Heliyon 10 (2024) e23670

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

High production *MBL2* polymorphisms protect against COVID-19 complications in critically ill patients: A retrospective cohort study

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ARTICLE INFO

Keywords:
Innate immunity
Complement system
Mannose-binding lectin
Mannan-binding lectin
Polymorphism
SARS-CoV-2

ABSTRACT

Mannose-binding lectin (MBL) binds to SARS-CoV-2, inhibits infection of susceptible cells, and activates the complement system via the lectin pathway. In this study, we investigated the association of *MBL2* polymorphisms with the risk of hospitalization and clinical worsening in patients with COVID-19. A total of 550 patients with COVID-19 were included (94 non-hospitalized and 456 hospitalized). Polymorphisms in *MBL2* exon 1 (codons 52, 54 and 57) and promoter region (−550, −221, and +4) were determined by real-time PCR. MBL and complement proteins were measured by Luminex. A higher frequency of the H/H genotype and the HYPA haplotype was observed in non-hospitalized patients when compared to hospitalized. In addition, critically ill patients carrying haplotypes associated with high MBL levels (HYPA/HYPA + HYPA/LYPA + HYPA/LYQA + LYPA/LYQA + LYPA/LYPA + LYQA/LYQA + LXPA/HYPA + LXPA/LYQA + LXPA/LYPA) were protected against lower oxygen saturation levels ($P = 0.02$), use of invasive ventilation use ($P = 0.02$, OR 0.38), and shock ($P = 0.01$, OR 0.40), independent of other potential confounders adjusted by multivariate analysis. Our results suggest that variants in *MBL2* associated with high MBL levels may play a protective role in the clinical course of COVID-19.

1. Introduction

COVID-19, a disease caused by the SARS-CoV-2 virus, has been responsible for one of the largest pandemics in human history, affecting more than 680 million people worldwide and causing an estimated 6.8 million deaths by early 2023 [1]. Despite advances in vaccination coverage worldwide, which have contributed to a reduction in the number of severe cases and deaths, COVID-19 is still a health problem, since mutations in the viral genome have caused new waves of cases and increased mortality in at-risk and

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https://doi.org/10.1016/j.heliyon.2023.e23670
Received 20 July 2023; Received in revised form 23 November 2023; Accepted 9 December 2023
Available online 13 December 2023

non-vaccinated groups [2].

Although most COVID-19 infections are mild, with symptoms such as fever and cough and recovery in 2–3 weeks, there are severe cases in which patients may develop clinical conditions requiring hospitalization and in extreme cases, death [3]. Multiple factors are involved in the evolution of the disease to severe forms, including advanced age, presence of comorbidities [4], viral variants [5], human genetic and immunological factors [6], and socioeconomic conditions [7].

Innate immunity plays a crucial role in the clinical course of COVID-19, favoring infection control in the early stages. However, it is known that the deregulated activation of pro-inflammatory components may contribute to an increase in lung injury and consequently worsen the clinical picture, especially in older individuals and those with comorbidities [8].

Mannose-binding lectin (MBL) is an important component of innate immunity, involved in pathogen recognition, activation of the complement system, and modulation of the inflammatory response [9]. The *MBL2* gene, which comprises 4 exons and is situated on chromosome 10, harbors 3 single nucleotide polymorphisms (SNPs) in exon 1 that exert a substantial influence on serum MBL levels. The three SNPs in codons 52 (Arg52Cys, allele D), 54 (Gly54Asp, allele B), and 57 (Gly57Glu, allele C), give rise to variant alleles, where the wild type is referred to as the "A" allele, and alleles B, C and D are collectively named “O.” This genetic modification impacts MBL’s capacity to oligomerize, decreasing its lifespan and ability to bind to mannan. Consequently, the decreased interaction with MBL-associated serine proteases results in reduced complement activation [10]. Furthermore, circulating MBL concentration is also influenced by three key SNPs (named "H/L″, "Y/X″, and "P/Q″) located in the promoter region that are in linkage disequilibrium with structural variants of exon 1 [10].

Previous evidence has shown that MBL was able to bind to SARS-CoV, inhibiting infection in susceptible cells [11]. In addition, mutations in the *MBL2* gene, related to low or deficient MBL levels, have been associated with susceptibility to SARS-CoV in Asian individuals [12,13]. In the context of COVID-19, it has been shown that MBL is able to bind to the SARS-CoV-2 spike protein independent of the variant of concern, activate the complement system via the lectin pathway, and perform an antiviral function in human lung-derived epithelial cell line and primary bronchial cells [14].

Studies investigating the role of polymorphisms in the *MBL2* gene with susceptibility or clinical outcome in COVID-19 have shown conflicting results. A previous study in a cohort of Italian individuals showed that genetic variants near or in the *MBL2* gene were associated with the severity of COVID-19 [14]. On the other hand, no association was found between variants in the *MBL2* gene and the risk of hospitalization or intensive care unit (ICU) admission in a Swedish study. However, haplotypes associated with intermediate MBL levels have been shown to be protective against the development of thrombotic complications and pulmonary embolism in critically ill patients [15]. Other studies conducted in small samples, have associated the B allele with the risk of severity and death or symptomatic outcome in pediatric patients [16–19]. In addition, recent studies have failed to demonstrate an association of polymorphisms in *MBL2* with long COVID [20,21].

Although previous studies have examined the role of MBL and its polymorphisms in the risk of hospitalization, few have examined its role in the risk of complications in hospitalized patients. In addition, most have focused on European and Asian populations, which have different genetic backgrounds than other populations, such as Latin Americans. Therefore, the aim of this study was to evaluate the association of functional polymorphisms in *MBL2* and their levels with the risk of hospitalization and clinical complications of COVID-19 in a cohort of Brazilian individuals. This study stands out for its in-depth evaluation of the main functional variants of *MBL2* described in the literature, through allelic, genotypic and haplotypic analysis, in addition to evaluating their relationship with plasma levels of MBL and other components of the complement system. The results of this study may contribute to the understanding of the role of MBL in the clinical course of COVID-19 and may allow the early identification of cases at higher risk for better clinical management of the disease.

2. Methods

2.1. Study participants

The present study enrolled individuals aged 18 years and older, with a laboratory-confirmed diagnosis of COVID-19, presenting with severe acute respiratory syndrome. The study focused on those admitted to the University Hospital of the Universidade Federal do Vale do São Francisco (HU-UNIVASF/EBSERH, acronym in Portuguese) and the Field Hospital of the Municipality of Petrolina between August 2020 and July 2021. Both are reference centers for treatment of COVID-19 in the Vale do São Francisco region, Petrolina, Northeast Brazil. Hospitalized individuals were divided into two categories: those in the Intensive Care Unit (ICU) and those in non-ICU settings (general wards). The study also investigated individuals with mild symptoms who did not require hospitalization, but who tested positive for COVID-19. These individuals, opting to present themselves at the university hospital for blood collection post complete remission, were also included.

This study obtained ethical approval from the Ethics Committee of the Hospital das Clínicas of the Federal University of Pernambuco (HC/UFPE, acronym in Portuguese), under registration number CAAE: 38,196,620.0.0000.8807. The research was conducted in accordance with the provisions of the Declaration of Helsinki and the Good Clinical Practice guidelines.

2.2. DNA extraction and genotyping

A 4-mL peripheral blood sample was collected in an EDTA tube (Vacuette K3EDTA tube, Greiner Bio-One, Kremsmünster, Austria) within the first 24 h after hospital admission. These samples were then centrifuged at 3500 rpm for 10 min to obtain plasma. Genomic DNA was then extracted from the peripheral blood samples using a commercially available extraction kit (ReliaPrep Blood gDNA

Miniprep System, Promega, Madison, WI, USA), according to the manufacturer's instructions. DNA samples were eluted to a final volume of 100 μL and quantified using a NanoDrop OneC spectrophotometer (Thermo Scientific, Waltham, MA, USA). The genetic material obtained was stored in a freezer at −20 °C until the genotyping procedures were initiated.

Six polymorphisms were selected in the *MBL2* gene, including three structural SNPs located in codons 52 (allele D, Arg52Cys, rs5030737), 54 (allele B, Gly54Asp, rs1800450), and 57 (allele C, Gly57Glu, rs1800451) in exon 1, and three SNPs (termed "H/L [rs11003125]", "Y/X [rs7096206]", and "P/Q [rs7095891]") in promoter regions −550, −221, and +4, respectively. The wild-type allele in exon 1 was denoted as "A," while alleles B–C were collectively referred to as "O", as described elsewhere.

Polymorphisms were detected by real-time PCR (QuantStudio 5, Thermo Fisher) using pre-designed TaqMan assays. The reaction mixture contained 5 μL of GoTaq Probe qPCR Master Mix (2 × , Promega, Madison, WI, USA), 0.25 μL of TaqMan assay, 2.75 μL of sterile water (DNase- and RNase-free), and 2.0 μL of genomic DNA, in a final volume of 10 μL. The qPCR thermal conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, 40 cycles at 95 °C for 15 s, and 60 °C for 1 min.

2.3. Quantification of MBL plasma levels and complement proteins

MBL levels and the concentrations of complement proteins, including C2, C4b, C5, C5a, adipsin (factor D), and factor I, were measured in plasma samples using the MILLIPLEX MAP Human Complement Panel 1 (Millipore Corporation, Billerica, MA, USA; Cat. Number: HCMP1MAG-19K), a Luminex xMAP technology-based multiplex assay that utilizes magnetic beads. The experiments were performed according to the manufacturer's instructions. The analyses were performed on a Luminex 200 system, and the median fluorescence intensity (MFI) was obtained.

2.4. Statistical analysis

The frequencies of *MBL2* genotypes, haplotypes, and alleles were obtained by direct counting. Haplotypes were analyzed using the expectation-maximum algorithm implemented in the Arlequin v. 3.11 software and deviations from Hardy–Weinberg equilibrium were tested.

For analysis, *MBL2* haplotypes pairs were divided into those associated with high expression of MBL (HYPA/HYPA + HYPA/LYPA + HYPA/LYQA + LYPA/LYQA + LYPA/LYPA + LYQA/LYQA + LXPA/HYPA + LXPA/LYQA + LXPA/LYPA), intermediate (HYPA/HYPD + HYPA/LYPB + HYPA/LYQC + HYPD/LYPA + HYPD/LYQA + LYPA/LYPB + LYPA/LYQC + LYPB/LYQA + LYQA/LYQC + LXPA/LXPA), and low (LXPA/LYPB + LXPA/LYQC + HYPD/LYPB + HYPD/HYPD + LYPB/LYQC + LYPB/LYPB + LYQC/LYQC).

Data analysis was performed using JASP version 0.16.4 (JASP Team 2022). GraphPad Prism version 8.0 (GraphPad, San Diego, CA, USA) was utilized to generate the graphs. Categorical data were expressed in terms of absolute frequency and percentage, while continuous variables were presented as median with interquartile range.

The Shapiro–Wilk test was used to verify normal distribution of continuous variables. Continuous variables were compared using the Kruskal–Wallis test with post hoc Dunn's test for pairwise comparisons. For categorical variables, Pearson's chi-square test and Fisher's exact test were used. A Spearman correlation test was conducted to examine the correlation between MBL plasma levels and other continuous variables. The results were presented using a heat map, with the strength of the correlation coefficients displayed in a color scale.

A multivariate regression model adjusted for confounding factors was used to analyze the association of *MBL2* haplotype pairs with complications of COVID-19. The odds ratio (OR) was estimated with a 95 % confidence interval (CI). *P* values < 0.05 were considered significant and were corrected (*Pc*) for multiple comparisons with Bonferroni's correction.

Table 1
Baseline characteristics.

Variable	Hospitalized				
	Non-hospitalized (N = 94)	Non-ICU (N = 272)	ICU (N = 184)	*P* value[a]	*P* value[b]
Age (mean ± SD)	36.7 ± 10.5	52.0 ± 14.1	56.5 ± 17.0	<0.001	<0.001
Sex					
Male (n, %)	36 (38.3)	162 (59.6)	111 (60.3)	<0.001	<0.001
Female (n, %)	58 (61.7)	110 (40.4)	73 (39.7)		
Comorbidities					
Hypertension (n, %)	8 (8.5)	119 (43.7)	100 (54.3)	<0.001	<0.001
Diabetes mellitus (n, %)	3 (3.1)	64 (23.5)	67 (36.4)	<0.001	<0.001
Obesity (n, %)	3 (3.1)	58 (21.3)	47 (25.5)	<0.001	<0.001
Chronic kidney disease (n, %)	0	2 (0.7)	14 (7.6)	0.07	0.006
Chronic heart disease (n, %)	0	4 (1.4)	10 (5.4)	0.08	0.02
COPD (n, %)	0	6 (2.2)	10 (5.4)	0.07	0.02
Cancer (n, %)	0	1 (0.3)	5 (2.7)	0.26	0.11
Death (n, %)	0	0	73 (39.6)	-[c]	-[c]

[a] Non-hospitalized versus hospitalized.
[b] Non-hospitalized versus ICU.
[c] Could not be calculated. COPD: chronic obstructive pulmonary disease; ICU: intensive care unit.

3. Results

3.1. Baseline characteristics

A total of 456 hospitalized patients were included in the study (184 ICU and 272 non-ICU). Additionally, 94 individuals who had mild symptoms and did not require hospitalization were included. Table 1 summarizes the baseline characteristics of these groups. For analysis purposes, non-hospitalized versus hospitalized (ICU + non-ICU) and non-hospitalized versus ICU patients were compared (Table 1).

In summary, non-hospitalized patients were younger; they had a higher frequency of females and a lower prevalence of comorbidities than patients who were hospitalized or admitted to the ICU. Hypertension, diabetes, and obesity were the most prevalent characteristics in the group of hospitalized patients. There was a 39.6 % prevalence of deaths in the ICU group. There were no deaths in the other groups (Table 1).

3.2. Association between MBL2 polymorphisms and hospitalization

The frequencies of the *MBL2* genotypes were in accordance with Hardy–Weinberg equilibrium. We investigated the allelic and genotypic distribution of polymorphisms in exon 1, promoter −550, −221, and +4 between the groups (Table 2). A higher frequency of the H/L and H/H genotypes, related to intermediate and high MBL levels, was observed in the group of non-hospitalized patients when compared to those hospitalized ($P = 0.01$, $Pc = 0.07$) or admitted to ICU ($P = 0.02$, $Pc = 0.14$). This association was also verified by allelic distribution analysis, where the H allele was more frequent in non-hospitalized patients (non-hospitalized versus hospitalized, *P*

Table 2
Allele and genotype distribution of *MBL2* polymorphisms among non-hospitalized and hospitalized individuals

MBL2		Hospitalized			
	Non-hospitalized (N = 94/188)	Non-ICU (N = 272/544)	ICU (N = 184/368)	*P (Pc)* value[a]	*P (Pc)* value[b]
Exon 1					
A/A	55 (58.5)	158 (58.0)	97 (52.7)	AA vs. AO + OO	AA vs. AO + OO
A/O	31 (32.9)	92 (33.8)	71 (38.5)	0.64 (0.99)	0.36 (0.97)
A/B	21 (22.3)	48 (17.6)	44 (23.9)		
A/C	7 (7.4)	30 (11.0)	22 (11.9)		
A/D	3 (3.1)	14 (5.1)	5 (2.7)		
O/O	8 (8.5)	22 (8.0)	16 (8.7)		
B/B	2 (2.1)	8 (2.9)	6 (3.2)		
B/C	3 (3.1)	6 (2.2)	5 (2.7)		
B/D	2 (2.1)	5 (1.4)	2 (1.0)		
C/C	1 (1.0)	1 (0.3)	3 (1.6)		
D/D	0	2 (0.7)	0		
Allele					
A	141 (75.0)	408 (75.0)	265 (72.0)	A vs. O	A vs. O
O	47 (25.0)	136 (25.0)	103 (28.0)	0.73 (0.99)	0.45 (0.99)
B	30 (16.0)	75 (13.8)	63 (17.1)		
C	12 (6.4)	38 (7.0)	33 (9.0)		
D	5 (2.6)	23 (4.2)	7 (1.9)		
Promoter −550					
L/L	36 (38.3)	141 (51.8)	98 (53.2)	LL vs. HL + HH	LL vs. HL + HH
H/L	49 (52.1)	108 (39.7)	70 (38.0)	0.01 (0.07)	0.02 (0.14)
H/H	9 (9.5)	23 (8.4)	16 (8.7)		
Allele					
L	121 (64.3)	390 (71.7)	266 (72.2)	L vs. H	L vs. H
H	67 (35.7)	154 (28.3)	102 (27.8)	0.03 (0.21)	0.05 (0.33)
Promoter −221					
Y/Y	70 (74.4)	187 (68.7)	132 (71.7)	YY vs. YX + XX	YY vs. YX + XX
Y/X	21 (22.3)	74 (27.2)	46 (25.0)	0.38 (0.97)	0.63 (0.99)
X/X	3 (3.1)	11 (4.0)	6 (3.2)		
Allele					
Y	161 (85.6)	448 (82.3)	310 (84.2)	Y vs. X	Y vs. X
X	27 (14.4)	96 (17.7)	58 (15.8)	0.39 (0.98)	0.66 (0.99)
Position + 4					
P/P	53 (56.3)	131 (48.1)	96 (52.1)	PP vs. PQ + QQ	PP vs. PQ + QQ
P/Q	36 (38.3)	109 (40.0)	71 (38.5)	0.24 (0.88)	0.51 (0.99)
Q/Q	5 (5.3)	32 (11.7)	17 (9.2)		
Allele					
P	142 (75.5)	371 (68.1)	263 (71.4)	P vs. Q	P vs. Q
Q	46 (24.5)	173 (31.9)	105 (28.6)	0.09 (0.52)	0.30 (0.94)

[a] Non-hospitalized versus hospitalized.
[b] Non-hospitalized versus ICU·ICU: intensive care unit; *Pc*: corrected *P*-value (Bonferroni).

= 0.03, *Pc* = 0.21), but did not remain significant after multiple testing correction. No association was observed among the other polymorphisms (Table 2).

The polymorphisms studied are in high linkage disequilibrium. Therefore, we decided to evaluate the distribution of haplotypes and haplotype pairs, classified according to MBL levels, among the patient groups. A higher frequency of the HYPA haplotype, related to high MBL levels, was observed in the group of non-hospitalized individuals compared to those hospitalized, but the association did not remain significant after correction for multiple testing ($P = 0.01$; $Pc = 0.09$). No significant association was observed for other haplotypes or haplotype pairs (Table 3).

3.3. Evaluation of plasma levels of MBL and complement proteins

MBL and complement protein levels were assessed in 231 plasma samples from hospitalized patients (111 ICU and 120 non-ICU) collected within 24 h of admission. Fig. 1 demonstrates the association between MBL levels (MFI) and the genotypes of the exon 1 and promoter region of *MBL2*. The wild-type A/A genotype in exon 1 was associated with higher MBL levels when compared to the A/O or O/O genotypes (median, AA: 2907, AO: 460, OO: 59, Kruskal–Wallis, $P < 0.0001$) (Fig. 1A). Supplementary Fig. 1 demonstrates the distribution of MBL levels according to exon 1 genotypes stratified by codon (Fig. S1).

Regarding the −550 promoter region, the H/H genotype was associated with higher MBL levels (median, H/H: 3281, H/L: 1642, L/L: 597, Kruskal–Wallis $P = 0.0001$) (Fig. 1B). At the −221 position, MBL levels were higher in individuals carrying the Y/X genotype (median, Y/X: 1687, Y/Y: 1192, X/X: 406, Kruskal–Wallis $P = 0.04$) (Fig. 1C). Finally, at the +4 position, the highest MBL levels were observed in individuals carrying the Q/Q genotype (median, Q/Q: 3043, P/Q: 1226, P/P: 892, Kruskal–Wallis $P = 0.02$) (Fig. 1D).

We then investigated the association of plasma MBL levels according to individual haplotype pairs (Fig. 2A) or grouped according to high, intermediate, or low levels (Fig. 2B). For both analyses, a significant difference between the groups could be observed (Kruskal–Wallis $P < 0.0001$). Additionally, we observed no correlation between MBL levels and components C2, C4b, C5, C5a, factor D, and factor I (Supplementary Fig. S2). There was also no association between *MBL2* genotypes or haplotypes and levels of complement proteins (data not shown).

3.4. Association of MBL2 haplotypes and clinical worsening in critically ill patients

Since evidence has suggested that *MBL2* polymorphisms are associated with COVID-19 complications in critically ill patients, we decided to check the association of *MBL2* haplotypes, classified as high and intermediate/low, with clinical parameters and complications developed after ICU admission. In line with our findings on the risk of hospitalization, it was shown that haplotypes associated with high MBL levels were associated with protection against lower oxygen saturation rate ($P = 0.02$), invasive ventilation use ($P = 0.02$, OR 0.38, 95 % CI 0.16 to 0.89), and shock ($P = 0.01$, OR 0.40, 95 % CI 0.19 to 0.83). These associations remained significant after correction for possible confounding factors in multivariate analysis (Table 4). There was no correlation between plasma MBL levels and post-admission clinical worsening (data not shown).

4. Discussion

Our results demonstrated that variants in the *MBL2* gene associated with high MBL levels may play a protective role on the clinical course of COVID-19, especially in critically ill patients. Our findings reinforce previous evidence found in European populations and

Table 3
Haplotype analysis of *MBL2* polymorphisms in exon 1, -550, -221 and +4 among non-hospitalized and hospitalized individuals.

MBL2		Hospitalized			
	Non-hospitalized (N = 94/188)	Non-ICU (N = 272/544)	ICU (N = 184/368)	*P (Pc)* value[a]	*P (Pc)* value[b]
Haplotypes					
$HYPA_{(High)}$	62 (33.0)	131 (24.1)	95 (25.8)	0.01 (0.09)	0.07 (0.51)
$LYQA_{(High)}$	34 (18.1)	135 (24.8)	72 (19.6)	0.16 (0.82)	0.67 (0.99)
$LYPA_{(High)}$	18 (9.6)	46 (8.5)	40 (10.9)	0.95 (1.00)	0.63 (0.99)
$LXPA_{(Low)}$	27 (14.3)	96 (17.6)	58 (15.7)	0.39 (0.99)	0.66 (0.99)
$HYPD_{(Low)}$	5 (2.6)	23 (4.2)	7 (1.9)	0.65 (0.99)	0.55 (0.99)
$LYPB_{(Low)}$	30 (16.0)	75 (13.8)	63 (17.1)	0.77 (1.00)	0.72 (0.99)
$LYQC_{(Low)}$	12 (6.4)	38 (7.0)	33 (9.0)	0.50 (0.99)	0.29 (0.96)
Haplotype Pair[c]					
High	52 (55.3)	147 (54.0)	91 (49.5)	0.58 (0.99)	0.35 (0.98)
Intermediate	32 (34.0)	85 (31.3)	63 (34.2)	0.76 (0.99)	0.97 (1.00)
Low	10 (10.7)	40 (14.7)	30 (16.3)	0.23 (0.92)	0.20 (0.89)

[a] Non-hospitalized versus hospitalized.
[b] Non-hospitalized versus ICU.
[c] High: *HYPA/HYPA + HYPA/LYPA + HYPA/LYQA + LYPA/LYQA + LYPA/LYPA + LYQA/LYQA + LXPA/HYPA + LXPA/LYQA + LXPA/LYPA*; intermediate: *HYPA/HYPD + HYPA/LYPB + HYPA/LYQC + HYPD/LYPA + HYPD/LYQA + LYPA/LYPB + LYPA/LYQC + LYPB/LYQA + LYQA/LYQC + LXPA/LXPA*; low: *LXPA/LYPB + LXPA/LYQC + HYPD/LYPB + HYPD/HYPD + LYPB/LYQC + LYPB/LYPB + LYQC/LYQC*. ICU: intensive care unit; *Pc*: corrected *P*-value (Bonferroni).

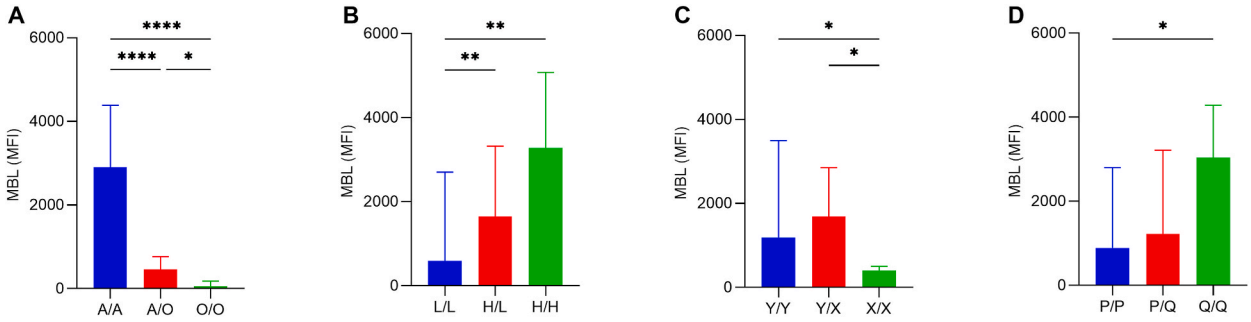

Fig. 1. Association of MBL plasma levels and polymorphisms in *MBL2* (A) exon 1, (B) promoter regions −550 H/L, (C) −221 Y/X, and (D) +4 P/Q. Data are shown as median and interquartile range. Statistical significance was determined using Kruskal–Wallis test with post hoc Dunn's test. MFI: median fluorescence intensity. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

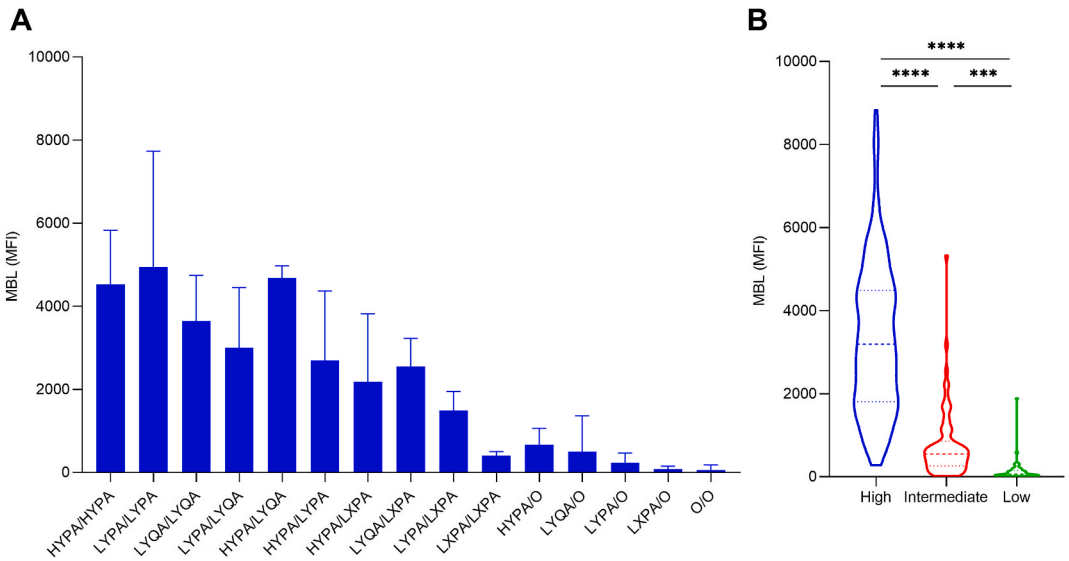

Fig. 2. Association of MBL plasma levels and (A) *MBL2* haplotype pairs and (B) haplotypes categorized according to high, intermediate, and low MBL levels (violin plots). Columns represents median and interquartile range. Statistical significance was determined using Kruskal–Wallis test with post hoc Dunn's test. MFI: median fluorescence intensity. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

bring new data about the role of *MBL2* polymorphisms on the risk of complications during hospitalization, including lower oxygen saturation rates, higher chance of mechanical ventilation use, and higher risk of shock.

Studies investigating the role of polymorphisms in the *MBL2* gene in patients with COVID-19 are scarce and show conflicting results. Stravalaci et al. (2022) [14], investigated 332 individuals with COVID-19 admitted to wards and ICU of two Italian hospitals, and 1668 healthy individuals with unknown COVID-19 status. They observed a significant lower frequency of the CCGGCC haplotype (also known as HYPA), associated with high MBL levels, in the group of individuals hospitalized with COVID-19 compared to the healthy group (OR = 0.78, $P = 0.02$). Furthermore, they found a significant predisposing effect in individuals carrying two disruptive alleles among rs5030737, rs1800450, and rs1800451 (O/O genotype). Other studies in small sample populations have associated polymorphisms in exon 1 with the risk of severe COVID-19 [16–18,20].

In the present study, we also observed a protective effect of the HYPA haplotype and the H variant in the promoter region −550 against the risk of hospitalization. Although the significance was lost after correction for multiple testing, it is important to consider that multiple testing corrections can also lead to type 2 errors, especially in small sample sizes. In the Italian cohort, with a larger control group, only a borderline association between HYPA haplotype and hospitalization was found [14].

Hultström et al. (2022) [15], evaluating data extracted from summary statistics of the COVID-19 Host Genetics Initiative (HGI), did not find any significant association between *MBL2* polymorphisms and risk of COVID-19 requiring hospitalization or advanced respiratory support. On the other hand, they observed a protective effect of *MBL2* haplotypes associated with intermediate MBL levels on the risk of thrombotic complications and pulmonary embolism in 426 Swedish critically ill ICU patients.

We then decided to evaluate whether *MBL2* haplotypes, classified according to their influence on MBL levels, would be associated with clinical worsening in critically ill ICU patients. Although we did not have data on thrombotic events in our cohort, we observed

Table 4
Association between haplotype pair and complications during ICU hospitalization.

	MBL2 haplotype pair		Univariate analysis[a]		Multivariate analysis[b]	
	High (N = 91)	Intermediate/Low (N = 93)	*P* value	OR (95 % CI)	*P* value	OR (95 % CI)
Clinical signs upon hospitalization						
Highest heart rate, median, (IQR)	122.0 (37.0)	123.0 (37.5)	0.69	–	–	–
Highest respiratory rate, median (IQR)	29.0 (11.0)	29.5 (10.2)	1.00	–	–	–
Lowest oxygen saturation (%), median (IQR)	91.0 (7.0)	88.0 (7.2)	**0.003**	–	**0.02**	- (0.41–6.59)
Maximum body temperature (°C), median (IQR)	38.0 (1.3)	38.2 (1.4)	0.62	–	–	–
Complications					–	–
Invasive ventilation (n, %)	69.0 (75.8)	83 (89.2)	**0.01**	2.64 (1.19–5.82)	**0.02**	0.38 (0.16–0.89)
Acute kidney injury (n, %)	31 (35.2)	38 (43,1)	0.28	1.40 (0.76–2.57)	–	–
Shock (n, %)	17 (20.0)	35 (40.2)	**0.003**	2.69 (1.34–5.14)	**0.01**	0.40 (0.19–0.83)
Cardiorespiratory arrest (n, %)	17 (28.3)	23 (38.9)	0.22	1.62 (0.75–3.48)	–	–
Death (n, %)	31 (34.0)	42 (45.6)	0.11	1.63 (0.90–2.95)	–	–

[a] Mann–Whitney test for continuous variables and chi-squared test for categorical variables.
[b] Adjusted for age, sex, diabetes, hypertension, chronic kidney disease, chronic heart disease, chronic obstructive pulmonary disease, and obesity.
CI: confidence interval; IQR: interquartile range; OR: odds ratio.

that haplotypes related to high MBL levels decreased the risk of complications such as lower oxygen saturation, use of mechanical ventilation, and shock.

The conflicting results found among the studies can be explained by differences in the frequency of SNPs in *MBL2* between the populations. Interestingly, we demonstrated that variant D (rs5030737) in heterozygosity has a lower impact on MBL levels when compared to variants B (rs1800450) and C (rs1800451) (Fig. S1). This occurs because the efficacy for variant B and C chains to create stable triple helixes with A chains, which can oligomerize into higher order structures, seems to be lower than for the D variant chain [10]. Therefore, it is possible that the antiviral activity of MBL is more compromised in populations where the B and C alleles are more frequent, than in populations with a higher frequency of the D allele. Additionally, other factors may also explain the different results among the studies, such as different criteria for defining the study groups, control populations with unknown COVID-19 status, vaccine status, and circulation of different SARS-CoV-2 strains.

The mechanisms by which MBL acts in the clinical course of COVID-19 are not yet fully understood, but they may be related to a potential fusion-neutralizing mechanism through competition with *C*-type lectins, as demonstrated previously with SARS-CoV [11]. Corroborating this hypothesis, a previous study has shown that transmembrane lectins act as attachment receptors for SARS-CoV-2, facilitating infection via the canonical ACE2 pathway [22]. Although MBL binding to SARS-CoV-2 spike proteins triggers activation of the complement system via the lectin pathway, in vitro experiments have demonstrated that the antiviral activity of MBL is independent of complement system activation [14]. Additionally, in the present study we found no association between the levels of MBL and its polymorphisms with the plasma concentration of complement system proteins, corroborating previous studies in COVID-19 and dengue [23,24].

The present study has limitations, including the lack of information on other polymorphisms in the *MBL2* gene, the lack of data on MBL levels in non-hospitalized patients, and the lack of a health control group with negative serology for COVID-19. On the other hand, our strengths include the inclusion of a control group of patients known to be positive for COVID-19 with mild symptoms, the availability of clinical data after admission, as well as the evaluation of *MBL2* polymorphisms in a highly mixed population such as the Brazilian, which confirms the results found in other countries regarding the role of *MBL2* variants in COVID-19.

In summary, our findings suggest that variants associated with high MBL production are associated with protection against COVID-19 hospitalization and clinical worsening in critically ill patients. Our results confirm previous findings in European populations and pave the way for new studies evaluating the role of *MBL2* polymorphisms in the clinical worsening of patients with severe COVID-19.

Funding statement

This work was supported by the Foundation for the Support of Science and Technology of the State of Pernambuco (FACEPE, Brazil acronym in Portuguese) (grant numbers APQ-0422–2.02/19 and IBPG-1902-4.03/22), by the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES, Brazil acronym in Portuguese), Finance Code 001, and by the Health Secretariat of Pernambuco, Brazil. (Agreement 10/2021).

Conflict of interest disclosure

Prof. Rodrigo Feliciano do Carmo is associate editor at Heliyon.

Ethics approval statement

This study received approval from the Ethics Committee of the Hospital das Clínicas of the Federal University of Pernambuco (HC/UFPE, acronym in Portuguese) under number CAAE: 38,196,620.0.0000.8807, and it was conducted in accordance with the provisions of the Declaration of Helsinki and the Good Clinical Practice guidelines. Informed consent to participate in this study was provided by the participants.

Data availability statement

The data presented in this manuscript will be available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Lorena Viana de Andrade: Writing - original draft, Investigation, Formal analysis. **Mirela Vanessa de Souza Sá:** Writing - original draft, Investigation. **Beatriz Vasconcelos:** Investigation. **Luydson Richardson Silva Vasconcelos:** Writing - review & editing, Validation, Methodology. **Ricardo Khouri:** Writing - review & editing, Validation, Methodology. **Carlos Dornels Freire de Souza:** Writing - review & editing, Validation, Data curation. **Anderson da Costa Armstrong:** Writing - review & editing, Funding acquisition, Conceptualization. **Rodrigo Feliciano do Carmo:** Writing - review & editing, Writing - original draft, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Rodrigo Feliciano do Carmo reports financial support was provided by Foundation for Support of Science and Technology of Pernambuco State. Rodrigo Feliciano do Carmo reports financial support was provided by Coordination of Higher Education Personnel Improvement. Anderson da Costa Armstrong reports financial support was provided by Secretaria Estadual de Saúde de Pernambuco. Associate editor at Heliyon - RFC.

Acknowledgements

The Multi-User Research Laboratory of HU-UNIVASF (EBSERH) for the support provided.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23670.

References

[1] COVID - Coronavirus Statistics - Worldometer. https://www.worldometers.info/coronavirus/#countries. (Accessed 20 April 2023).
[2] K. Dhama, F. Nainu, A. Frediansyah, M.I. Yatoo, R.K. Mohapatra, S. Chakraborty, et al., Global emerging Omicron variant of SARS-CoV-2: impacts, challenges and strategies, J Infect Public Health 16 (2023) 4, https://doi.org/10.1016/J.JIPH.2022.11.024.
[3] W. Guan, Z. Ni, Y. Hu, W. Liang, C. Ou, J. He, et al., Clinical characteristics of coronavirus disease 2019 in China, N. Engl. J. Med. 382 (2020) 1708–1720, https://doi.org/10.1056/NEJMOA2002032.
[4] A. Izcovich, M.A. Ragusa, F. Tortosa, M.A.L. Marzio, C. Agnoletti, A. Bengolea, et al., Prognostic factors for severity and mortality in patients infected with COVID-19: a systematic review, PLoS One 15 (2020), e0241955, https://doi.org/10.1371/JOURNAL.PONE.0241955.
[5] M.L. Robinson, C.P. Morris, J.F. Betz, Y. Zhang, R. Bollinger, N. Wang, et al., Impact of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants on inpatient clinical outcome, Clin. Infect. Dis. (2022), https://doi.org/10.1093/CID/CIAC957.
[6] Q. Zhang, P. Bastard, A. Karbuz, A. Gervais, A.A. Tayoun, A. Aiuti, et al., Human genetic and immunological determinants of critical COVID-19 pneumonia, Nat 2022 6037902 603 (2022) 587–598, https://doi.org/10.1038/s41586-022-04447-0.
[7] C.D.F. De Souza, M.F. Machado, R.F. Do Carmo, Human development, social vulnerability and COVID-19 in Brazil: a study of the social determinants of health, Infect Dis Poverty 9 (2020) 1–10, https://doi.org/10.1186/S40249-020-00743-X.
[8] M.S. Diamond, T.D. Kanneganti, Innate immunity: the first line of defense against SARS-CoV-2, Nat Immunol 2022 232 23 (2022) 165–176, https://doi.org/10.1038/s41590-021-01091-0.
[9] E.W.K. Ip, K. Takahashi, R. Alan Ezekowitz, L.M. Stuart, Mannose-binding lectin and innate immunity, Immunol. Rev. 230 (2009) 9–21, https://doi.org/10.1111/J.1600-065X.2009.00789.X.
[10] P. Garred, F. Larsen, H.O. Madsen, C. Koch, Mannose-binding lectin deficiency—revisited, Mol. Immunol. 40 (2003) 73–84.
[11] Y. Zhou, K. Lu, S. Pfefferle, S. Bertram, I. Glowacka, C. Drosten, et al., A single asparagine-linked glycosylation site of the severe acute respiratory syndrome coronavirus spike glycoprotein facilitates inhibition by mannose-binding lectin through multiple mechanisms, J. Virol. 84 (2010) 8753–8764, https://doi.org/10.1128/JVI.00554-10.
[12] W.K.E. Ip, H.C. Kwok, H.K.W. Law, G.H.W. Tso, E.K.P. Kong, W.H.S. Wong, et al., Mannose-binding lectin in severe acute respiratory syndrome coronavirus infection, J. Infect. Dis. 191 (2005) 1697–1704, https://doi.org/10.1086/429631.
[13] H. Zhang, G. Zhou, L. Zhi, H. Yang, Y. Zhai, X. Dong, et al., Association between mannose-binding lectin gene polymorphisms and susceptibility to severe acute respiratory syndrome coronavirus infection, J. Infect. Dis. 192 (2005) 1355–1361, https://doi.org/10.1086/491479.
[14] M. Stravalaci, I. Pagani, E.M. Paraboschi, M. Pedotti, A. Doni, F. Scavello, et al., Recognition and inhibition of SARS-CoV-2 by humoral innate immunity pattern recognition molecules, Nat Immunol 2022 232 23 (2022) 275–286, https://doi.org/10.1038/s41590-021-01114-w.

[15] M. Hultström, R. Frithiof, J. Grip, L. Lindelöf, O. Rooijackers, S. Pigazzini, et al., Genetic determinants of mannose-binding lectin activity predispose to thromboembolic complications in critical COVID-19, Nat Immunol 2022 236 23 (2022) 861–864, https://doi.org/10.1038/s41590-022-01227-w.
[16] Y. Kashiwagi, S. Suzuki, R. Takahashi, G. Yamanaka, Y. Hirai, H. Kawashima, Association of the mannose-binding lectin 2 BB genotype with COVID-19-related mortality, Life 2023 13 (2023) 382, https://doi.org/10.3390/LIFE13020382, 13, Page 382.
[17] M. Speletas, K. Dadouli, A. Syrakouli, N. Gatselis, G. Germanidis, V.A. Mouchtouri, et al., MBL deficiency-causing B allele (rs1800450) as a risk factor for severe COVID-19, Immunobiology 226 (2021), 152136.
[18] A. Medetalibeyoglu, G. Bahat, N. Senkal, M. Kose, K. Avci, G.Y. Sayin, et al., Mannose binding lectin gene 2 (rs1800450) missense variant may contribute to development and severity of COVID-19 infection, Infect. Genet. Evol. 89 (2021), 104717.
[19] D. Yilmaz, M. Soyoz, A. Sahin, B. Cerci-Alkac, H.I. Karahan-Coven, Y. Ekemen-Keles, et al., Association between mannose binding lectin gene polymorphisms and clinical severity of COVID-19 in children, Mol. Biol. Rep. 50 (2023) 5871–5877, https://doi.org/10.1007/S11033-023-08524-Z.
[20] M.A.F. Queiroz, A.M. Santiago, S. Brito Wr dos, K.A.S. Pereira, W.B. de Brito, S. Torres Mk da, et al., Polymorphisms in the MBL2 gene are associated with the plasma levels of MBL and the cytokines IL-6 and TNF-α in severe COVID-19, Front. Immunol. 14 (2023), 1151058.
[21] L. Hurler, Szilágyi Á, F. Mescia, L. Bergamaschi, B. Mező, G. Sinkovits, et al., Complement lectin pathway activation is associated with COVID-19 disease severity, independent of MBL2 genotype subgroups, Front. Immunol. 14 (2023), 1162171.
[22] F.A. Lempp, L.B. Soriaga, M. Montiel-Ruiz, F. Benigni, J. Noack, Y.J. Park, et al., Lectins enhance SARS-CoV-2 infection and influence neutralizing antibodies, Nat 2021 5987880 598 (2021) 342–347, https://doi.org/10.1038/s41586-021-03925-1.
[23] J.C. Holter, S.E. Pischke, E. de Boer, A. Lind, S. Jenum, A.R. Holten, et al., Systemic complement activation is associated with respiratory failure in COVID-19 hospitalized patients, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 25018–25025, https://doi.org/10.1073/PNAS.2010540117.
[24] N.T. Giang, H. van Tong, D. Quyet, N.X. Hoan, T.H. Nghia, N.M. Nam, et al., Complement protein levels and MBL2 polymorphisms are associated with dengue and disease severity, Sci Reports 2020 101 10 (2020) 1–13, https://doi.org/10.1038/s41598-020-71947-2.