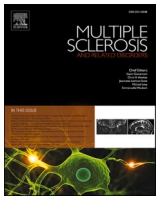
Multiple Sclerosis and Related Disorders 71 (2023) 104556

Contents lists available at ScienceDirect

Multiple Sclerosis and Related Disorders

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



Predictors of hospitalization due to infection in rituximab-treated MS patients

Jakob Rishovd Karlowicz [a,1], Mattias Klakegg [a,1], Jan Harald Aarseth [b], Lars Bø [a,c], Kjell-Morten Myhr [a,c], Hilde Marie Torgauten [a,c], Øivind Torkildsen [a,c], Stig Wergeland [a,b,c,*]

[a] Department of clinical medicine, University of Bergen, Norway
[b] Norwegian MS-registry and biobank, Dept of Neurology, Haukeland University Hospital, Bergen, Norway
[c] Neuro-SysMed, Haukeland University Hospital, Bergen, Norway

ARTICLE INFO

Keywords:
Multiple sclerosis
Rituximab
Serious infections
Hospitalization
Hypogammaglobulinemia

ABSTRACT

Background: Rituximab is extensively used off-label to treat multiple sclerosis (MS), and long-term vigilance for adverse events is needed. This study was conducted to determine frequencies and predictors of hematological adverse events, including hypogammaglobulinemia, severe lymphopenia, neutropenia, and infections leading to hospitalization.

Methods: This retrospective cohort study included all patients with MS initiating rituximab treatment at Haukeland University Hospital between January 1st, 2017, and July 1st, 2021. Patients were followed by clinical monitoring and repeated blood sampling every six months. Clinical outcomes and laboratory results were retrieved from the Norwegian MS Registry and Biobank and the patient administrative system at Haukeland University Hospital.

Results: Five hundred and fifty-six patients were included, 515 with relapsing-remitting MS (RRMS) and 41 with progressive MS. Overall, 33 patients (5.9%) experienced 56 episodes of infections requiring hospital admission. Sixty patients (10.8%) had confirmed hypogammaglobulinemia, 17 (3.1%) had confirmed severe lymphopenia, and 10 (1.8%) had confirmed severe neutropenia. Predictors of infection requiring hospital admission were progressive MS (adjusted OR (aOR): 4.81; 95%CI: 1.25-18.48), duration of treatment with rituximab (aOR: 1.52; 95%CI: 1.11-2.09) and confirmed severe lymphopenia (aOR: 13.58; 95%CI: 3.41-54.06) and neutropenia (aOR: 13.40; 95%CI: 2.93-61.25). Of the hematological abnormalities, only hypogammaglobulinemia was associated with treatment duration (aOR: 1.35; 95%CI: 1.09-1.69).

Conclusion: The risk of hospitalization due to infection is associated with time on rituximab treatment, in patients with lympho- or neutropenia, and in patients with primary progressive MS. We observed a time-dependent decline in IgG values, in contrast to neutrophil and lymphocyte count, suggesting a cumulative dose-dependent response. These predictors can assist clinicians in assessing and monitoring MS patients receiving rituximab.

1. Introduction

Rituximab is a monoclonal antibody targeting CD20+ B lymphocytes similar to ocrelizumab and ofatumumab. Anti-CD20 antibodies are effective in treating several inflammatory neurological diseases, including multiple sclerosis (MS) (Granqvist et al., 2018; Hauser et al., 2017; Hauser et al., 2020; Hauser et al., 2008; Hawker et al., 2009; Montalban et al., 2017; Salzer et al., 2016). Studies on the effect and safety of anti-CD20 antibodies in treating patients with autoimmune diseases and hematological cancers show that many patients develop hypogammaglobulinemia and neutropenia, which again increase the risk of infections (Barmettler et al., 2018; Md Yusof et al., 2019; Tesfa and Palmblad, 2011; van Vollenhoven et al., 2013). The same has been found in recent studies on rituximab- and ocrelizumab-treated MS patients (Hauser et al., 2021; Perriguey et al., 2022; Vollmer et al., 2020; Zoehner et al., 2019). In addition to hypogammaglobulinemia and

* Corresponding author at: Department of Neurology, Haukeland University Hospital, 5021 Bergen, Norway.
E-mail address: stig.wergeland@helse-bergen.no (S. Wergeland).
[1] Karlowicz, J.R. and Klakegg, M. contributed equally to this work, and are co-first authors.

https://doi.org/10.1016/j.msard.2023.104556
Received 9 September 2022; Received in revised form 30 December 2022; Accepted 7 February 2023
Available online 11 February 2023

neutropenia, lymphopenia has been reported as an individual risk factor for infection in rituximab-treated MS and NMOSD-patients (Vollmer et al., 2020). A higher risk of infection has been reported for treatment with rituximab, compared to other commonly used disease-modifying treatments (DMTs) in MS (Luna et al., 2020).

In MS, studies on the long-term safety of rituximab are warranted, due to off-label use in many countries. Therefore, we have performed a single-center retrospective cohort study on the risk of hospitalization due to infectious diseases in rituximab-treated MS patients, and how this risk is associated with patient clinical characteristics, hypogammaglobulinemia, and white blood cell counts.

2. Material and methods

2.1. Study population and design

This study is a retrospective cohort study of all MS patients who initiated rituximab treatment from January 2017 to July 2021 at the Department of Neurology, Haukeland University Hospital, Bergen, Norway. Participants were identified through the Norwegian MS Registry and Biobank, and followed by clinical monitoring and repeated blood sampling, including differential white blood cell counts and immunoglobulin (IgG, IgM, and IgA) quantification every six months for a median of 32 months. The standard regimen of rituximab treatment was a single infusion of 1000 mg intravenously at initiation, and subsequently 500 mg every six months. Only minor adjustments of the infusion schedule were implemented during the COVID-19 pandemic.

2.2. Data collection

MS cases were identified from the Norwegian MS Registry and Biobank, including information on age, sex, date of disease onset, previous DMTs, Expanded Disability Status Scale (EDSS) score at baseline, MS disease phenotype at inclusion (relapsing-remitting or progressive disease), dates of rituximab initiation and discontinuation and death during follow-up. From January 1st, 2017, until December 31st, 2021, all available hospital laboratory data were retrieved from the patient records, including white blood cell counts with differential counts, IgG-, IgA- and IgM concentrations. In addition, data on hospitalizations due to infection were automatically extracted from the electronic medical records, including International Classification of Diseases-10 (ICD-10) codes, admission- and discharge dates. If an ICD-10 code for an infectious disease was registered either as primary- or bi-diagnosis, the medical records from each of the identified hospitalizations were reviewed manually to verify that the main reason for hospitalization was indeed an infection. Infections were categorized by site of infection. We categorized previously received DMT-therapies into treatment naïve; platform therapy (interferon beta or glatiramer acetate), teriflunomide, dimethyl fumarate, natalizumab, fingolimod; or induction therapy (alemtuzumab, cladribine, mitoxantrone, or hematopoietic stem cell transplantation). We defined baseline laboratory measures from blood samples collected closest to rituximab initiation, within one year prior to start. Observation time was set from the date of treatment initiation until either the end of follow-up or six months after the patient ceased the treatment, or six months after the longest treatment period for patients that had paused their therapy for more than twelve months. The cause of treatment termination was collected by reviewing the medical record of patients who discontinued therapy with rituximab. The cumulative dose of rituximab was estimated based on the standard treatment regimen of 1000 mg rituximab as the first dose, followed by 500 mg at each subsequent infusion every six months.

2.3. Outcome measures

The primary outcome was hospitalization due to infection. Secondary outcomes were hematological laboratory abnormalities defined as 1) hypogammaglobulinemia with IgG values <6 g/liter, 2) severe lymphopenia $<0.5x10^9$ cells/liter (corresponding to Common Terminology Criteria for Adverse Events (CTCAE) 3 and above) and 3) severe neutropenia $<1.0x10^9$ cells/liter (CTCAE grade 3 and above). IgM and IgA values were considered low when <0.3 g/liter and <0.8 g/liter, respectively.

To account for analytical and biological variance, deviating laboratory values were defined as confirmed abnormalities when having two consecutive blood samples below lower limit of normal (LLN) or when the last registered blood sample was below LLN. For confirming severe lymphopenia and neutropenia, the maximum time between consecutive samples was set to 3 months, as these cell counts can change considerably within each patient in a short period of time. Longer intervals could thus decrease the identification of lympho- or neutropenia. The minimum time between two consecutive samples was set to be 3 months or more to confirm hypogammaglobulinemia, to identify patients with sustained hypogammaglobulinemia. Only confirmed laboratory abnormalities that occurred before hospitalization were used in the analyses of associations between hematological abnormalities and severe infection.

2.4. Statistical methods

Analysis of baseline characteristics, follow-up characteristics, and outcomes were based on descriptive statistics, i.e., frequencies, mean, median and range. Infection-free survival was analyzed by Kaplan-Meier survival curves. Patients with treatment termination, death, or end of follow-up were censored in the analysis. Stepwise logistic regression was performed to analyze the risk of infection leading to hospitalization in patients with confirmed hypogammaglobulinemia, severe lymphopenia, and neutropenia (Table 4). Variables with a p-value <0.20 or of clinical relevance were included in the analyses. Covariates included treatment duration, gender, age per year, disease duration, type of MS, and all previously received DMTs. Similarly, this was done to assess risk factors for hypogammaglobulinemia, severe lymphopenia, and neutropenia. Statistical analyses were conducted in SPSS version 26 (IBM Corp., Armonk, NY).

2.5. Approval and patient consent

All included patients had consented to participation in the Norwegian MS Registry and Biobank. The study was approved by the Regional Committee for Medical Research Ethics, Western Norway (REK no 87388). The study was undertaken with the understanding and written consent of each subject, and that the study conforms with World Medical Association Declaration of Helsinki.

2.6. Data availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

3. Results

3.1. Patient characteristics

A total of 593 patients with MS received rituximab at Haukeland University Hospital during the defined study period, of whom 556 (93.3 %) had consented to participation in the Norwegian MS Registry and Biobank. 515 (92.6%) had relapsing-remitting MS (RRMS) and 41 (7.4%) had progressive forms of MS (21 primary progressive MS (PPMS), 20 secondary progressive MS (SPMS)). Baseline characteristics are provided in Table 1. EDSS scores recorded within one year before rituximab initiation existed for 253 patients (45.5%). Mean and median cumulative doses of rituximab were 3304 mg and 3500 mg, respectively,

Table 1
Baseline and follow-up characteristics.

Baseline characteristics	Total study population n=556		Type of MS RRMS n=515		Progressive MS n=41	
Sex						
Female, n, %	398	71.2%	378	73.4%	20	48.8%
Male, n, %	158	28.2%	137	26.6%	21	51.2%
Age (years), mean, SD	42.1	12.0	41.0	11.4	56.4	10.9
Disease duration (years), mean, SD	8.2	9.1	7.6	8.7	15.3	11.6
EDSS at inclusion, mean, SD	2.2	1.8	2.0	1.5	5.3	1.7
Death, n, %	5	0.8%	1	0.2%	4	9.8%
Observation time (months), mean, SD	32.8	15.0	33.2	15.5	29.0	15.4
Estimated cumulative dose (mg), mean, SD	3303.9	1276.3	3332	1271.6	2951	1298.1
Estimated infusions, median, SD	5.6	2.6	6	2.5	4.9	2.6
Rituximab pause >365 days, n, %	17	3.1%	16	3.1%	1	2.4%
Rituximab discontinuation, n, %	46	8.3%	38	7.4%	8	19.5%
Previous DMT						
Treatment naïve, n, %	234	42.1%	210	40.8%	24	58.5%
Interferon beta and glatiramer acetate, n, %	192	34.5%	180	35.0%	12	29.3%
Teriflunomide, n, %	103	18.3%	100	19.4%	3	7.3%
Dimethyl fumarate, n, %	115	21.0%	111	21.6%	4	9.8%
Natalizumab, n, %	70	12.4%	65	12.5%	5	12.2%
Fingolimod, n, %	113	20.2%	105	20.4%	8	19.5%
Induction treatment*, n, %	23	4.1%	21	4.1%	2	4.9%

n = number, SD = standard deviation, MS = multiple sclerosis, RRMS = relapsing remitting MS, DMT = disease modifying therapy, EDSS = Expanded Disability Status Scale.
* Induction treatment consisted of alemtuzumab, HSCT, cladribine, or mitoxantrone.

and ranged from 1000 mg to 5500 mg.

In total, 50 patients (9.0%) discontinued rituximab during the study period, mainly due to adverse events (n=26, 4.7%) such as recurrent infections (n=13, 2.3%) and muscle- and skeletal pain (n=13, 2.3%). Three patients (0.5%) experienced disease activity (relapse, n=2, disease progression in SPMS, n=1). Three patients (0.5%) had possible serious adverse reactions, consisting of myocardial infarction (n=1), severe acute allergic reaction (n=1) and atrial flutter (n=1). Five patients (0.9%) died during follow-up, the registered causes were Alzheimer's disease (n=1), suicide (n=1), covid-19 infection (n=1) and unknown (n=2).

Near half of the study population (n=234, 42.1%) were treatment-naïve before rituximab treatment initiation, and the majority of them (n=204, 87.2%) were diagnosed with MS within three months prior to treatment start. Table 1 shows the number of all previously used DMTs. The latest DMT received prior to rituximab initiation was interferon-beta 1a/1b or glatiramer acetate (n=43, 7.7%), dimethyl fumarate (n=69, 12.4%), fingolimod (n=68, 12.2%), teriflunomide (80, 14.4%), natalizumab (n=43, 7.7%), and induction therapy (n=2, 0.4%. Both patients had been treated with HSCT). In total, 129 patients (23.2%) received one previous DMT, 116 (20.9%) two, and 77 (13.8%) three or more.

3.2. Serious infections

Infection outcomes are provided in Tables 2 and 4 and Fig. 1. Overall, 57 infections requiring hospitalization were identified during follow-up in 33 (5.9%) unique patients, of whom 19 (57.6%) had multiple admissions (Table 2). This corresponds to an incidence rate of 21.7 per 1000 person-years. Crude incidence rates of infection did not vary noticeably by treatment year up to five years, although the power to detect a difference was low, especially with longer treatment duration (data not shown). The mean time to first infection requiring hospitalization was 20.2 (± 16.9) months, ranging from <1 to 57 months. The most frequent categories of infections were lower and upper urinary tract infections (n=18, 32.1% and n=9, 16.1% respectively), respiratory tract infections (n=12, 21.4%) and sepsis (n=9, 16.1%). Logistic regression analysis for assessing the risk of infections requiring hospitalization showed an increased odds ratio (OR) for a progressive type of MS (adjusted OR (aOR): 4.81; 95% CI: 1.25-18.5) and treatment duration (per year) (aOR: 1.52; 95% CI: 1.11-2.09). Age and disease duration were not associated with the risk of serious infection. Previous use of dimethyl fumarate showed a protective effect against developing infection (aOR: 0.24; 95% CI: 0.07-0.86). On the contrary, previous use of any other DMTs revealed no significant effect on the risk of infections requiring hospitalization.

Table 2
Serious infections requiring hospital admission among multiple sclerosis patients receiving rituximab therapy.

Outcomes	Total study population n=556	Type of MS RRMS n=515	Progressive MS n=41
Hospital admission due to infection			
Episodes	57	41	16
Patients	33 (5.9%)	28 (5.4%)	5 (12.2%)
Patients with ≥2 episodes	19 (3.4%)	15 (2.9%)	4 (9.7%)
Types of infection			
Lower urinary tract infection	18 (32.1%)		
Lower respiratory tract infection	12 (21.4%)		
Sepsis	9 (16.1%)		
Upper urinary tract infection	9 (16.1%)		
Upper respiratory tract infection	3 (5.4%)		
Gastrointestinal infection	2 (3.6%)		
Other	4 (7.0%)		

Other serious infections included 1 dacryocystitis, 1 gynecologic infection, 1 ehrlichiosis, and 1 intervertebral disc infection.
n = number, MS = multiple sclerosis, RRMS = relapsing remitting MS.

3.3. Hematological laboratory abnormalities

An overview of hematological laboratory abnormalities and predictors are shown in Tables 3 and 4 and Fig. 1 (Panel D, F, H). Thirty-three patients (5.9%) experienced severe lymphopenia, of whom 17 (3.1%) with confirmed analyses, as defined in the method section. The mean time to first confirmed lymphopenia was 20.2 (±20.8) months, with a range of <1 to 51 months. Risk factors for severe lymphopenia were increasing age (aOR: 1.06; 95%CI: 1.01-1.12) and the use of induction therapy prior to rituximab (OR: 5.56; 95%CI: 1.48-20.91, aOR: 4.57; 95%CI: 0.90-23.12).

Of 79 patients (14.2%) with hypogammaglobulinemia, 60 (10.8%) were confirmed at the next blood sample time-point. The mean time to the first confirmed episode was 19.2 (±14.0) months, ranging from <1 to 52 months. Risk factors for developing hypogammaglobulinemia were treatment duration (per year) (aOR: 1.35; 95%CI: 1.09-1.69) and use of natalizumab prior to rituximab (aOR: 2.54; 95%CI: 1.12-5.76). Previous use of interferon beta-1a/1b or glatiramer acetate revealed a lower risk for hypogammaglobulinemia (aOR: 0.36; 95%CI: 0.16-0.83).

Furthermore, 14 patients (2.5%) had severe neutropenia, of whom

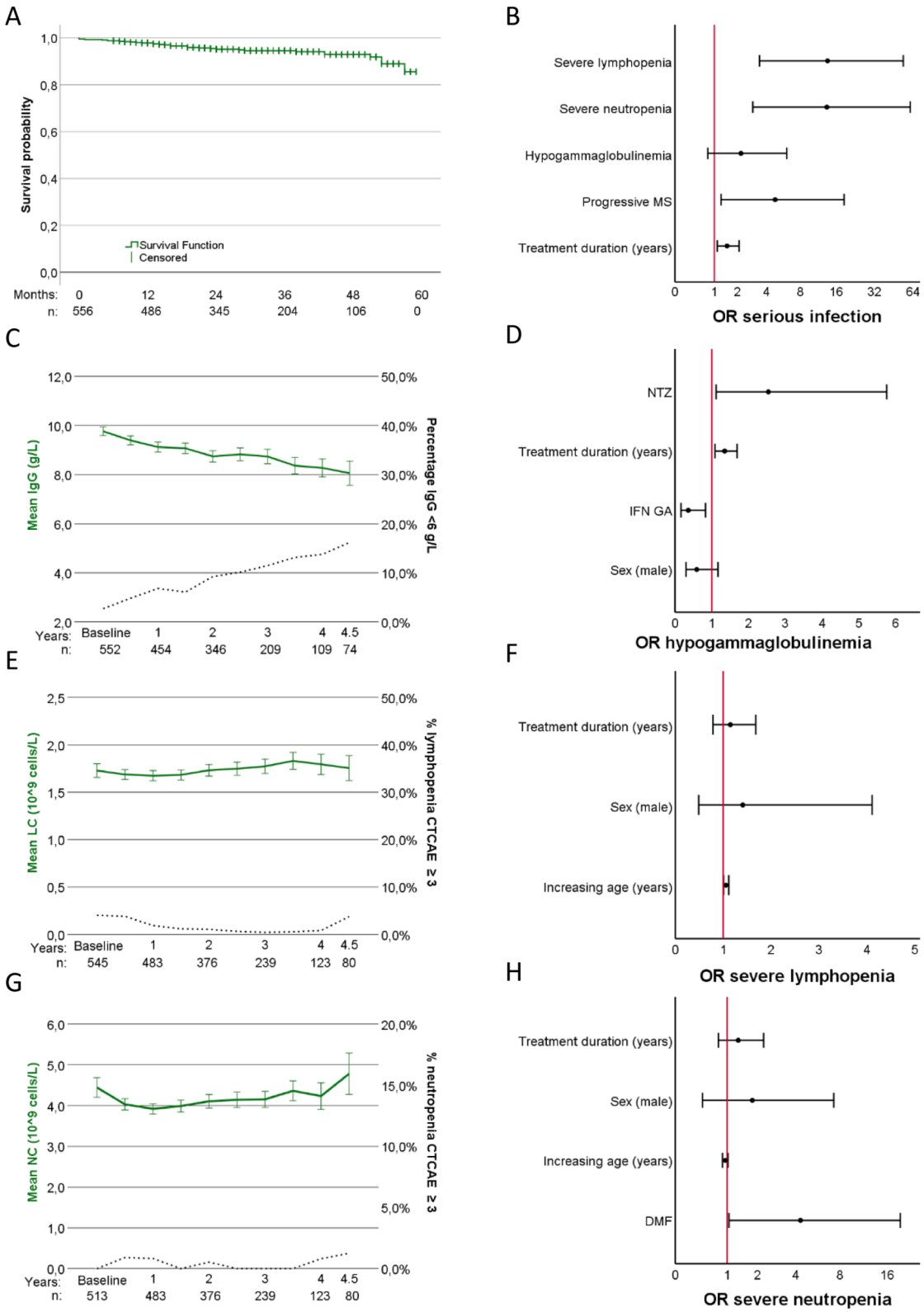


Fig. 1. Overview of main outcomes and their predictors. Time in months to first serious infection requiring hospital admission (A), predictors of serious infection (B), mean IgG value with standard deviation and proportion of patients with IgG below 6 g/L by year of rituximab treatment (C), hypogammaglobulinemia (D), mean lymphocyte count with standard deviation and proportion of patients with lymphopenia CTCAE grade 3 and above by year of rituximab treatment (E), severe lymphopenia (F), mean neutrophil count with standard deviation and proportion of patients with neutropenia CTCAE grade 3 and above by year of rituximab treatment (G), and severe neutropenia (H). Data in B, D, F, and H are adjusted odds ratios (OR).
n = number, IgG = immunoglobulin G, LC = lymphocyte count, NC = Neutrophil count, NTZ = previous use of Natalizumab, IFN GA = previous use of either interferon beta-1a/1b or glatiramer acetate and DMF = dimethyl fumarate, CTCAE = Common Terminology Criteria for Adverse Events.

Table 3
Hematological abnormalities among multiple sclerosis patients receiving rituximab therapy.

	Total study population n=556	Type of MS RRMS n=515	Progressive MS n=41
Hypogammaglobulinemia, <6 g/l IgG			
Confirmed	60 (10.8%)	56 (10.9%)	4 (9.8%)
Total	79 (14.2%)	75 (14.6%)	4 (9.8%)
Lymphopenia <0.5× 10^9 cells/l			
Confirmed	17 (3.1%)	17 (3.3%)	0
Total	34 (6.1%)	31 (6.0%)	3 (7.3%)
Neutropenia <1.0 × 10^9 cells/l			
Confirmed	10 (1.8%)	9 (1.5%)	1 (2.4%)
Total	14 (2.5%)	13 (2.5%)	1 (2.4%)
IgM <0.3 g/l unconfirmed	144 (25.9%)	136 (26.4%)	8 (19.6%)
IgA <0.8 g/l unconfirmed	69 (12.4%)	65 (12.6%)	4 (9.8%)

Hematological abnormalities were defined as confirmed when having two consecutive blood samples below the set threshold or when the last registered blood sample was pathological. The total includes both confirmed and unconfirmed abnormalities.
n = number, MS = multiple sclerosis, RRMS = relapsing remitting MS, Ig = immunoglobulin.

10 (1.8%) were confirmed. The mean time to first confirmed episode was 22.5 (±20.7) months with a range of <1 to 57 months. Of the ten patients with confirmed neutropenia, six continued standard treatment regimens. The dosing interval was increased for two patients due to malignant melanoma and recurrent infections, respectively. Rituximab treatment was terminated due to non-neutropenia-related events in two patients. All cases of confirmed severe neutropenia occurred within 1-4 months after the latest rituximab infusion. Previous use of dimethyl fumarate showed an increased risk of severe neutropenia (aOR: 4.49; 95%CI: 1.05-19.24). Rituximab treatment duration was not a risk factor for acquiring severe neutropenia (aOR: 1.32; %CI: 0.78-2.26) or severe lymphopenia (aOR: 1.15; 95%CI: 0.79-1.68).

In one patient with lymphopenia and in 21 patients with hypogammaglobulinemia, the abnormal lab finding occurred in the last available lab sample, and thus no follow-up samples were available for confirming the results. When excluding these cases the risk factors for infection did not change. Previous use of platform therapy (aOR: 0,45; 95%CI: 0.17-1.15) or natalizumab (aOR: 2.33; 95%CI: 0.93-5.86) were no longer significant risk factors for hypogammaglobulinemia, however other risk factors did not change. Previously received induction therapy (aOR: 6.38; 95%CI: 1.23-33.23) became a significant risk factor for lymphopenia, while other risk factors did not change.

3.4. Laboratory values as predictors of serious infections

Predictors of serious adverse events are demonstrated in Fig. 1 and Table 4. Six of the 17 patients (35.3%) with confirmed severe lymphopenia during follow-up experienced hospitalization due to an infection at any time. Of these, severe lymphopenia was detected in five patients at admission, and one was revealed after hospitalization and thus excluded from further calculations. Patients with severe lymphopenia had a significantly increased risk of serious infection resulting in hospitalization with an aOR of 13.58 (95%CI: 3.41-54.1). Seven of 60 patients (11.7%) with confirmed hypogammaglobulinemia experienced an infection requiring hospitalization during follow-up. Of these, five were revealed before admission, one was detected at admission, and one was revealed after hospitalization and thus excluded. When including the time relationship between confirmed hematological abnormality and hospitalization, hypogammaglobulinemia was no longer a significant predictor of serious infections (aOR:2.19; 95%CI: 0.78-6.12). However, when disregarding the time relationship, hypogammaglobulinemia was a significant risk factor for infections requiring hospitalization (aOR: 2.81; 95%CI: 1.05-7.55). Furthermore, four of the ten patients with severe neutropenia experienced an infection and were registered with neutropenic fever. These were all detected at admission. Patients with severe neutropenia had a significantly increased risk of infection requiring hospitalization (aOR: 13.40; 95%CI: 2.93-61.25).

4. Discussion

The risk of hospitalization due to infection is associated with time on rituximab treatment, in patients with lympho- or neutropenia, and in patients with primary progressive MS. Overall, 33 (5.9%) of rituximab-treated patients with MS were hospitalized due to infection (57 episodes) in the follow-up period for up to five years. The median treatment duration until first infection was 16 months. These findings are consistent with a large Swedish cohort study in RRMS patients with a similar prevalence of 4.6% (Luna et al., 2020) and two recent studies with a prevalence of 5.9% (Perriguey et al., 2022) and 6.5% (Vollmer et al., 2020). The latter showed a slightly higher prevalence, possibly caused by a high number of participants with impaired mobility (37.9%). The most common infections were respiratory and urinary tract infections, in line with a recent large review (Chisari et al., 2022).

The risk of infection leading to hospital admission was significantly elevated in patients with progressive MS, increasing treatment duration, and confirmed severe lymphopenia and neutropenia. Confounders such as age and disability may explain the 4.8-fold increased infection risk in progressive MS due to older and more disabled patients in this group. Usually, increasing age and disability correlate with risk of infection,

Table 4
Risk factors for serious infections, hypogammaglobulinemia, lymphopenia, and neutropenia among multiple sclerosis patients receiving rituximab therapy.

	Infections n=33		**Hypogammaglobulinemia n=60**		**Lymphopenia n=17**		**Neutropenia n=10**	
	OR (95% CI)*	p-value	OR (95% CI)*	p-value	OR (95% CI)*	p-value	OR (95% CI)*	p-value
Lymphopenia	**13.6 (3.41-54.1)**	**0.000**						
Hypogammaglobulinemia	2.19 (0.78-6.12)	0.136						
Neutropenia	**13.4 (2.93-61.3)**	**0.001**						
Treatment duration (y)	**1.52 (1.11-2.09)**	**0.010**	**1.35 (1.09-1.69)**	**0.007**	1.15 (0.79-1.68)	0.447	1.32 (0.78-2.26)	0.304
Sex (male)	0.48 (0.18-1.25)	0.131	0.59 (0.30-1.17)	0.131	1.41 (0.49-4.11)	0.527	1.80 (0.44-7.32)	0.414
Increasing age (y)	0.99 (0.95-1.03)	0.568	1.01 (0.98-1.04)	0.631	**1.06 (1.01-1.12)**	**0.018**	0.95 (0.88-1.02)	0.178
Progressive type of MS	**4.81 (1.25-18.5)**	**0.022**	0.82 (0.24-2.78)	0.745	0.52 (0.05-5.01)	0.568	5.77 (0.34-83.5)	0.199
Increasing disease duration (y)	0.99 (0.93-1.05)	0.718	1.02 (0.99-1.06)	0.241	0.95 (0.88-1.02)	0.174	0.96 (0.82-1.13)	0.618

Bold values indicate statistically significant values, P<0.05.
n = number, OR = odds ratio, y = year, MS = multiple sclerosis, CI = Confidence interval.
* logistic regression adjusted for previous disease-modifying therapies.

thus explaining the high OR. However, neither age per year nor age categorized as younger or above 60 years were independent risk factors for infection in our material. A confounding factor might be that the older patients are carefully selected for treatment by the clinician and thus cautiously treated due to presumed infection risk. Unfortunately, data on disability were missing in nearly half the population, and this variable was excluded from the analysis.

An elevated risk for infections in patients with severe lymphopenia and severe neutropenia is concurrent with studies on MS and rheumatic diseases (Subesinghe et al., 2020; Tesfa and Palmblad, 2011; Vollmer et al., 2020). The rather high ORs in our study may relate to the analysis of confirmed abnormal blood values (two consecutive samples under the set threshold), thus selecting patients with hospital admissions more often. Furthermore, five of the 33 patients with hospital admissions due to infection had confirmed lymphopenia, and four had confirmed neutropenia, of which all were disclosed at admission. Thus, another possible reason for the high ORs is that severe lymphopenia and neutropenia are markers for serious infection rather than predictors of serious infection, as discussed in a recent study on lymphopenia in rheumatoid arthritis (Subesinghe et al., 2020). A limitation of our study is that blood samples were taken routinely every six months, and asymptomatic episodes of neutropenia or lymphopenia would not be captured by our study design. Patients admitted to the hospital who were found to have a hematological abnormality may cause a sampling bias in our study, possibly overestimating the risk of infection requiring hospitalization when confirmed severe lymphopenia or severe neutropenia was present. An elevated risk of infection with increasing treatment duration is concurrent with previous studies on MS and patients with other diseases receiving rituximab (Barmettler et al., 2018; Hawker et al., 2009; Md Yusof et al., 2019; van Vollenhoven et al., 2013; Vollmer et al., 2020).

About 10 % of the patients were recorded with hypogammaglobulinemia, and only one-tenth of those were subsequently hospitalized due to an infection. Nevertheless, confirmed hypogammaglobulinemia at any time (i.e., no relation in time between hypogammaglobulinemia and infection) was significantly associated with an increased risk of infections, corroborating the findings in studies with the same methodological approach (Barmettler et al., 2018; Md Yusof et al., 2019; Perriguey et al., 2022; Vollmer et al., 2020). However, when analyzing the data prospectively, we found that hypogammaglobulinemia was not a significant predictor for subsequently experiencing an infection requiring hospitalization. This difference from previous studies might be due to our smaller sample size and study design that only accepted confirmed laboratory abnormalities before infection. In contrast, these previous studies defined no time relation between infection and blood sample. Importantly, the data suggest that the risk of infection when having hypogammaglobulinemia is relatively low in absolute numbers.

Hypogammaglobulinemia was the most frequent hematological abnormality observed in our study population. In concordance with previous observational data and a recent study on ocrelizumab (Vollmer et al., 2020; Zoehner et al., 2019), we found a progressive decline in IgG-value over time, suggesting a cumulative dose-dependent response. Moreover, previous use of interferon beta-1a/1b or glatiramer acetate seemed to be a protective factor against developing hypogammaglobulinemia, supporting that these drugs may increase IgG levels (Pavelek et al., 2021). On the other hand, previous use of natalizumab showed an increased risk of hypogammaglobulinemia in our population, also confirming previous real-world observations (Cuculiza Henriksen et al., 2021).

Overall, a low number of patients experienced severe lymphopenia and neutropenia during follow-up. In contrast to IgG levels, neutrophil and lymphocyte counts did not show a dose-dependent response to rituximab, consistent with previous studies (Graves et al., 2014; Rigal et al., 2020; Vollmer et al., 2020). For severe lymphopenia, increasing age (per year) was the only significant risk factor. It is well known that rituximab may lead to neutropenia, the majority being of the late-onset type (Moore, 2016), and thus in line with our findings. Only previous use of dimethyl fumarate was shown to increase the risk of severe neutropenia, possibly influenced by the small sample size. Two patients experienced severe neutropenia twice, whereas the other eight patients only had one episode, suggesting that recurrence of neutropenia on rituximab is rare. The absolute number of patients with severe neutropenia was low; however, clinicians need to be aware of the possibility of neutropenic fever.

The main strength of our study is the methodological approach to confirm abnormal laboratory results and the criteria of time relationship between an infection requiring hospitalization and laboratory outcomes, allowing a prospective and clinically relevant design. Another strength is that the patients were followed at a single center, with standardized dosing- and monitoring regimen. Furthermore, patients were recruited through the Norwegian MS registry, which has a high response rate, limiting selection bias and increasing case validation.

There are several limitations in the present study. Importantly, the absence of a control group and the study's observational design prevent firm conclusions regarding the incidence of infections and hematological abnormalities directly related to treatment. Furthermore, it is possible that an elevated risk of infections or pathological blood samples could be detected with a longer treatment duration and follow-up. In addition, data on mild infections were lacking as we do not have access to patient records from primary care. Thus, overall infection risk may be underestimated. Importantly, a larger sample size may be required to avoid biases when assuming risk factors, as the absolute number of patients with adverse events was low. When assessing the associations between lympho- and neutropenia, there is a risk of surveillance bias since samples typically are collected when evaluating symptoms that can preceed an infection leading to hospitalization. Hematological status is not routinely collected for safety monitoring other than every six months. Lastly, relevant confounding factors were not adjusted for in the analysis, including disability levels, body mass index, comorbidities, smoking status, and socioeconomic factors, including education.

In conclusion, our data indicate that rituximab is a safe therapy for MS patients, at least for a treatment period up to 4.5 years. The frequency of severe infection requiring hospital admission, severe lymphopenia, and severe neutropenia was low. The most common serious adverse event was hypogammaglobulinemia, but the risk of serious infection among patients with hypogammaglobulinemia was still low. Nevertheless, serum IgG levels showed a decreasing trend over time, indicating that monitoring of IgG levels during treatment is needed. Further studies should investigate the long-term effects on IgG levels and the risk of infections.

Credit author statements

Jakob Rishovd Karlowicz: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data. **Mattias Klakegg**: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data. **Jan Harald Aarseth**: Major role in the acquisition of data; Analysis or interpretation of data. **Lars Bø**: Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data. **Hilde Marie Torgauten**: Major role in the acquisition of data; Study concept or design. **Øivind Torkildsen**: Major role in the acquisition of data; Study concept or design. **Kjell-Morten Myhr**: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data. **Stig Wergeland**: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data

Conflict of interests

J.R. Karlowicz report no disclosures relevant to the manuscript.

M. Klakegg report no disclosures relevant to the manuscript.

J.H. Aarseth report no disclosures relevant to the manuscript.

L. Bø has received unrestricted research grants to his institution and/or scientific advisory board or speakers honoraria from Biogen, Genzyme, Merck, NOvartis, Roche, and Teva, and has participated in clinical trials organized by Biogen, Merck, Novartis and Roche.

H. Torgauten report no disclosures relevant to the manuscript.

Ø. Torkildsen has received speaker honoraria from and served on scientific advisory boards for Biogen, Sanofi-Aventis, Merck and Novartis.

K-M. Myhr has received unrestricted research grants to his institution, scientific advisory board or speaker honoraria from Biogen, Novartis, and Sanofi, and has participated in clinical trials organized by Biogen, Merck, Novartis, Roche and Sanofi.

S. Wergeland has received honoraria for lecturing and advice from Biogen, Janssen and Sanofi.His department has received unrestricted institutional grants from Biogen, Merck and Novartis. He is currently collaborating on research projects funded by Merck and EMD Serono.

References

Barmettler, S., Ong, M.S., Farmer, J.R., Choi, H., Walter, J., 2018. Association of immunoglobulin levels, infectious risk, and mortality with rituximab and hypogammaglobulinemia. JAMA Netw. Open 1 (7), e184169.

Chisari, C.G., Sgarlata, E., Arena, S., Toscano, S., Luca, M., Patti, F., 2022. Rituximab for the treatment of multiple sclerosis: a review. J. Neurol. 269 (1), 159–183.

Cuculiza Henriksen, A., Ammitzbøll, C., Petersen, E.R., McWilliam, O., Sellebjerg, F., von Essen, M.R., Romme Christensen, J., 2021. Natalizumab differentially affects plasmablasts and B cells in multiple sclerosis. Mult. Scler. Relat. Disord. 52, 102987.

Granqvist, M., Boremalm, M., Poorghobad, A., Svenningsson, A., Salzer, J., Frisell, T., Piehl, F., 2018. Comparative effectiveness of rituximab and other initial treatment choices for multiple sclerosis. JAMA Neurol. 75 (3), 320–327.

Graves, J., Vinayagasundaram, U., Mowry, E.M., Matthews, I.R., Marino, J.A., Cheng, J., Waubant, E., 2014. Effects of rituximab on lymphocytes in multiple sclerosis and neuromyelitis optica. Mult. Scler. Relat. Disord. 3 (2), 244–252.

Hauser, S.L., Bar-Or, A., Comi, G., Giovannoni, G., Hartung, H.P., Hemmer, B., Lublin, F., Montalban, X., Rammohan, K.W., Selmaj, K., Traboulsee, A., Wolinsky, J.S., Arnold, D.L., Klingelschmitt, G., Masterman, D., Fontoura, P., Belachew, S., Chin, P., Mairon, N., Garren, H., Kappos, L., 2017. Ocrelizumab versus interferon Beta-1a in relapsing multiple sclerosis. N. Engl. J. Med. 376 (3), 221–234.

Hauser, S.L., Kappos, L., Arnold, D.L., Bar-Or, A., Brochet, B., Naismith, R.T., Traboulsee, A., Wolinsky, J.S., Belachew, S., Koendgen, H., Levesque, V., Manfrini, M., Model, F., Hubeaux, S., Mehta, L., Montalban, X., 2020. Five years of ocrelizumab in relapsing multiple sclerosis: OPERA studies open-label extension. Neurology 95 (13), e1854–e1867.

Hauser, S.L., Kappos, L., Montalban, X., Craveiro, L., Chognot, C., Hughes, R., Koendgen, H., Pasquarelli, N., Pradhan, A., Prajapati, K., Wolinsky, J.S., 2021. Safety of Ocrelizumab in patients with relapsing and primary progressive multiple sclerosis. Neurology 97 (16), e1546–e1559.

Hauser, S.L., Waubant, E., Arnold, D.L., Vollmer, T., Antel, J., Fox, R.J., Bar-Or, A., Panzara, M., Sarkar, N., Agarwal, S., Langer-Gould, A., Smith, C.H., 2008. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. N. Engl. J. Med. 358 (7), 676–688.

Hawker, K., O'Connor, P., Freedman, M.S., Calabresi, P.A., Antel, J., Simon, J., Hauser, S., Waubant, E., Vollmer, T., Panitch, H., Zhang, J., Chin, P., Smith, C.H., 2009. Rituximab in patients with primary progressive multiple sclerosis: results of a randomized double-blind placebo-controlled multicenter trial. Ann. Neurol. 66 (4), 460–471.

Luna, G., Alping, P., Burman, J., Fink, K., Fogdell-Hahn, A., Gunnarsson, M., Hillert, J., Langer-Gould, A., Lycke, J., Nilsson, P., Salzer, J., Svenningsson, A., Vrethem, M., Olsson, T., Piehl, F., Frisell, T., 2020. Infection risks among patients with multiple sclerosis treated with fingolimod, natalizumab, rituximab, and injectable therapies. JAMA Neurol. 77 (2), 184–191.

Md Yusof, M.Y., Vital, E.M., McElvenny, D.M., Hensor, E.M.A., Das, S., Dass, S., Rawstron, A.C., Buch, M.H., Emery, P., Savic, S., 2019. Predicting severe infection and effects of hypogammaglobulinemia during therapy with rituximab in rheumatic and musculoskeletal diseases. Arthrit. Rheumatol. 71 (11), 1812–1823.

Montalban, X., Hauser, S.L., Kappos, L., Arnold, D.L., Bar-Or, A., Comi, G., de Seze, J., Giovannoni, G., Hartung, H.P., Hemmer, B., Lublin, F., Rammohan, K.W., Selmaj, K., Traboulsee, A., Sauter, A., Masterman, D., Fontoura, P., Belachew, S., Garren, H., Mairon, N., Chin, P., Wolinsky, J.S., 2017. Ocrelizumab versus placebo in primary progressive multiple sclerosis. N. Engl. J. Med. 376 (3), 209–220.

Moore, D.C., 2016. Drug-induced neutropenia: a focus on rituximab-induced late-onset neutropenia. P t 41 (12), 765–768.

Pavelek, Z., Novotny, M., Soucek, O., Krejsek, J., Sobisek, L., Sejkorova, I., Masopust, J., Kuca, K., Valis, M., Klimova, B., Stourac, P., 2021. Multiple sclerosis and immune system biomarkers: Novel comparison in glatiramer acetate and interferon beta-1a-treated patient groups. Mult. Scler. Relat. Disord. 53, 103082.

Perriguey, M., Maarouf, A., Stellmann, J.P., Rico, A., Boutiere, C., Demortiere, S., Durozard, P., Pelletier, J., Audoin, B., 2022. Hypogammaglobulinemia and infections in patients with multiple sclerosis treated with rituximab. Neurol. Neuroimmunol. Neuroinflamm. 9 (1).

Rigal, J., Ciron, J., Lépine, Z., Biotti, D., 2020. Late-onset neutropenia after RITUXIMAB therapy for multiple sclerosis, neuromyelitis optica spectrum disorders and MOG-antibody-associated diseases. Mult. Scler Relat. Disord. 41, 102019.

Salzer, J., Svenningsson, R., Alping, P., Novakova, L., Björck, A., Fink, K., Islam-Jakobsson, P., Malmeström, C., Axelsson, M., Vågberg, M., Sundström, P., Lycke, J., Piehl, F., Svenningsson, A., 2016. Rituximab in multiple sclerosis: a retrospective observational study on safety and efficacy. Neurology 87 (20), 2074–2081.

Subesinghe, S., Kleymann, A., Rutherford, A.I., Bechman, K., Norton, S., Benjamin Galloway, J., 2020. The association between lymphopenia and serious infection risk in rheumatoid arthritis. Rheumatology (Oxford) 59 (4), 762–766.

Tesfa, D., Palmblad, J., 2011. Late-onset neutropenia following rituximab therapy: incidence, clinical features and possible mechanisms. Expert Rev. Hematol. 4 (6), 619–625.

van Vollenhoven, R.F., Emery, P., Bingham 3rd, C.O., Keystone, E.C., Fleischmann, R.M., Furst, D.E., Tyson, N., Collinson, N., Lehane, P.B., 2013. Long-term safety of rituximab in rheumatoid arthritis: 9.5-year follow-up of the global clinical trial programme with a focus on adverse events of interest in RA patients. Ann. Rheum. Dis. 72 (9), 1496–1502.

Vollmer, B.L., Wallach, A.I., Corboy, J.R., Dubovskaya, K., Alvarez, E., Kister, I., 2020. Serious safety events in rituximab-treated multiple sclerosis and related disorders. Annal. Clin. Transl. Neurol. 7 (9), 1477–1487.

Zoehner, G., Miclea, A., Salmen, A., Kamber, N., Diem, L., Friedli, C., Bagnoud, M., Ahmadi, F., Briner, M., Sédille-Mostafaie, N., Kilidireas, C., Stefanis, L., Chan, A., Hoepner, R., Evangelopoulos, M.E., 2019. Reduced serum immunoglobulin G concentrations in multiple sclerosis: prevalence and association with disease-modifying therapy and disease course. Ther. Adv. Neurol. Disord. 12, 1756286419878340.