MAJOR ARTICLE

Incidence and Predictive Risk Factors of Infective Events in Patients With Multiple Sclerosis Treated With Agents Targeting CD20 and CD52 Surface Antigens

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Objective. Monoclonal antibodies (MAbs) directed against the CD20 and CD52 antigens are used increasingly in patients with multiple sclerosis (MS). Several life-threatening opportunistic infections have been reported in postmarketing case series. The aim of this study was to investigate the incidence of infections and associated prognostic factors during the first year of treatment in patients receiving anti-CD20 (ocrelizumab or rituximab) or anti-CD52 MAbs (alemtuzumab).

Methods. A retrospective study was conducted in patients with MS referring to the Neurodegenerative Diseases Center at the University of Naples Federico II who received MAbs between November 2015 and June 2018.

Results. A total of 163 patients were enrolled. Approximately 40% of patients experienced lymphocytopenia during treatment. Eighty-six infective events were reported in 67 patients (41%). Bacterial infections were significantly more frequent with anti-CD20, whereas viral infections prevailed with alemtuzumab. Cytomegalovirus reactivation rates were significantly higher in the alemtuzumab group than in patients on anti-CD20 (51% vs 6%, $P < .001$). The overall annualized infection rate was 1.1 per patient-year, higher in patients on anti-CD52 versus those on anti-CD20 regimens (1.5 vs 0.8 per patient-year). Alemtuzumab treatment, prior exposure to ≥2 MS drugs, and iatrogenic immune impairment significantly and independently predicted an infection event (adjusted hazard ratio [aHR], 2.7; $P = .013$; aHR, 1.7; $P = .052$; and aHR, 2.9; $P = .004$; respectively).

Conclusions. Given their considerable infection risk, MS patients receiving MAbs should undergo timely follow up and tailored preventive interventions. Anti-CD52–based treatment, prior exposure to MS drugs, and on-treatment immune impairment are significant predictive factors of infection and their evaluation could help clinicians to stratify a patient's risk of infection.

Key words. Alemtuzumab; CMV; infection; monoclonal antibodies; multiple sclerosis.

INTRODUCTION

The advent of monoclonal antibodies (MAbs)-based regimens was a milestone in the treatment of multiple sclerosis (MS). However, although the efficacy of MAbs is well established, the magnitude of their infective risk remains a matter of debate [1, 2]. Indeed, the risk of infection depends both on the mechanisms of action of drugs, and host-related factors (age, comorbidities, exposure to corticosteroids or to other immune-modulating agents, neurologic dysfunctions, and mobility). As

yet, there is no standardized consensus regarding pretreatment testing, vaccinations, patient education or counseling before and during therapy, or infection monitoring strategies [3, 4].

In addition to cases of John Cunningham virus (JCV) infection and progressive multifocal leukoencephalopathy (PML) reported in patients on anti-VLA4 directed agents (ie, natalizumab), major infectious complications, in terms of both frequency and severity, have been observed in patients on MAbs that target lymphoid cell surface antigens, namely anti-CD52 (alemtuzumab [ALM]) and anti-CD20 agents (ocrelizumab [OCR] and rituximab [RTX]) [1, 5]. Despite the relatively low infection rate recorded in randomized controlled trials [6–8], potentially life-threatening infections have been reported in patients receiving these drugs [9–14].

We conducted a retrospective study to evaluate the incidence and predictive factors of infectious adverse events (IAE) in patients affected by MS-related disorders during the first year of treatment with MAbs directed against CD20 and CD52 antigens.

METHODS

Patients

All patients referring to the Centre of Neurodegenerative Diseases of Naples at the University of Naples Federico II who started ALM (Lemtrada®), OCR (Octrevus®) or RTX (Mabthera®, off-label use) from November 1, 2015, to June 1, 2018, were retrospectively recruited. Inclusion criteria were a diagnosis of MS spectrum disorder (relapsing-remitting MS, primary-progressive MS, secondary-progressive MS, or neuromyelitis optica), age ≥18 years, and availability of clinical and laboratory follow-up data. Ongoing infection at enrollment was an exclusion criterion. The MAb schedules are reported in the online supplementary material. Of note, in Italy, ALM was approved for MS treatment in April 2015 while OCR was approved in January 2018. With regard to RTX, this drug was approved for the treatment of neuromyelitis optica in December 2017, while it still warrants off-label administration in MS patients.

Data Collection

Data were collected from paper charts and electronic medical records. All patients consented to anonymous data collection for scientific purposes upon initiation of MAb therapy. Given the retrospective nature of the study, specific informed consent was not required. All data were processed according to current privacy regulations and the standards of good clinical practice. The following data were collected at baseline: age, gender, diagnosis, MAb therapy start date, disease duration, Expanded Disability Status Scale (EDSS) scores, lesion accrual on brain magnetic resonance imaging, previous exposure to disease-modifying therapies (DMTs), comorbidities, total lymphocyte and CD4+ T-cell count, data regarding exposure to infection (namely cytomegalovirus [CMV], hepatitis B virus [HBV], hepatitis C virus [HCV], HIV, and varicella zoster virus [VZV]), JCV serostatus, and Mycobacterium tuberculosis (MTB) exposure (based on Quiagen, ADA s.r.l., Padua, Italy). At the discretion of the physician, patients with resolved HBV infection (HB surface antigen -/HB core antibody +/HBV DNA negative) received prophylaxis with lamivudine (100 mg/day). Patients with latent MTB infection received isoniazid 300 mg/day for 6 months starting at the onset of MAb treatment.

Duration of follow up was defined as the interval between the MAb start date and the last visit in the following year (up to 365 days). In patients who did not experience an infective event, duration of follow up was defined as the observation period. In patients with infective complications, the observation period corresponded to the interval between the MAb start date and the date of the first infective event. In case of subsequent infective events, the date of the first event was considered the observation end-date. Laboratory variables were collected at baseline, +1 month, +3 months, +6 months, +12 months, and in case of infection. New onset of lymphocytopenia (<800 cells/μl) and/or hypogammaglobulinemia (IgG <7 g/dl) and/or neutropenia (<1000 cells/μl) during treatment were considered indicative of iatrogenic immune impairment.

Infective adverse events were defined as new infections that persisted for more than 24 hours. Infection could be either microbiologically or clinically documented. Diagnostic criteria are reported in the online supplementary material. The following bacterial infections were recorded: bacteraemia and sepsis, urinary tract infection (UTI), respiratory tract infection (RTI), endocarditis, acute gastroenteritis, intra-abdominal infection, skin-soft tissues or bone infection, central nervous system infection, and MTB infection [15–18]. Fungal infections were recorded according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group 2008 criteria of possible, probable, or certain invasive fungal infection [19]. Viral infections caused by the following agents were recorded: HSV, VZV, CMV, HBV, and JCV (PML) [20–22]. Cytomegalovirus reactivation was defined as detectable viremia (>85 copies/ml on serum) regardless of signs of CMV disease. Serum CMV viral load was evaluated at +1 month, +3 months, and +6 months after treatment onset.

In case of infection, we evaluated aetiology, severity, outcome, site of infection, and time of infection onset after starting treatment. An infection was considered severe (versus mild or moderate) when it was fatal and/or required hospitalization or intravenous anti-infective drug use. For each patient, we calculated the total number of infective events and infective recurrences (ie, when the same agent caused the same clinical event during follow up). The immunological and virologic tests used are reported in the online supplementary material.

Statistical Analysis

The Gaussian distribution of quantitative variables was evaluated with the Kolmogorov-Smirnov test. Quantitative variables are reported using the mean and standard deviation (SD) in the case of Gaussian distribution and median and interquartile range (IQR) in the case of non-Gaussian distribution. The *t* test was used for comparisons between parametric quantitative variables. Comparisons between nonparametric and nonpaired continuous variables were assessed with the Mann-Whitney test while paired continuous variables were assessed with the Wilcoxon signed rank test. The χ2 test with Yates correction (or Fisher exact test when appropriate) was used for comparisons between categorical variables. The Kaplan-Meier method was used to evaluate the crude time-to-infection. The effect of the single variables was evaluated using the log rank test. The association between infective events and a variety of potential predictors was investigated with a univariate Cox regression analysis. All results were expressed as adjusted hazard ratios (aHR) with 95% confidence intervals (CI). To evaluate the individual contribution of each independent factor, variables that showed a significant association at univariate analysis were included in a multivariate Cox regression model, together

with clinically relevant covariates according to the physician's judgement. For all tests, P values < .05 were considered statistically significant. Statistical analyses were performed using the software package SPSS version 18.0 (PASW Statistics, Inc., Chicago, IL).

RESULTS

Baseline Characteristics

 A total of 163 MS patients were enrolled in the study. Of these, 82 patients (41%) received ALM, 38 patients (23%) received OCR, and 58 patients received RTX (36%). Demographic characteristics are reported in Table 1 and are stratified according to drug class. Patients were equally affected by relapsing-remitting and primary MS phenotypes (48%). The median baseline EDSS score was 5.5 (IQR, 4–6.5). Median lymphocyte and CD4+ T-cell counts were within normal ranges both in patients receiving anti-CD20 and in patients receiving anti-CD52. No patient was HIV or HCV seropositive. No patient had active HBV infection; 20 patients had HBV resolved infection, but only 1 (on RTX-treatment) received lamivudine prophylaxis. No patient had active MTB infection; 4/5 patients with latent MTB infection received isoniazid (2/2 on anti-CD20 drugs and 2/3 on ALM).

Treatment Follow up

As shown in Table 1, median follow up was 226 days (IQR, 96–365 days; 60 patient years). Follow up was longer in patients treated with anti-CD52 than in patients treated with anti-CD20 (365 days; IQR, 345–365 vs 133 days; IQR, 64–231) (P < .001, χ^2 test). Regarding iatrogenic immune impairment, 67 patients (41%) experienced lymphocytopenia, 11 (7%) had concomitant hypogammaglobulinemia, and 2 (1%) developed concurrent neutropenia. The iatrogenic immune impairment rate was significantly higher in patients receiving anti-CD52 regimens than in patients receiving anti-CD20 regimens (87% vs 22%; P ≤ .001, χ^2 test). Nearly all patients (92%) developed an immune impairment during the first month of treatment with no difference between the 2 treatment groups (P = 1.00, χ^2 test).

Figure 1 shows the median lymphocyte and CD4+ T-cell counts at baseline and at 1, 3, 6, 12 months, and thereafter, stratified according to drug class. Both total lymphocyte and CD4+ T-cell levels significantly decreased during the first month of treatment (median lymphocytes, 1410 cells/µl; IQR, 1050–2050 at baseline vs 900 cells/µl; IQR, 290–1430 at +1 month, P < .001; median CD4+ T-cells, 674 cells/µl; IQR, 371–1020 at baseline vs 185 cells/µl; IQR, 56–370 at +1 month, P < .001; Wilicoxon signed rank test). At +1 month, the decrease in lymphocyte counts was greater in anti-CD52–treated patients than in anti-CD20–treated patients (Δ median lymphocytes, -505 cells/µl; IQR, -1180–-1945 vs -103 cells/µl; IQR, -60–-230, respectively [P < .001]; Δ median CD4+ T-cells, -544 cells/µl; IQR, -87–-992 vs -379 cells/µl; IQR, -155–-734 cells/µl, respectively [P = .188;

Mann-Whitney test]). Almost all patients recovered the total lymphocyte count within +6 months after the first drug infusion, whereas CD4+ T-cell levels remained significantly lower than pretreatment levels in anti-CD52–treated patients even a year later (P < .001, χ^2 test).

Infective Adverse Events

Eighty-six IAEs occurred in 67 (41%) patients. Fifty-one of the 67 patients developed a single infection and 16 patients experienced multiple events (2 events in 14 patients, and 4 and 3 events in 2 patients, respectively). Nineteen infections, which occurred in 15 patients, were considered severe, and 67 infections, which occurred in 52 patients, were considered mild to moderate. Outcome was favorable in all patients, except for a case of fatal aspergillosis. The overall annualized infection rate was 1.1 per patient-year. The rate of IAEs was higher in patients receiving ALM than in those receiving anti-CD20 agents (1.5 vs 0.8 per patient-year). As shown in Figure 2, the mean crude time-to-infection onset was significantly shorter in ALM-treated patients (171 days; 95% CI, 134–208 than in anti-CD20–treated patients (263 days; 95% CI, 229–297; P = .001, log-rank test).

The main characteristics of the 86 IAEs observed in the 2 groups of patients (anti-CD52 vs anti-CD20) are reported in Table 2. The IAE rate was significantly higher in the anti-CD52 arm (43/67 patients, 64%) than in the anti-CD20 arm (24 /86, 25%; P < .001, χ^2 test). At the time of infection, anti-CD52–treated patients had lower median lymphocyte counts (550 cells/µl; IQR, 220–910 vs 1205 cells/µl; IQR, 588–1580; P = .007, Mann-Whitney test) and higher rates of lymphocytopenia (71% vs 32%; P = .001, χ^2 test). The median time-to-any-infection did not differ significantly in terms of drug class and infection etiology (median time to bacterial infection onset, 55 days; IQR, 10–179 vs viral infection onset, 30 days; IQR, 28–87; P = .083, χ^2 test). However, infections of any type occurred more frequently during the first posttreatment month, especially in the anti-CD52 arm (32 of 58 infective events, 55%). Bacterial infections were more frequent in patients receiving anti-CD20 treatment than in those receiving anti-CD52 treatment (P < .001, χ^2 test), whereas viral infections were more frequent in anti-CD52–treated patients (P < .001, χ^2 test).

Regarding clinical features, UTI and RTI were more frequent in patients on anti-CD20 therapy than in patients on anti-CD52 therapy (P = .004 and .013, respectively, χ^2 test) (Table 2). Severity and recurrence of IAEs were similar in the 2 groups (Table 2). Eight severe infections requiring hospitalization and/or intravenous anti-infective treatment occurred in RTX-treated patients (1 case of *Serratia marcescens* pneumonia, 1 case of pneumonia responsive to cotrimoxazole, 2 complicated UTI, 1 pyelonephritis, 1 acute prostatitis, 1 cellulitis, and 1 probable invasive pulmonary aspergillosis). In the ALM group, 11 patients required hospitalization and/or anti-infective treatment (1 case of *Haemophilus influenzae type B* pneumonia, 2 cases of

Table 1. Main Characteristics of Patients Receiving Anti-CD20 or Anti-CD52 Agents for Multiple Sclerosis Spectrum Disorders[a]

	Total (n = 163) n (%)	Anti-CD20 (n = 96) n (%)	Anti-CD52 (n = 67) n (%)	P (χ2 test)
Female sex	100 (61)	50 (52)	50 (75)	.004
Age (years, mean ± SD)	44.5 ± 11.4	48.4 ± 10.3	38.9 ± 10.5	<.001 (t test)
Comorbidity burden				
No comorbidity	53 (34)	23 (25)	30 (48)	.003
1 comorbidity	47 (30)	25 (27)	22 (35)	.264
2–3 comorbidities	31 (20)	23 (25)	8 (13)	.069
>3 comorbidities	26 (17)	23 (25)	3 (5)	.001
Median disease duration [years]	9.8 [4.4–15.8]	11.1 [5.5–18.1]	7.5 [4.0–13.5]	.005 (Mann-Whitney test)
Lesion accrual on brain MRI[b]				
Low	7 (5)	4 (5)	3 (6)	1.000
Medium	24 (18)	12 (15)	12 (24)	.187
High	100 (76)	65 (80)	35 (70)	.180
EDSS scores				
<3.5	29 (18)	5 (5)	24 (36)	<.001
3.5–5	39 (24)	18 (19)	21 (31)	.064
5–7	61 (37)	42 (44)	19 (28)	.046
≥7	33 (20)	30 (31)	3 (5)	<.001
Diagnosis				
RRMS	78 (48)	20 (21)	58 (87)	<.001
PPMS	24 (15)	24 (25)	0 (0)	<.001
SPMS	55 (34)	46 (48)	9 (13)	<.001
NMO	5 (3)	5 (5)	0 (0)	.079
DMT exposure				
Naïve	17 (10)	10 (10)	7 (10)	0.995
Single	31 (19)	17 (18)	14 (21)	0.610
Two-three lines	66 (41)	38 (40)	28 (42)	0.778
Four or more lines	49 (30)	31 (32)	18 (27)	0.457
MAbs-experienced	61 (38)	25 (26)	36 (55)	<0.001
Median wash out time from last DMT [days]	37 [0–134]	68 [0–178]	16 [1–67]	0.087 (Mann-Whitney test)
Infections				
CMV seropositivity (IgG)	128 (79)	78 (81)	50 (75)	0.311
VZV seropositivity (IgG)	155 (95)	92 (96)	63 (94)	0.718
HBV serostatus				
HBV seronegative	101 (62)	68 (71)	33 (49)	0.005
Resolved HBV	20 (12)	14 (15)	6 (9)	0.281
HBV vaccination	42 (26)	14 (15)	28 (42)	<0.001
TBC serostatus				
LTBI	5/112 (4)	2/65 (3)	3/48 (6)	0.652
JCV seropositivity (IgG)	111/132 (84)	56/72 (78)	55/60 (92)	0.030
Baseline immune status				
Median lymphocyte count [cells/μl]	1410 [1050–2050]	1390 [1120–1815]	1500 [850–2300]	0.170 (Mann-Whitney test)
Lymphocyte count > 800 cells/μl	133 (82)	83 (87)	50 (75)	0.055
Lymphocyte count 800-500 cells/μl	22 (14)	11 (12)	11(16)	0.362
Lymphocyte count 500-200 cells/μl	8 (5)	2 (2)	6 (9)	0.065
Median C4+ T-cell count	674 [371–1020]	654 [376–954]	704 [347–1104]	0.652 (Mann-Whitney test)
C4+ T-cell count < 200 cells/μl	21/150 (14)	6/90 (7)	15/60 (25)	0.002
Follow-up data				
Median follow-up days	226 [96–365]	133 [64–231]	365 [345–365]	<0.001 (Mann-Whitney test)
Suspension rate	12 (7)	4 (4)	8 (12)	0.073

Abbreviations: CMV, cytomegalovirus; DMT, disease modifying therapies; EDSS, Expanded Disability Status Scale; HBV, hepatitis B virus; Ig, immunoglobulin; JCV, John Cunningham virus; LTBI, latent tuberculosis infection; MAbs, monoclonal antibodies; MRI, magnetic resonance imaging; NMO, neuromyelitis optica, PPMS, primary progressive MS; RRMS, relapsing-remitting MS; SD, standard deviation; SPMS, secondary progressive MS; TBC, tuberculosis; VZV, varicella zoster virus.

[a]Data are expressed as number (percentage) for qualitative variables or median (interquartile range) or mean ± SD for quantitative variables.

[b]Thirty-two records missing.

complicated urinary tract infection, 1 case of cutaneous HSV infection and 1 of ocular HSV infection, 1 case of cutaneous zoster infection, 3 CMV diseases, 1 proven invasive pulmonary aspergillosis, and 1 case of acute toxoplasmosis during pregnancy). Four infections were reported in patients receiving OCR (4/38[6%], namely, 3 UTI and 1 CMV reactivation), all
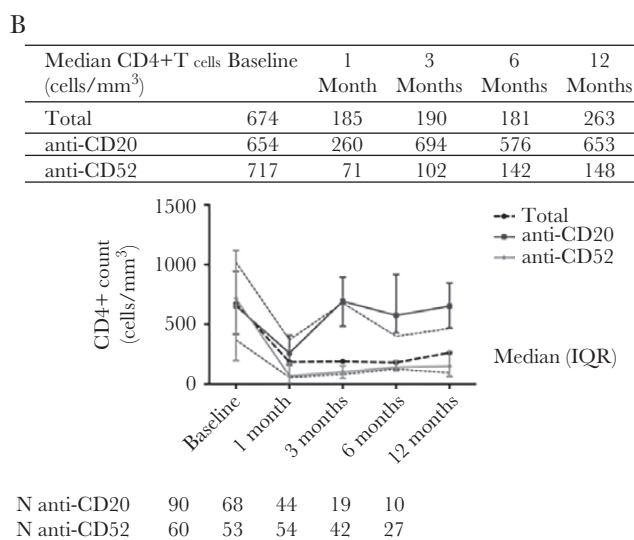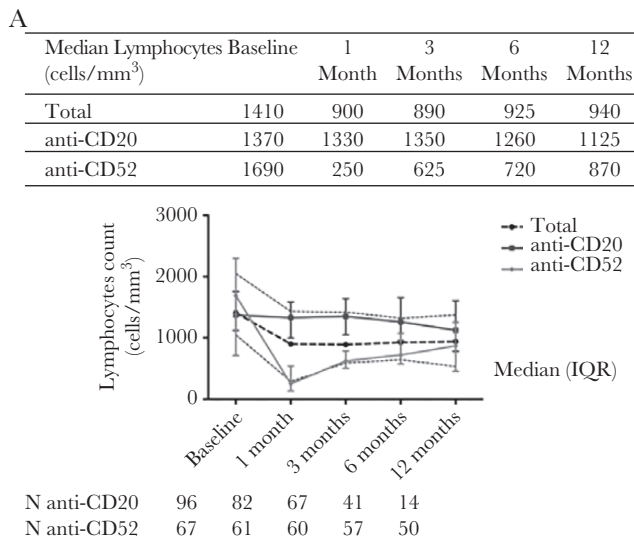
A

Median Lymphocytes Baseline (cells/mm^3)	Baseline	1 Month	3 Months	6 Months	12 Months
Total	1410	900	890	925	940
anti-CD20	1370	1330	1350	1260	1125
anti-CD52	1690	250	625	720	870

	Baseline	1 month	3 months	6 months	12 months
N anti-CD20	96	82	67	41	14
N anti-CD52	67	61	60	57	50

B

Median CD4+T cells Baseline (cells/mm^3)	Baseline	1 Month	3 Months	6 Months	12 Months
Total	674	185	190	181	263
anti-CD20	654	260	694	576	653
anti-CD52	717	71	102	142	148

	Baseline	1 month	3 months	6 months	12 months
N anti-CD20	90	68	44	19	10
N anti-CD52	60	53	54	42	27

Figure 1. Total lymphocytic (A) and CD4+ T cells (B) counts during treatment.

non-severe. The IAE rate was higher in subjects receiving RTX than in those receiving OCR (34.5% vs 10.5%; $P = .008$, χ^2 test).

During the first month of treatment, approximately half of the patients receiving ALM experienced CMV reactivation (24/50, 48%). No case of JCV disease (PML) was reported. Furthermore, no patient with resolved HBV infection experienced viral reactivation (mean follow up, 182 days; SD, 156). Similarly, no patient with latent MTB infection developed active disease (median follow up, 61 days; IQR, 21–270). The occurrence of infection caused treatment delay in 9 patients (all on RTX) and suspension of treatment in 6 patients (3 on ALM and 3 on RTX).

CMV Reactivation

As shown in Figure 3, 80 patients were screened for CMV viral load at the end of the first month (30/96 patients on anti-CD20 and 50/67 patients on anti-CD52), 94 patients were screened at
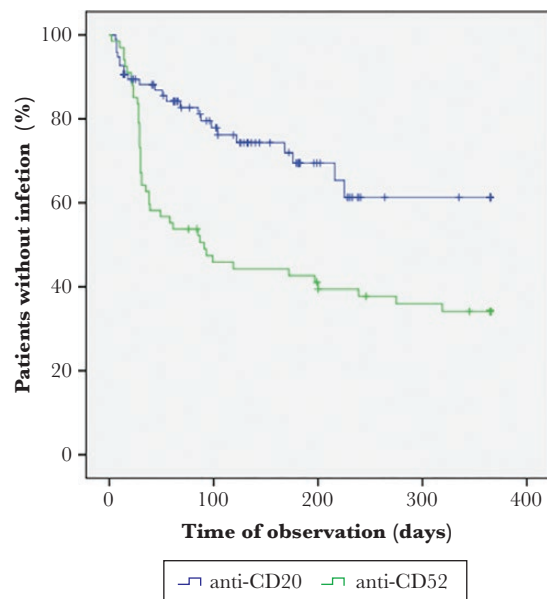

Figure 2. Crude time-to-infection onset per drug class ($P = .001$; log-rank test).

the end of the third month (45/96 patients on anti-CD20 and 49/67 patients on anti-CD52), and 80 patients were screened at the end of the sixth month (24/96 patients on anti-CD20 and 56/67 patients on anti-CD52). Cytomegalovirus reactivation rates fluctuated in both groups during follow up (Figure 3). Episodes of CMV reactivation were significantly more frequent in patients receiving anti-CD52 MAbs than in those receiving anti-CD20 MAbs (34 /67, 50.7% *vs* 5/96, 5.2%; $P < .001$, χ^2 test). No patient in the anti-CD20 group had a viral load >160 copies/ml or experienced active disease during reactivation. In the ALM-treated group, 3 patients experienced 2 reactivation events, 10/34 patients (29%) had a CMV viral load >1000 copies/ml (8 patients with CMV DNA > 3000 copies/ml), and 4/34 (12%) developed active disease requiring antiviral treatment (1 with pneumonia, 2 patients with fever and thrombocytopenia, and 1 reactivation during pregnancy, which caused congenital CMV infection and sensorineural hearing loss in the newborn).

Predictors of Infection

As shown in Table 3, at univariate Cox analysis, women and subjects affected by relapsing-remitting MS or with a history of more than 2 DMTs were significantly more prone to experience an IAE, whereas patients with primary-progressive MS were less likely to have an IAE. Administration of ALM was associated with an increased risk of subsequent infections, whereas the rate of infections was significantly lower in OCR-treated patients. Consequently, statistically significant covariates and the development of immune impairment were included in the multivariate Cox regression model. As shown in Figure 4, ALM and prior MS treatment were predictive of infection (aHR, 2.7;

**Table 2.** Infective Events According to Monoclonal Antibodies Administered[a]

	Total (n = 86) *n (%)*	Anti-CD20 (n = 28) *n (%)*	Anti-CD52 (n = 58) *n (%)*	*P* value (χ2 test)
Recurrent IAE	9 (11)	4 (14)	5 (9)	.325
Etiology				
Bacterial	31 (36)	19 (68)	12 (21)	<.001
Viral	49 (57)	7 (25)	42 (72)	<.001
Fungal	5 (6)	2 (7)	3 (5)	.527
Severity				
Mild-moderate	67 (78)	20 (71)	47 (81)	.314
Severe	19 (22)	8 (29)	11 (19)	—
Type of infection				
UTI	23 (27)	13 (46)	10 (17)	.004
RTI	8 (9)	6 (21)	2 (3)	.013
CMV reactivation	42 (49)	5 (18)	37 (64)	<.001
HSV or VZV reactivation	4 (5)	1 (4)	3 (5)	.607
Median time of onset (days) [IQR]	31 [23–92]	48 [10–103]	30 [28–91]	.567 (Mann-Whitney test)
First month onset	44 (51)	12 (43)	32 (55)	.284
2–6 months onset	28 (33)	10 (36)	18 (31)	.664
7–12 months onset	14 (16)	6 (21)	8 (14)	.274
Immune status at infective event				
Median lymphocyte count [cells/µl, IQR]	670 [250–1350]	11205 [588–1580]	5550 [220–910]	.007 (Mann-Whitney test)
Lymphocytopenia (<800 cells/µl)	50 (58)	9 (32)	441 (71)	.001
C4+ T-cell count < 200 cells/µl	50/62 (81)	110/13 (77)	440/49 (82)	.485
Median C4 T-cell count[b] [cells/µl, IQR]	84 [22–177]	1108 [0–427]	178 [23–174]	.762 (Mann-Whitney test)
Median C8 T-cell count[b] [cells/µl, IQR]	128 [41–231]	1149 [0–271]	1114 [47–235]	.986 (Mann-Whitney test)

Abbreviations: CMV, cytomegalovirus; DMT, disease-modifying therapies; HSV, herpes simplex virus; IAE, infective adverse events; IQR, interquartile range; NS, not significant; RTI, respiratory tract infections; UTI, urinary tract infections; VZV, varicella zoster virus.

[a]Data are expressed as number (percentage) or median (IQR).

[b]Twenty-four patient records missing (15 on anti-CD20 and 9 on anti-CD52).

Reactivation Rate	1 Month	3 Months	6 Months
Total	36%	6%	4%
anti-CD20	3%	9%	0%
anti-CD52	48%	6%	5%
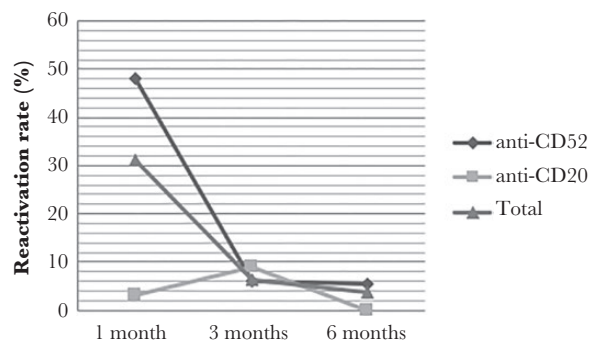
Number of reactivations			
	1 Month	3 Months	6 Months
Total	29/80	6/94	3/80
anti-CD20	1/30	4/45	0/24
anti-CD52	24/50	3/49	3/56

Figure 3. CMV reactivation rates during treatment according to drug class.

CI, 1.2–5.9; *P* = .012 and aHR, 1.7; CI, 1.0–2.8; *P* = .052, respectively, Cox test), while IAEs were significantly less common in OCR-treated patients (aHR, 0.2; CI, 0.0–0.8; *P* = .024, Cox test). Furthermore, iatrogenic immune impairment had an additional effect on IAE rate (aHR, 2.9; CI, 1.4–6.0; *P* = .004, Cox test). We also evaluated risk factors for IAEs not including cases of CMV reactivation with spontaneous resolution. At multivariate Cox analysis, only female sex was a significant predictive factor of infection (aHR, 2.447; CI, 1.173–5.104; *P* = .017), whereas patients receiving OCR were significantly less likely to develop infective complications (aHR, 0.183; CI, 0.044- 0.763; *P* = .020).

DISCUSSION

In this retrospective study, we investigated the incidence and main predictors of infection in patients with MS treated with biological agents that target lymphocyte cell surface antigens. To the best of our knowledge, this is the first study to compare the infective risk of the various classes of MAbs in patients affected by MS. We recruited 163 patients who started targeted therapy within a 28-month period (86 on anti-CD20 MAb and 67 on anti-CD52 MAb). Median follow up was longer in patients receiving ALM (365 days) than in patients receiving anti-CD20 agents (133 days; *P* < .001, Mann-Whitney test), probably because ALM was approved for MS almost 3 years before anti-CD20 drugs. Approximately 40% of patients experienced 1 or more IAE, irrespective of demographic and neurological

Table 3. Risk Factors for the Development of Infective Adverse Events (n = 86)

	Patients without IAE (n = 96) n (%)	Patients with IAE (n = 67) n (%)	HR	95% CI		P (Cox-Mantel test)
Female sex	51 (53)	49 (73)	1.93	1.12	3.31	.017
Age > 40 years	64 (67)	38 (57)	0.95	0.58	1.55	.835
Disease features						
Disease duration > 10 years	49 (51)	32 (48)	0.96	0.60	1.56	.877
EDSS > 5	59 (62)	36 (54)	0.72	0.36	1.38	.304
Presence of comorbidities	63/93 (68)	41/64 (64)	1.18	0.71	1.96	.529
Diagnosis						
RRMS	36 (38)	42 (63)	1.83	1.11	3.01	.018
PPMS	21 (22)	3 (5)	0.29	0.09	0.91	.035
SPMS	34 (35)	21 (31)	0.85	0.51	1.42	.535
NMO	4 (4)	1 (2)	0.53	0.07	3.87	.528
Prior DMT						
More than two prior DMT	36 (38)	38 (57)	1.67	1.03	2.70	.039
MAbs exposure	30 (31)	31 (46)	1.30	0.80	2.12	.285
Less than 4 weeks wash out time	34/80 (43)	33/57 (58)	1.13	0.66	1.92	.656
MAbs						
Alemtuzumab-based regimen	24 (25)	43 (64)	2.24	1.35	3.72	.002
Ocrelizumab-based regimen	34 (35)	4 (6)	0.20	0.07	0.55	.002
Rituximab-based regimen	38 (40)	20 (30)	0.93	0.55	1.58	.792
Immune status						
BL lymphocytopenia (<800 cells/μl)	14 (15)	16 (24)	1.13	0.64	1.98	.677
BL CD4+ T-cell count < 200 cells/μl	9/88 (10)	12/62 (19)	1.25	0.66	2.35	.491
Iatrogenic immune impairment[a]	38 (40)	41 (61)	1.21	0.73	2.00	.463

Abbreviations: BL, baseline; CI, confidence intervals; DMT, disease-modifying therapies; EDSS, Expanded Disability Status Scale; HR, hazard ratio; IAE, infective adverse events; MAbs, monoclonal antibodies; NMO, neuromyelitis optica; PPMS, primary progressive multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.

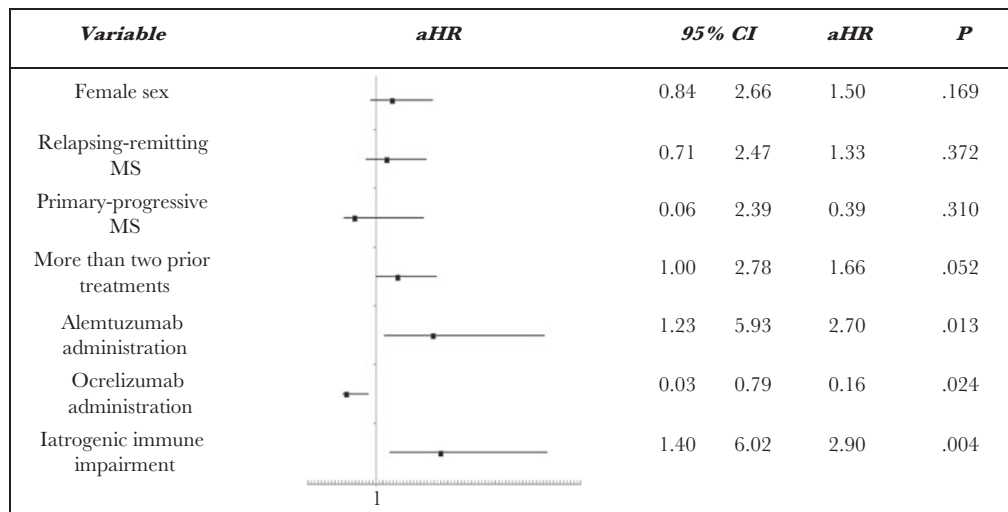[a]Defined as new onset of lymphocytopenia and/or hypogammaglobulinemia and/or neutropenia during treatment.

Variable	aHR	95% CI		aHR	P
Female sex		0.84	2.66	1.50	.169
Relapsing-remitting MS		0.71	2.47	1.33	.372
Primary-progressive MS		0.06	2.39	0.39	.310
More than two prior treatments		1.00	2.78	1.66	.052
Alemtuzumab administration		1.23	5.93	2.70	.013
Ocrelizumab administration		0.03	0.79	0.16	.024
Iatrogenic immune impairment		1.40	6.02	2.90	.004

Figure 4. Predictive risk factors of infections at multivariant Cox regression analysis.

clinical features at baseline. Severe infections were mainly of bacterial origin (53%). Outcome was favorable in all patients, except in a young woman who developed severe neutropenia and lymphopenia 1 month after ALM infusion and died from fulminant necrotizing pulmonary aspergillosis. Several opportunistic infections were reported, namely, mucocutaneous candidiasis (3 cases), invasive pulmonary infection caused by *Aspergillus fumigatus* (2 cases), primary *Toxoplasma gondii* infection (1 case), *Herpes simplex* reactivation (3 cases), VZV reactivation (1 case), and CMV reactivation (41 cases). Most of these complications occurred soon after drug infusion and could be favored by the considerable decrease of lymphocytes

(total and CD4+ T-cells) and premedication with high doses of corticosteroids. Although we did not observe any case of PML, HBV, or MTB reactivation, the relatively small sample size and the limited follow up preclude any firm conclusion regarding these infections.

Notably, the infective risk was significantly higher in patients receiving anti-CD52 regimens who had a history of DMT or who developed iatrogenic immune impairment (namely, lymphocytopenia) during therapy. Patients treated with ALM experienced mainly viral infections (42 out of 58 IAEs). Remarkably, the CMV reactivation rate was 55% in patients treated with ALM (37/67). This finding is in agreement with the results of studies conducted in hematologic settings. In fact, Laurenti et al observed a comparable rate of CMV reactivation (66%) in a small cohort of patients receiving ALM for chronic lymphocytic leukaemia [23]. Similarly, Vallejo and colleagues found a CMV reactivation rate of about 40% in patients treated with ALM for lymphoproliferative disorders, and the reactivation rate was even higher (46%) when corticosteroids were co-administered [24]. Such high reactivation rates may be biased by missing samples or differences in viral monitoring strategies (eg, timing or type of assays). Nevertheless, given recent postmarketing reports of CMV disease in MS patients [10–12, 25], this complication should invariably be considered in case of suggestive clinical features in the MS setting.

Most of the infections in the anti-CD20–treated patients were of bacterial origin, which coincides with the finding of Trivin et al that 79% of all infections were bacterial in a cohort of patients receiving RTX for autoimmune renal syndromes [26]. Moreover, among CD20-treated patients, the infective rate was significantly lower in patients receiving OCR than in those receiving RTX, which further supports the concept that safety profiles differ between the 2 agents in terms of IAEs.

As in most retrospective studies, missing data constitute a possible limitation of our study. Mild infections may have been underreported because they did not require treatment or a medical consultation. The lack of a control group, which was due to different screening and follow-up protocols in historical cohorts of our institute, is another potential limitation of our work. The strengths of the study are the real-life profile of the examined population, irrespective of potential differences in disease activity, comorbidities and prior treatments, and the collection of data regarding all types of infection. In addition, the finding of an elevated IAE rate in MAb-experienced patients, who are generally excluded from randomized clinical trials, sheds new light on drug safety and tolerability in this difficult-to-treat subset of patients.

Finally, the evidence that MAbs therapy is associated with a significant infective risk in patients affected by MS, particularly during the early months after infusion, reinforces the need to set-up specific management protocols. Because on-treatment immune impairment is a more effective predictor of IAEs than is baseline immune status, follow up should be customized for each patient. Moreover, given the high CMV reactivation rate reported in ALM-treated patients, serial serum CMV DNA monitoring may be advisable shortly after drug infusion. In fact, viral load monitoring could encourage timely reporting of CMV reactivation in these patients, helping to better estimate the probability of such events.

In conclusion, our findings provide new insights into infectious safety issues of anti-CD20 and -CD52 agents in an MS setting. These results can facilitate the assessment of infective risk in this population, thereby helping clinicians to stratify patients and optimize preventive interventions.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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