

Seroconversion for SARS-CoV-2 in Rheumatic Patients on Synthetic and Biologics Disease Modifying Anti-Rheumatic Drugs in São Paulo, Brazil

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Abstract

Introduction: There is a lack of information on how immunomodulatory drugs for autoimmune rheumatic diseases (ARDs) impair humoral immune response following SARS-CoV-2 exposure.

Methods: A prospective study was performed with ARD patients on synthetic or biologic DMARDs (sDMARDs or bDMARDs) classified into three groups (antimalarial monotherapy, antimalarial plus bDMARD, antimalarial plus sDMARD) and a fourth group (control). All patients underwent a clinical baseline interview, anti-SARS-CoV-2 IgG/IgM tests at baseline and three months later, monitored for incident respiratory symptoms at follow-up, with rRT-PCR in suspected cases.

Results: One hundred patients were included. Fewer than half who turned IgG positive (42.8%) remained asymptomatic. All three positive rRT-PCR patients showed seroconversion for anti-SARS-CoV-2 IgG. There was also a trend for significant association for more frequent use of bDMARDs in IgG-positive patients (42.9% vs. 19.8%, $p=0.056$). Although patients on bDMARDs were also on antimalarial drugs, most of the patients who were not on bDMARDs were also on antimalarial drugs (group 1 and 3). Hence antimalarial use was widely present in both comparator groups. On the other hand, none of the patients on non-antimalarial sDMARD had detectable anti-SARS-CoV-2 IgG compared to 35.4% the remaining sample (0.0% vs. 35.4%, $p=0.050$).

Conclusion: Although anti-SARS-CoV-2 IgG positivity was quite common (14% incidence), half evolved asymptotically. Temporally withholding bDMARD therapy in ARD patients during the pandemic based on possible humoral response impairment seems not suitable. sDMARD was associated with a lower incidence of anti-SARS-CoV-2 IgG positivity, although the study was not properly designed to clarify this matter.

Keywords: Autoimmune diseases • Virus diseases • Antirheumatic agents • Biologic agents

Introduction

Coronavirus disease 2019 (COVID-19), caused by a newly described beta coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 virus), has spread worldwide since the first official case was reported in Wuhan, Hubei Province, central China, in December 2019 [1,2]. Since then, with a well-marked feature of fast dissemination by inter-human contact, in addition to its high level of virulence, the disease has brought people to an unprecedented health crisis and has forced the World Health Organization (WHO) to declare that COVID-19 has become pandemic [3].

Currently, the entire world registers over 108 million infected people and a number of lethal cases of approximately 2.4 million [4]. However, despite being classified as a major public health problem, coronavirus disease is usually characterized by the presence of mild respiratory symptoms (cough, fever, dyspnea and fatigue) accompanied by lymphopenia. Nevertheless, in the most severe cases, it might evolve to pneumonia with an acute respiratory syndrome and sometimes lead to death [5].

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In this pandemic setting, for the purpose of identifying susceptible groups, it has been shown that patients with severe SARS-CoV-2 infection share some comorbidities, such as diabetes mellitus, arterial hypertension, coronary heart disease, and previous lung disease [6]. Since all these conditions are characterized by underlying inflammation, it was reasonable to presume that chronic inflammatory rheumatic diseases might also arise as a risk factor for COVID-19 infection [7]. This was particularly true because of the already known increased risk for viral infections in this group of patients [8]. Furthermore, most synthetic and biologic disease-modifying antirheumatic drugs (DMARDs) currently used in ARD patients have already been shown to increase both the incidence and severity of infections; thus, COVID-19 could additionally run a more severe course in these patients [9,10]. However, with the increase in coronavirus scientific data, from the initial case reports to well-designed longitudinal studies on risk factors for COVID-19, it became clear that inflammatory rheumatic diseases were seldom included as a risk factor either for incident or severe SARS-CoV-2 infection [11]. In addition, there is evidence of some biologic DMARDs being used for the treatment of severe cases of COVID-19 [12-14].

The diagnosis of COVID-19 acute infection is based on clinical features, but preferably confirmed by the detection of viral RNA in naso/oropharyngeal swabs by nucleic acid amplification methods such as real-time reverse transcription-polymerase chain reaction (rRT-PCR) and loop-mediated isothermal amplification (LAMP) [15-17]. Serologic tests for IgG, IgA and IgM anti-SARS-CoV-2, targeting different viral antigens, have recently been implemented in clinical practice. Its value resides in confirming exposure to SARSCoV-2, including patients with negative RT-PCR results, being more

effective particularly after 10 days of symptoms onset [18]. Serology may also prove to be important for identifying the development of persistent COVID-19 immunity, as detected by the persistence of serum antibody positivity, particularly for IgG; however, whether it could prevent recurrent infection is still unknown [19].

The ability to produce detectable levels of anti-SARS-CoV-2 antibodies after COVID-19 exposure seems to vary among patients. Some patients will develop high titers of IgM/IgA and most importantly IgG, while a substantial amount of them will not present any serum antibody detected by current methods, even after a PCR-confirmed COVID-19 infection [20]. The factors, either clinical or demographic, that determine one person to produce detectable antibodies after exposure are unclear. Likewise, it is also unknown whether rheumatic patients and the use of conventional or biologic DMARDs have any effect on anti-SARS-CoV-2 antibody development.

This study aimed to assess the serologic performance of rheumatic patients, for both non-autoimmune rheumatic diseases and autoimmune rheumatic diseases (ARDs), on synthetic and biologic DMARD during the COVID-19 pandemics in São Paulo, Brazil.

Materials and Methods

Patient selection

One hundred patients (≥ 18 yrs) with a diagnosis of rheumatic diseases followed by four rheumatologists (members of this research team: FMS, MOP, JBL, JFC) were enrolled in this prospective study from March 2020 to August 2020 in São Paulo, Brazil.

To ensure representativeness of using multiple different synthetic and biologic DMARDs, a convenience sampling method was performed by selecting patients according to medication use into four groups: Group 1 (antimalarial monotherapy), Group 2 (antimalarial plus biologic DMARD), Group 3 (antimalarial plus any other synthetic DMARD) and Group 4 (no antimalarial/DMARD). The latter consisted of a control group of patients currently not on any synthetic or biologic DMARD, including antimalarials. To proceed with the identification of potential patients for enrollment, the rheumatologists performed an electronic systematic search in their medical records searching for common names of antimalarials, non-antimalarial sDMARDs and bDMARDs. Diseases known not to use any of these medications, such as Fibromyalgia, Osteoarthritis and Osteoporosis, were searched for the control group (Group 4). Patients were then sequentially invited from these lists and, once informed consent signed, they were allocated into each group according to medication use.

Clinical and demographic data

Patients underwent a baseline clinical interview by telephone, email or office appointment to confirm medical information. Demographic and disease clinical data were collected. Patients were also asked at baseline whether they had any respiratory symptoms suggestive of COVID-19 at any time since the beginning of the pandemic. They were then weekly assessed for a total period of 12 weeks using a specific questionnaire to monitor symptoms such as cough, rhinorrhea, dyspnea, anosmia, fatigue, diarrhea and fever, as well as the need for hospitalization.

Laboratory data assessment

Study participants were scheduled for two at-home blood sample collections for anti-SARS-CoV-2 IgM and IgG identification. An automated chemiluminescence immunoassay (CLIA) for the qualitative determination of IgG and IgM antibodies against the spike (S) and nucleocapsid (N) proteins from SARS-CoV-2 in human serum or plasma was run in the MAGLUMI analyzer (Snibe Diagnostics, Shenzhen China) according to the manufacturer's instructions. The results are presented in aleatory units per mL (AU/mL) in comparison to calibrators also provided in the kit.

The first blood collection was drawn at baseline and the second one up to twelve weeks later. Between these two procedures, all patients were monitored

through weekly telephone contact actively searching for new-onset respiratory symptoms. Symptomatic patients were referred to their treating rheumatologist to judge whether these symptoms could not be otherwise explained by previous chronic respiratory conditions. If the acute respiratory syndrome was deemed to be highly suggestive of COVID-19 infection by the treating physician, then the patient was submitted to at-home naso/oropharyngeal swab collection for SARS-CoV-2 rRT-PCR testing. The combined naso/oropharyngeal swabs were immersed in 3 mL of sterile saline 0.9% and transported to the lab.

RT-PCR: An aliquot of 200 μ L was extracted by the DSP Virus/Pathogen kit in the automated platform QIASymphony and eluted in 60 μ L. Five microliters of eluate was subjected to rRT-PCR with primers and probe from the viral E gene in duplex to the cellular control RNaseP, as described, employing TaqMan Fast Virus 1-Step Master Mix (ThermoFisher, Brazil). A Ct value of 35 was adopted as the cut-off [21]. The limit of detection was determined as 408 copies/mL by probit analysis using the ACCUPLX SARS-COV-2 reference material (0505-0126, Seracare, USA).

Patients whose serologic test resulted in IgG positivity at baseline were censored and thus not submitted to the second blood collection.

Statistical analysis

All demographic and clinical variables were compared between patients according to serologic status, which was assessed in four different scenarios: positivity for any immunoglobulin (Ig) at any time, positivity for IgG at any time, seroconversion for any Ig throughout the follow-up and seroconversion for IgG throughout the follow-up. Seroconversion was defined as the absence of the respective antibody at baseline followed by a later positive test.

All analyses were performed using R software version 3.5.2 (R Development Core Team, 2005). Chi-square, Fisher's exact, Mann-Whitney, Student's T, and Welch's T tests were used as appropriate. A univariate analysis was performed between baseline variables for the different serologic classifications. The significance level was set at 5% ($P=0.05$).

The study was approved by the local ethical board (Ethics Committee from Hospital Santa Paula) and by the national ethical board (CONEP-National Commission on Ethics and Research) at the register number CAAE: 30444020.3.0000.0008. All patients signed a written informed consent form before enrollment, and the study was conducted in accordance with the Declaration of Helsinki [22].

Results

A total of 100 patients were selected and included in the final analysis (Figure 1). The demographic data are described in Table 1. The cohort was largely represented by autoimmune rheumatic diseases (ARDs). Systemic lupus erythematosus (SLE) was the most common diagnosis (19%), followed by psoriatic arthritis (PsA) (16%) and rheumatoid arthritis (RA) (15%). The remainder ARDs consisted of Undifferentiated Connective Tissue Diseases (UCTDs), Ankylosing Spondylitis (ASs), other spondyloarthritis (SpAs), Sjogren Syndromes (SS), Antiphospholipid Syndrome (APS) and Kikuchi Disease. Overall, the cohort of ARDs consisted of patients in remission of disease activity (76.3%). Those with mild and moderate disease activity represented 14.5% of ARDs patients. Percentage of patients in disease remission according to specific diagnosis were as follows: UCTD (100%), AS (100%), Kikuchi Disease (100%), APS (100%), PsA (94%), other SpAs (83%), RA (64%), SLE (63%), and SS (60%). The sample size for each group was as follows: Group 1 ($n=28$), Group 2 ($n=23$), Group 3 ($n=23$) and Group 4 ($n=26$). Twenty-six (26%) patients were not on any synthetic or biologic DMARD, including antimalarial drugs. These individuals represented a miscellaneous combination of non-ARDs including Fibromyalgia, Osteoporosis and Osteoarthritis. They served the purpose of a control group (Group 4). Antimalarials used included Hydroxychloroquine Sulphate and Chloroquine Diphosphate. Non-antimalarial synthetic DMARD (sDMARD) included Methotrexate, Sulfasalazine, Azathioprine, 6-mercaptopurine and Mycophenolate Mofetil. Biologic DMARDs (bDMARD) included Abatacept, Secukinumab, Infliximab, Golimumab and Adalimumab.

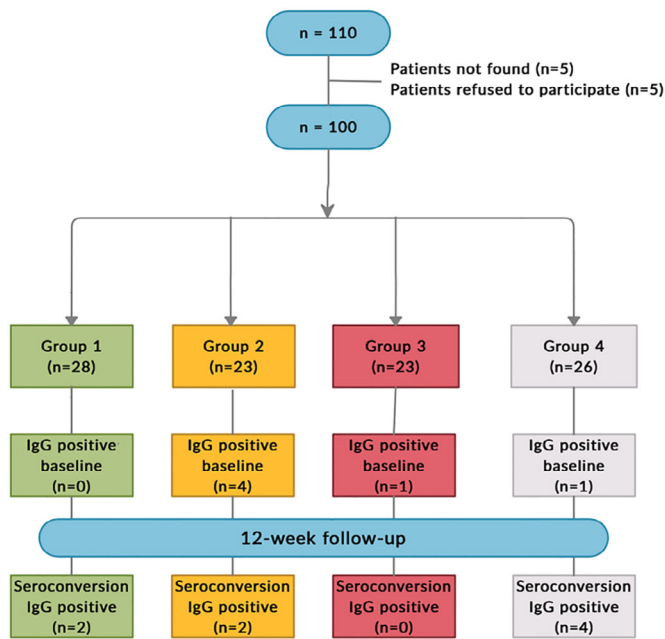


Figure 1. Schematic overview of the study design. Prospective study with four treatment arms. A total of 100 patients with rheumatic diseases were enrolled. Six of these patients had detectable anti-SARS-CoV-2 IgG at baseline. All patients were followed for up to 12 weeks with regular weekly telephone contact actively searching for incident respiratory symptoms. Seroconversion for anti-SARS-CoV-2 IgG was found in eight patients.

Table 1. Demographic and clinical characteristics of RD patients enrolled in the study.

	N = 100
Age (years), mean (SD)	46.5 (14.2)
Sex, n (%)	
Male	15 (15.0%)
Female	85 (85.0%)
Months since RD diagnosis, median (IQR)	36 (21-80)
RD activity, n (%)	
Remission	49 (73.1%)
Mild	8 (11.9%)
Moderate	6 (9.0%)
Severe	4 (6.0%)
Respiratory symptoms, n (%)	33 (33.0%)
Groups according to therapy, n (%)	
Group 1	28 (28.0%)
Group 2	23 (23.0%)
Group 3	23 (23.0%)
Group 4	26 (26.0%)
Antimalarial use, n (%)	74 (74.0%)
sDMARD use, n (%)	31 (31.0%)
Months on sDMARD use, median (IQR)	7 (3-31)
bDMARD use, n (%)	23 (23.0%)
Months on bDMARD use, median (IQR)	16 (1-45)
GC use, n (%)	43 (43.0%)
GC dose ¹ , median (IQR)	0 (0-3)

Note: SD, standard deviation; RD, rheumatic disease; IQR, interquartile range; sDMARD, synthetic DMARD; bDMARD, biologic DMARD; GC, glucocorticoid.¹prednisone or equivalent to prednisone.

At baseline, 7 (7%) patients tested positive for anti-SARS-CoV-2 antibodies, either IgG, IgM or both. Of these, 6 were positive for IgG and, hence, were censored. None except for 1 could recall any respiratory symptoms since the beginning of the pandemics. The patient who did recall respiratory symptoms presented four weeks before study enrollment with

typical COVID-19 symptoms, including fever, fatigue, cough and dyspnea. By that time, her chest CT confirmed a highly likely COVID-19 pneumonia, and although she was admitted for a few days, no oxygen supplementation was warranted. Her recovery was unremarkable. The remaining 94 (94%) patients were submitted to weekly follow-up and finally to the second blood test.

Thirty-three (33%) patients presented respiratory symptoms, mostly mild, during the follow-up. None of them required admission. Nine of these cases were considered highly suggestive of COVID-19 infection and were then submitted to SARS-CoV-2 rRT-PCR testing. Three (33.3%) were positive, and six (66.7%) were negative. Notably, all three positive rRT-PCR patients later had detectable anti-SARS-CoV-2 IgG. Additionally, two suspected patients whose rRT-PCR results were negative also had detectable anti-SARS-CoV-2 IgG in the follow-up.

Twenty-one (21%) individuals tested positive for some anti-SARS-CoV-2 Ig at some point of the study. As expected, there was a trend for a higher incidence of respiratory symptoms among those who tested positive for some Ig compared to those who did not (52.6% vs. 29.1%, $P=0.062$). No other significant difference or trend was found when Ig-positive patients were compared to Ig-negative patients (Table 2). Fourteen (14%) patients tested positive for anti-SARS-CoV-2 IgG at some point of the study. These patients were significantly older (54.3 yrs \pm 8.2 vs. 45.2 yrs \pm 14.6, $p=0.002$) than their IgG-negative counterparts. There was also a trend for significant association for more frequent use of bDMARDs in IgG-positive patients (42.9% vs. 19.8%, $P=0.056$) (Figure 2). It is remarkable to note that fewer than half of the patients (42.8%) who tested IgG positive (6/14) in the study reported no respiratory symptoms (Table 3). In Figure 3, the final results for any time anti-SARS-CoV-2 positivity in the entire sample is depicted.

Potential predictors for any Ig seroconversion and specifically for IgG seroconversion were also assessed. Fourteen (14%) patients subsequently tested positive for some anti-SARS-CoV-2 Ig at follow-up after negative baseline serology. These patients presented more frequently with respiratory symptoms during the follow-up compared to those patients who remained persistently Ig negative (64.3% vs. 27.7%, $P=0.012$) (Table 2). Eight (8%) patients developed detectable IgG in the second serology after testing negative at baseline. A trend for a higher incidence of respiratory symptoms was found in these patients compared to those who showed no IgG seroconversion (62.5% vs. 31.4%, $P=0.075$). While none of these patients were on use of non-antimalarial sDMARD, nearly one-third of patients who remained IgG negative during the follow-up were on non-antimalarial sDMARD, resulting in a trend for statistical significance (0.0% vs. 35.4%, $P=0.050$) (Table 3). IgG seroconversion according to specific groups are shown in Figure 1.

Discussion

This was a prospective study in which all patients underwent the same standardized protocol, with blood serology by a highly accurate method at two different time points. This study assessed the pattern of anti-SARS-CoV-2 antibodies during the pandemics of COVID-19 in Brazilian rheumatic patients, and fourteen percent were infected by SARS-CoV-2, as confirmed by anti-SARS-CoV-2 IgG positivity. Herein, although infected patients presented more often with respiratory symptoms, it is remarkable to note that asymptomatic COVID-19 infections were fairly frequent in this population (50.0%). None of the patients showed severe COVID-19, and all patients who presented with respiratory symptoms in the study fully recovered. A higher use of bDMARD and a lower use of sDMARD in those patients who turned SARS-CoV-2 IgG positive were also found, even among asymptomatic COVID-19 infections. To date, this is the first prospective study to assess anti-SARS-CoV-2 seroconversion in rheumatic disease patients.

Synthetic and biologic DMARDs are well known for increasing both the frequency and severity of infections in rheumatic disease patients who are on chronic use [10]. Although the magnitude and propensity for specific pathogens may vary among different drugs, on average, this has been true for both bacterial and viral etiologies [8,23]. In this scenario, COVID-19 started

Table 2. Baseline characteristics of patients according to immunoglobulin (Ig) positivity at any time point and seroconversion to Ig during the study.

	Ig Neg (n = 79)	Ig Pos (n = 21)	p	Seroconv.Ig Neg (n = 85)	Seroconv.Ig Pos (n = 14)	p
Age (years), mean (SD)	45.6 (14.4)	49.8 (13.0)	0.238	46.4 (14.3)	46.7 (14.3)	0.936
Sex, n (%)			1.000			1.000
Men	12 (15.2%)	3 (14.3%)		13 (15.3%)	2 (14.3%)	
Women	67 (84.8%)	18 (85.7%)		72 (84.7%)	12 (85.7%)	
Months since RD diagnosis, median (IQR)	36 (64-20)	60 (24-96)	0.399	36 (19-69)	60 (28-105)	0.219
RD activity, n (%)			0.680			
Remission	36 (70.6%)	13 (81.2%)		40 (71.4%)	8 (80.0%)	
Mild	7 (13.7%)	1 (6.2%)		7 (12.5%)	1 (10.0%)	
Moderate	4 (7.8%)	2 (12.5%)		5 (8.9%)	1 (10.0%)	
Severe	4 (7.8%)	0 (0.0%)		4 (7.1%)	0 (0.0%)	
Respiratory symptoms, n (%)	23 (29.1%)	10 (52.6%)	0.062	23 (27.7%)	9 (64.3%)	0.012
Groups according to therapy, n (%)			0.655			0.888
Group 1	24 (30.4%)	4 (19.0%)		24 (28.2%)	4 (28.6%)	
Group 2	17 (21.5%)	6 (28.6%)		20 (23.5%)	2 (14.3%)	
Group 3	17 (21.5%)	6 (28.6%)		19 (22.4%)	4 (28.6%)	
Group 4	21 (26.6%)	5 (23.8%)		22 (25.9%)	4 (28.6%)	
Antimalarial use, n (%)	58 (73.4%)	16 (76.2%)	1.000	63 (74.1%)	10 (71.4%)	1.000
NAM sDMARD use, n (%)	24 (32.0%)	8 (38.1%)	0.609	28 (34.6%)	4 (28.6%)	0.767
Months on NAM sDMARD use, median (IQR)	7 (3-25)	7 (5-60)	0.490	1 (0-9)	5 (1-7.5)	0.499
bDMARD use, n (%)	17 (21.5%)	6 (28.6%)	0.562	20 (23.5%)	2 (14.3%)	0.729
Months on bDMARD use, median (IQR)	24 (2-48)	12 (0-36)	0.515	15 (2-48)	9 (0-25)	0.433
GC use, n (%)	36 (42.4%)	7 (50.0%)	0.772	34 (40.5%)	8 (57.1%)	0.260
GC dose ¹ , median (IQR)	0 (0-3)	3 (0-3)	0.171	0 (0-3)	3 (0-4)	0.373

Note: Seroconv.Ig Neg: Patients who remained negative for ANY immunoglobulin (Ig) during follow-up. Seroconv.Ig Pos: Patients who were negative for ANY Ig at baseline and turned positive for ANY Ig during follow-up

SD, standard deviation; RD, rheumatic disease; IQR, interquartile range; NAM, Non-antimalarial; sDMARD, synthetic DMARD; bDMARD, biologic DMARD; GC, glucocorticoid.

¹ prednisone or equivalent to prednisone.

Results that reached statistical significance ($P < 0.05$) or a trend toward it ($P = 0.05-0.1$) are highlighted in bold.

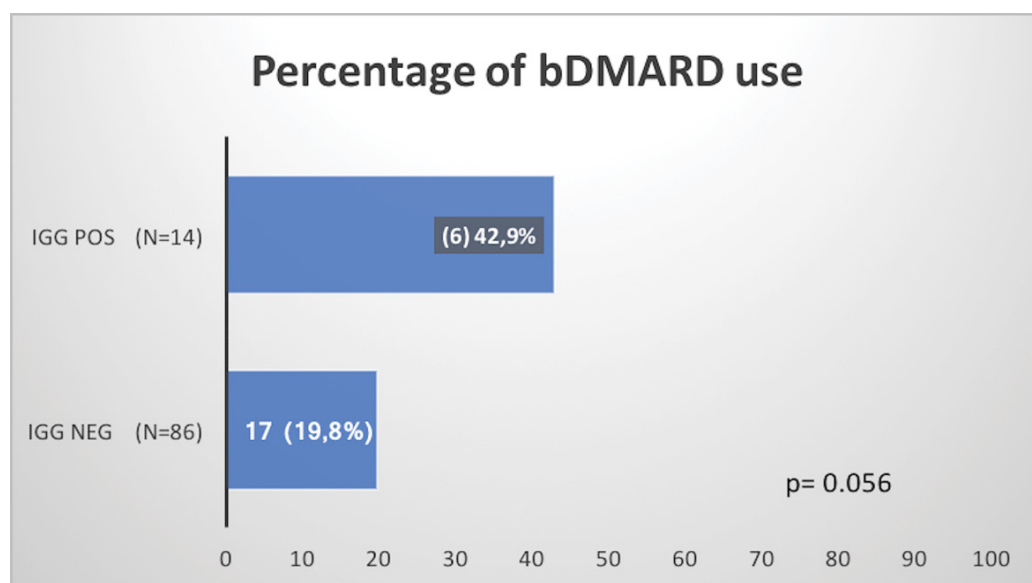


Figure 2. Distribution of biologic disease-modifying anti-rheumatic drug (bDMARD) users in anti-SARS-CoV-2 IgG seroconverted patients.

to be a challenge to rheumatologists: whether the rheumatic diseases or their own treatment could be a risk factor for SARS-CoV-2 infection or either for the outcome of coronavirus disease in those infected rheumatic patients. At first, it was reasonable to expect that autoimmune rheumatic disease patients on synthetic and/or biologic DMARDs would be particularly vulnerable to more frequent and severe COVID-19 infections. Recently, different cohorts with rheumatic patients infected by SARS-CoV-2 have been published, and this idea has been contradicted [24-27]. However, some authors have shown that the clinical course and disease severity of COVID-19 in these patients are closely related to what overtakes the general population. Therefore, risk

factors such as age and previous cardiovascular and pulmonary diseases are likely to play a major role in determining the risk for infection severity in rheumatic disease patients [28]. Accordingly, in this study, despite synthetic and biologic DMARD users, no severe clinical manifestations were found in infected patients. However, how the immune system in synthetic and biologic DMARD users reacts to SARS-CoV-2 exposure and the degree to which its antibody production capacity is affected is vastly unknown.

To contribute to filling in the knowledge gap on the matter, this cohort was able to show some seroconversion patterns in rheumatic disease patients on synthetic and biologic DMARDs after SARS-CoV-2 exposure. Fourteen

Table 3. Baseline characteristics of patients according to immunoglobulin G (IgG) positivity at any time point and seroconversion to IgG during the study.

	IgG Neg (n=86)	IgG Pos (n=14)	p	Seroconv.IgG Neg (n=86)	Seroconv.IgG Pos (n=8)	p
Age (years), mean (SD)	45.2 (14.6)	54.3 (8.2)	0.002	45.2 (14.6)	51.4 (9.3)	0.246
Sex, n (%)			0.687			1.000
Men	14 (16.3%)	1 (7.1%)		14 (16.3%)	1 (12.5%)	
Women	72 (83.7%)	13 (92.9%)		72 (83.7%)	7 (87.5%)	
Months since RD diagnosis, median (IQR)	36 (19-67)	54 (25-105)	0.384	36 (19-67)	54 (28-111)	0.361
RD activity, n (%)			0.401			1.000
Remission	39 (68.4%)	10 (100.0%)		39 (68.4%)	5 (100.0%)	
Mild	8 (14.0%)	0 (0.0%)		8 (14.0%)	0 (0.0%)	
Moderate	6 (10.5%)	0 (0.0%)		6 (10.5%)	0 (0.0%)	
Severe	4 (7.0%)	0 (0.0%)		4 (7.0%)	0 (0.0%)	
Respiratory symptoms, n (%)	27 (31.4%)	6 (42.8%)	0.211	27 (31.4%)	5 (62.5%)	0.075
Groups according to therapy, n (%)			0.111			0.253
Group 1	26 (30.2%)	2 (14.3%)		26 (30.2%)	2 (25.0%)	
Group 2	17 (19.8%)	6 (42.9%)		17 (19.8%)	2 (25.0%)	
Group 3	22 (25.6%)	1 (7.1%)		22 (25.6%)	0 (0.0%)	
Group 4	21 (24.4%)	5 (35.7%)		21 (24.4%)	4 (50.0%)	
Antimalarial use, n (%)	65 (75.6%)	9 (64.7%)	0.511	65 (75.6%)	4 (50.0%)	0.202
NAM sDMARD use, n (%)	29 (35.4%)	3 (21.4%)	0.373	29 (35.4%)	0 (0.0%)	0.050
Months on NAM sDMARD use, median (IQR)	6 (4-24)	60 (30-66)	0.456	1 (0-7.5)	0 (0-0)	0.180
bDMARD use, n (%)	17 (19.8%)	6 (42.9%)	0.056	17 (19.8%)	2 (25.0%)	0.661
Months on bDMARD use, median (IQR)	24 (2-48)	12 (0-36)	0.515	24 (2-48)	9 (0-25)	0.391
GC use, n (%)	36 (42.4%)	7 (50.0%)	0.772	36 (42.4%)	3 (37.5%)	1.000
GC dose ¹ , median (IQR)	0 (0-4)	1 (0-3)	0.846	0 (0-4)	0 (0-3)	0.610

Note: SD, standard deviation; RD, rheumatic disease; IQR, interquartile range; NAM, Non-antimalarial; sDMARD, synthetic DMARD; bDMARD, biologic DMARD; GC, glucocorticoid.

¹prednisone or equivalent to prednisone.

Results that reached statistical significance ($P < 0.05$) or a trend toward it ($P = 0.05-0.1$) are highlighted in bold.

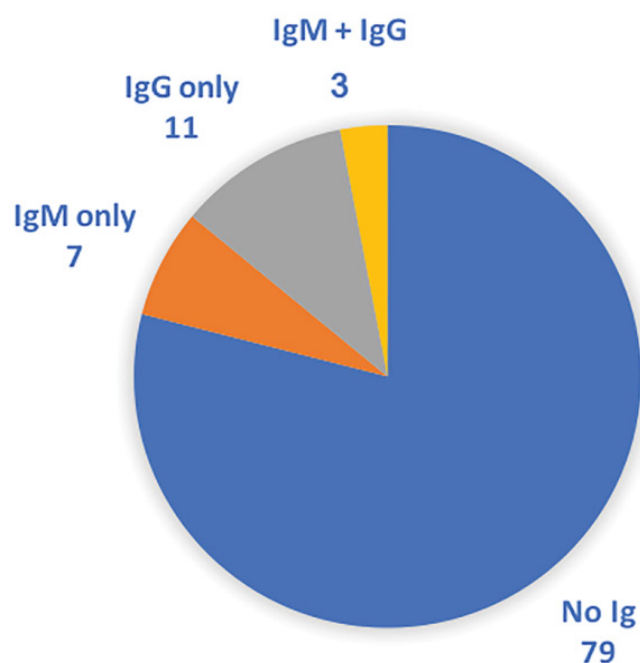


Figure 3. Prevalence of any time anti-SARS-CoV-2 antibody positivity in study patients. Only 20% of them evolved to anti-SARS-CoV-2 positivity for either IgG, IgM or both.

(14.0%) percent eventually had anti-SARS-CoV-2 IgG detected by CLIA, which has been shown to be highly specific for diagnosing COVID-19 [29]. Supporting this is the fact that all PCR-confirmed COVID-19 infections had a later IgG titer above the upper limits and were hence considered IgG positive. Anti-SARS-CoV-2 IgM positivity was not considered as a surrogate of COVID-19 infection because of the cross reaction with rheumatoid factor IgM, present in part of

the sample [30]. Notably, the only patient who initially tested positive for IgM and negative for IgG further tested negative for both antibodies in the follow-up blood collection. He remained asymptomatic throughout the study. A second patient whose serology was negative in the first blood exam tested positive for isolated IgM in the follow-up test. She also remained asymptomatic during the study and ever since. IgM titers can be detected before IgG increases in acute COVID-19 infections; however, persistent or transient positivity for IgM not followed by IgG detection is rather common in the authors' experience, and false positivity must be considered in these cases [20].

A statistical trend was found for a higher prevalence of bDMARD use in patients who tested positive for anti-SARS-CoV-2 IgG when compared to patients not on bDMARDs. This difference must be analyzed carefully, since it might simply represent a more frequent use of health services by bDMARD users than their counterparts. Hence, it should not be automatically taken as an immune promoting influence or as any sort of COVID-19 infection protective role by bDMARDs. It is, however, reassuring to notice that slightly over one quarter (26.0%) of bDMARD patients in the study adequately produced anti-SARS-CoV-2 IgG, and none evolved into severe COVID-19 infection. Although no definitive conclusion can be drawn from these data, it does seem that bDMARD users retain their humoral immunity against SARS-CoV-2. These results are in line with the recently published data from the COVID-19 Global Rheumatology Alliance, where bDMARD use was associated with less severe COVID-19 infection in autoimmune rheumatic disease patients [24].

In the opposite direction, the absence of non-antimalarial sDMARD users in those patients who seroconverted for anti-SARS-CoV-2 IgG during the follow-up must be interpreted with caution, as confounding factors might have influenced this result. For instance, different levels of SARS-CoV-2 exposure may exist between sDMARD users and non-sDMARD users. Furthermore, the lack of anti-SARS-CoV-2 production may not necessarily be associated with a lack of immune response to COVID-19, as cellular immunity has been studied and seems to play a protective role in COVID-19 infection [31-33].

Nine patients (9.0%) developed highly suggestive symptoms of COVID-19

infection during the follow-up. All of them underwent a single rRT-PCR test, but only three (33.0%) tested positive. These three patients later had detectable anti-SARS-CoV-2 IgG antibodies. Two additional patients who initially tested negative by rRT-PCR later also developed anti-SARS-CoV-2 IgG antibodies. Hence, anti-SARS-CoV-2 serology proved a more sensitive method for COVID-19 than a single rRT-PCR test from oropharyngeal swab. This finding does not come to us as a surprise, since previous studies have reported the sensitivity for a single oropharyngeal rRT-PCR test as close to 60% for detecting COVID-19 infection [34]. Reported sensitivity for serologic tests, however, have ranged from 88% to 100% [35].

It is noteworthy to mention that, although 43% of the cohort was on glucocorticoid (GC) use, no significant difference was found for any pattern of serologic status between patients currently on use of GC and those who were not. Use of GC had no statistical influence on anti-SARS-CoV-2 IgG or IgM positivity during the follow-up. However, this study was not appropriately designed to assess this association. Since immunosuppressive effects of GC are well-known, further studies are needed to assess this relationship [36].

The strength of this cohort is based on the fact of being a prospective study analyzing the region with one of the highest COVID-19 infection incidences during the peak rate and the overwhelming health system; data reliability, as the responsible treating physicians were also members of the research team; the sensitivity and specificity of the serologic tests; and the feasibility in assessing patients suspected for COVID-19 infection with PCR throughout the protocol.

The limitations of this study include the convenience, non-random sample, which included patients diagnosed with a wide range of different rheumatic diseases, some of which were not autoimmune diseases. Thus, a role for each of these conditions on SARS-CoV-2 seroconversion could not be assessed separately. Similarly, both sDMARD and bDMARD use encompassed many different drugs, and the distinct role of SARS-CoV-2 seroconversion for each of these drugs is expected and could not be assessed due to the small sample size. Furthermore, due to few observations in the anti-SARS-CoV-2 IgG seroconverted patients, running a multivariate analysis was not feasible.

Conclusion

Although anti-SARS-CoV-2 IgG positivity was quite common in the rheumatic patients of the sample (14% incidence); half of these patients evolved asymptotically, with no clinical detectable COVID-19 infection. Moreover, none of the patients presented with severe clinical manifestations and this is reassuring. Although the study was not designed to answer this question, temporally withholding rheumatic patient treatment during the pandemic based on this concern may not be warranted. Furthermore, bDMARD use seems not to hamper the humoral immune response to SARS-CoV-2 although no final conclusion about this matter can be drawn from this study. Non-antimalarial sDMARD use was associated with a lower incidence of anti-SARS-CoV-2 IgG positivity, although no causal effect can be drawn from this result due to the study design. Whether sDMARD hampers the humoral immune response, switches humoral to cellular immunity or even impacts COVID-19 infection remains to be elucidated.

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Conflicts of Interest

None to disclose

Availability of Data and Materials

The data are available upon reasonable request.

Ethics Approval

The study was approved by the local ethical board (Ethics Committee from Hospital Santa Paula) and by the national ethical board (CONEP-National Commission on Ethics and Research) at the register number CAAE: 30444020.3.0000.0008.

Consent to Participate

All patients signed a written informed consent form before enrollment.

Consent for Publication

All authors listed here agreed to be accountable for all aspects of the work and allow the publication of this version.

Contribution Statement

All authors contributed to the study conception and design. Conceptualization: Felipe M Santana, Jayme F Cobra and Camille P Figueiredo; Methodology: Felipe M Santana, Jaqueline B Lopes, Mariana O Perez, Jayme F Cobra and Camille P Figueiredo; Formal analysis and investigation: Felipe M Santana, Jose Eduardo Levi, Jayme F Cobra and Camille P Figueiredo; Original draft preparation: Felipe M Santana, Jaqueline B Lopes, Mariana O Perez and Camille P Figueiredo; Writing – review and editing: Gustavo Campana, Jose Eduardo Levi, Flavia PPL Lopes, Otavio Gebara, Jayme F Cobra and Camille P Figueiredo; Funding acquisition: Gustavo Campana, Flavia PPL Lopes, Otavio Gebara and Jayme F Cobra; Resources: Felipe M Santana, Jaqueline B Lopes, Mariana O Perez and Jayme F Cobra.

References

- Holshue, Michelle L, Chas DeBolt, Scott Lindquist and Kathy H Lofy, et al. "First Case of 2019 Novel Coronavirus in the United States." *N Engl J Med* 382: 929-936.
- Burki, Talha Khan. "Coronavirus in China." *Lancet Respir Med* 8 (2020): 238.
- Liu, Jia, Xin Zheng, Qiaoxia Tong and Wei Li, et al. "Overlapping and Discrete Aspects of the Pathology and Pathogenesis of the Emerging Human Pathogenic Coronaviruses SARS-CoV, MERS-CoV, and 2019-nCoV." *J Med Virol* 92 (2020): 491-494.
- <https://COVID19.who.int>.
- Huang, Chaolin, Yeming Wang, Xingwang Li and Lili Ren, et al. "Clinical Features of Patients Infected with 2019 Novel Coronavirus in Wuhan, China." *Lancet* 395 (2020): 497-506.
- Tian, Wenjie, Wanlin Jiang, Jie Yao and Christopher J Nicholson, et al. "Predictors of Mortality in Hospitalized COVID-19 Patients: A Systematic Review and Meta Analysis." *J Med Virol* 92 (2020): 1875-1883.
- Figueroa-Parra, Gabriel, Gloria Mayela Aguirre-Garcia, Carmen Magdalena Gamboa-Alonso and Adrian Camacho-Ortiz, et al. "Are My Patients with Rheumatic Diseases at Higher Risk of COVID-19?" *Ann Rheum Dis* 79 (2020): 839-840.
- Tinsley, Andrew, Seyedehsan Navabi, Emmanuelle D Williams and Guodong Liu, et al. "Increased Risk of Influenza and Influenza-Related Complications among 140,480 Patients with Inflammatory Bowel Disease." *Inflamm Bowel Dis* 25 (2019): 369-376.
- Chiu, Ying-Ming and Der-Yuan Chen. "Infection Risk in Patients Undergoing Treatment for Inflammatory Arthritis: Non-Biologics versus Biologics." *Expert Rev Clin Immunol* 16 (2020): 207-228.
- Caporali, Roberto, Marta Caprioli, Francesca Bobbio-Pallavicini and Carlomaurizio Montecucco. "DMARDS and Infections in Rheumatoid Arthritis." *Autoimmun Rev* 8 (2008): 139-143.

11. Santana, Felipe M, Jaqueline B Lopes, Mariana O Perez and Gustavo Campana, et al. "Seroconversion for SARS-CoV-2 in Rheumatic Patients on Synthetic and Biologics Disease Modifying Anti-Rheumatic Drugs in São Paulo, Brazil." *20 (2020): 30118-30119.*
12. Xu, Xiaoling, Mingfeng Han, Tiantian Li and Wei Sun, et al. "Effective Treatment of Severe COVID-19 Patients with tocilizumab." *Proc Natl Acad Sci USA 117 (2020): 10970-10975.*
13. Luo, P, Y Liu, L Qiu and X Liu, et al. "Tocilizumab Treatment in COVID-19: A Single Center Experience." *J Med Virol 92: 814-818.*
14. Toniati, Paola, Simone Piva, Marco Cattalini and Emirena Garrafa, et al. "Tocilizumab for the Treatment of Severe COVID-19 Pneumonia with Hyperinflammatory Syndrome and Acute Respiratory Failure: A Single Center Study of 100 Patients in Brescia, Italy." *Autoimmun Rev (2020): 102568.*
15. Zou, Lirong, Feng Ruan, Mingxing Huang and Lijun Liang, et al. "SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients." *New Eng J Med 382 (2020): 1177-1179.*
16. Wang, Wenling, Yanli Xu, Ruqin Gao and Roujian Lu, et al. "Detection of SARS-CoV-2 in Different Types of Clinical Specimens." *JAMA 323 (2020): 1843-1844.*
17. Romano, CM, A Chebabo and JE Levi. "Past, Present, and Future of COVID-19: A Review." *Braz J Med Biol Res 53 (2020): e10475.*
18. Lauer, Stephen A, Kyra H Grantz, Qifang Bi and Forrest K Jones, et al. "The Incubation Period of Coronavirus Disease 2019 (COVID-19) from Publicly Reported Confirmed Cases: Estimation and Application." *Ann Int Med 172 (2020): 577-582.*
19. Younes, Nadin, Duaa W Al-Sadeq, Hadeel Al-Jighefee and Salma Younes, et al. "Challenges in Laboratory Diagnosis of the Novel Coronavirus SARS-CoV-2." *Viruses 12 (2020): 582.*
20. Long, Quan-Xin, Bai-Zhong Liu, Hai-Jun Deng and Gui-Cheng Wu, et al. "Antibody Responses to SARS-CoV-2 in Patients with COVID-19." *Nat Med 26 (2020): 845-848.*
21. Corman, VM, O Landt, M Kaiser and R Molenkamp, et al. "Detection of 2019 Novel Coronavirus (2019-nCoV) by Real-Time RT-PCR." *Euro Surveill 25: 2000045.*
22. <https://www.wma.net/policy/current-policies>
23. Davies, Rebecca, Taunton R Southwood, Lianne Kearsley-Fleet and Mark Lunt, et al. "Medically Significant Infections are increased in Patients with Juvenile Idiopathic Arthritis Treated with Etanercept: Results from the British Society for Paediatric and Adolescent Rheumatology Etanercept Cohort Study." *Arthritis Rheumatol 67 (2015): 2487-2494.*
24. Gianfrancesco, Milena, Kimme L Hyrich, Sarah Al-Adely and Loreto Carmona, et al. "Characteristics associated with Hospitalisation for COVID-19 in People with Rheumatic Disease: Data from the COVID-19 Global Rheumatology Alliance Physician-Reported Registry." *Ann Rheum Dis 79 (2020): 859-866.*
25. Nuñez, Dalifer D Freites, Leticia Leon, Arkaitz Mucientes and Luis Rodriguez-Rodriguez, et al. "Risk Factors for Hospital Admissions related to COVID-19 in Patients with Autoimmune Inflammatory Rheumatic Diseases." *Ann Rheum Dis 79 (2020): 1393-1399.*
26. Pablos, Jose L, María Galindo, Loreto Carmona and Ana Lledó, et al. "Clinical Outcomes of Hospitalised Patients with COVID-19 and Chronic Inflammatory and Autoimmune Rheumatic Diseases: A Multicentric Matched Cohort Study." *Ann Rheum Dis 79 (2020): 1544-1549.*
27. Zhong, Jixin, Guifen Shen, Huiqin Yang and Anbin Huang, et al. "COVID-19 in Patients with Rheumatic Disease in Hubei Province, China: A Multicentre Retrospective Observational Study." *Lancet Rheumatol 2 (2020): e557-e564.*
28. Fredi, Micaela, Ilaria Cavazzana, Liala Moschetti and Laura Andreoli, et al. "COVID-19 in Patients with Rheumatic Diseases in Northern Italy: A Single-Centre Observational and Case-Control Study." *Lancet Rheumatol 2 (2020): e549-e556.*
29. Xie, Xingzhi, Zheng Zhong, Wei Zhao and Chao Zheng, et al. "Chest CT for Typical Coronavirus Disease 2019 (COVID-19) Pneumonia: Relationship to Negative RT-PCR testing." *Radiol 296 (2020): E41-E45.*
30. Wang, Qiang, Qin Du, Bin Guo and Daiyong Mu, et al. "A Method to Prevent SARS-CoV-2 IgM False Positives in Gold Immunochromatography and Enzyme-Linked Immunosorbent Assays." *J Clin Microbiol 58 (2020): e00375- e00420.*
31. Tay, Matthew Zirui, Chek Meng Poh, Laurent Rénia and Paul A MacAry, et al. "The Trinity of COVID-19: Immunity, Inflammation and Intervention." *Nat Rev Immunol 20 (2020): 363-374.*
32. Manners, Christopher, Erick Larios Bautista, Hannah Sidoti and Osvaldo J Lopez. "Protective Adaptive Immunity against Severe Acute Respiratory Syndrome Coronaviruses 2 (SARS-CoV-2) and Implications for Vaccines." *Cureus 12 (2020): e8399.*
33. Vabret, Nicolas, Graham J Britton, Conor Gruber and Samarth Hegde, et al. "Immunology of COVID-19: Current State of the Science." *Immunity 52 (2020): 910-941.*
34. Yang, Yang, Minghui Yang, Chenguang Shen and Fuxiang Wang, et al. "Laboratory Diagnosis and Monitoring the Viral Shedding of SARS-CoV-2 Infection." *Innov 1 (2020): 100061.*
35. Deeks, Jonathan J, Jacqueline Dinnes, Yemisi Takwoingi and Clare Davenport, et al. "Antibody Tests for Identification of Current and Past Infection with SARS CoV-2." *Cochrane Database Syst Rev 6 (2020): 2-4.*
36. Coutinho, Agnes E and Karen E Chapman. "The Anti-Inflammatory and Immunosuppressive Effects of Glucocorticoids, Recent Developments and Mechanistic Insights." *Mol Cell Endocrinol 335 (2011): 2-13.*

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