Rituximab is a monoclonal antibody that targets CD20+ B cells. After rituximab infusion, B-cell depletion usually ensues, mainly of B memory cells, with a subsequent reduction of IgG production and of B effector cells, resulting in a lower production of IgM. Furthermore, a secondary effect on T helper cell response has also been described. Rituximab is widely used for treating rheumatic diseases (RMDs) such as systemic sclerosis, systemic lupus erythematosus, Sjögren syndrome, and idiopathic inflammatory myopathies, although it has only been approved for treating rheumatoid arthritis (RA), microscopic polyangiitis, and granulomatosis with polyangiitis.

The effect of rituximab on B-cell response has been studied, focusing on the possible associated higher rates of infection and the lower seroconversion rates after vaccination in treated patients. A lower IgG response has been previously described after influenza and pneumococcal vaccination in patients with RA.2 Retrospective studies have reported a higher infection rate in RA patients treated with rituximab compared with other biologies such as abatacept or tocilizumab.3

Whether patients with RMD have a higher risk for severe COVID-19, especially those receiving rituximab treatment, has been a matter of debate since the start of the pandemic. Initial case reports and small cohorts reported a possible increase in the risk of severe SARS-CoV-2 infection in patients treated with rituximab.4 This finding has not been consistently observed in larger cohorts.5 Only the COVID-19 Global Rheumatology Alliance registry, which evaluated factors associated with COVID-19–related deaths, found rituximab exposure to be an independent risk factor.6

A recent case report highlighted the lack of seroconversion and possibility of reinfection in patients treated with rituximab.7 After the first COVID-19 wave (polymerase chain reaction [PCR] detection was not widely available initially), Madrid was a registered “high impact area” with official data showing more than 390,242 infected people and 19,291 infection-related deaths until December 22, 2020.8 After the first COVID-19 wave (PCR determination were documented at inclusion.

Seroconversion after possible SARS-CoV-2 infection, in a cohort of unvaccinated RMD patients treated with rituximab in a high impact area, has not previously been described.

**METHODS**

**Study Design**

Medical records review study of a cohort of patients with an RMD followed up at the Rheumatology Department of the Ramón y Cajal University Hospital (Madrid, Spain), who had undergone a serological test for anti–SARS-CoV-2 IgG between April 15, 2020 and December 22, 2020. Positivity rate for anti–SARS-CoV-2 IgG and predictors of a positive serological result were analyzed in rituximab-treated patients and compared with those not treated with rituximab.

The current study was a subanalysis of a larger study of SARS-CoV-2 infection in RMD patients (study number 136/20), approved by the local ethics committee (Comité de Ética de Investigación con Medicamentos del Hospital Universitario Ramón y Cajal) on May 5, 2020. All patients provided informed consent to participate and for publication of data before their inclusion. The research was conducted in compliance with the Helsinki Declaration.

**Patients**

Patients aged >16 years, regardless of previous COVID-19 history, were included. Patients with an RMD treated with targeted synthetic disease-modifying antirheumatic drugs (tsDMARDs) or biological tsDMARDS (bsDMARDs), rather than rituximab or TNF inhibitor were excluded (Figure). Patients were classified into 2 groups according to rituximab treatment in the previous year (RTX group and n-RTX group). History of confirmed/suspected COVID-19 and serological test result for anti–SARS-CoV-2 were recorded. Patients treated with corticosteroids, conventional DMARDs (cDMARDs), and/or TNF inhibitor constituted the control group (n-RTX group).

Rheumatic disease diagnosis, age at diagnosis, age at time of serological test for SARS-CoV-2, comorbidities, interstitial lung involvement, previous suspected/confirmed COVID-19, and previous PCR determination were documented at inclusion.

Rheumatic disease diagnosis was divided into 2 subgroups for the purpose of data analysis: arthropathies and connective tissue diseases. Arthropathies included RA (and secondary Sjögren syndrome), psoriatic arthritis, juvenile idiopathic arthritis, spondyloarthropathies, gout, and polymyalgia rheumatica. Connective tissue diseases included systemic sclerosis, inflammatory myopathies, systemic lupus erythematosus, vasculitis, and primary Sjögren syndrome.

**Variables and Operative Definitions**

Rate of anti–SARS-CoV-2 IgG positivity was considered the primary endpoint of the study, defined as the percentage of patients having a positive serological result in each group (also calculated according to previous history of confirmed or suspected COVID-19). Confirmed COVID-19 was considered in patients with at least 2 symptoms: a positive PCR for SARS-CoV-2 and/or a compatible chest x-ray. Suspected COVID-19 was diagnosed in patients presenting with at least 2 symptoms suggestive of SARS-CoV-2 infection.

**Sero logical Test**

One SARS-CoV-2 antibody assay was available in the hospital’s routine laboratory and was used during the study. A chemiluminescent microparticle immunoassay for SARS-CoV-2 IgG was used (SARS-CoV-2 IgG for use with ARCHITECT; Abbott Laboratories, Abbott Park, IL; reference 06R8620). This is a qualitative
assay for the detection of IgG antibodies against the SARS-CoV-2 nucleocapsid protein (N-IgG) in human serum and plasma. Positivity of anti N-IgG is defined by an index >1.40.

Statistical Analysis

Categorical variables were reported as proportions and/or percentages, whereas continuous variables were expressed as the mean and standard deviation (SD) or median values and interquartile ranges (IQRs), for normally or nonnormally distributed variables, respectively. The Mann-Whitney U test, Student t test, and χ² test were used to compare data (RTX and n-RTX groups), when appropriate. A multivariate logistic regression model was plotted to identify the association of rituximab treatment and a positive anti–SARS-CoV-2 IgG result. Odds ratios (ORs) were calculated with 95% confidence interval and adjusting for potential confounding factors. Variables were selected if they modified the crude OR by more than 10%. Statistical significance was assumed at a p value <0.05. Independent variables were selected for the multivariate model based on clinical judgment or if the p value was <0.20 in the bivariate analysis. Multicollinearity among independent variables was also explored, using Pearson and Spearman correlations to build the model. All the analyses were performed using the SPSS 25.0 statistical program.

RESULTS

One-hundred fifty-two patients were included, 48 of whom were on rituximab treatment. The demographic and clinical characteristics of patients included in the study and the bivariate analysis comparing the RTX and n-RTX groups are summarized in Table 1.

Median age at inclusion was similar in the groups, 73.7% of patients were female. Regarding diagnosis, this was equally distributed, almost half of the patients were diagnosed with a connective tissue disease in both groups, but a higher rate of interstitial lung disease (ILD) was reported in the RTX group (RTX, 35.4%; n-RTX, 7.7%; p < 0.0001).

More than half of the patients in the RTX group were treated with corticosteroids, but median dose was not different between groups. Conventional DMARDs were more frequently prescribed to n-RTX patients, and 53.9% were neither treated with rituximab nor TNF inhibitor.

Only 33.5% of the cohort had a history of suspected and confirmed COVID-19. Rates of confirmed COVID-19 were similar between groups; however, a higher percentage of n-RTX patients had a history of suspected disease (n-RTX, 17.3%; RTX, 6.3%; p = 0.079).

Positivity Rate

Overall, seropositivity rate for anti–SARS-CoV-2 IgG was 25.7%. Among RTX and n-RTX groups, 8.3% (4/48) and 33.7% (35/104) (p = 0.01) had a positive anti–SARS-CoV-2 IgG, respectively. Four of 104 (3.8%) n-RTX patients tested positive without previous symptoms. No asymptomatic infections were diagnosed in the RTX group.
Univariable analysis showed a lower rate of positive anti–SARS-CoV-2 IgG in the RTX group with both confirmed (40%) and suspected (0%) infection compared with the n-RTX group, 80% and 83.3%, respectively (\(p = 0.045\) and \(p = 0.015\)).

A multivariate analysis was plotted to identify the effect of rituximab treatment on a negative anti–SARS-CoV-2 IgG result (Table 2). Rituximab treatment was the main factor associated with a negative IgG result, followed by older age at inclusion. Male sex and a previous positive SARS-CoV-2 PCR were identified as independent factors associated with a positive anti–SARS-CoV-2 IgG. The presence of ILD and cDMARDs use was retained in the model as confounding factors, whereas corticosteroids and previous chronic obstructive pulmonary disease were not included as they did not influence the main variable (RTX group).

**DISCUSSION**

The current study found a lower seroconversion rate in the RTX group, regardless of previous COVID-19 history. To the au-

### TABLE 1. Demographic and Clinical Characteristics

| Patients, n (%) | Rituximab (RTX = 48) | No Rituximab (n-RTX = 104) | Total Cohort (n = 152) | p value |
|----------------|----------------------|---------------------------|------------------------|---------|
| Age at inclusion, mean (SD), y | 62.3 (14.9) | 58.4 (17.5) | 59.6 (16.8) | 0.190 |
| Female, n (%) | 38 (79.2) | 74 (71.2) | 112 (73.7) | 0.297 |
| Diagnosis, n (%) | 25 (52.1) | 58 (55.8) | 83 (54.6) | 0.727 |
| Connective tissue diseases | 23 (47.9) | 46 (44.2) | 69 (45.4) | 0.992 |
| Comorbidities, n (%) | 18 (37.5) | 34 (32.7) | 52 (34.2) | 0.561 |
| Diabetes | 5 (10.4) | 10 (9.6) | 15 (9.9) | 0.878 |
| Dyslipidemia | 18 (37.5) | 30 (28.8) | 48 (31.6) | 0.286 |
| COPD/asthma | 6 (12.5) | 4 (3.8) | 10 (6.6) | 0.045* |
| ILD, n (%) | 11 (22.9) | 25 (24) | 36 (23.7) | 0.831 |
| CVD, n (%) | 17 (35.4) | 8 (7.7) | 25 (16.4) | 0.0001* |
| CCs, n (%) | 23 (47.9) | 46 (44.2) | 69 (45.4) | 0.992 |
| CCs, median (IQR), mg/d | 5 (5–10) | 5 (3.8–8.8) | 5 (5–10) | 0.217 |
| cDMARDs, n (%) | 27 (56.3) | 73 (70.2) | 100 (65.8) | 0.092 |
| bDMARDs, n (%) | 0 (0) | 82 (78.8) | 82 (53.9) | 0.0001* |
| Previous PCR, n (%) | 29 (60.4) | 72 (69.2) | 101 (66.4) | 0.191 |
| Negative | 11 (22.9) | 12 (11.5) | 23 (15.1) | 0.574 |
| Positive | 8 (16.7) | 20 (19.2) | 28 (18.4) | 0.356 |
| Previous symptoms, n (%) | 43.5 (10.5–89.3) | 62.5 (18.3–88.3) | 63 (19–90.5) | 0.181 |
| COVID-19, n (%) | 13 (27.1) | 36 (34.6) | 49 (32.2) | 0.181 |
| No suspected | 107 (44–238-5) | 80.5 (32.8–124.3) | 92 (35.5–155) | 0.001 |
| Suspected | 35 (72.9) | 66 (63.5) | 101 (66.4) | 0.183 |
| Confirmed | 3 (6.3) | 18 (17.3) | 21 (13.8) | 0.001 |
| n* Two patients had a negative PCR but compatible chest x-ray and symptoms requiring hospitalization.

cDMARDs, conventional disease-modifying anti-rheumatic drugs; CCs, corticosteroids; CI, confidence interval; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; IQR, interquartile ranges; ILD, interstitial lung disease; PCR, Polymerase chain reaction; SD, standard deviation.

### TABLE 2. Multivariate Analysis

| Total Cohort (n = 152) | OR (95% CI) | p value |
|------------------------|-------------|---------|
| Sex (ref female) | 4.23 (1.55–11.50) | 0.005 |
| Age at inclusion, y | 0.97 (0.94–0.99) | 0.026 |
| ILD (ref no ILD) | 0.35 (0.08–1.47) | 0.151 |
| cDMARD (ref no cDMARD) | 1.61 (0.58–4.44) | 0.359 |
| Previous PCR (ref no previous PCR) | 1.98 (0.51–7.70) | 0.325 |
| Negative | 18.72 (5.20–67.43) <0.0001 |
| Positive | 0.08 (0.02–0.37) | 0.001 |

cDMARDs, conventional disease-modifying anti-rheumatic drugs; CI, confidence interval; ILD, interstitial lung disease; OR, Odds Ratio; PCR, Polymerase chain reaction; Ref, Reference; RTX, rituximab.
In this cohort, rituximab treatment was the main factor associated with a negative anti–SARS-CoV-2 IgG. A lower positivity rate of anti–SARS-CoV-2 IgG was found in the RTX group, regardless of previous COVID-19 history. No asymptomatic infections were diagnosed, and no suspected COVID-19 cases were confirmed in the RTX group. Therefore, seroconversion should be assessed after COVID-19, and vaccination strategies should be reviewed in patients treated with rituximab.

ACKNOWLEDGMENTS

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by all authors. The first draft of the manuscript was written by A. G.-F and PM-A., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. The authors wish to thank M.A. Martín-Martínez for her outstanding help in conducting the statistical design and analysis.

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