with rituximab. IgG⁺ memory B cells were isolated and immortalized with Epstein–Barr virus (EBV), as described previously,¹⁶ and culture supernatants were tested for binding to rituximab by ELISA. Heavy chain and light chain variable regions were sequenced,¹⁷ and all monoclonal antibodies (mAbs) were produced recombinantly as IgG1 molecules.

Rituximab ELISA for validation of human monoclonal anti-rituximab antibodies

Rituximab was produced recombinantly as human IgG with murine IgG2a CH2-CH3 domains (here referred as mRituximab) to avoid cross-reaction with secondary antibody. ELISA plates were coated with 1 μ g/mL of mRituximab, blocked and incubated with titrated antibodies, followed by alkaline phosphatase (AP)-conjugated anti-human IgG Fc γ -specific antibody (Jackson ImmunoResearch, West Grove, PA, USA). Substrate (*p*-nitrophenylphosphate (*p*-NPP), Sigma, Missouri, USA) was added to plates that were read at 405 nm. EC50 (ng/mL) was calculated for every sample by nonlinear regression analysis using GraphPad Prism 5 software.

Rituximab inhibition of binding assay

Titred anti-rituximab mAbs were incubated with a fixed concentration of mRituximab (0.1 μ g/mL). The mixtures were then used to stain irrelevant CD20⁺ B cells, followed by R-phycoerythrin (RPE)-conjugated anti-mouse IgG antibody (SouthernBiotech, Birmingham, AL, USA). Samples were read by fluorescence-activated cell sorting (FACS), and mean fluorescence intensity values were used to calculate inhibition of rituximab binding (%) to B cells by mAbs.

Comparison of sensitivity of the ECL and commercial ELISA

The three human monoclonal anti-rituximab antibodies and the rat anti-rituximab positive control were then titrated and analysed both with ECL and ELISA

Table 1. Frequency of ADAs against rituximab in MS.

	RR	Progressive forms	Total
Total patients (<i>n</i>)	238	101	339
Positive, <i>n</i> (%)	88 (37)	27 (26.5)	115 (33.9)
Positive >5 AU/mL, <i>n</i> (%)	46 (19)	15 (14.9)	61 (18)
Negative, <i>n</i> (%)	150 (63)	74 (73.5)	224 (66.1)

ADAs: anti-drug antibodies; MS: multiple sclerosis; RR: relapsing–remitting; AU: arbitrary unit.
Risk of ADA positivity in RRMS patients compared to progressive MS patients, odds ratio (OR): 1.9 (95% confidence interval (CI): 1.04–3.5, $p=0.038$).

kit (Promonitor). Sensitivity was determined as the last dilution in which a signal above the cut-point could be detected.

Statistical analyses

The primary outcome measure was ADA status categorized as follows: ADA negative, ADA positive and highly positive (titre: >5 AU/mL). The >5 AU/mL threshold is equivalent to the low-positive control used in the assay (3 ng/mL). Chi-square or Fisher's exact tests (sample sizes <5) were used in the analyses of associations between categorical variables. Depending on the distribution of data, *t* test or Mann–Whitney tests were used to compare the differences in mean values or medians between groups.

Clinical outcomes of the study included IR, AE, BCC/BCD, brain MRI Gd+ lesions, disease severity scores and relapses. To account for variation in time between rituximab infusions, a treatment index was calculated by dividing treatment duration by number of previous infusions at the time of ADA sampling. The study follow-up period for analysis of disease progression using clinical outcomes was from treatment initiation to completion of data collection (November 2016). Fisher's exact test was used to analyse differences in proportion of patients experiencing IR and AE between ADA categories. Wilcoxon signed rank sum test (paired test) was used for comparing MRI Gd+ lesions at baseline and follow-up. When analysing the BCC as binary variable (complete and incomplete depletion), logistic regression models adjusted for confounding effects of age, sex, disease duration, treatment index and MS type were used. The delta changes in number of Gd+ lesions on MRI, global MSSS and ARMSS score¹³ from baseline (i.e. start of rituximab) to the most recent measurement were also calculated. Linear regression models adjusted for age, sex, disease duration, treatment index and MS type were used to investigate whether changes in the severity scores differed by ADA status.

In all analyses, patients with incomplete data were excluded from the relevant analysis. If patients had >1 treated sample analysed, the most recent sample was used for the analyses except when analysing IR/AE where all collected samples were included for the analysis. In these analyses, all samples were treated as individual events to determine whether the variability in ADA status correlated with AE/IR. Statistical analyses were performed using Stata (StataCorp, 2009, Stata Statistical Software: Release 14; StataCorp LP, College Station, TX, USA).

Results

Frequency of ADAs

After application of the exclusion criteria, 387 samples from 339 patients with MS, RRMS ($n=238$) and progressive MS ($n=101$) were included in the analysis (Figure 1). Rituximab ADAs were detected in 34% of all MS patients, and 18% of patients had positive titres greater than 5 AU/mL (Table 1). Stratified by MS type, RRMS had a higher frequency of rituximab ADAs (37%) compared to progressive forms (26%). When adjusted for age, sex, disease duration and treatment index, RRMS had almost two times greater risk (odds ratio (OR): 1.9, 95% confidence interval (CI): 1.04–3.5, $p=0.038$) of becoming ADA positive compared to progressive MS. No difference was found in clinical outcomes at baseline between ADA categories or titres (Table 2). The number of previous infusions at the time of ADA testing ranged from one to eight in this MS cohort, with an average treatment duration of 1.54 ± 0.84 years (range: 0.4–5.3). There was a clear significant decrease in frequency of ADA positivity with increasing number of infusions in both categories of positive patients ($p<0.001$) (Figure 2).

Longitudinal samples were obtained from 46 patients: 45 patients with two samples and 1 with three samples. The majority of these patients had two negative ($n=27$, 59%) or two positive ($n=10$, 22%) samples

Table 2. Patient characteristics at treatment initiation.

	RRMS		Progressive forms of MS			
	Negative	Positive	Total positive	Titres >5 AU/mL	Negative	Positive
Total patients (<i>n</i>)	150	88	88	46	74	27
Sex (female, <i>n</i> (%))	100 (66.7)	60 (68.2)	60 (68.2)	37 (80.4)	54 (71.6)	19 (70.4)
Sex (male, <i>n</i> (%))	50 (33.3)	28 (31.8)	28 (31.8)	9 (19.6)	21 (28.4)	8 (29.6)
Age (mean years, SD)	39.5 ± 9.8	42.2 ± 10.9	42.2 ± 10.9	31.9 ± 10.2	48.6 ± 8.6	51 ± 6.8
Age at disease onset (mean years, SD)	30.5 ± 9.6	32 ± 10.7	32 ± 10.7	42 ± 10.6	31.8 ± 8.7	30.5 ± 10
Disease duration (mean years, SD)	8.9 ± 6.8	9.4 ± 6.9	9.4 ± 6.9	11.2 ± 6.85	17 ± 8.8	20 ± 10
1+ Gd+ lesions on MRI (% <i>n</i> /total)	33 (35/106)	32 (19/58)	32 (19/58)	29.6 (8/27)	23.2 (13/56)	23.8 (5/21)
EDSS (median, IQR)	2 ± 2.5 (<i>n</i> = 103)	2 ± 2.5 (<i>n</i> = 62)	2 ± 2.5 (<i>n</i> = 62)	2 ± 2.5 (<i>n</i> = 33)	5 ± 2.5 (<i>n</i> = 44)	3.5 ± 1.5 (<i>n</i> = 13)
MSSS (median, IQR)	4 ± 3.6 (<i>n</i> = 96)	4.16 ± 3.8 (<i>n</i> = 62)	4.16 ± 3.8 (<i>n</i> = 62)	4.1 ± 3.9 (<i>n</i> = 32)	5.8 ± 3.6 (<i>n</i> = 65)	5.5 ± 4 (<i>n</i> = 20)
GARMS (median, IQR)	4.4 ± 3.72 (<i>n</i> = 103)	3.75 ± 3.3 (<i>n</i> = 62)	3.75 ± 3.3 (<i>n</i> = 62)	4.2 ± 3.4 (<i>n</i> = 32)	6 ± 2.9 (<i>n</i> = 65)	6.3 ± 2.75 (<i>n</i> = 20)
BCC (cells/L, mean, SD)	0.31 ± 0.28 (<i>n</i> = 68)	0.35 ± 0.34 (<i>n</i> = 49)	0.35 ± 0.34 (<i>n</i> = 49)	0.42 ± 0.41 (<i>n</i> = 27)	0.32 ± 0.25 (<i>n</i> = 32)	0.30 ± 0.31 (<i>n</i> = 14)
RRMS: relapsing–remitting multiple sclerosis; MS: multiple sclerosis; AU: arbitrary unit; SD: standard deviation; 1+ Gd+: one or more gadolinium-positive lesions; MRI: magnetic resonance imaging; EDSS: Expanded Disability Status Scale; IQR: interquartile range; MSSS: multiple sclerosis severity score; GARMS: global age-related multiple sclerosis severity score; BCC: B-cell count.						

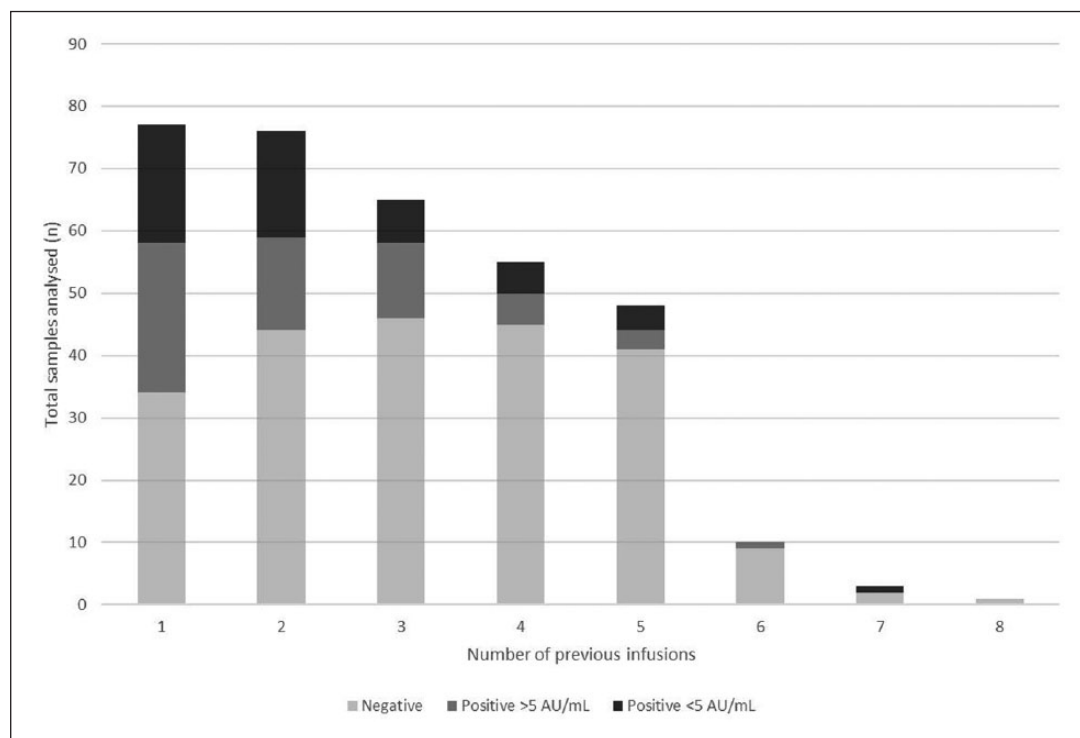


Figure 2. Graph demonstrating the change in frequency of ADA status with increasing number of infusions.

over time, and thus, the later test result confirmed the first. Of the eight patients (17%) who showed transient positivity (positive in the first sample and negative in the second), all titres in the initial positive sample were low, less than 4 AU/mL. Only one patient had a negative sample followed by a positive, and this patient developed ADAs following the fourth infusion. All four groups were similar in regard to demographic and clinical characteristics.

Isolation and characterization of human monoclonal anti-rituximab antibodies

Three rituximab-specific human B-cell clones were identified after screening by ELISA of EBV-immortalized IgG⁺ memory B cells isolated from the blood of an ADA-positive pemphigus patient. The three mAbs showed high-affinity binding to rituximab by ELISA with EC₅₀ values ranging from 11 to 24 ng/mL. Moreover, the three mAbs potently neutralized rituximab, by inhibiting its binding to CD20⁺ B cells (table and figure demonstrating results in supplementary file).

Assay comparison

A subgroup of 321 samples was analysed using both ECL and ELISA methods. The ECL method was

significantly more sensitive than the ELISA method, with 5.6% (18/321) of the cohort detected as positive with ELISA compared to 32.7% (105/321) with ECL. A moderate correlation was seen between ELISA and ECL titres in positive samples ($r=0.4$, $p<0.001$). All samples detected positive for ADAs on the ELISA were also positive on the ECL; however, the ELISA had a false-negative rate of 27% (87/321). Furthermore, the three human monoclonal anti-rituximab antibodies (RTX-1, RTX-3, and RTX-10) titrated with both methods showed a limit of detection of 4, 0.8, and 4 ng/mL for Promega ELISA kit and 0.8, 0.16, and 0.16 ng/mL for ECL, respectively.

Association between BCC and ADA

In total, 236 patients had BCC within a 30-day period prior to ADA sampling and rituximab infusion and were thus included in the analysis of BCC and ADA association. A significantly greater proportion ADA-positive patients displayed incomplete or unmainained BCD at this time point ($\geq 0.01 \times 10^9$ cells/L), with a mean of detectable BCC in 45% in all ADA-positive patients compared to 26% in ADA-negative patients (OR: 3.8, $p<0.001$) (Table 3). The proportion of patients with incomplete BCD increased with ADA titres >5 AU/mL (OR: 5.8, $p<0.001$). Patients with low-positive titres were found to be significantly

Table 3. Association between BCC and ADA categories.

	Negative	Positive		
		Total positive	Titres <5 AU/mL	Titres >5 AU/mL
Total patients (<i>n</i>)	148	88	41	48
BCC (cells/L $\pm$ SD)	0.014 $\pm$ 0.028	0.038 $\pm$ 0.054	0.02 $\pm$ 0.29	0.05 $\pm$ 0.065
Complete BCD (%)	74.3	54.5	61 ^a	33
Incomplete BCD (%)	25.6	45.5 ^b	39	67 ^c

BCC: B-cell count; ADA: anti-drug antibody; AU: arbitrary unit; BCD: B-cell depletion; SD: standard deviation.
^aLikelihood of having complete BCD in patients with low-positive titres compared to patients with higher titres ($p < 0.008$).
^bRisk of incomplete BCD in ADA-positive compared to ADA-negative patients (OR: 3.8, $p < 0.001$).
^cRisk of incomplete BCD in patients with >5 AU/mL ($p < 0.001$) compared to ADA-negative patients.

Table 4. Association between infusion reactions and ADA categories.

	Negative	Positive		Total cohort
		Total positive	Titre >5 AU/mL	
Total patients (<i>n</i>)	248	131	66	380
No IR, <i>n</i> (%)	201 (81)	106 (80.9)	52 (78.8)	308 (81.1)
Grade 1, <i>n</i> (%)	45 (18.1)	23 (17.6)	13 (19.7)	68 (17.9)
Grade 2, <i>n</i> (%)	2 (0.8)	2 (1.5)	1 (1.5)	4 (1.1)
Total IR, <i>n</i> (%)	47 (19.0)	25 (19.1)	14 (21.2)	72 (19.0)

ADA: anti-drug antibody; AU: arbitrary unit; IR: infusion reactions.

more likely to have maintained complete BCD compared to those with higher titres ($p < 0.008$) and did not significantly differ from the ADA-negative cohort ($p = 0.128$). No significant differences were found between MS types and BCC.

Association of ADA and clinical outcomes

A total of 19% of the patient cohort experienced either a grade 1 or 2 IR (Table 4) and 7% of all patients experienced a grade 1 to 3 AE (Table 5). No serious AE (grade 4) occurred in this cohort during follow-up. No difference in total IR ($p = 0.6$) and AE ($p = 0.28$) or severity of either were found between ADA-positive and ADA-negative patients. Furthermore, there was also no association between IR/AE and ADA titres nor with MS type or rituximab dose. However, the power to detect a difference in dose was limited by the small number of patients who had received 1000 mg at the most recent infusion ($n = 33$).

At the time of sampling for ADA, patients had a mean treatment duration of 1.54 ± 0.84 (0.4–5.3) years and an average follow-up time of 2.2 ± 0.9 (0.7–5.7) years of clinical data. No difference was observed between ADA categories in age, sex, disease duration and treatment index at baseline, with the exception of MS

type, as shown in Table 1. Stratification by MS type was limited by the low number of progressive patients; however, this was accounted for in the logistic regression model.

Disease activity was strongly suppressed with initiation of rituximab in both the ADA-positive and ADA-negative groups, with a reduction in average Gd+ lesions on MRI over the follow-up period (ADA-positive mean: -0.92 , 95% CI: -0.33 to 1.5 and ADA-negative mean: -1.2 , 95% CI: -0.61 to 1.8) and in improvement in both global MSSS and ARMSS score. However, when adjusting for demographics and clinical characteristics, no significant difference was found between ADA categories. Only 10 relapses in 9 patients were recorded during the follow-up period. Three of the nine patients were ADA positive, and the patient with two documented relapses was ADA positive. Due to the small number of relapses, this outcome was not included in the analyses.

Of the 339 included patients, five (1.5%) discontinued rituximab treatment during the study period (Table 6). Four of these patients belonged to the ADA-positive group (3.5%, 4/115) and only one was ADA negative (0.4%, 1/224, $p = 0.033$). Of the ADA-positive patients, all four discontinued due to lack of

Table 5. Association between adverse events and ADA categories.

	Negative	Positive		Total cohort
		Total positive	Titre >5 AU/mL	
Total patients (<i>n</i>)	247	130	66	378
No AE, <i>n</i> (%)	229 (92.7)	122 (93.8)	60 (90.9)	352 (93)
Grade 1, <i>n</i> (%)	11 (4.5)	7 (5.4)	5 (7.6)	18 (5)
Grade 2, <i>n</i> (%)	6 (2.4)	0	0	6 (1.5)
Grade 3, <i>n</i> (%)	1 (0.4)	1 (0.8)	1 (1.5)	2 (0.5)
Total AE, <i>n</i> (%)	18 (7.3)	8 (6.2)	5 (7.6)	26 (6.9)

ADA: anti-drug antibody; AU: arbitrary unit; AE: adverse events.

treatment effect, with one patient also experiencing a grade 3 IR. The one patient who was ADA negative discontinued due to a grade 1 AE.

Discussion

This prospective, cross-sectional study provides clinical level of evidence IV regarding the frequency and clinical relevance of ADAs against rituximab. The results demonstrate that rituximab-treated MS patients had a high degree of ADAs compared to other diseases treated with the same drug. Consistent with previous studies, a lower frequency of ADAs was seen in progressive forms of MS, compared to RR forms.^{1,2} The reason for this is not clear; however, it could relate to the higher level of immunological activity found in the earlier relapsing stage of the disease. This could also account for the variation of immunogenicity of rituximab in other disease types such as SLE relative to RA and NHL, underscoring the importance of taking caution when extrapolating data between diseases.

The higher frequencies of ADAs found in this study indicate that rituximab is more immunogenic in MS than previously reported.¹⁻³ As seen in the comparison of the ECL and ELISA kit in this study, a higher frequency of ADAs was attributed to the use of a more sensitive method. These results highlight the need to ensure that assays used to detect ADAs have adequate sensitivity before assessing the biological and clinical relevance of ADAs and need for routine testing.

The biological significance of ADAs with rituximab treatment is not clearly understood in any disease cohort. We here demonstrate a strong association between ADA positivity, higher BCC and thus incomplete BCD at the time of next rituximab infusion, notably greater with higher titres of ADAs (>5AU/mL).

The primary mode of action for rituximab is BCD, but the correlation between degree of depletion and clinical efficacy has not been investigated in detail. However, recent data from a dose finding study of another anti-CD20 drug (ofatumumab) suggest a cut-off at 0.032×10^9 cells/L for abrogating neuroradiological disease activity defined as reduction in disease activity to ≤ 1 lesion per year.¹⁸ This might implicate a possible negative impact on therapeutic efficacy in ADA-positive patients with high titres (>5AU/mL), where the mean BCC was 0.05×10^9 cells/L.

Consistent with previous studies, an overall improvement was seen with rituximab treatment; however, no significant difference in treatment efficacy was found between ADA groups. Still, the fact that four of the five patients who discontinued rituximab treatment during the study period due to disease progression or to secondary loss of response were ADA positive indicates that there could possibly be a clinically relevant effect of ADAs in a subgroup of patients. This notion is supported by the fact that one of the four ADA-positive patients who had incomplete BCD discontinued rituximab therapy due to two relapses and was switched to Arzerra (ofatumumab, humanized monoclonal anti-CD20 antibody) and maintained complete BCD and no further disease activity over a 10-month follow-up (patient 2, Table 6). Interestingly, a negative relationship found between number of infusions and the frequency of ADAs suggests that the risk of developing ADAs and experiencing lack of effect might diminish over time. However, it is not possible to ascertain a causal relationship due to the cross-sectional design of the study, but this warrants further investigation in prospective longitudinal cohorts.

New anti-CD20 treatments are expected to soon reach the market, with ocrelizumab already approved by the Food and Drug Administration (FDA) for progressive MS and pending approval for RRMS. An expected

Table 6. Characteristics of patients who discontinued rituximab treatment.

Age (years)	1	2	3	4	5
	57	44	53	52	62
Sex	Male	Woman	Woman	Woman	Woman
MS type	SPMS	RRMS	SPMS	RRMS	PPMS
Disease duration (years)	8.5	9.7	20.0	1.9	1.5
Baseline EDSS (median, IQR)	n/a	3	6.5	2	n/a
End of treatment EDSS (median, IQR)	5	2	6.5	3.5	6
Total number of infusions (<i>n</i>)	6	7	3	2	3
Reason for discontinuation	Grade 1 adverse event	Lack of effect (5 Gd+ lesions)	Lack of effect (worsening SPMS)	Lack of effect/grade 3 infusion reaction	Lack of effect (worsening PPMS)
BCC at the time of ADA sampling (cells/L)	0	0.02	0.12	0.13	0
ADA sample result	Negative	Positive ^a	Positive	Positive	Positive
ADA titre (AU/mL)	0	160	8	4	8

SPMS: secondary-progressive multiple sclerosis; RRMS: relapsing–remitting multiple sclerosis; MS: multiple sclerosis; EDSS: Expanded Disability Status Scale; IQR: interquartile range; BCC: B-cell count; ADA: anti-drug antibody; AU: arbitrary unit; PPMS: primary-progressive multiple sclerosis.
^aDiscontinued rituximab therapy due to two relapses and was switched to Arzerra (ofatumumab, humanized monoclonal anti-CD20 antibody).

improvement over rituximab is that ocrelizumab is humanized and therefore likely to be less immunogenic. As confirmed in other biological therapies such as interferon beta, the immunogenicity of biosimilars varies regarding ADA frequency and titre.¹¹ These results are important for comparative purposes of ADAs against rituximab to new biosimilars in a real-world setting, not least since newer treatment will command a substantially higher price.

Conclusion

Rituximab treatment is associated with a high degree of ADAs that correlates with efficacy of BCD. However, the clinical relevance of ADAs remains uncertain. Further longitudinal studies using sensitive ADA detection methods are required to determine the true effect of ADAs, specifically those with higher titres of ADAs.

Acknowledgements

The authors would like to thank Anne Schwartz from GSK, Gail Calvert from MSD and Anna Mattsson for the support in the ECL assay validation and Carl Piehl and Peter Alping for help with collection of primary data. The research leading to these results was conducted as part of the ABIRISK consortium (Anti-Biopharmaceutical Immunization: Prediction and Analysis of Clinical Relevance to Minimize the Risk). For further information, please refer to www.abirisk.

eu. N.D. and A.J. are the co-first authors. F.P. and A.F-H. are the co-senior authors.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: M.R. has received research support from Biogen Idec and Sanofi-Aventis and received speaker honoraria from Biogen Idec. K.F. has received an unrestricted research grant from Biogen and travel compensation for lectures from Biogen and Novartis. F.P. has received unrestricted academic research grants from Biogen, Genzyme and Novartis and travel support and/or compensation for lectures and/or participation in advisory boards from Biogen, Merck Serono, Novartis, Genzyme and Teva, which have been exclusively used for the support of research activities. A.F-H has received unrestricted funding speaking honoraria from Biogen Idec and Pfizer. N.D., A.J., A.M., and L.P. disclose no conflicts of interest.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The research reported in this article was partially funded through a Patient-Centered Outcomes Research Institute (PCORI) Award (MS-1511-33196). The statements in this article are solely the responsibility of the authors and do not necessarily represent the views of the Patient-Centered Outcomes

Research Institute (PCORI), its Board of Governors or Methodology Committee.

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