Circulating sTREM-1 as a predictive biomarker of pediatric multisystemic inflammatory syndrome (MIS-C)

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ABSTRACT

The exacerbation of the inflammatory response caused by SARS-CoV-2 in adults promotes the production of soluble mediators that could act as diagnostic and prognostic biomarkers for COVID-19. Among the potential biomarkers, the soluble triggering receptor expressed on myeloid cell-1 (sTREM-1) has been described as a predictor of inflammation severity. The aim was to evaluate sTREM-1 and cytokine serum concentrations in pediatric patients during the acute and convalescent phases of COVID-19. This was a prospective study that included 53 children/adolescents with acute COVID-19 (Acute-CoV group); 54 who recovered from COVID-19 (Post-CoV group) and 54 controls (Control group). Preexisting chronic conditions were present in the three groups, which were defined as follows: immunological diseases, neurological disorders, and renal and hepatic failures. The three groups were matched by age, sex, and similar preexisting chronic conditions. No differences in sTREM-1 levels were detected among the groups or when the groups were separately analyzed by preexisting chronic conditions. However, sTREM-1 analysis in the seven multisystemic inflammatory syndrome children (MIS-C) within the Acute-Cov group showed that sTREM-1 concentrations were higher in MIS-C vs non-MIS-C acute patients. Then, the receiver operating curve analysis (ROC) performed with MIS-C acute patients revealed a significant AUC of 0.870, and the sTREM-1 cutoff value of > 5781 pg/mL yielded a sensitivity of 71.4 % and a specificity of 91.3 % for disease severity, and patients with sTREM-1 levels above this cutoff presented an elevated risk for MIS-C development in 22.85-fold (OR = 22.85 [95 % CI 1.64–317.5], *p = 0.02*). The cytokine analyses in the acute phase revealed that IL-6, IL-8, and IL-10 concentrations were elevated regardless of whether the patient developed MIS-C, and those levels decreased in the convalescent phase, even when compared with controls. Spearman correlation analysis generated positive indexes between sTREM-1 and IL-12 and TNF-α concentrations, only within the Acute-CoV group. Our findings revealed that sTREM-1 in pediatric patients has good predictive accuracy as an early screening tool for surveillance of MIS-C cases, even in patients with chronic underlying conditions.

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1. Introduction

The COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected the global population [1–4]. Unlike adults, adolescents and children seem to be less susceptible to this infection (there is a 17.8 % rate of infection in the population aged 0–15 years old) and usually have better prognoses, although severe/critical COVID-19 has been described in some cases of children and immunocompromised pediatric patients [5,6].

The clinical spectrum of pediatric COVID-19 is very broad, ranging from asymptomatic to critically ill patients. The most common signs and symptoms in children and adolescents are fever and cough, followed by headache, rhinorrhea, myalgia, dyspnea, conjunctivitis, nausea, abdominal pain, vomiting, and diarrhea [1,3–5,7–11]. Other clinical features have also been reported in pediatric populations with COVID-19, such as cardiovascular, renal, thrombotic, cutaneous, olfactory, gustatory, neurological, and ocular manifestations. Leukopenia, lymphopenia, and increased inflammatory markers were the most frequently reported laboratory abnormalities in these patients [1–4,7,8,10–12].

The pathogenic mechanism of SARS-CoV-2 is based on the affinity of its spike glycoprotein to the angiotensin-converting enzyme receptor (ACE2) [13], which is expressed in multiple tissues, such as the airways, esophagus, ileum, colon, liver, cornea, heart, kidney and testis [14]. The reason why COVID-19 is less prevalent and less severe in pediatric patients is still unclear, but according to Patel et al. (2020) [15], children and adolescents present lower concentrations of ACE2 on the cell surface, leading to the hypothesis that this group has fewer severe cases of the disease due to a decrease in ACE2 expression.

In COVID-19, inflammatory mechanisms are crucial for clinical presentation. In patients with severe infection, there is an increase in the concentrations of a series of inflammatory mediators, such as C-reactive protein, D-dimer, TNF-α, and IL-6. Critically ill patients with acute COVID-19, particularly children with multisystemic inflammatory syndrome (MIS-C), may have an increase in proinflammatory mediators, mainly TNF-α, IL-1β, IL-6, IP-10, MCP-1, MIP-1A, and granulocyte colony-stimulating factor (G-CSF) [1,2,16]. According to Pereira et al. (2020) [4], MIS-C occurred in approximately 9 % of children and adolescents at our tertiary Brazilian Hospital.

Among the potential biomarkers of the diagnosis and prognosis of severe infections (including MIS-C), the soluble triggering receptor expressed on myeloid cell-1 (sTREM-1) is highlighted as a predictor of inflammation severity [17,18]. TREM-1 is an immunoglobulin-like membrane receptor member of the TREM family that is selectively expressed on neutrophil and monocyte/macrophage surfaces [19]. sTREM-1 is a soluble factor released after proteolytic cleavage of the membrane-anchored TREM-1 by metalloproteinases and after stimulation of TREM-1 by proinflammatory mediators [20]. Shreds of evidence suggest that TREM-1 amplifies the innate immune response [18,19] via involvement in the signaling pathways of the immunopathogenesis of viral infection [21]. Activation of the TREM-1 pathways results in neutrophil degranulation, cell survival, and potent production of TNF-α, IL-6, IL-1β, IL-8, MCP-1, IL-12p40, and GM-CSF by monocytes/macrophages and dendritic cells, increasing inflammation during infection by different pathogens, such as influenza, dengue, and hepatitis C, as well as in chronic inflammatory diseases [22–24].

Soluble TREM-1 can be measured in human body fluids. A general increase is seen in severe cases of inflammatory diseases and is considered an inflammatory and prognostic biomarker of sepsis [18,21]. Reports carried out in adult patients indicate that the increase in sTREM-1 plasma levels occurs mainly in severe and critical forms of COVID-19, suggesting that this molecule can be used as an important indicator of a poor prognosis [23,25].

However, there is no evidence of the role of sTREM-1 in the pediatric population with COVID-19. The identification of a molecule that reflects the severity and prognosis of the disease is extremely important for the diagnosis and management of COVID-19 in the pediatric population. Therefore, the present study aims to quantify serum concentrations of sTREM-1 and inflammatory cytokines in pediatric patients with acute infection and in the convalescence phase and their relationship with disease severity.

2. Patients and methods

The present study was approved by the Institutional Review Board Ethics Committee for Analysis of Research Projects (CAAE: 37460620.8.0000.0068), and written consent was obtained from all participants and/or their parents.

2.1. Patients

This was a prospective study composed of 161 children and adolescents separated into three cohorts matched by sex, age, and similar comorbidities (Table 1). The acute COVID-19 group (Acute-CoV) comprised 53 children and adolescents with acute SARS-CoV-2 infection (samples collected at diagnosis). The Post-CoV group comprised 54 convalescent patients (samples collected 3–6 months postinfection), and the control group comprised 54 individuals (children and adolescents) without COVID-19. Within our cohort, 14 patients who had acute COVID donated their samples in the acute phase and in the convalescence phase, so it was possible to match their samples for soluble marker analysis. All COVID-19 patients (acute and convalescent) were followed-up at the Instituto da Criança e do Adolescente, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (ICr-HCFMUSP), Brazil; Instituto de Tratamento do Câncer Infantil (ITACI) of the HCFMUSP; and Instituto Central (IC-HCFMUSP).

Children and adolescents with COVID-19 were included in this study according to the following inclusion criteria: 1) patients diagnosed with COVID-19 between 2020 and 2022; 2) confirmation of SARS-CoV-2 infection by analyzing nasopharyngeal or oropharyngeal swabs using a genomic RNA assay with qRT–PCR or by anti-SARS-CoV-2 IgG serology and/or rapid immunochromatographic assay for anti-SARS-CoV-2 IgM and IgG antibodies [10]; and 3) age at diagnosis between 1 and 18 years old, being previously healthy or with a preexisting chronic condition.

COVID-19 was classified according to clinical features and chest X-ray imaging, being defined as mild, moderate, severe, and critical cases, as follows: Mild, symptoms of acute upper respiratory tract infection; Moderate, patients with pneumonia, without need of oxygen therapy; Severe, patients with pneumonia, dyspnea, with cyanosis and oxygen saturation below 92 %; Critical, acute respiratory distress syndrome or respiratory failure, could also present with organ dysfunction [26].

The control group was matched for age, sex, and preexisting chronic conditions. For the control group, previously selected individuals without COVID-19 were invited to participate in the study (assessed by negative qRT–PCR and negative serology), aged 1 to 18 years, being previously healthy or with some chronic condition, according to the clinical conditions found in the COVID-positive groups.

Preexisting chronic conditions in the three groups, COVID (acute and convalescent) and controls, were defined according to the duration of signs and symptoms greater than three months, and the diagnosis was established for each disease based on the physician's scientific knowledge and diagnostic criteria [27]. The following pediatric chronic conditions or specific situations were considered immunological alterations: immunodeficiencies, organ transplants or hematopoietic stem cells, neoplasms, chronic kidney diseases, autoimmune diseases, and use of immunosuppressants [28].

Blood samples were collected from a peripheral vein (2 mL) in special clot activator tubes for serum separation, which was aliquoted and immediately frozen at −80 °C for further analysis.

Table 1

Demographics, clinical manifestations, COVID classification and pre-existing conditions of patients with acute pediatric COVID-19 or MIS-C (Acute-CoV) at the time of diagnosis and convalescent patients of COVID-19 or MIS-C (Post-CoV) assessed in the acute phase and negative controls for COVID-19.

Acute-CoV Group

	Acute-CoV (n = 53)			Control (n = 54)		
	MISC (n = 7)	Non-MISC (n = 46)	MISC X Non-MISC (p-value)		MISC X Control (p-value)	Non-MISC X Control (p-value)
Demographic data						
Age (years), Med (95 % CI)	5 (1.5–12.4)	10 (8–12)	*0.22*	11 (9–12)	*0.13*	*0.75*
Male / Female (n)	4/3	22/24	*0.56*	27/27	*0.94*	*0.32*
Classification of COVID-19						
Mild, n (%)	–	32 (69.6)	–	–	–	–
Moderate, n (%)	–	4 (8.7)	–	–	–	–
Severe, n (%)	–	7 (15.2)	–	–	–	–
Critical, n (%)	–	3 (6.5)	–	–	–	–
MIS-C, n (%)	7 (13.2)	–	–	–	–	–
Clinical Data						
Duration of signs/symptoms before diagnosis (days), Med (95 % CI)	7 (2.4 – 14.8)	3 (2.8–6.5)	*0.21*	–	–	–
Fever, n (%)	6 (85.7)	31 (67.4)	*0.31*	15 (27.8)	***0.02***	***0.001***
Nasal discharge, n (%)	1 (14.3)	11 (23.9)	*0.49*	8 (14.8)	*0.97*	*0.15*
Cough, n (%)	0 (0)	19 (41.3)	*0.99*	12 (22.2)	*0.99*	*0.20*
Sore throat, n (%)	2 (28.6)	0 (0)	*0.99*	0 (0)	–	–
Dyspnea, n (%)	4 (57.1)	10 (21.7)	*0.31*	0 (0)	–	–
Headache, n (%)	2 (28.6)	5 (10.9)	*0.27*	4 (7.0)	*0.1*	*0.43*
Myalgia, n (%)	2 (28.6)	6 (13.0)	*0.48*	5 (9.3)	*0.15*	*0.26*
Nausea, n (%)	0 (0)	5 (10.9)	*0.99*	1 (1.8)	*1.00*	*0.07*
Vomit, n (%)	1 (14.3)	6 (13.0)	*0.98*	1 (1.9)	*0.14*	***0.04***
Diarrhea, n (%)	2 (28.6)	8 (17.4)	*0.59*	3 (5.6)	*0.06*	***0.04***
Others, n (%)	6 (85.7)	26 (56.5)	*0.31*	20 (37.0)	***0.04***	***0.01***
Pre-existing chronic conditions						
No comorbidity, n (%)	2 (28.5)	4 (8.7)	*1.00*	7 (12.9)	*1.00*	*1.00*
Immunological alterations, n (%)	2 (28.5)	24 (52.2)	*0.13*	23 (42.6)	*0.27*	*0.46*
Neurological diseases, n (%)	2 (28.5)	10 (21.7)	*0.48*	15 (27.8)	*0.49*	*0.95*
Kidney and liver failure, n (%)	1 (14.5)	8 (17.4)	*0.36*	9 (16.7)	*0.48*	*0.70*

Post-CoV Group

	Post-CoV (n = 54)			Control (n = 54)		
	MISC (n = 10)	Non-MISC (n = 44)	MISC X Non-MISC (p-value)		MISC X Control (p-value)	Non-MISC X Control (p-value)
Demographic data						
Age (years), Med (95 % CI)	7.5 (4–10)	13 (10–12)	***0.02***	11 (9–12)	*0.08*	*0.23*
Time between diagnosis and study entry (months), Med (95 % CI)	2.9 (1.2–5.6)	4.4 (3.6–5.3)	*0.12*	–	–	–
Male / Female (n)	5/5	21/23	*0.88*	27/27	*0.80*	*0.75*
Classification of COVID-19						
Mild, n (%)	–	26 (59.1)	–	–	–	–
Moderate, n (%)	–	9 (20.4)	–	–	–	–
Severe, n (%)	–	6 (13.6)	–	–	–	–
Critical, n (%)	–	3 (6.8)	–	–	–	–
MIS-C, n (%)	10 (18.5)	–	–	–	–	–
Clinical Data						
Duration of signs/symptoms before diagnosis (days), Med (95 % CI)	7.5 (4–23.2)	2 (3–7)	***0.02***	–	–	–
Fever, n (%)	9 (90)	32 (72.7)	*0.99*	15 (27.8)	*1.00*	***< 0.01***
Nasal discharge, n (%)	0 (0)	18 (40.9)	*0.99*	8 (14.8)	–	***0.005***
Cough, n (%)	3 (30)	20 (45.5)	*0.38*	12 (22.2)	*0.60*	***0.02***
Sore throat, n (%)	3 (30)	7 (15.9)	*0.22*	0 (0)	–	–
Dyspnea, n (%)	1 (10)	14 (31.8)	*0.19*	0 (0)	–	–
Headache, n (%)	5 (50)	16 (36.4)	*0.43*	4 (7.0)	***0.002***	***0.001***
Myalgia, n (%)	5 (50)	14 (31.8)	*0.22*	5 (9.3)	***0.004***	***0.01***
Nausea, n (%)	3 (30)	13 (29.5)	*0.98*	1 (1.8)	***0.01***	***0.004***
Vomit, n (%)	4 (40)	12 (27.3)	*0.43*	1 (1.9)	***0.003***	***0.005***
Diarrhea, n (%)	4 (40)	11 (25)	*0.52*	3 (5.6)	***0.006***	***0.004***
Others, n (%)	10 (100)	34 (77.3)	*0.99*	20 (37.0)	*1.00*	***< 0.01***
Pre-existing chronic conditions						
No comorbidity, n (%)	10 (100)	2 (4.5)	*1.00*	7 (12.9)	*1.00*	*1.00*
Immunological alterations, n (%)	–	23 (52.3)	*0.99*	23 (42.6)	*0.99*	*0.14*
Neurological diseases, n (%)	–	11 (25)	*0.99*	15 (27.8)	*0.99*	*0.29*
Kidney and liver failure, n (%)	–	8 (18.2)	*0.99*	9 (16.7)	*0.99*	*0.23*

Significant *p*-values are in bold. n: numbers; Med (95 % CI): Median (lower – upper 95 % confidence interval); Other symptoms: pneumonia, abdominal pain, cutaneous rash and hypoxemia.

2.2. MIS-C diagnosis

For the diagnosis of MIS-C, the criteria of the Centers for Disease Control and Prevention [12] and the World Health Organization [29] were applied.

In children and adolescents with MIS-C, the following laboratorial parameters were evaluated: inflammatory markers (C-reactive protein (CRP), ferritin); coagulopathy markers (elevated ᴅ-dimers, fibrinogen) and myocardial, renal and hepatic function tests (troponin-T, lactate dehydrogenase, serum creatinine, aspartate aminotransferase and alanine aminotransferase).

Of the 53 patients from the Acute-CoV group, seven patients presented with MIS-C, and of the 54 patients from the Post-CoV group, ten patients developed MIS-C during the acute phase.

2.3. sTREM-1 quantification

sTREM-1 was quantified using the Human TREM-1 DuoSet ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The results were expressed in pg/mL.

2.4. Cytokine analysis

The levels of the cytokines IL-1β, IL-8, IL-6, IL-10, IL-12, and TNF-α were measured in serum samples using the cytometric bead array technique (Inflammatory CBA, Becton Dickinson, BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions, using a detection range of 10 to 5000 pg/mL. The data were acquired using the LSRII Fortessa flow cytometer (BD Biosciences, San Jose, CA, USA), and the fluorescence intensity of each cytokine was measured and analyzed by FlowJo software (Tree Star, Ashland, OR, USA).

2.5. Statistical analysis

To synthesize the data, a descriptive analysis was initially applied for the calculation of means, medians, standard deviations, and confidence intervals. According to data normality, tested by the D'Agostino-Pearson normality test, comparisons were performed using the Kruskal–Wallis test, with Dunn's multiple comparisons *post hoc* test for the three groups or by Wilcoxon, used to examine differences between the matched samples. Spearman's correlation analysis was also performed. These analyses were carried out using GraphPad Prism version 7.0 software for Windows (GraphPad Software Inc., San Diego, CA, USA).

Univariate logistic regression was performed to obtain the Odds Ratio (OR) values and their respective 95 % confidence interval (CI) and were run to determine the association between demographic, clinical, and biochemical variables and risk of MIS-C development. Then, the multiple logistic regression analysis was performed to obtain the values of the adjusted OR by the variables previously associated with the risk of MIS-C development. These analyses were carried out on Statistical Package for the Social Sciences (SPSS - version 18.0 for Windows) software.

All statistical tests were performed considering a confidence interval of 95 % and a significance level of *p < 0.05*.

3. Results

3.1. Sample group characteristics

Table 1 presents demographic data, clinical manifestations, COVID classification and underlying conditions of 53 laboratory-confirmed pediatric COVID-19 acute patients with MIS-C vs without MIS-C, and 54 pediatric COVID-19 convalescent patients who had MIS-C or not in the acute phase, and 54 controls. The 161 subjects enrolled in this study were separated into three cohorts matched by sex, age, and similar comorbidities. The mean age was 10 years for Acute-CoV patients vs 13

years for Post-CoV patients vs 11 years for Controls, with no significant differences. MIS-C patients were younger than COVID groups, but significantly younger only in the Post-CoV group. No significant differences were detected in the numbers of males and females across COVID and control groups, including in MIS-C groups.

The clinical longitudinal follow-up matched the preexisting chronic conditions between the two COVID groups and both were similar to control group. Similarly, the predominant preexisting chronic conditions were immunological disorders for both groups of patients and controls (49.1 %, 52.3 %, 42.6 %, *p > 0.05*). Acute patients with MIS-C were evenly distributed among the different comorbidities, but the convalescent patients who had MIS-C were all previously healthy. In this latter group, the duration of signs/symptoms before diagnosis was significantly longer than in convalescent patients who did not progress to MIS-C (*p = 0.02*).

The main initial clinical symptoms reported in the Acute-CoV patients and convalescent patients assessed in the acute phase were fever, followed by cough and nasal discharge. Dyspnea, headache, and myalgia were also frequent. In the MIS-C groups, the main symptom was fever.

Laboratorial parameters of patients with acute COVID-19 and MIS-C (Acute-CoV), convalescent patients of COVID-19 and MIS-C (Post-CoV) evaluated in the longitudinal follow-up visit and negative controls for COVID-19 are presented in Table 2. Post-CoV MIS-C patients presented significantly lower neutrophil counts, CRP, ᴅ-dimer and ferritin levels than Acute-CoV MIS-C patients. Post-CoV non-MIS-C COVID-19 patients presented significantly lower CRP, ᴅ-dimer, ferritin, lactate dehydrogenase and Troponin T levels than Acute-CoV Non-MIS-C ones (data not shown).

In the MIS-C Acute-CoV group, 71.4 % of patients required intensive care unit (ICU) care, and the mean time hospitalization was 14.2 days. Most MIS-C patients (85.7 %) were treated with steroids and IVIg and 71.4 % needed mechanical ventilation. Two patients presented with shock, and vasoactive therapy was used, and no deaths were reported in our study. In the MIS-C Post-CoV group, during the acute phase, 40 % of patients required ICU care, and the mean time hospitalization was 6 days. Thirty percent (30 %) of patients were treated with steroids, 80 % with IVIg and 20 % needed mechanical ventilation. Three patients (30 %) presented with shock, in which vasoactive drugs were used, and no deaths were reported.

3.2. Determination of sTREM-1 concentrations

The sTREM-1 serum levels were compared among the study groups, and the median (95 % CI) concentrations of sTREM-1 were 1105 (1559–3061) in the Acute-CoV group, 940.6 (1187–2208) in the Post-CoV group, and 842.2 pg/mL (984.2–1907) in the Control group, without statistically significant differences among them.

In Fig. 1**A to 1D**, sTREM-1 concentrations were analyzed in the patients grouped by comorbidities as follows: without comorbidities (Fig. 1**A**), with immunological alterations (Fig. 1**B**), with neurological disorders (Fig. 1**C**), and with kidney and hepatic failures (Fig. 1**D**). Comparing median sTREM-1 concentrations by underlying disease in those groups (autoimmune, oncological, primary immunodeficiency, neurological, chronic kidney disease, etc.) or medications in use (calcineurin inhibitors, corticosteroids, etc.) we did not observe statistically significant differences.

3.2.1. MIS-C sTREM-1 analysis

The lack of significant differences in the sTREM-1 levels in the present study led us to separate the MIS-C cases from the acute and convalescent groups. The analysis of sTREM-1 serum levels in MIS-C vs non-MIS-C patients within the acute and convalescent groups showed that sTREM-1 concentrations were markedly higher in MIS-C acute patients than in non-MIS-C acute patients and controls. In the convalescence phase, although sTREM-1 concentrations were still elevated in the patients who had MIS-C, no significant differences were detected when

Table 2

Laboratorial parameters (Med, 95 % CI) of patients with acute pediatric COVID-19 and MIS-C (Acute-CoV) at the time of diagnosis and convalescent patients of COVID-19 or MIS-C (Post-CoV) in the longitudinal follow-up visit and negative controls for COVID-19.

Acute-CoV Group						
	Acute-CoV (n = 53)			Control (n = 54)		
	MISC (n = 7)	Non-MISC (n = 46)	MISC X Non-MISC		MISC X Control (*p-value*)	Non-MISC X Control (*p-value*)
			(p-value)			
Hematological parameters						
Leukocyte count/mm3	10500 (7329–16931)	7210 (6589–11361)	*0.049*	6880 (7230–10969)	*0.24*	*0.37*
Neutrophil count/ mm3	6860 (5252–11052)	3680 (3611–5739)	*0.09*	4688 (4737–7702)	*0.32*	*0.32*
Lymphocyte count/mm3	1660 (575–4699)	1900 (1605–3959)	*0.71*	1845 (1713–2803)	*0.61*	*0.96*
Thrombocyte count/mm3 x1000	211 (73–375)	233 (217–305)	*0.35*	265 (224–292)	*0.48*	*0.39*
Biochemical and Inflammatory markers						
C-reactive protein, mg/L	70.6 (9.2–220.1)	11.5 (14.3–71.4)	*0.20*	4.8 (10.7–33.4)	*0.02*	*0.12*
Fibrinogen, mg/dL	419 (167.2–750.8)	352.5 (303–519.6)	*0.91*	258 (226.5–353)	*0.13*	*0.05*
D-dimer, ng/mL	5361 (2063–14078)	1002 (0–12179)	*0.03*	584 (550–4844)	*0.09*	*0.97*
Ferritin, ng/mL	226 (0–1010)	214 (180–2994)	*0.41*	104 (133–472)	*0.46*	*0.04*
Lactate dehydrogenase, U/L	354.5 (0–713.4)	329.5 (244–567)	*0.79*	274 (239–347)	*0.71*	*0.38*
Aspartate aminotransferase, U/L	28 (8.4–101.5)	28 (24.3–71.4)	*0.39*	24 (24.1–34.9)	*0.04*	*0.33*
Alanine aminotransferase, U/L	24 (2.5–68.3)	19 (21.6–84.2)	*0.88*	15 (17.1–30.3)	*0.14*	*0.07*
Troponin T, ng/mL	0.005 (0–0.028)	0.0085 (0–0.13)	*0.99*	0.006 (0.006–0.01)	*0.99*	*0.78*
Serum creatinine, mg/dL	0.44 (0.17–0.7)	0.46 (0.46–2.08)	*0.22*	0.47 (0.44–0.57)	*0.18*	*0.61*
Post-CoV Group						
	Post-CoV (n = 54)			**Control (n = 57)**		
	MISC (n = 10)	**Non-MISC (n = 44)**	**MISC X Non-MISC**		**MISC X Control (*p-value*)**	**Non-MISC X Control (*p-value*)**
			(p-value)			
Hematological parameters						
Leukocyte count/mm3	7480 (6219–9555)	5920 (5946–7795)	*0.32*	6880 (7230–10969)	*0.54*	*0.05*
Neutrophil count/ mm3	2902 (2066–3779)	3006 (2931–4044)	*0.35*	4688 (4737–7702)	*0.04*	*0.003*
Lymphocyte count/mm3	3928 (2913–5469)	2325 (2187–2993)	*0.01*	1845 (1713–2803)	*0.02*	*0.33*
Thrombocyte count/mm3 x1000	348 (306–397)	257 (225–288)	*0.02*	265 (224–292)	*0.02*	*0.97*
Biochemical and Inflammatory markers						
C-reactive protein, mg/L	0.5 (0–3.9)	0.77 (1.8–6.2)	*0.34*	4.8 (10.7–33.4)	*0.15*	*0.02*
Fibrinogen, mg/dL	297.5 (245.1–400)	323.5 (299.7–356.6)	*0.88*	258 (226.5–353)	*0.25*	*0.07*
D-dimer, ng/mL	414.5 (0–41267)	374.5 (0–1973)	*0.25*	584 (550–4844)	*0.20*	*0.21*
Ferritin, ng/mL	35.5 (17.9–83.7)	64 (84–375)	*0.24*	104 (133–472)	*0.07*	*0.52*
Lactate dehydrogenase, U/L	253.5 (233–296)	241 (223–263)	*0.28*	274 (239–347)	*0.52*	*0.07*
Aspartate aminotransferase, U/L	26 (22.7–29.9)	23 (17–56.4)	*0.64*	24 (24.1–34.9)	*0.60*	*0.51*
Alanine aminotransferase, U/L	12.5 (11–18.4)	15 (13.2–58.6)	*0.33*	15 (17.1–30.3)	*0.31*	*0.34*
Troponin T, ng/mL	0.004 (0.003–0.005)	0.005 (0–0.05)	*0.99*	0.006 (0.006–0.01)	*0.99*	*0.57*
Serum creatinine, mg/dL	0.45 (0.34–0.49)	1.48 (0.3–1.7)	*0.08*	0.47 (0.44–0.57)	*0.08*	*0.62*

Significant *p*-values are in bold. n: numbers; Med (IC 95 %): Median (lower – upper 95 % confidence interval).
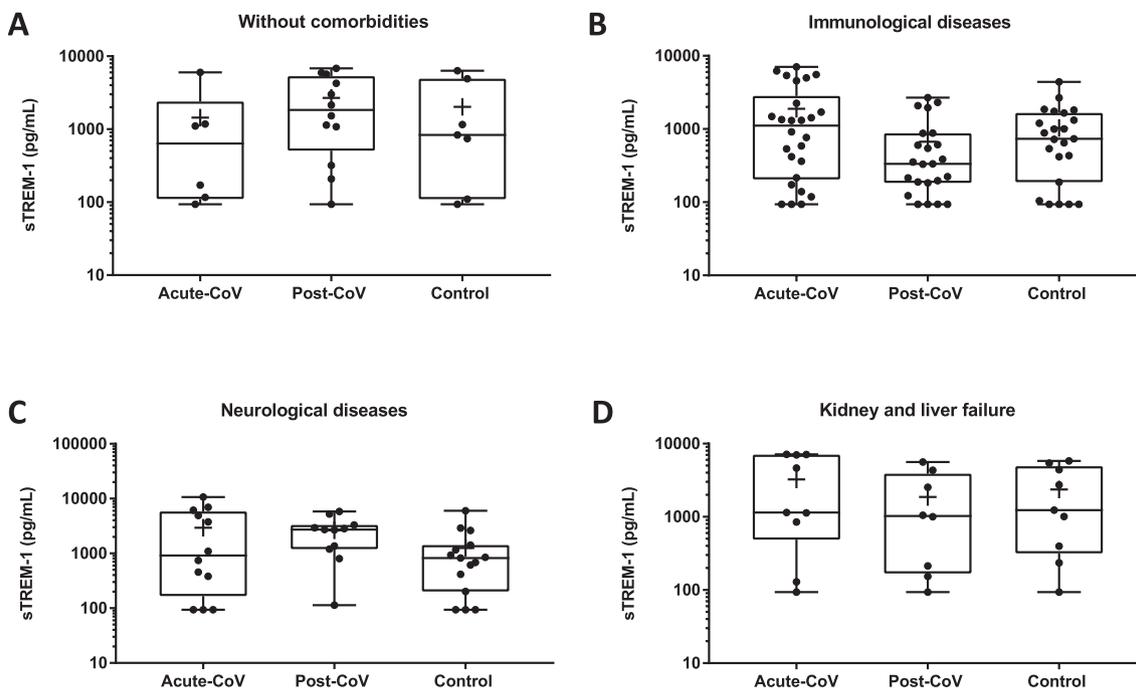
Fig. 1. sTREM-1 concentrations in serum samples from patients with acute COVID-19 infection (Acute-CoV), COVID-19 convalescent patients (Post-CoV) and control patients (control), separated by comorbidities: individuals without comorbidities (**3A**); with immunological diseases (**3B**); with neurological disorders (**3C**); and with kidney and liver failure (**3D**). A comparison of the data is presented as box and whisker plots with individual data points. The box represents the 25th–75th percentiles, and the median is represented by the line within the box. The whiskers represent the 5th–95th percentiles.
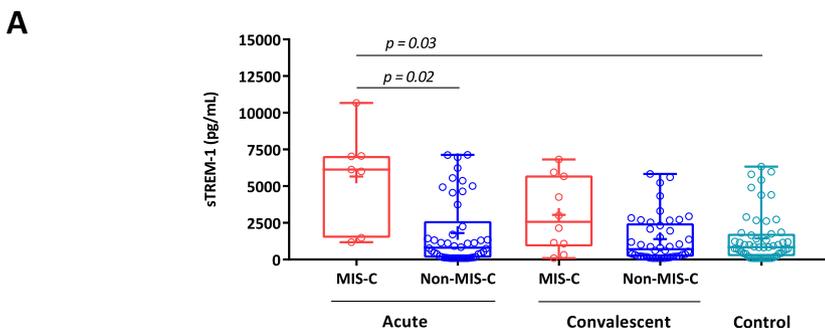
Fig. 2. **(A)** Serum concentrations of sTREM-1 in patients with MIS-C vs non-MIS-C within the acute COVID-19 group, COVID-19 convalescent group and controls. A comparison of the data is presented as box and whisker plots with individual data points. The box represents the 25th–75th percentiles, and the median is represented by the line within the box. The whiskers represent the 5th–95th percentiles. The significant adj p-values are demonstrated on the graphic. **(B)** Association between serum concentrations of sTREM-1 and MIS-C development among acute COVID-19 patients, COVID-19 convalescent group, and controls, showing the Adjusted Odds Ratio (AOR) values, 95 % Confidence Intervals (95 % CI) and adjusted p values (Adj p-value).
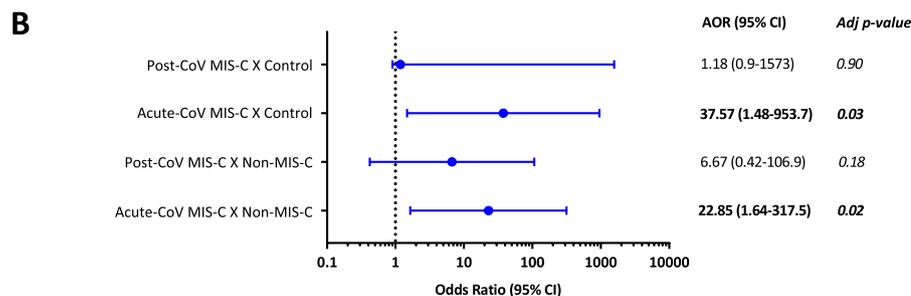
this group was compared to milder convalescent COVID-19 cases or controls (Fig. 2).

To investigate the efficiency of sTREM-1 as a severity biomarker, receiver operating curve analysis (ROC) was performed for MIS-C within the Acute-CoV group, and the area under the ROC curve (AUC) was determined. The AUC was 0.870, and the sTREM-1 cutoff value of > 5781 pg/mL revealed a sensitivity of 71.4 % (29–96.3 %), specificity of

91.3 % (79.2–97.6), PPV of 55.5 %, and NPV of 95.4 % for differentiating MIS-C from non-MIS-C acute patients. The ROC curve analysis for differentiating acute MIS-C patients from the control group was also performed and revealed an AUC of 0.904, and a sTREM-1 cutoff value of > 5992.0 pg/mL yielded a sensitivity of 71.4 % (29–96.3 %) and specificity of 98.2 % (90.1–100) (Fig. 3).

The univariate logistic regression analysis of the Acute-CoV group
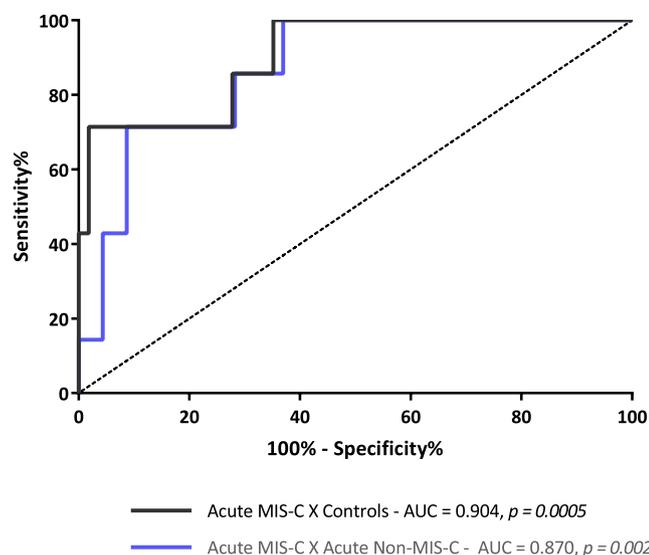
Fig. 3. sTREM-1 ROC curves from MIS-C vs non-MIS-C acute patients (blue) and from acute MIS-C vs controls (black) to predict COVID-19 disease severity in children and adolescents. The areas under the curve (AUCs) and the p values for each comparison were designated under the graphic. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

revealed that D-dimer levels and leukocyte numbers were the only variables significantly associated with MIS-C development and were included in the multiple logistic regression model as covariates. The adjusted multiple logistic regression analysis confirmed that high sTREM-1 concentrations (cut off > 5781 pg/mL) had a significant association with MIS-C development (OR = 22.85 [95 % CI 1.64–317.5], *p = 0.02*). All the other demographic and laboratory parameters, including sex, age and comorbidities did not achieve any association with MIS-C in the univariate logistic regression analysis and were not included in the model.

In the comparison of MIS-C acute patients vs controls, CRP and aspartate aminotransferase were associated with MIS-C development and were included in the multiple logistic regression model as covariates. The result confirmed the sTREM-1 association with MIS-C development (OR = 37.57 [95 % CI 1.48–953.71], *p = 0.03*) (Fig. 2).

3.3. Determination of cytokine concentrations

Given the pathophysiological role of cytokines as mediators of inflammation, the measurement of cytokine levels has been used as an early marker for the diagnosis of COVID-19. The concentrations of IL-1β, IL-8, IL-6, IL-10, IL-12, and TNF-α are shown in Table 3. Acute-CoV

patients did not show differences in cytokine levels compared to Controls (data not shown). However, Post-CoV patients revealed statistically lower IL-1β, IL-6, IL-8, and IL-10 levels than both the Acute-CoV and Control groups.

Additionally, Spearman correlation analysis generated positive indexes between sTREM-1 and IL-12 concentrations ($r = 0.47$; *p < 0.001*) and between sTREM-1 and TNF-α ($r = 0.50$; *p < 0.001*) within the Acute-CoV group. The Post-CoV and Control groups did not show significant correlation indexes among sTREM-1 and all cytokine levels.

3.3.1. MIS-C cytokine analysis

Cytokine levels were also analyzed in MIS-C vs non-MIS-C patients within the acute and convalescent groups (Fig. 4). Unlike what was observed for sTREM-1, the cytokines IL-6, IL-8, and IL-10 in the acute phase were similar to that observed in the control group, regardless of whether the patient developed MIS-C, but those levels were lower in the convalescent phase compared both with the Acute-Cov and control groups. ROC curves performed with IL-6, IL-8, and IL-10 concentrations in MIS-C vs non-MIS-C patients within the Acute-CoV group did not reveal significant results.

3.4. Matched acute and convalescent patients

In our sample, we had 14 patients whose samples were collected in the acute and convalescent phases. None of these patients developed MIS-C in the acute phase. The analysis revealed what we had already observed for the total series of acute and convalescent patients. The sTREM-1 levels were equivalent between the acute and convalescent phases, and the IL-1β, IL-6, IL-8, and IL-10 levels were significantly increased during the acute phase (Fig. 5).

4. Discussion

In this study, we reported the association of circulating sTREM-1 concentrations and MIS-C development. To our knowledge, this is the first study that highlights the importance of sTREM-1 levels as a predictor of MIS-C in children and adolescents.

Our study was designed in three cohorts matched by sex, age, and similar comorbidities. The majority of patients were diagnosed with mild COVID-19, which has also been reported by others [10,30,31]; nevertheless, the signs and symptoms of COVID-19 infection in children can be highly heterogeneous [32–34].

It is of particular interest that among our acute and convalescent patients, the majority (83.2 %) had some type of preexisting comorbidity, in contrast with healthy patients, accounting only for 16.8 % of our cases, certainly because of our tertiary referral hospital profile. To compose each group, we were concerned about choosing patients with similar comorbidities. The most prevalent ones observed in all three groups were preexisting immunological alterations, and of note, the

Table 3
IL-1β, IL-8, IL-6, IL-10, IL-12, and TNF-α concentrations (pg/mL) in serum samples from patients with acute COVID-19 infection (Acute-CoV), COVID-19 convalescent patients (Post-CoV) and control subjects (Control).

Cytokines					
	Acute-CoV (n = 53)	Post-CoV (n = 54)	Acute X Post-CoV *(p-value)*	Control (n = 54)	Post-CoV X Control *(p-value)*
IL-1β	0.4 (0.2–1.0)	0.2 (0.07–0.6)	***0.04***	0.7 (0.3–1.6)	***0.0004***
IL-6	8.7 (3.8–24.4)	1.5 (0.8–2.6)	***<0.0001***	7.2 (1.7–61.8)	***<0.0001***
IL-8	25.6 (15.3–83.3)	6.5 (3.6–10.0)	***<0.0001***	39.8 (11.1–110.4)	***<0.0001***
IL-10	2.1 (1.2–3.6)	0.5 (0.3–1.0)	***<0.0001***	1.3 (0.6–4.5)	***<0.0001***
IL-12	0.5 (0.02–1.6)	0.1 (0–0.7)	*0.08*	0.4 (0.1–0.9)	*0.16*
TNF-α	0.3 (0.02–1.4)	0.2 (0–0.8)	*0.35*	0.3 (0.1–0.9)	*0.60*

Values expressed in median (interquartile range). No differences were detected between Acute-CoV and Control groups. Significant p-values are in bold.

Fig. 4. Serum concentrations of IL-1β, IL-8, IL-6, IL-10, IL-12, and TNF-α in samples from patients with MIS-C vs non-MIS-C within the acute COVID-19 group and in the COVID-19 convalescent group. A comparison of the data is presented as box and whisker plots with individual data points. The box represents the 25th–75th percentiles, and the median is represented by the line within the box. The whiskers represent the 5th–95th percentiles. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

most severe patients in our study were those with immunological diseases, followed by patients with neurological diseases. This finding indicates that these chronic diseases are a potential risk factor for COVID-19 infection in pediatric patients. In agreement, a systematic review and *meta*-analysis of 42 studies composed of children with COVID-19, with or without preexisting comorbidities, showed that 5.1 % of children with comorbidities had severe COVID-19, in contrast with 0.21 % of severe patients without comorbidities [34]. Those authors concluded that children with comorbidities have a greater predisposition for mild or severe COVID-19 than healthy children, which was also reported by other studies [10,31]. In light of the foregoing, our results indicate that children with preexisting chronic conditions are a vulnerable population for developing mild to severe COVID-19 infection.

In contrast, we noticed in our study that all MIS-C cases in the convalescent group occurred in healthy children, which we did not observe in the acute group. Other studies have already shown that healthy children comprise 60–70 % of MIS-C cases, and children with underlying conditions comprise 25–75 % of COVID-19 patients, whether mild or severe [32,35,36]. This was probably due to the characteristics of our tertiary hospital, in which the acute MIS-C cases were composed of children with underlying conditions who were already being followed-up in our hospital. However, in contrast, healthy children from the convalescent group who developed MIS-C in the acute phase were treated at the local hospital close to their homes.

An accurate triage tool is critical for the early and rapid distinction of patients with mild COVID-19 from those with severe/critical COVID-19 or even MIS-C, which would certainly aid in clinical decision making. sTREM-1 has been used as a prognostic marker in septic shock and other inflammatory states, but few studies have associated this soluble marker with COVID-19 patients [23,25], and currently, there is no study regarding this association in children and adolescents. In sepsis, sTREM-1 levels are increased due to inflammation resulting from the cytokine storm in the host organism, which is more pronounced according to the severity of sepsis [37,38]. Based on this information and the similarity between sepsis and severe COVID-19 concerning the inflammatory state, we decided to investigate sTREM-1 levels in serum from COVID-19 pediatric patients and whether those levels were still altered in the convalescent phase, with samples collected three to six months after diagnosis.

In our work, no statistically significant differences were found in sTREM-1 concentrations between the acute and convalescent groups or when those groups were compared to controls. It would be expected that patients using immunosuppressive or anti-inflammatory medications as a routine pharmacological treatment would have lower sTREM-1 levels, but this was not observed in our study. Nevertheless, we did observe a slight trend of higher sTREM-1 levels between the comorbidity subgroups of the three matched groups: (1) in immunological alterations, higher sTREM-1 concentrations in the Acute-CoV group; (2) in
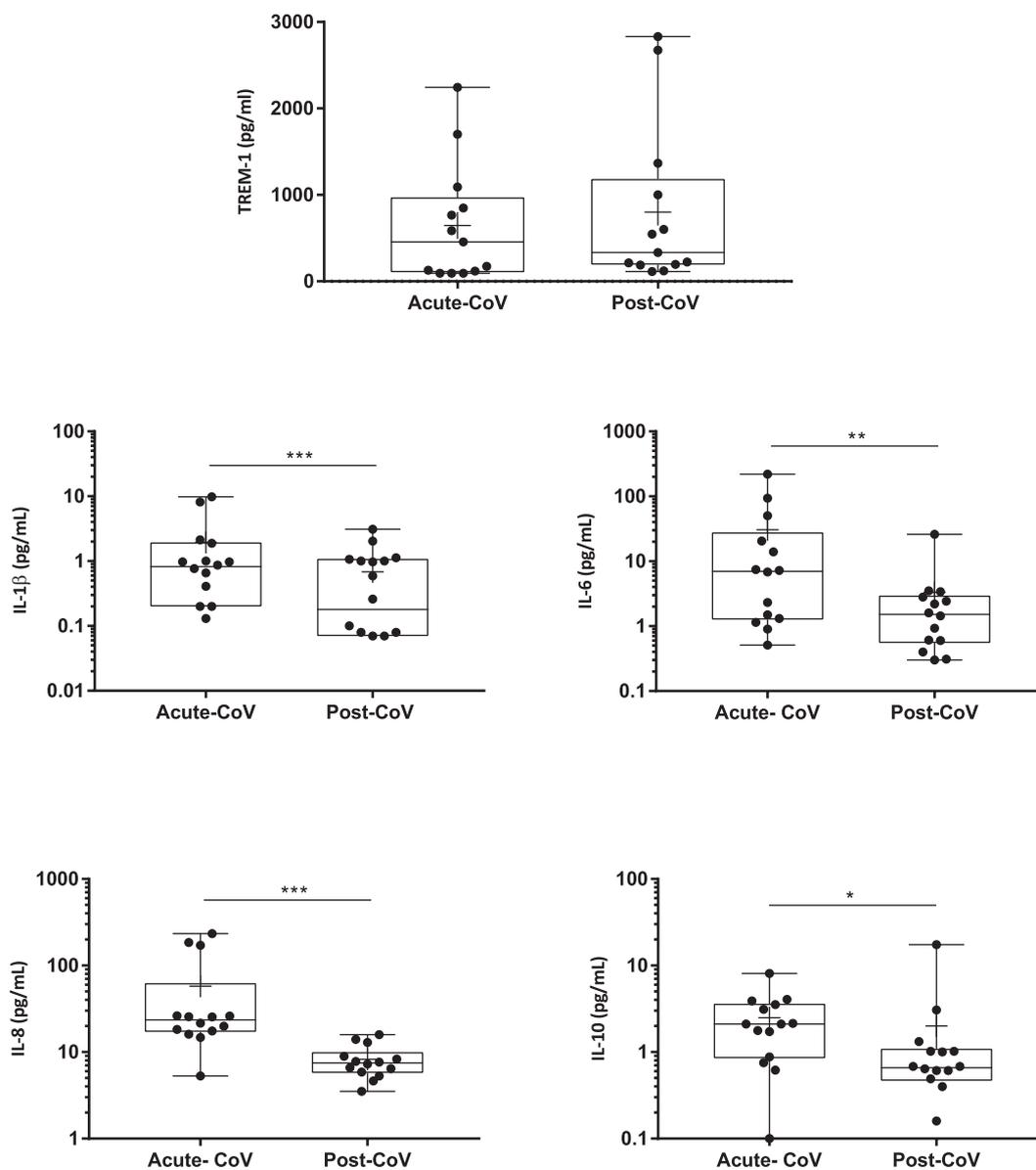
Fig. 5. Serum concentrations of sTREM-1, IL-1β, IL-8, IL-6, and IL-10 in patients whose samples were collected in the acute (Acute-CoV) and convalescent (Post-CoV) phases. A comparison of the data is presented as box and whisker plots with individual data points. The box represents the 25th–75th percentiles, and the median is represented by the line within the box. The whiskers represent the 5th–95th percentiles (data compared by Wilcoxon matched-pairs signed rank U test; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

neurological diseases and patients without comorbidities, sTREM-1 concentrations were slightly higher in the Post-CoV group. It is important to emphasize that all the cases that progressed to MIS-C during the acute phase of the convalescent group were healthy children; therefore, this trend toward higher levels of sTREM-1 in this group is probably due to a delay in the decay of sTREM-1 levels. The lack of significant differences among our study groups is probably impacted by the inflammatory profile of our cohort due to the preexisting comorbidities in the majority of patients, including those in the Control group, leading to a lack of power to show significant differences, which requires further validation studies.

It is also important to draw attention to the way in which some comorbidities can induce elevated sTREM-1 levels, such as chronic kidney disease, liver failure, and autoimmune hepatitis; further, sTREM-1 is also correlated with disease activity in SLE and rheumatoid arthritis, which are diseases presented by children included in our series [19,39–43]. This information explains why we did not observe significant differences in the comparison of sTREM-1 concentrations among

the milder COVID cases and control patients.

In contrast, it is noteworthy that sTREM-1 levels in MIS-C patients in the acute phase were markedly higher than those of milder COVID-19 cases, and those levels were still moderately higher in MIS-C patients during the convalescent phase, as discussed above. These results indicate that sTREM-1 is persistently increased during the disease course in MIS-C patients and could be used as a predictor biomarker of the severity of COVID-19 pediatric infection. Similarly, de Nooijer et al. (2021) [24], in an observational study of two adult cohorts (healthy and COVID-19 patients), demonstrated that increased sTREM-1 plasma concentrations are correlated with worsening of the clinical outcomes for patients with COVID-19, mainly to mortality, suggesting that sTREM-1 plays an important role in the disease course. In agreement, an analysis based on predictive algorithms such as respiratory rate and oxygen saturation (intubation necessity) demonstrated that circulating sTREM-1 and IL-6 concentrations in COVID-19 patients have higher specificity and good predictive accuracy for severity and mortality, with the increase in sTREM-1 levels having the best prognostic accuracy for

intubation necessity and for death [25]. In our study, we demonstrated that sTREM-1 is a good biomarker for MIS-C in the acute phase, which elevated the risk of MIS-C development in 22.85-fold in our patients. Regarding sTREM-1 as prognostic biomarker, no association was observed because none of the patients progressed to death. In fact, UNICEF [44] states that until March 2022, the mortality rate in children and adolescents due to COVID-19 infection was 0.4 % of all deaths (over 13,400 deaths).

Silva-Neto et al. (2021) [23] revealed that sTREM-1 was predictive of in-hospital disease severity in adults using a cutoff value of 116.5 pg/mL, but unlike our study, their COVID-negative control patients were healthy, which explains the great difference in the cutoff values between this study and ours. Both our ROC curves, calculated with MIS-C cases compared with non-MIS-C patients in the acute group or with control patients, revealed lower AUC values than those described by these authors, probably due to the high sTREM-1 concentrations observed in our subjects, including controls.

In our study, IL-1, IL-6, IL-8 and IL-10 concentrations were not increased in patients with acute COVID-19 compared to controls. In contrast, Curatola et al. (2021) [45] observed higher TNF-α and IL-6 levels in children with COVID-19 than in patients without COVID or any infection, but those cytokine levels did not correlate with disease severity. These different results, once again, are probably due to the inflammatory state of our control patients.

In agreement with previous reports, we did not detect altered IL-12 and TNF-α concentrations in our COVID-19 or MIS-C patients [46,47], but we did observe significant correlations between sTREM-1 and IL-12 levels and TNF-α, which have been directly associated with the systemic inflammatory response. Grangeiro de Carvalho et al. (2011) [48] have shown that activation of the Th1-type response with the release of IL-12, IL-18 and TNF-α induces TREM-1 signaling. It seems that the TREM-1 signaling pathway together with inflammatory cytokines form a cycle that may represent a positive feedback loop for perpetuating the inflammatory response. Indeed, it has been demonstrated that cytokines related to inflammasome activation and the IFN-γ pathway are significantly higher in MIS-C patients than in severe or critical COVID-19 patients and healthy controls, whereas CXCL10 and IL-10 were higher in both MIS-C patients and severe/critical COVID-19 patients than in healthy controls [47].

Interestingly, we observed that convalescent patients presented lower levels of IL-1β, IL-6, IL-8 and IL-10, which was also observed in the analysis of patients whose matched samples were collected in the acute and convalescent phases. In contrast, in adults, increased levels of inflammatory cytokines were reported, with a slower decay of IL-6, suggesting a more inflammatory profile in COVID-19 convalescents [49]. Our results suggest that after the acute episode of COVID-19, the immune response may suffer some type of exhaustion/immunosuppression that leads to a decrease in cytokine secretion. Apparently, after episodes of intense activation of the immune response due to chronic antigenic exposure, transient immunosuppression occurs. This fact is evidenced in adult and neonatal sepsis and apparently occurs in the most severe cases of COVID-19 [50–52].

However, unlike cytokines, we observed a longer decay of sTREM-1 levels in patients with MIS-C. It is not yet known whether this could be related to some post-acute sequelae of COVID-19 or post-COVID condition, terms used to define long-term COVID, which has also been observed in the pediatric population [53,54]. A longer follow-up of these patients could tell us how long it might take for the sTREM-1 levels to return to baseline. All of these findings lead us to reflect on whether these children could present abnormalities in the immune response after infection by SARS-CoV-2 and point us to the importance of studying this issue more deeply.

5. Conclusion

Our findings revealed that sTREM-1 in pediatric patients has good predictive accuracy and can be useful as an early screening tool for surveillance of MIS-C cases, even in patients with chronic underlying conditions, and could help in the management of these cases earlier to avoid a more severe disease course.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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