HLA-B27 did not protect against COVID-19 in patients with axial spondyloarthritis – data from the ReumaCov-Brasil Registry

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Abstract

Background Some studies have suggested the HLA-B27 gene may protect against some infections, as well as it could play a benefit role on the viral clearance, including hepatitis C and HIV. However, there is lack of SARS-CoV-2 pandemic data in spondyloarthritis (SpA) patients.

Aim To evaluate the impact of HLA-B27 gene positivity on the susceptibility and severity of COVID-19 and disease activity in axial SpA patients.

Methods The ReumaCoV-Brasil is a multicenter, observational, prospective cohort designed to monitor immune-mediated rheumatic diseases patients during SARS-CoV-2 pandemic in Brazil. Axial SpA patients, according to the ASAS classification criteria (2009), and only those with known HLA-B27 status, were included in this ReumaCov-Brasil's subanalysis. After pairing them to sex and age, they were divided in two groups: with (cases) and without (control group) COVID-19 diagnosis. Other immunodeficiency diseases, past organ or bone marrow transplantation, neoplasms and current chemotherapy were excluded. Demographic data, managing of COVID-19 (diagnosis, treatment, and outcomes, including hospitalization, mechanical ventilation, and death), comorbidities, clinical details (disease activity and concomitant medication) were collected using the Research Electronic Data Capture (REDCap) database. Data are presented as descriptive analysis and multiple regression models, using SPSS program, version 20. P level was set as 5%.

Results From May 24th, 2020 to Jan 24th, 2021, a total of 153 axial SpA patients were included, of whom 85 (55.5%) with COVID-19 and 68 (44.4%) without COVID-19. Most of them were men (N=92; 60.1%) with mean age of 44.0 ± 11.1 years and long-term disease (11.7 ± 9.9 years). Regarding the HLA-B27 status, 112 (73.2%) patients tested positive. There were no significant statistical differences concerning social distancing, smoking, BMI (body mass index), waist circumference and comorbidities. Regarding biological DMARDs, 110 (71.8%) were on TNF inhibitors and 14 (9.15%) on IL-17 antagonists. Comparing those patients with and without COVID-19, the HLA-B27 positivity was not different between groups (n=64, 75.3% vs. n=48, 48%, respectively; *p*=0.514). In addition, disease activity was similar before and after the infection. Interestingly, no new episodes of arthritis, enthesitis or extra-musculoskeletal

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manifestations were reported after the COVID-19. The mean time from the first symptoms to hospitalization was 7.1 ± 3.4 days, and although the number of hospitalization days was numerically higher in the B27 positive group, no statistically significant difference was observed (5.7 ± 4.11 for B27 negative patients and 13.5 ± 14.8 for B27 positive patients; $p = 0.594$). Only one HLA-B27 negative patient died. No significant difference was found regarding concomitant medications, including conventional or biologic DMARDs between the groups.

Conclusions No significant difference of COVID-19 frequency rate was observed in patients with axial SpA regarding the HLA-B27 positivity, suggesting a lack of protective effect with SARS-CoV-2 infection. In addition, the disease activity was similar before and after the infection.

Trial registration This study was approved by the Brazilian Committee of Ethics in Human Research (CONEP), CAAE 30186820.2.1001.8807, and was registered at the Brazilian Registry of Clinical Trials – REBEC, RBR-33YTQC. All patients read and signed the informed consent form before inclusion.

Keywords COVID-19, SARS-CoV-2, HLA-B27, Axial spondyloarthritis, Disease activity, Prospective study

Introduction

Patients with immune-mediated rheumatic diseases (IMRD) have higher risk of infections, particularly associated with immunossupresion and disease activity. However, patients with spondyloarthritis (SpA) have no increased risk for severe infection, even after exposing to the TNF (TNFi) and IL17 inhibitors (IL17i) [1], differently of individuals with systemic erythematous lupus (SLE) and rheumatoid arthritis (RA) [1–4]. Some aspects could be highlighted to try to explain these findings when the IMRDs are compared, including lower use of glucocorticosteroids and other immunosuppressive drugs [5], as well as lower cellular immune response impairment [6]. However, there are scarce data regarding SpA in COVID-19.

Since that Brewerton and Schlosstein [7] reported a close relationship between ankylosing spondylitis and HLA-B27, many other aspects have been pointed out over time, especially related with some protective role regarding infections (occurrence and severity) [7–20]. Considering the HLA-B27 is ubiquitous worldwide, some authors have addressed this potential advantage during the humanity evolution among B27-carriers regarding influenza virus, herpes simplex type 2 virus, malaria, Epstein-Barr virus, HIV, HCV and reactivation of latent toxoplasmosis infection after TNFI [8–10].

Noteworthy, the cytotoxic T lymphocyte (CTL) responses, activated by HLA antigen presentation, are implicated in the control of viral replication, more effectiveness regarding the clearance of microrganisms, and a specific and strong CTL response against some conservative not easily mutate epitopes. On the other hand, it is involved with beginning and chronification of reactive arthritis in predisposed patients and in experimental models, showing a definitive proof of concept, particularly regarding Chlamydia and other pathogens from gut microbiota [11–20]. Therefore, the antigen presenting properties and/or to the co-inheritance of gene variants may contribute to an altered homeostasis in case of microbial infections or tissue injury.

Regarding endemic malaria, the geographic distribution of HLA-B27 has been demonstrated as an inverse latitude-related gradient, suggesting that Plasmodium falciparum may have exerted a negative selection on this gene. In addition, a higher susceptibility to severe forms of malaria, associated with HLA-B27 or other close gene, as well as other factors associated with lower HLA-B27 prevalence in some regions may have contributed for newly generated and more advantageous B27 haplotypes for surviving and natural selection [21].

In addition, there are some pieces of evidence supporting the association between HLA-B27 and lower viral load and long-term non-progression in HIV infection as well as spontaneous clearance of hepatitis C virus (HCV) infection, particularly genotype 1 infection, but not genotype 3, suggesting different immune response, according to single amino acid residues sequence epitopes [8, 22–24].

Some mechanisms are postulated, including complicated pathways of viral escape from immunodominant HLA-B27-restricted virus-specific CD8+T-cell epitopes, CD8+T-cell polyfunctionality and functional avidity, thymic selection of CD8+T-cell precursors, specific T-cell receptor repertoires and clonotypes, efficient antigen processing, and evasion from regulatory T-cell-mediated suppression [25].

Considering the ability of HLA-B27 to confer resistance to the progression of several viral infection, as well as to promote a spontaneous CD8+T cell-mediated viral clearance and a potential protective role in pandemic times, we hypothesized if HLA-B27 positive SpA patients could have lower occurrence and a less severe course of COVID-19 than those HLA-B27 negative. In addition, we explored the disease activity during the COVID-19 in this clinical setting.

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Methods

This is a prospective analysis from ReumaCoV-Brasil Registry using only axial SpA patients enrolled from May 24th, 2020 to January 24th, 2021. Details of the registry design were described elsewhere recently. Briefly, the ReumaCoV-Brasil is a multicenter, observational, ongoing prospective cohort study carried out to monitor adult patients with immune-mediated rheumatic diseases (IMRD) plus COVID-19 diagnosis comparing to IMRD without COVID-19 [26–28].

Using a nationwide sampling strategy, it is a two-phase study: [1] Cross-sectional evaluation (inclusion) with information about previous or current symptoms of COVID-19 and clinical characteristics at the baseline, which can be performed by telephone call (preferred because the social distancing) or by a face-to-face visit, if possible; [2] Prospective follow-up concerning the SpA details with two face-to-face visits, every three months (3-month and 6-month assessment), after viral infection. The primary outcomes were the specific SpA disease activity changes after COVID-19, at four time points: [1] At baseline; [2] Within 4–6 weeks after the SARS-CoV-2 infection; [3] 3 months after the inclusion (±15 days); [4] 6 months after inclusion (±15 days). If the patient is unavailable to perform a face-to-face visit at baseline because social distancing, the physician may use the clinical data within the last 6 months (a period without any COVID-19 evidence).

The present study enrolled axial SpA patients from the ReumaCoV trial, including those with and without COVID-19 diagnosis (case and control group respectively), paired to sex and age. Moreover, they were selected based on the identification of a case by the researcher, through telephone contact, outpatient appointment or during hospitalization related to COVID-19. The inclusion criteria were: [1] age over 18 [2], COVID-19 diagnosis, based on clinical symptoms AND/OR polymerase chain reaction for SARS-CoV-2, AND/OR antibody against SARS-CoV-2 (IgM or IgG), based on the Brazilian Ministry of Health criteria [3], prior diagnosis of SpA, according to usual ASAS classification criteria (2009), and [4] previously known HLA-B27 status. The exclusion criteria were other immunodeficiency diseases, past organ or bone marrow transplantation, neoplasms within the last 5 years, current chemotherapy, HIV diagnosis and thymus diseases [29, 30].

This study was registered at the Brazilian Registry of Clinical Trials – REBEC (RBR-33YTQC). Also, it was approved by the National Research Ethics Commission (CONEP) [Approval number 3,955,206, on April 5th, 2020]. It is pertinent to emphasize that the ReumaCoV-Brazil study was conceived and conducted within the context of an ongoing pandemic, with restrictions on in-person medical assessments and logistical challenges due to social distancing guidelines. Consequently, a significant portion of the data was gathered via telephone interviews and retrospective retrieval of information from electronic patient records (EPR), accompanied by prospective updates on COVID-19 progression and disease activity. Therefore, verbal consent was initially obtained through telephone communication, and subsequently, during a in-person evaluation, the formal signature of the consent form was acquired.

The disease activity assessment was performed using a global physician assessment (GPA), using a numerical visual analog scale (VAS), ranging from 0 to 10 (being zero no activity and 10 great activity), as well specific and validated disease activity measurements. Pre-COVID data were obtained from the notes of the patient's medical record, in a consultation carried out in the last 6 months and the post-COVID data were obtained at the time of data collection for this study. For patients with axSpA, the disease activity measurement considered were BASDAI (Bath Ankylosing Spondylitis Disease Activity Index), ASDAS-ESR (Ankylosing Spondylitis Disease Activity Score using erythrocyte sedimentation rate) and ASDAS-CRP (Ankylosing Spondylitis Disease Activity Score using Protein C Reactive) as well the frequency of isolated clinical manifestations before (cases and controls) and after COVID-19 (only for cases) [31, 32].

Outcomes related to COVID-19 severity were assessed and classified according to the care needed for each patient. Mild COVID-19 required only ambulatory care, moderate COVID-19 required non-intensive hospital treatment, including emergency room and stay for more than 24 h; and severe COVID-19 required admission to an ICU, MV, or led to death. All participants included in this analysis had been prospectively monitored until complete endpoints resolution.

Demographic data such as age, sex, work situation and social distancing during the pandemic, as well diagnosis and treatment of IMRD, comorbidities, clinical characteristics, COVID-19 management and their endpoints, were collected using a Research Electronic Data Capture (REDCap) database (https://www.project-redcap.org/), through telephone call or face-to-face interview, if permitted by local health recommendations. Alcohol intake was considered as 15 servings per week for men and 10 servings per week for women. One serving is approximately 285 ml of beer, 120 ml of wine, or 30 ml of distilled spirits (whiskey, vodka, and cachaça). Active smoking was considered when the person smoked more than 1 cigarette per day every day currently and obesity was defined when the body mass index was over 30 kg/m^2.

In case of hospitalization, the data were collected directly with the patient, if possible, or from medical

records. In cases where the death was notified, data were collected directly from a family member, who authorized the data inclusion. Different methodologies were used for HLA typing: PCR-SSO (Sequence-Specific Oligonucleotide), PCR-SSP (Sequence-specific amplification), Sanger sequencing and RT-PCR sequencing and Real Time (RT-PCR).

Statistical analysis

To characterize the patient profile, the frequencies percentage and mean and standard deviation (SD) of variables were calculated. Comparisons of means between two groups were performed using the Student's t test for independent samples. To verify normality data, it was applied the Kolmogorov-Smirnov test. In case normality violation, it was used the Mann-Whitney non-parametric test. The chi-square association test was used to assess the association among categorical variables with standardized adjusted residual calculation, or Fischer's exact test for small samples. The final logistic regression model used moderate/ severe forms as dependent variable and appropriate adjustments were performed considering all independent variables that had statistical significance up 10% in the univariate analysis. P value was set as significant if below 5%. Statistical analyzes were performed using the SPSS 20.0 statistical software.

Results

A total of 153 axial SpA patients were included, of whom 85 (55.5%) with COVID-19 and 68 (44.4%) without COVID-19. Most of them were men (N=92; 60.1%) with mean age of 44.0±11.1 years and long-term disease (11.7±9.9 years), and 112 (73.2%) patients had positivity status for HLA-B27. All patients included were COVID-19 confirmed cases, according to Brazilian Ministry of Healthy recommendations, most of them classified according to lab criterion (80.1%), mostly through RT-PCR (58.5%). Regarding biological DMARDs, 110 (71.8%) were on TNF inhibitors and 14 (9.15%) on IL-17 antagonists. No significant difference was found regarding concomitant medications, including conventional or biologic DMARDs between the groups There were no significant statistically differences concerning social distancing, smoking, BMI, waist circumference and comorbidities. Also, there were no significant difference concerning ASDAS-CRP or ASDAS-ESR, but SpA patients HLA-B27 negative had higher score related to pain and other PROs (patient-reported outcomes) measured though the VAS and BASDAI (Table 1). However, when stratifying the relationship between the disease activity, measured by these variables (VAS and BASDAI), and the COVID-19 severity, no significant differences were observed (Table 2). Interestingly, no new episodes of arthritis,

enthesitis or extra-musculoskeletal manifestations were reported after the COVID-19.

Comparing SpA patients with and without COVID-19, the HLA-B27 positivity was not different between groups (n=64, 75.3% vs. n=48, 48%, respectively; p=0.514). Similarly, when comparing mild, moderate, and severe COVID-19, according to the HLA-B27 status, no significant difference was found (Table 3). The mean time from the first symptoms to hospitalization was 7.1±3.4 days, and although the number of hospitalization days has been numerically higher in the B27 positive group than in those negative status, no statistically significant difference was observed (13.5±14.8 vs. 5.7±4.11, respectively; p=0.594). Only one HLA-B27 negative patient died. The hospitalization frequency, considered as moderate COVID, was higher in the B27 positive patient group (p=0.031) (Table 3). Disease activity was similar before and after the infection the control group had significantly higher disease activity score, according to ASDAS-CRP (3.8 vs. 3.2, p=0.08).

Discussion

Our data demonstrated no significant difference of COVID-19 frequency rate or severity pattern in patients with axial SpA regarding the HLA-B27 positivity status, suggesting a lack of protective effect against the SARS-CoV-2 infection. In addition, the disease activity was similar before and after the infection. Our data emphasize that the disease behavior in axial SpA patients is similar to the general population, regardless biologic therapy and HLA-B27 status.

Similarly, Rosenbaum et al. using an online self-reported survey in 3,435 SpA patients from 65 countries (85% with AS; 76.1% were HLA-B27 positive from 2,836 aware of HLA-B27 status) from the Spondylitis Association of America (SAA) between 10 and 2020 and 26 April 2021, showed also similar rate of infection between HLA-B27 positive (1.4%) and negative (1.5%), as well as only in those tested (0,6% in both groups) and without significant difference related to self-reported COVID-19 severity. In addition, they did not find any significant difference the incident rate ratio for COVID-19 compared to the US population adjusted for age and sex, regardless HLA-B27, diagnosis of SpA or its treatment. On the other hand, it is important to note that some potential bias, such as responders' profile, low number of infected individuals (n=41), self-reported diagnosis of SpA and HLA-B27 status, as well as COVID-19, including confirmatory lab testing in only 36.9% of sample and severity based on patient's opinion), and some limitations, such as lack of information regarding the disease activity data. Thus, our prospective cohort study provides more consistent data regarding the relationship between HLA-B27 and COVID-19 susceptibility and severity [33–35].

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Table 1 Clinical and demographic data of the sample, comparing HLA-B27 positive with HLA-B27 negative patients

	HLA-B27 negative (N = 41)	HLA-B27 positive (N = 112)	OR (95% CI)	*P* value
Cases	21 (24.7)	64 (75.3)	1.27 (0.62–2.60)	0.514**
Controls	20 (29.4)	48 (70.6)	-	
Mean age (SD)	43.9 (11.6)	44.0 (10.9)	0.01 (-0.36-0.34)ξ	0.95
Sex				
Male	15 (16.3)	77 (83.7)	3.81 (1.8–8.07)	**0.001***
Female	26 (42.6)	35 (57.4)		
Disease duration (years)	8.9 (7.2)	11.7 (10.7)		0.068#
Comorbidities				
Cardiopathy	0 (0)	5 (100)	0.73 (0.64–0.84)	0.33**
Diabetes	2 (22.2)	7 (77.8)	1.16 (0.22–6.10)	1.0**
Lung disease	0 (0)	2 (100)	0.74 (0.65–0.84)	1.0**
Kidney disease	0 (0)	1 (100)	0.75 (0.66–0.84)	1.0**
Hypertension	7 (25.9)	20 (74.1)	0.90 (0.31–2.59)	0.86*
Obesity (BMI > 30 kg/m^2)	2 (20)	8 (80)	1.35 (0.26–6.95)	1.0**
No comorbidities	8 (24.2)	25 (75.8)	1.04 (0.37–2.87)	0.94*
Current lifestyle habits				
Active smoking	1 (16.7)	5 (83.3)	1.69 (0.18–15.3)	1.0**
Alcohol intake	1 (16.7)	5 (83.3)	1.69 (0.18–15.3)	1.0**
Medications				
IL17 inhibitors	5 (35.7)	9 (64.9)	0.62 (0.19-2.0)	0.43*
TNF inhibitors	30 (27.3)	80 (72.7)	0.91 (0.41–2.04)	0.83**
Current corticosteroids (yes or no))	3 (42.9)	4 (57.1)	0.46 (0.10–2.19)	0.39**
Oral CE < 10 mg/day	2 (33.3)	4 (66.6)	0.33 (0.10–1.03)ξ	0.429
Methotrexate	2 (18.2)	9 (81.8)	1.55 (0.30–7.84)	0.72**
Sulfasalazine	5 (25.0)	15 (75.0)	1.11 (0.37–3.28)	0.85*
Disease activity				
VAS	4.1 (2.7)	2.6 (2.6)	0.72 (0.30–1.13)**	**0.007#**
BASDAI	4.2 (2.2)	2.5 (2.4)	0.34 (-0,18-0.87)**	0.01#
ASDAS-ESR	5.7 (13.8)	2.8 (3.6)	-0.06 (-0.55-0.43)**	0.20#
ASDAS-CRP	3.2 (2.4)	3.8 (11.6)		0.08#

BMI: Body Mass Index; VAS: Visual Analog Scale; CE: corticosteroids; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; ASDAS: Ankylosing Spondylitis Disease Activity Score; ESR: Erythrocyte Sedimentation Rate; CRP: C Reactive Protein; *Chi-square test (95%CI); **Fisher exact test (95%CI); #t test for independent samples (95%CI); ξCohen's d test for comparing effect size between means (CI 95%)

Table 2 Disease activity, measured by VAS and BASDAI, and the COVID-19 severity in SpA patients

	VAS 0–4		VAS 5–10		OR (95% CI)	*P* value
	n	%	n	%		
Hospitalization	5	83.3	1	16.7	0.63 (0.06–5.82)	0.570
Intensive unit care	3	75.0	1	25.0	1.09 (0.10-11.25)	1.000
	BASDAI 0–4		BASDAI 5–10		OR (95% CI)	*P* value
	n	%	n	%		
Hospitalization	2	66.7	1	33.3	1.35 (0.11–15.9)	1.000
Intensive unit care	1	50.0	1	50.0	2.76 (0.16–46.6)	0.474

VAS: visual Analog Scale; BASDAI: bath Ankylosing Spondylitis Disease Activity Index

During human evolution, the existence of conserved HLA haplotypes is resulting from a selective pressure by some pathogens that have edited the immune response genes and provided higher survival probability over time. Considering that the virus-specific T cell response is critical for determining the infection severity, as well as viral clearance, interferon release, duration of immunity and efficacy of vaccines, our initial hypothesis was driven to the protective role of HLA-B27 against SARS-CoV-2 infection in axial SpA patients, a similar finding found in other viral infections. However, our hypothesis was not confirmed in this prospective face-to-face analysis [36].

Regarding specifically the SARS-CoV-2, the infectivity, severity, and mortality rates related to COVID-19

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Table 3 Outcomes related to COVID-19 (only case group), according to the HLA-B27 status in spondyloarthritis patients

Outcomes	HLA-B27 negative (n=21)		HLA-B27 positive (n=64)		OR (CI 95%)	*P* value
	n	%	n	%		
Hospital care	5	29.4	12	76.5	0.73 (0.22–2.41)	0.754*
Hospitalization	4	66.7	2	33.3	0.13 (0.02–0.81)	0.031**
Intensive unit care	2	50.0	2	50.0	0.30 (0.04–2.32)	0.254**
Mechanical ventilation	2	66.7	1	33.3	0.50 (0.02–8.95)	1.000**
Lenght of hospitalization (mean, SD)	5.7 (4.11)		13.5 (14.8)		-1,07 (-2,97-0.96)$^{\xi}$	0.594#
Severity of COVID – 19$^{\delta}$						
Mild	12	20.0	48	80.0	0.44 (0.15–1.24)	0.119*
Moderate	4	66.7	2	33.3	0.13 (0.02–0.81)	0.031**
Severe	2	50.0	2	50.0	0.30 (0.04–2.32	0.254**
Death	1	100	0	0	-	1.000**

$^{\delta}$Mild activity was considered for patients who did not require hospital care, moderate for those who were hospitalized and severe for those who were admitted to the intensive care unit. *Chi-square test (95%CI); **Fisher exact test (95%CI); #t test for independent samples (95%CI); $^{\xi}$Cohen's d test for comparing effect size between means (CI 95%)

have not affected all countries equally, suggesting that several aspects could be involved in different populations. Beyond traditional risk factors, such as age, comorbidities, lack of masking/ social distancing and viral load, some authors highlight the relevance of genetic biomarkers as potential drivers for ethnic susceptibility and unfavorable prognosis [37, 38]. Although the HLA systems have a strong linkage disequilibrium that can result in conserved multi-locus haplotypes depending on how the alleles are arranged in adjacent loci of a specific region of the chromosome, it is the best way to characterize the host genetic variance concerning the innate immunity, especially related to MHC class I, which plays a major role in susceptibility to viral infections [39]. Thus, these aspects may contribute to the difference in mortality among various countries [40]. However, a recent review found heterogeneous findings in the association of interindividual genetic variants with COVID-19 susceptibility and severity COVID-19, but without any relationship with HLA-B27 [41].

Furthermore, NK and dendritic cells, neutrophils and other antigen presenting cells with different signaling ways, including PD-1 and cytokine storm, and exhausted lymphocytes are involved in the COVID-19 pathophysiology [36]. Recently, it was demonstrated CD8^{+} T cells derived from SARS-CoV-2 survivors exhibited polyfunctional effector responses to two novel NC-derived peptides identified as HLA-binders, although not specified [36, 42, 43].

Considering previous outbreaks, Lin et al. [44] reported higher risk and severity of SARS-CoV infection in health care workers carrying HLA-B*4601 (risk and severity) and HLA-B*5401 (only risk) in Taiwan [45]. On the other hand, no significant association was observed in Chinese individuals regarding genotypic patterns of HLA-A, -B and -DRB1 loci in SARS patients when compared to a co-resident population. In Saudi individuals, Hajeer at al demonstrated significant association with MHC class II

(HLA-DRB1*11:01 and DQB1*02:02) and susceptibility to severe MERS-CoV (Middle East respiratory syndrome) [45, 46].

Regarding the COVID-19, Leite et al. evaluated data sets of HLA-B alleles, KIR genes and functional single nucleotide polymorphisms (SNPs) in cytokines related to COVID-19 cytokine storm and found significant correlation between eight HLA-B alleles and polymorphisms in three cytokine genes (IL6, IL10, and IL12B) and daily death rates across countries [47]. Sakuraba et al. demonstrated significant association between HLA-C*05 and higher COVID-19 mortality in 74 countries, after multiple adjustments. A 1% increase in the allele frequency of HLA-C*05 was associated with an increase of 44 deaths/ million. Noteworthy, the combination of HLA-C*05 to its receptor *KIR2DS4fl*, expressed on NK cells, causes NK cell-induced hyperactive immune response and higher mortality rate. Countries with similar ethnic and/or geographic background responded in a similar pattern to each pandemic [48].

Although the HLA genotypes might affect the susceptibility to SARS-CoV-2 infection or severity of COVID-19, these aspects are not still clear up to present day. Toyoshima et al. identified a total of 1234 mutations by comparing with the reference SARS-CoV-2 sequence after analyzing 12,343 SARS-CoV-2 genome sequences of individuals from 28 countries in six geographic areas. They found that ORF1ab 4715 L and S protein 614G variants and the frequency of several HLA alleles, including HLA-A*11:01, were associated with number of infected cases and not as an independent factor for fatality rate in each country [49].

More recently, Pretti et al., evaluating 140 HLA alleles and the landscape of 3,723 potential HLA-I A and B restricted SARS-CoV-2-derived antigens in 37 countries, added a new information regarding three potential antigens coverage of S and N derived peptides and the number of deaths [50]. Similarly, other authors have also

reported same findings related to SARS-CoV-2 immune responses, susceptibility and different genetic backgrounds. Interestingly, Guo et al. found diverse capacities of S protein specific epitope presentation by different HLA alleles with very limited number of predicted epitopes for HLA-B*2705, HLA-B*4402 and HLA-B*4403 and as high as 132 epitopes for HLA-A*6601, suggesting individuals with HLA-B*44 are more prone to SARS-CoV-2 infection. These findings could help us to better understanding the role of HLA-B27 in the COVID-19 [25, 51].

Thus, some points could be highlighted to explain the lack of association between COVID-19 and HLA-B27. Firstly, only Guo study showed some relationship with B27 in general population database across the countries. Secondly, the cytokines pattern and inflammatory signaling ways involved with severe COVID do not seem to be related to Th17 activation, an immune-inflammatory via strongly associated with SpA. Thirdly, the low plasticity of epitopes found by Guo et al. in HLA-B27 could not be crucial to influence the antigen presentation to the spike proteins from SARS-CoV-2 and other coronavirus family. Fourthly, severe COVID-19 have been reported in all IMRDs and the unfavorable outcome has been more related to age and comorbidities than underlying rheumatic disease. On the other hand, the SARS-CoV-2 could work as a trigger to a myriad of musculoskeletal syndromes. Although some authors have reported anecdotal cases of reactive arthritis after COVID-19, the most recent systematic review showed that the manifestations with consistent association with SARS-CoV-2 infection were autoimmune cytopenia, cutaneous vasculitis, encephalitis, and Guillain-Barre syndrome [52]. Considering the inclusion period ended before the massive vaccination against the COVID-19, it is important to mention that our data are related to the SARS-CoV-2 itself and HLA-B27 positivity without any interference of adenovirus vectors, inactivated full virus or mRNA vaccines.

Another important thing to be considered is related to endoplasmatic reticulum aminopeptidases (ERAPs) [53]. As they are pivotal enzymes for trimming peptides and to generate optimal-length antigens to fit into the MHC class I groove, the SARS-CoV-2 likely must not cause any important impairment in this cell step neither related to repertoire of antigens presented by HLA-B27. The inadequate activation of NK and CD8$^+$ T cells could have other pathophysiological mechanisms. Nonetheless, these points are merely speculative because there is no information on the ERAP1, ERAP2, and LNPEP, as well as co-segregation with HLA-B27 subtypes, which are involved in antigen presentation and peptide trimming and the COVID-19 occurrence and severity. Interestingly, it is important to note these multifaceted aminopeptidases

regulates the renin–angiotensin system and higher susceptibility to hypertension, other aspects associated with severe COVID-19 [54]. Considering that an **i**ncreased risk of some comorbidities has been reported in patients with spondyloarthritis, including hypertension and other cardiovascular diseases, more attention could be given to these aspects to understand better this complex interaction among genetic, environmental and several cellular processes [55].

Additionally, the concomitant conventional and biological DMARDs are also related to higher risk of infections, particularly related to immune response impairment against several pathogens, we did not find any significant association with severe COVID-19 in our SpA database, regardless HLA-B27 status. Considering all forms of COVID-19, we observed 19 cases in B27 negative SpA patients and 52 in those B27 positive, suggesting lack of protective role regarding the SARS-CoV-2 infection. However, when evaluating only hospitalized patients (moderate COVID-19), there was a greater number of patients in the B27 negative group. It is important emphasizing that the effect size was very small (OR=0.13; 95%CI 0.02–0.81) in only 6 patients (4 HLA-B27 negative and 2 HLA-B27 positive), which does not allow us to draw conclusions about that.

To our best knowledge, our database is the largest and robust database regarding SpA patients during COVID-19 pandemic and comparing to SpA patients no infected by SARS-CoV-2. However, our ongoing observational prospective registry has some limitations, such as short period of follow-up (until infection resolution).

In conclusion, our data did not support the protective role of HLA-B27 on SARS-CoV-2 infection, including occurrence, ability to assert immune control of novel coronavirus and COVID-19 severity. More studies are needed to understand the role of HLA-B27 and variants of SARS-CoV-2.

Authors' contributions
Mota GD: The main author, responsible of performing this sub analysis, regarding the role of HLAB27 on COVID19 infection among patients with axial spondyloarthritis. Also, responsible for the data collection in one of the participating centers of the original trial ReumaCoV Brasil. Pinheiro, MM: Project supervisor and a scientific advisor responsible for the design and development of the thesis, also the researcher responsible for data collection in one of the participating centers of the original trial ReumaCoV Brasil. Marques CL, Ribeiro SL, Albuquerque C, Castro G, Fernandino D, Omura F, Ranzolin A, Resende G, Silva N, Souza M, Studart S, Xavier R, Yazbek M: particpating members of the other centers among the ReumaCov trial. All authors read and approved the final manuscript.

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Data Availability
All clinical and laboratory data was collected and stored on the REDCAP (Research Electronic Data Capture) platform, such as demographic data, details of infection and treatment of COVID-19 (diagnosis, test performed, treatment, epidemiology and outcomes including hospitalization, mechanical ventilation and death). Also, comorbidities and clinical characteristics, including disease time, disease activity parameters: BASDAI [Bath Ankylosing Spondylitis Disease Activity Index], ASDAS-CRP and ASDAS-ESR [Ankylosing Spondylitis Disease Activity Score], current drug therapy).

Declarations

Ethics approval and consent to participate
Our study was designed as a sub analysis from a national multicenter study (ReumaCoV-Brazil) with the idealization, support, and supervision of the Brazilian Society of Rheumatology during the emergency period of the COVID-19 pandemic and with the Federal University of Pernambuco as the coordinating center and managing of ethical demands. The central and original project was approved by CONEP (CAAE 30186820.2.1001.8807) on April 5, 2020.

The research team contacted all patients identified by the eligibility criteria by telephone, giving initial authorization and permission for the collection of clinical information. When the restrictions of the pandemic allowed face-to-face consultation in the respective center, they all read and signed the consent form.

As mentioned above, we used the same database of patients that had already been approved by CONEP as an unfolding. We emphasize that there is no need for further collection of clinical or laboratory data because the HLA-B27 positivity and evaluation with the metrics used in the study are part of the follow-up routine of patients with spondyloarthritis.

In addition, it is important to emphasize that the original ReumaCoV-Brazil study was conceived and developed in full pandemic context, with restrictions for face-to-face medical evaluations and logistical difficulties, in view of the sanitary recommendations of social distancing. Thus, many data needed to be collected by telephone and by searching for previous information, retrospectively, in the patient's electronic medical record (PEP), with prospective updates on the outcomes of the evolution of COVID-19 and disease activity.

Consent for publication
The authors agreed with the publication.

Competing interests
Not applicable.

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