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Incidence, Clinical Presentation, Relapses and Outcome of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection in Patients Treated With Anti-CD20 Monoclonal Antibodies

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Background. Our objective is to describe the presentation and complications, including relapses, of coronavirus disease 2019 (COVID-19) in patients under anti-CD20 treatments. In addition, to describe viral clearance and determine the safety of reintroducing anti-CD20 treatment.

Methods. Retrospective cohort study of 422 patients under anti-CD20 treatment that was administered from 1 January 2019 to 31 December 2020.

Results. Fifty-seven patients were diagnosed with COVID-19 (13.5%). Twenty-five patients (43.9%) required hospital admission. Five patients died (8.8%), and 10 developed severe COVID-19 and acute respiratory distress syndrome. Mortality rate was higher among patients infected during the first 3 months following the last dose of anti-CD20 (14.7% vs 0%, $P = .046$). The median time of persistence of positive reverse transcription polymerase chain reaction (RT-PCR) was 22 days (IQR 13–40).

Nine out of 52 survivors (17.3%) presented relapses. All of them received the last dose of anti-CD20 less than 6 months before the COVID-19 episode. Clinical presentation was fever (n = 8; 88.9%), dyspnea (n = 7; 77.8%), cough (n = 7; 77.8%), worsening of previous infiltrates (n = 5; 55.6%) and new pulmonary infiltrates (n = 8; 88.9%). An increase in lymphocytes with CD4/CD8 ratio inversion was observed in all cases. Among the 25 patients who resumed anti-CD20 drug, 4 (16.0%) presented relapses vs 5/28 among those who did not (17.9%), ($P = .857$).

Conclusions. Patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) during the 6 months after anti-CD20 administration had a worse outcome and a higher mortality rate. The duration of infectivity may be longer. Relapses of COVID-19 occurred in more than 15% and were associated with viral replication. Once the infection is resolved, it is safe to restart treatment with anti-CD20.

Keywords. rituximab; anti-CD20 monoclonal antibodies; COVID-19; SARS-CoV-2 infection; COVID-19 relapse.

A subset of coronavirus disease 2019 (COVID-19) patients develop an exaggerated inflammatory response by innate immunity, responsible for acute respiratory distress syndrome [1–3]. The humoral immune response and production of neutralizing antibodies by B-lymphocytes are essential in viral clearance [4, 5], and contribute to reduce the inflammatory damage.[1, 4, 6]

During the last 2 decades, monoclonal anti-CD20 antibodies have been available for treatment of different diseases. These antibodies act selectively on B-lymphocytes and decrease antibody production.[7–9],which in turn leads to a depressed humoral response [5, 10, 11]. It is biologically plausible that these patients may have inability for virus clearance, leading to an increased inflammation. Hence, patients treated with these drugs could have a higher risk of severe COVID-19 and death [12–14]. Case reports of atypical presentation, persistence of viral replication, protracted symptomatology, and relapses have been reported [15, 16]. These patients could benefit from a specific therapeutic approach, to counterbalance the absence of antibodies production [16]. Despite these findings, to our knowledge there is no published series of patients treated with anti-CD20 describing COVID-19 in this population, nor have risk factors for severe infection, death and relapses been identified.

Our primary objectives were to describe the incidence, clinical presentation and complications (including relapses) of COVID-19 in patients under anti-CD20 treatments. We aimed

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to identify patients at risk for severe infection or death. Our secondary objectives were to describe the immune response and infectivity in these patients, and to determine the safety of resuming anti-CD20 treatment once the infection was overcome.

METHODS

A retrospective observational study was performed from February 2020 to March 2021 in a tertiary teaching hospital.

We retrospective reviewed the electronic medical records of all patients aged 18 years or older treated with any anti-CD20 drug from 1 January 2019 through 31 December 2020. All patients with confirmed COVID-19 after at least 1 dose of anti-CD20 treatment were included. Patients were followed-up until 30 April 2021 or death.

Antigenic rapid tests were performed by Panbio COVID-19 Ag Rapid Test Device (Abbott®, USA), which is based on human immunoglobulin G (IgG) specific to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Serological tests were performed by chemiluminescence immunoassay method: Liaison®XL (DiaSorin) that detects IgG anti-S1/S2 and immunoglobulin M (IgM) specific antibodies to SARS-CoV-2. For reverse transcriptase-polymerase chain reaction (RT-PCR), several automatized or semiautomatic systems were employed, such as Cobas® SARS-CoV-2 System (Roche Diagnostics), which uses target-specific forward and reverse primers for ORF1 a/b nonstructural region, a region that is unique to SARS-CoV-2, and a conserved region in the structural protein envelope E-gene, or Lightmix® Modular SARS-CoV-2 (Roche Diagnostics), where only E-gene is detected. Some of these systems reported the cycle threshold (Ct), which refers to the number of cycles of an RT-PCR required to amplify RNA until detection. A Ct <30 was considered infective [17, 18].

Definition and Variables

Any patient with a positive viral test, including antigenic test and RT-PCR, was considered to have confirmed COVID-19. Due to healthcare system overload secondary to a high incidence of infection during March and April 2020, outpatient diagnosis of COVID-19 was based on clinical and radiological findings consistent with COVID-19. Therefore, these patients were also considered as confirmed COVID-19.

Data on baseline characteristics, anti-CD20 treatment, clinical, microbiological, complications, and prognosis variables were extracted from patient`s electronic medical records.

A recurrence was defined as a clinical episode of symptoms consistent with acute COVID-19, accompanied by re-positive/persisting RT-PCR in respiratory samples. According to previously published definitions [19], a relapse was defined as a clinical recurrence occurring within 90 days of primary infection and supported by the absence of epidemiological exposure. A reinfection was considered as a clinical recurrence occurring >90 days after the initial episode with an epidemiological exposure. Because patients under anti-CD20 treatment had confirmed relapses with the same strain more than 90 days after the initial episode and without new epidemiological exposure have been described [20], we included these patients as possible relapses. We described as a possible relapse a consistent episode with no alternative etiology and without a microbiological test of SARS-CoV-2 infection or tested with inconclusive results.

Severity of disease was defined per the National Institutes of Health (NIH) criteria [21].

Statistical Analysis

Data are presented as median and interquartile range (IQR) for quantitative variables and as percentage and absolute value for qualitative variables. Inferential analysis was performed using χ^2 test (or Fisher exact test when necessary) for qualitative variables and Mann-Whitney's *U* for quantitative variables. Bilateral *P*-values of <.05 were considered statistically significant. All statistical analyses were performed using SPSS version 25 software package (SPSS Inc., IBM, Chicago, Illinois, USA).

RESULTS

During the study period, 422 patients received anti-CD20 treatment. Of these, 67 patients were diagnosed with COVID-19 but only in 57 patients after the introduction of anti-CD20 treatment (13.5%). Figure 1 shows the flowchart of patients.

Baseline characteristics, anti-CD20 therapy, and clinical presentation are shown in Table 1. Among these 57 patients, 17 (29.8%) were under concomitant treatment with corticosteroids, 10 patients (17.5%) were under another immunosuppressive treatment (4 R-CHOP protocol, 2 tacrolimus, and 1 case each of the following: mycophenolate, azathioprine, methotrexate, and belimumab).

Risk Factors for Severe COVID-19

Twenty-five patients (43.9%) required hospital admission due to COVID-19. All of them fulfilled NIH criteria for severe disease. Table 1 compares characteristics of severe versus nonsevere patients. There were no differences in concomitant treatment with corticosteroids or other immunosuppressant therapies prior to COVID-19. Patients who were diagnosed with COVID-19 during the 6 months after the last dose of anti-CD20 developed severe disease more frequently (48.9% vs 18.0%, *P* = .090).

Complications and Outcomes

Five patients (8.8%) died. All deaths occurred during hospital admission (20% of admitted patients) and the cause of death was SARS-CoV-2 related pulmonary injury. During admission, 10 (37.5%) developed critical COVID-19, 8 (36.4%) bacterial pneumonia, and 4 (13.6%) pulmonary thromboembolism. Heart failure, acute renal failure, and bacteremia occurred in 3 patients each (12.0%).

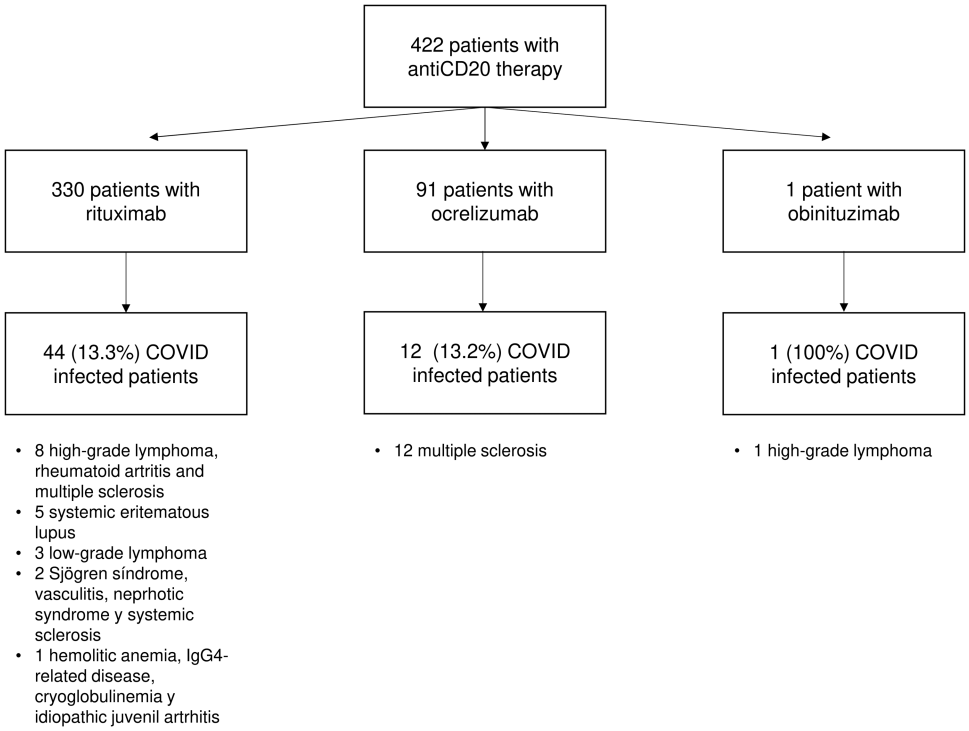

Figure 1. Patient's flowchart. Abbreviation: COVID, coronavirus disease.

Treatment with corticosteroids was indicated in 24 (41.3%), including 21 inpatients (84.0% of admitted patients). Tocilizumab was administrated in 12 (48.0%). Three patients were treated with convalescent plasma. No patient received remdesivir. No significant differences in mortality were found between treatments.

Table 2 summarizes factors associated with mortality. Concomitant treatment with corticosteroids or other immunosuppressant was not associated with mortality ($P = 1.000$ in both cases). None of the patients who developed COVID-19 6-month past from the last infusion died versus 10.8% of those who acquired COVID-19 within 6 months of infusion ($P = .035$).

Time of RT-PCR Positivity and Immunity

Among the 19 patients with more than 1 RT-PCR determination, the median duration of positivity was 22 days (IQR 13–40), with a maximum of 65 days. Six patients had positive RT-PCR for more than 30 days. Three of them presented a Ct suggestive of infectivity (day 43 Ct 26, day 52 Ct 23, day 56 Ct 28), and 1 unlikely infective (day 35 Ct 34). Persistence of positive RT-PCR for more than 30 days was associated with mortality (33.3% (2/6) vs 0% (0/13), $P = .043$). Only 1 out of the 6 (16.7%) with persistently positive RT-PCR was under chronic corticosteroids.

Antibodies were detected in 6 out of 28 patients who had a serology determination (20.7%). The presence of serum antibodies was more likely if the episode of COVID-19 occurred 6 months after the last anti-CD20 dose as compared to less than 3-month past from the last dose (40.0% vs 14.2%, $P = .072$). Three of the 6 patients with positive serology were on chronic corticosteroid treatment (50.0%) and 2 (33.3%) on other immunosuppressant treatment ($P = .448$ and $P = .440$, respectively). None of the patients with positive serology presented relapses, as compared to 9 out of the 22 (40.9%) with negative serology ($P = .071$).

Time From Last Anti-CD20 Dose

The median interval between the last dose and COVID-19 was 2 months (IQR 0.8–4.1). In total, 35 out of 57 patients (61.4%) had SARS-CoV-2 infection within 3 months from the last dose, 11 (19.3%) between 3 and 6 months, and 11 (19.3%) after 6 months.

Clinical characteristics and outcomes according to time from the last dose of anti-CD20 drug are shown in Table 3. There was a trend to a higher frequency of acute respiratory distress, and a higher mortality among patients infected during the first 3 months (23.5% vs 8.7%, $P = .104$, and 14.7% vs 0%, $P = .046$, respectively).

Use of Anti-CD20 Treatment After Recovery

Thirty-five patients received an anti-CD20 drug after the episode of COVID-19, in 10 cases this treatment was started after COVID-19 recovery.

One out of the 35 patients died after receiving anti-CD20 (rituximab); however, death was not considered attributable to rituximab (but to refractory status epilepticus).

Table 1. Comorbidity, Anti-CD20 Therapy, and Clinical Presentation of Coronavirus Disease 2019 (COVID-19) in Patients Treated With Anti-CD20 Drug

Variable		Total (n = 57)	Severe-Critical COVID-19 (n = 25)	Mild-Moderate COVID-19 (n = 32)	*P*	Missing
Demographic and comorbidity						
Age		51 (41–60)	56 (42–69)	46 (40–53)	.026	0
Gender (female)		70.2% (40)	64,0% (16)	75.0% (24)	.397	0
Charlson comorbidity index		2 (1–3)	3 (1–5)	1 (0–3)	.026	0
Arterial hypertension		22.8 (13)	36.0% (9)	12.5% (4)	.036	0
Dyslipidemia		17.5% (10)	28.0% (7)	9.4% (3)	.087	0
Diabetes mellitus		1.5% (6)	16.0% (4)	6.3% (2)	.388	0
Heart failure		8.8% (5)	12.0% (3)	6.3% (2)	.645	0
Ischemic heart disease		1.8% (1)	4.0% (1)	0	-	0
COPD		1.8% (1)	1.7% (1)	0	-	0
Liver cirrhosis		3.5% (2)	4.0% (1)	3.1% (1)	1.000	0
Chronic kidney disease		15.8% (8)	20.0% (5)	12.5% (4)	.485	0
Anti-CD20 therapy						
Drug	Rituximab	77.2% (44)	84.0% (21)	71.9% (23)	.149	0
	Ocrelizumab	21.1% (12)	12.0% (3)	28.1% (9)		
	Obinutuzumab	1.7% (1)	4.0% (1)	0		
Indication	Hematological malignancy	21.1% (12)	32.0% (8)	12.5% (4)	.337	0
	Neurological	33.3% (19)	32.0% (8)	34.3% (11)		
	Systemic	42.1% (24)	32.0% (8)	50.0% (16)		
	Hematological non-malignant	3.5% (2)	4.0% (1)	3.1% (1)		
Anti-CD20 duration (months)		11 (0.7–27)	8.6 (2–23)	12.7 (0.5–31)	.792	0
Chronic corticosteroids		29.8% (17)	24.0% (6)	34.4% (11)	.561	0
Other immunosuppressant		17.5% (10)	16.0% (4)	18.8% (6)	.786	
Group	<6 months	80.7% (46)	92.0% (23)	71.8% (23)	.090	0
	>6 months	19.3% (11)	8.0% (2)	28.1% (9)		
Post-COVID anti-CD20 dose		43.1% (25)	30.0% (6)	59.4% (19)	.016	5
Clinical presentation and outcomes						
Cough		70.2% (40)	79.2% (19)	67.7% (21)	.380	2
Expectoration		14.5% (8)	12.5% (3)	13.3% (4)	1.000	3
Fever		67.3% (37)	95.8% (23)	45.2% (14)	.001	2
Thoracic pain		13.2% (7)	16.7% (4)	10.3% (3)	.688	4
Anosmia		29.2% (14)	30.0% (6)	28.6% (8)	1.000	9
Asthenia		61.2% (30)	76.2% (16)	50.0% (14)	.081	8
Arthromyalgia		40.4% (21)	47.8% (11)	34.5% (10)	.400	5
Dyspnea		34.5% (19)	62.5% (15)	12.9% (4)	.001	2
Asymptomatic		8.6% (5)	0	8.8% (5)	-	9
Pulmonary infiltrate		70.0% (28)	95.8% (23)	31.3% (5)	.001	17
COVID-related death		8.8% (5)	20.0% (20)	0	.013	0
Relapse		15.8% (9)	30.0% (6)	9.4% (3)	.105	5

Chronic corticosteroids: a systemic chronic therapy with corticoids with at least 5 mg/kg of prednisone or equivalent at time of SARS-CoV-2 infection.
Abbreviations: COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

None of the 10 patients who started anti-CD20 de novo after COVID-19 presented recurrences. Among the 25 patients who restarted the anti-CD20 drug after COVID-19, 4 (16.0%) presented recurrences versus 5 out of the 28 who did not restart treatment (17.9%), without significant differences ($P = .857$)

Recurrences

Nine (17.3%) out of 52 survivors of the first COVID-19 episode presented recurrences.

Table 4 shows risk factors for recurrence. No recurrence occurred in patients who received the last dose of anti-CD20 more than 6 months before COVID-19. Patients with fever and dyspnea in the first episode presented recurrence more often (100% vs 59.5%, $P = .021$: and 77.8% vs 53.8%, $P = .019$, respectively). Chronic treatment with corticosteroids or other immunosuppressant was not associated with recurrence ($P = .705$ and $P = .183$, respectively).

Clinical data, treatment, and outcome of recurrences are summarized in Table 5 at patient-level data. The time from initial diagnosis to the first recurrence was 51 days (IQR 40–56 days), with a minimum of 36 and a maximum of 105. Four patients had more than 1 episode (2, 3, 5, and 6 episodes each).

In 6 patients recurrences were classified as relapses due to microbiological confirmation within 90 days from the first episode, 2 cases of them by RT-PCR in nasopharyngeal exudate, 3 by RT-PCR in bronchoalveolar lavage, and 1 case by positive

Table 2. Factors Associated With Mortality in COVID-19 Patients With Previous Treatment by Anti-CD20

Variable		Total (n = 57)	Survivor (n = 52)	Nonsurvivor (n = 5)	*P*	Missing
Demographic and comorbidity						
Age		51 (41–60)	47 (40–60)	71 (60–84)	.001	0
Gender (female)		70.2% (40)	73.1% (38)	40.0% (2)	.151	0
Charlson comorbidity index		2 (1–3)	2 (1–3)	5 (3–9)	.001	0
Arterial hypertension		22.8% (13)	15.4% (8)	100% (5)	.001	0
Diabetes mellitus		10.5% (6)	9.6% (5)	20.0% (1)	.458	0
Heart failure		8.8% (5)	5.8% (3)	40.0% (2)	.056	0
Chronic kidney disease		15.8% (9)	13.5% (7)	40.0% (2)	.173	0
Anti-CD20 therapy						
Indication	Hematological malignancy	21.1% (12)	17.3% (9)	60.0% (3)	.082	0
	Neurological	33.3% (19)	36.5% (19)	0		
	Systemic	42.1% (24)	42.3% (22)	40.0% (2)		
	Hematological nonmalignant	3.5% (2)	3.8% (2)	0		
Anti-CD20 duration (months)		11 (0.7–26)	12 (0.5–30)	7 (3–9)	.274	0
Chronic corticosteroids		29.8% (17)	30.8% (16)	20.0% (1)	1.000	0
Other immunosuppressant		17.5% (10)	17.3% (9)	20.0% (1)	1.000	0
Clinical presentation						
Cough		72.7% (40)	72.0% (36)	80.0% (4)	1.000	2
Fever		67.3% (37)	64.0% (32)	100% (5)	.102	2
Asthenia		61.2% (30)	60.0% (27)	75.0% (5)	.649	4
Arthromyalgia		39.6% (21)	39.6% (19)	40.0% (2)	1.000	5
Dyspnea		34.5% (19)	32.0% (16)	60.0% (3)	.209	2
Pulmonary infiltrate		70.0% (41)	65.7% (23)	100% (5)	.118	17
LDH (UImL)		292 (262–411)	286 (261–398)	377 (247–675)	.611	32
CRP (mg/L)		76 (36–152)	75 (19–145)	106 (52–199)	.667	32
Lymphocytes (cell $\times 10^3$/mL)		0.7 (0.5–1.2)	0.8 (0.6–1.3)	0.4 (0.1–0.6)	.042	32
D-Dimer (ng/mL)		855 (392–2200)	745 (315–4375)	990 (705–1495)	.505	32
Ferritin (ng/mL)		528 (214–1646)	458 (142–1343)	1208 (473–2217)	.357	32
Complications						
Acute respiratory distress		17.9% (10)	9.8% (5)	100% (5)	.001	1
Bacterial pneumonia		15.1% (8)	10.2% (5)	75.0% (3)	.009	4

Chronic corticosteroids: a systemic chronic therapy with corticoids with at least 5 mg/kg of prednisone or equivalent at time of SARS-CoV2 infection. Bacterial pneumonia: a microbiological confirmed bacterial coinfection or superinfection during the COVID-19 episode.
Abbreviations: COVID-19, coronavirus disease 2019; CRP, C-reactive protein; LDH, lactate dehydrogenase; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

immunohistochemistry of protein-S in pneumocytes in a surgical lung biopsy. Three recurrences were classified as possible relapses: a patient with no evidence of an alternative cause but without diagnostic test for SARS-CoV-2 (patient 4); a patient with first clinical recurrence 105 day after the initial episode, microbiologically confirmed with RT-PCR in nasopharyngeal exudate (Ct 21) without new epidemiological exposure (patient 6); and a patient with a RT-PCR in nasopharyngeal exudate with Ct 35, presenting symptoms consistent with viral infection and radiological worsening, without an alternative cause (patient 9). We did not identify any case of suspected reinfection.

Eight patients (88.9%) presented fever, 7 (77.8%) dyspnea, 7 (77.8%) cough. 5 patients (55.6%) showed worsening of previous infiltrates, and 8 (88.9%) occurrence of new ones. The 4 cases in which lymphocyte populations on bronchoalveolar lavage were available, showed a lymphocyte increase with CD4/CD8 ratio inversion. One of the episodes was complicated with acute respiratory distress, another with pulmonary thromboembolism and a third with pulmonary superinfection (*Pneumocystis jirovecii*). There were no further complications or deaths.

DISCUSSION

Our results show that patients treated with anti-CD20 monoclonal antibodies have a higher rate of severe COVID-19, death, a more protracted clinical course than general population, and frequent recurrences.

These patients do not seem to have an increased risk of SARS-CoV-2 infection compared to general population. We identified 13.5% of patients under anti-CD20 treatment with COVID-19, whereas the last prevalence study available in our setting showed 13.2% in the general population [22].

However, these patients are at high risk for poor clinical outcome, as described previously [14]. Nearly half of our patients required admission, with 1 out of 5 presenting acute respiratory distress syndrome, and a mortality rate close to 10%. Hospitalization rate was higher than in the general population

Table 3. Demographic, Clinical Presentation, and Complications of COVID-19 According to Time From Last Infusion to Infection Diagnoses

Variable	Total (n = 57)	< 3 months (n = 35)	3–6 months (n = 11)	> 6 months (n = 11)	*P*	Missing
Demographic and comorbidity						
Age	51 (41–60)	52 (44–61)	40 (30–57)	49 (30–69)	.247	0
Gender (female)	70.2% (40)	62.9% (22)	63.6% (7)	100%	.101	0
Charlson comorbidity index	2 (1–3)	2 (1–3)	1 (0–3)	3 (1–4)	.264	
Clinical presentation						
Cough	72.7% (40)	66.7% (22)	100%	63.6% (7)	.111	2
Fever	67.3% (37)	63.6% (21)	90.9% (10)	54.5% (6)	.175	2
Asthenia	61.2% (30)	63.3% (19)	88.9% (8)	30.0% (3)	.038	8
Arthromyalgia	40.4% (21)	35.5% (11)	60.0% (6)	36.4% (4)	.451	6
Dyspnea	34.5% (19)	39.4% (13)	36.4% (4)	18.2% (2)	.436	3
Creatinine (mg/dL)	0.76 (0.50–0.90)	0.73 (0.49–1.22)	0.97 (0.40–1.83)	[a]	.532	32
LDH (UI/mL)	299 (263–402)	264 (246–267)	332 (138–431)	[a]	.825	32
CRP (mg/L)	58 (48–152)	55 (17–83)	58 (41–103)	[a]	.675	32
Lymphocytes (cell $\times 10^3$/mL)	0.70 (0.41–1.20)	0.41 (0.32–0.96)	0.66 (0.36–1.21)	[a]	.320	32
D-Dimer (ng/mL)	910 (400–1600)	920 (445–1011)	1140 (265–2125)	[a]	.437	32
Ferritin (ng/mL)	287 (186–842)	313 (184–740)	279 (179–971)	[a]	.547	32
IL-6 (UI/mL)	31.7 (9.7–258)	21.6 (8.8–163)	32.7 (21–553)	[a]	.330	32
Pulmonary infiltrate	70.0% (28)	66.7% (16)	70.0% (7)	83.3% (5)	.888	18
Complications and outcomes						
Acute respiratory distress	17.9% (10)	23.5% (8)	9.1% (1)	9.1% (1)	.094	0
Bacterial pneumonia	15.1% (8)	21.2% (7)	9.1% (1)	0	.063	0
COVID-related death	8.8% (5)	14.3% (5)	0	0	.035	0

Chronic corticosteroids: a systemic chronic therapy with corticoids with at least 5 mg/kg of prednisone or equivalent at time of SARS-CoV2 infection. Bacterial pneumonia: a microbiological confirmed bacterial coinfection or superinfection during the COVID-19 episode.

Abbreviations: COVID-19, coronavirus disease 2019; CRP, C-reactive protein; ICU, intensive care unit; IL-6, interleukin 6; LDH, lactate dehydrogenase ; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Median and interquartile range (IQR) of blood determinations for patients with COVID-19 more than 6 months after anti-CD20 are not calculable because there are only 2 determinations available.

Table 4. Risk Factors for Relapses During the First Episode of SARS-CoV-2 Infection

Variable		Relapse (n = 9)	Non-relapse (n = 43)	*P*	Missing
Age		53 (35–60)	46 (40–57)	.755	0
Gender (female)		44.4% (4)	76.7% (32)	.052	0
Charlson comorbidity index		2 (0–6)	2 (0–3)	.562	0
Drug	Rituximab	77.8% (7)	76.7% (33)	1.000	0
	Ocrelizumab	22.2% (2)	23.2% (10)		
	Obinutuzumab	0	0		
Indication	Hematological Malignancy	11.1% (1)	16.3% (7)	.840	0
	Neurological	44.4% (4)	34.9% (15)		
	Systemic	33.3% (3)	41.9% (18)		
	Hematological nonmalignant	11.1% (1)	6.9% (3)		
Group[a]	< 6 months	22.0% (9)	78.0% (32)	.047	0
	> 6 months	0	100% (11)		
Post-COVID anti-CD20 dose		44.4% (4)	48.8% (20)	.909	5
Chronic corticosteroids		22.2% (2)	31.7% (13)	.705	0
Other immunosuppressant		0	22.0% (9)	.183	0
Persistent positive RT-PCR		37.8% (3)	27.3% (3)	.600	34
Serum antibody detection		0	33.3% (6)	.004	27
Cough		77.8% (7)	69.0% (29)	.704	1
Fever		100% (9)	59.5% (25)	.021	1
Asthenia		77.8% (7)	53.8% (21)	.077	6
Arthromyalgia		33.3% (3)	40.0% (16)	1.000	5
Dyspnea		77.8% (7)	23.8% (10)	.019	2
Pulmonary infiltrate		77.8% (7)	64.3% (18)	1.000	15
Acute corticoid treatment		77.8% (7)	35.7% (15)	.024	1

Chronic corticoid was defined as a systemic chronic therapy with corticoids at least with 5 mg/kg of prednisone or equivalent at time of SARS-CoV2 Infection.

Abbreviations: COVID, coronavirus disease; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Percentages in this variable are calculated across rows. For the rest of variables, percentages are calculated across columns.

Table 5. Individual Characteristics of Patients Experiencing a Relapse Episode After a SARS-CoV-2 Infection

Patient	Recurrence Classification	Demographic	Anti-CD20	Group	No.	Time to Relapses	Status Between Episodes	Clinical Presentation	Diagnoses	Management	Outcomes
1	Relapse	35 years old, female	Multiple sclerosis	3–6 months.	2	38 days 112	Dyspnea of moderate exertion	Fever, dyspnea, cough	Nasopharyngeal RT-PCR (Ct unknown)	Corticoid	Radiological improvement. Persistent dyspnea
		No comorbidity	ocrelizumab	164 days		days		Worsening and new pulmonary infiltrates			
2	Relapse	56 years old, male	High-grade lymphoma	< 3 months	6	34 days	Asymptomatic	Fever, dyspnea, cough	Pulmonary biopsy and BAL RT-PCR (Ct 22)	Corticoid convalescent plasma remdesivir	Since plasm and remdesivir, asymptomatic and radiological resolution
		No comorbidity	rituximab	33 days		143 days		New pulmonary infiltrates			
								Superinfection			
3	Relapse	24 years old, female	Multiple sclerosis	3–6 months	5	58 days	Asymptomatic	Fever	BAL RT-PCR (Ct 23)	Corticoid	Continues with migratory infiltrates.
								Worsening and new pulmonary infiltrates			Asymptomatic
		DM	rituximab	135 days		287 days					
4	Possible relapse	35 years old, male	Hemolytic anemia	3–6 months	1	51 days	Asymptomatic	Fever, dyspnea, cough	No confirmation (no test done)	No specific treatment	Radiological resolution
		No comorbidity	rituximab	154 days				New pulmonary infiltrates			Asymptomatic
5	Relapse	55 years old, male	Multiple sclerosis	< 3 months	1	36 days	Dyspnea of moderate exertion	Fever, dyspnea, cough	Nasopharyngeal RT-PCR (Ct 29)	No specific treatment	Radiological improvement. Persistent dyspnea
		CF	ocrelizumab	65 days				Worsening of pulmonary infiltrates			
6	Possible relapse	53 years old, male	Multiple sclerosis	< 3 months	1	105 days	Asymptomatic	Dyspnea	Nasopharyngeal RT-PCR (Ct 21)	Corticoid	Asymptomatic
		No comorbidity	Rituximab	10 days				Worsening and new pulmonary infiltrates		Immunoglobulin	No radiological follow-up
7	Relapse	48 years old female	Rheumatoid arthritis	< 3 months	3	47 days	Asymptomatic	Fever, dyspnea, cough	BAL RT-PCR (Ct 34)	Corticoid convalescent plasma	Asymptomatic
		No comorbidity	Rituximab	7 days		83 days		New pulmonary infiltrates		Immunoglobulin	No radiological follow-up
8	Relapse	60 years old, male	Sjogren's syndrome	<3 months	1	54 days	Dyspnea of moderate exertion	Fever, dyspnea, cough,	BAL RT-PCR (Ct 23)	Corticoid	Radiological improvement. Asymptomatic
		Liver cirrhosis, CF, CRF	Rituximab	86 days				New pulmonary infiltrates		Remdesivir convalescent plasma tocilizumab	
								Acute respiratory distress			
9	Possible relapse	80 years old, female	Vasculitis	3–6 months	1	51 days	Asymptomatic	Fever, dyspnea, cough, new pulmonary infiltrates	Nasopharyngeal RT-PCR (Ct 35)	Corticoid	Asymptomatic
		HT, DM, CRF	Rituximab	95 days				Thromboembolism			No radiological follow-up

Abbreviations: BAL, bronchoalveolar lavage; CF, cardiac failure; CRF, chronic renal failure; Ct, cycle threshold; DM, diabetes mellitus; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

in our setting (43.9% vs 14.7%) [23]. Mortality rate was higher than that registered worldwide (8.8% vs 2.1%) or in our country (8.8% vs 2.6%) for general population [24]. For hospitalized patients, mortality was higher than previously described in our institution for general population (20.0% vs 13.8%) [25, 26] and critical illness was higher than described in a nationwide register (37.9% vs 17.8%) [27]. We also found a higher proportion of bacterial superinfections (36.4% vs 11.1%) [27]. Mortality rate was also beyond that of other immunosuppressed patients described in a similar scenario (20.0% vs 11.5%) [28, 29]. Of note, the severity of COVID-19 was not influenced by other immunosuppressant treatments. Our data support the importance of the interval between the last dose of anti-CD20 drug and the diagnosis of COVID-19 in the probability of developing severe

disease and death, as previously speculated [11] in keeping with the pharmacokinetics of the anti-CD20 treatment. Rituximab is detectable in serum during 3 to 6 months and B-cell function recovers after 6 months [30, 31]. This would produce a deeper humoral immunosuppression in the first 6 months, especially in the first 3 months after its administration [7, 30]. In fact, in our study, the prognosis of patients who acquired COVID-19 beyond 6 months from last infusion was similar to that of the general population (hospitalization 18.0% vs 14.7% [23] and mortality 0% vs 2.6%) [24]. They also had a higher prevalence of positive serum antibodies than patients who acquired COVID-19 in the first 6 months from the last administration (40.0% vs 14.3%). We propose that patients with SARS-CoV-2 infection during the first 6 months from the last anti-CD20 dose, and particularly, during the first 3 months, may require additional therapeutic interventions to avert their potentially worse COVID-19 prognosis [16, 32–35]; however, the efficacy of such interventions in this population requires further study.

We demonstrated that a significant percentage of patients persisted with positive RT-PCR months after infection, which had been observed in previous small case reports [20]. The positivity of these tests, often with low Ct values, along with negative serology, suggest that these patients represent a persistent infectious source for a longer time. Accordingly, isolation period should be longer and probably based on the negativity of RT-PCR [35].

A striking fact of our work was the high proportion of patients presenting clinical recurrence of the viral infection, and specifically of relapses (17.3% vs 0.1%), as compared to the general population [36]. Although re-positivity may be a common phenomenon [37], clinical episodes of relapses have been previously described only sporadically [32, 38–40]. One out of 5 of our patients presented at least 1 episode of clinical relapse, most of them occurring within 3 months following the initial infection. In our series, risk factors for relapse included developing COVID-19 during the first months from last anti-CD20 infusion and presenting an initial infection with more severe symptoms. Chronic corticoid or concomitant immunosuppressant drugs were not associated with relapse. The usual clinical presentation and possible complications of relapses were consistent with acute COVID-19. A common feature in bronchoalveolar lavage was the increase in lymphocyte count, with CD4/CD8 ratio inversion. It is important to consider the possibility of a relapse COVID-19 in patients with these features and pursue the diagnosis, including repeated RT-PCR in lower airway tract and, in cases without an alternative etiology, a lung biopsy with immunohistochemistry techniques. Treatment of these episodes could include the use of hyperimmune plasma and/or antiviral drugs [16, 32, 33].

Another controversial issue is the management of immunosuppression once the infection has resolved in patients that require anti-CD20 therapy [11, 41–44]. Our data suggest that, in patients who have recovered from COVID-19, it is safe to resume anti-CD20 therapy. Likewise, it is safe to initiate de novo an anti-CD20 drug in patients recovered from SARS-CoV-2 infection. Therefore, we recommend continuing or initiating anti-CD20 treatment once the symptoms have receded and RT-PCR determinations are negative.

Although our study does not provide specific information about serologic response to SARS-CoV-2 vaccination, we did observe that the more distant from the last dose of anti-CD-20 drug, the higher the serologic response to infection. Thus, we speculate that the COVID-19 vaccine may be more effective the longer it is separated from the last dose, suggesting at least 3 months delay, in line with other authors [44, 45]. The optimal approach to vaccination requires clinical studies in this subgroup of patients.

Our study has several limitations. First, it is a retrospective single center study. Second, since we did not have a control group, comparisons with the general and immunocompromised population are based on data from other studies. These comparisons convey 2 possible biases: first, no statistical tools were used to confirm whether the numerical difference was statistically significant; additionally, characteristics and comorbidities of general and immunocompromised populations may differ from our study population. However, this bias was reduced choosing population-based studies made in our area. Besides, regarding the need to prolong the isolation of these patients, it was not possible to perform viral cultures, so we cannot confirm infectivity. Despite this, the frequent low Ct found in our patients has been associated to a high probability of infectivity [17, 18, 46], providing validation to our conclusions. Fourth, we did not have access to whole genome analysis to confirm that recurrent episodes were caused by the same strain than the first one. However, based on previously published experience [12, 31, 40] and the clinical profile of our patients, we hypothesized that the majority of recurrence episodes in these patients, in the absence of a new epidemiological exposure, were due to relapses. Finally, the relatively low number of patients and the observational nature of the study prevent us from drawing causal conclusions regarding treatment. Nevertheless, we believe that the data we obtained are valuable and may lead to hypotheses for future therapeutic trials.

CONCLUSION

SARS-CoV-2 infection in patients under treatment with anti-CD20 antibodies shows an incidence similar to that of the general population, although with higher rates of hospital admission and mortality, because the risk is higher the closer the infection is to the last dose of anti-CD20, mainly in the first 3 months. The duration of infectivity may be longer than in other groups. Relapses of COVID-19 occur in patients infected during the first 6 months after anti-CD20 infusion. The use of convalescent plasma may be an option. Once the infection is resolved, it is safe to resume treatment with anti-CD20. Further

studies are needed to evaluate the management of SARS-CoV-2 infection in patients treated with these drugs.

Note

Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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