Article published online: 2022-10-04

Serum Vitamin D Levels in Relation to Development of Multisystem Inflammatory Syndrome in Pediatric COVID-19

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J Pediatr Infect Dis 2022;17:308–316.

Abstract

Objective The aim of the study is to evaluate vitamin D (vit D) levels in children with and without development of multisystem inflammatory syndrome in children (MIS-C) after coronavirus disease 2019 (COVID-19) and also between those with severe and moderate MIS-C.

Methods This comprises retrospective data of 68 patients including 34 patients with MIS-C and admitted into the pediatric intensive care unit (MIS-C group) and 34 patients without MIS-C (non-MIS-C group) were analyzed for their presenting characteristics, serum vit D levels, ventilatory needs, and prognostic scores.

Results Vit D levels were significantly lower in patients with versus without MIS-C [9 (2–18) vs. 19 (10–43) ng/mL, $p < 0.001$], and also in patients with severe versus moderate MIS-C [7.5 (2–17) vs. 9 (5–18) ng/mL, $p = 0.024$]. Vit D deficiency (levels <12 ng/mL) was more common in the MIS-C versus non-MIS-C group (79.4 vs. 11.8%, $p < 0.001$) and in severe versus moderate MIS-C (92.9 vs. 70.0%, $p < 0.001$). The severe versus moderate MIS-C was associated with significantly higher levels of procalcitonin [7.6 (0.9–82) vs. 1.7 (0.2–42) ng/mL, $p = 0.030$] and troponin [211 (4.8–4,545) vs. 14.2 (2.4–3,065) ng/L, $p = 0.008$] and higher likelihood of reduced ejection fraction (75.0 vs. 15.4%, $p = 0.004$).

Conclusion Our findings indicate the higher prevalence of vit D deficiency in pediatric COVID-19 patients with versus without MIS-C, as well as in those with severe versus moderate MIS-C. Higher troponin and procalcitonin levels and dyspnea at presentation seem also to be risk factors for severe MIS-C, more pronounced cardiac dysfunction, and poorer prognosis.

Keywords
- multisystem inflammatory syndrome
- pediatric COVID-19
- vitamin D
- cardiac dysfunction
- procalcitonin
- prognostic scores

received
May 19, 2022
accepted after revision
August 8, 2022
published online
October 4, 2022

DOI https://doi.org/10.1055/s-0042-1756713.
ISSN 1305-7707.

Introduction

The initial reports during the early phase of coronavirus disease 2019 (COVID-19) pandemic indicated a mildly symptomatic course of COVID-19 in children, and severe illness only in 2 to 6% of them.[1–3] However, starting from mid-April 2020, clusters of pediatric cases epidemiologically linked with COVID-19 have been reported as presenting with fever, hypotension, severe abdominal pain, and cardiac dysfunction.[4,5] Centers for Disease Control and Prevention (CDC) and the World Health Organization named this new hyper-inflammatory syndrome that emerged in older school-aged children and adolescents as SARS-Cov-2-associated multisystem inflammatory syndrome in children (MIS-C).[4,6–8]

The observational studies in adult COVID-19 patients suggested a link between reduced levels of circulating form of vitamin D [25-hydroxyvitamin D; 25(OH)D] and COVID-19 illness severity and mortality.[9,10] Children with vitamin D (vit D) deficiency or insufficiency are considered more susceptible to respiratory infections,[11] while pediatric COVID-19 patients were also reported to have significantly lower vit D levels than healthy controls.[12]

The proposed risk factors for increased susceptibility to MIS-C, such as certain comorbidities (i.e., obesity and asthma) and ethnic origin are also known to be independently associated with vit D deficiency.[13] Hence, vit D deficiency has been suggested to be associated with an increased incidence of MIS-C in these children.[10]

Vit D has also been reported to inhibit several cytokines that are used to monitor MIS-C patients undergoing treatment.[10,14] Accordingly, the influence of vit D in MIS-C is proposed to be mediated through its well-known role in the modulation of adaptive and innate immunity, including the regulation of inflammatory cytokine release.[10,14] However, despite the more extensive investigation regarding the role of vit D in adult COVID-19 patients, only a few studies to date have addressed the relationship between vit D and MIS-C in pediatric COVID-19.[10,15]

Identification of the children who are at risk to develop MIS-C after an asymptomatic/mild COVID-19 and development of prognostic biomarkers to identify those at risk for severe MIS-C are important for better management and an improved outcome of MIS-C patients.[10]

This study aimed to evaluate vit D levels in pediatric patients with versus without development of MIS-C after COVID-19 and determine the clinical and laboratory correlates of more severe disease courses in those with MIS-C.

Methods

A total of 34 pediatric patients were admitted to pediatric intensive care unit (PICU) due to post-COVID-19 development of MIS-C (MIS-C group; $n = 34$) within a 12-month study period and 34 pediatric control subjects with the previous history of non-hospitalized mild symptomatic COVID-19 and no progression to MIS-C within the first 6-months (Non-MIS-C group; $n = 34$) were included in this retrospective study. The COVID-19 diagnosis was based on virological (viral genome-RNA detection by polymerase chain reaction [PCR]) and serological (IgM/IgG antibodies against the virus) tests. The MIS-C diagnosis was based on the clinical presentation including fever $\geq$3 days plus presence of two of the five criteria: (1) rash or bilateral non-purulent conjunctivitis or mucocutaneous inflammation signs, (2) hypotension or shock, (3) cardiac dysfunction on echocardiography or elevated troponin/ N-terminal (NT)-pro hormone BNP (NT-proBNP), (4) evidence of coagulopathy, and (5) acute gastrointestinal problems, as well as elevated markers of inflammation, evidence of infection, or contact with patients who have COVID-19, and exclusion of other obvious microbial causes of inflammation.[7,16]

The study was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the institutional ethics committee along with permission for the use of patient data for publication purposes (Date of Approval: November 08, 2021, Reference number/Protocol No: 20.478.486/889).

Data on patient demographics, body mass index (kg/m^2), and serum vit D levels (at the time of initial COVID-19 diagnosis in the non-MIS-C group and at the time of PICU admission in the MIS-C group) were recorded in all patients. Data on presenting characteristics, laboratory findings, echocardiography findings, need for invasive mechanical ventilator (IMV) and non-invasive mechanical ventilator (NIMV) support, length of PICU stay, hypotension and related vasoactive inotropic scores (VIS), and the prognostic scores including Pediatric Risk of Mortality 2 (PRISM 2), Pediatric Index of Mortality 2 (PIM 2), and Pediatric Logistic Organ Dysfunction (PELOD) were recorded only in patients with moderate and severe MIS-C. Patients with hemodynamic instability and those who need NIV/IMV were considered to have severe MIS-C. Patients with predicted clinical worsening and requiring PICU admission were considered to have moderate MIS-C.

Serum levels of 25 OH vit D were measured using a DXI800 instrument (Beckman Coulter, Brea, California, United States) via an immuno-inhibition assay, and classified as vit D deficiency ($<$12 ng/mL), vit D insufficiency (12–20 ng/mL), and normal Vit D ($>$20 ng/mL) levels.[17] Echocardiography was performed on the first day of hospital admission in patients with inotropic support indication, and on the second day of hospitalization in other patients.

Statistical analysis was made using MedCalc Statistical Software version 19.7.2 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021). Chi-square test, Yates continuity correction, and Fisher exact test were used for analysis of categorical data, while Mann-Whitney U test and Student's *t*-test were used for analysis of numerical variables. Data were expressed as mean (SD, standard deviation), median (min–max), and percent (%) where appropriate. $p < 0.05$ was considered statistically significant.

Results

Overall, the mean (SD; IQR) age of patients was 98.6 (55.5; 75.0) months in the MIS-C group and 103.6 (71.2; 118.0) months in the non-MIS-C group; and the girls composed of 52.9 and 47.1% of patients in the MIS-C and non-MIS-C groups, respectively. No significant difference was noted between MIS-C and non-MIS-C groups in terms of age and gender (►**Table 1**).

Patients with severe MIS-C were significantly older than those with moderate MIS-C [median (min–max) 114 (60–204) vs. 78 (11–180) months, $p = 0.022$], while no significant gender differences were noted between patients with severe and moderate MIS-C (►**Table 1**).

All patients with MIS-C were RT-PCR negative for SARS-CoV-2 virus, but were antibody positive, indicating past infection, while posteroanterior chest X-ray findings suggestive of pulmonary congestion were evident in three (8.8%) of 34 patients.

Overall comorbidity was evident in six (17.6%) of 34 patients with MIS-C (five were in severe group) including obesity in five patients and cerebral palsy in one patient. The previous history of contact with a COVID-19 positive patient was confirmed in 18 patients, not known in 11 patients, and COVID-19 positivity was noted in five patients.

Treatments included intravenous immunoglobulin (IVIG, 2 g/kg for 12 hours IV infusion), IV methylprednisolone (2 mg/kg in 18 patients, 10 mg/kg in 13 patients, and 30 mg/kg in three patients), oral aspirin (median 3 mg/kg, ranged 3–5 mg/kg), and antibiotherapy (with empiric antibiotics suggested by pediatric infection department including vancomycin and ceftriaxone in all patients with severe MIS-C), and low-molecular-weight heparin (only for four patients with severe MIS-C in accordance with suggestions by pediatric cardiology and pediatric hematology specialists), and inotropic agents (adrenalin and milrinone) in patients with hypotension. None of the patients received anakinra, while all patients initiated vit D supplementation at the time of diagnosis in accordance with current pediatric endocrinology guidelines.

Median (min–max) vit D levels were significantly lower in the MIS-C group compared with non-MIS-C group [9 (2–18) vs. 19 (10–43) ng/mL, $p < 0.001$], and also in patients with severe versus moderate MIS-C [7.5 (2–17) vs. 9 (5–18) ng/mL, $p = 0.024$] (►**Table 1**).

More patients with MIS-C had vit D deficiency (levels <12 ng/mL) than those without MIS-C (79.4 vs. 11.8%, $p < 0.001$). Vit D deficiency was also more common in patients with severe versus moderate MIS-C (92.9 vs. 70.0%, $p < 0.001$) (►**Table 1**).

Overall, 41.2% of patients in the non-MIS-C group but none of patients in the MIS-C group had normal vit D levels (>20 ng/mL) (►**Table 1**).

In the MIS-C group, fever (100.0%), weakness (100.0%), and abdominal pain (76.5%) were the most common

Table 1 Baseline characteristics and vitamin D levels in study groups

		MIS-C			Non-MIS-C ($n = 34$) (D)	*p*-Value	
		Total ($n = 34$) (A)	Moderate ($n = 20$) (B)	Severe ($n = 14$) (C)		B vs. C	A vs. D
Age (months)	**Mean (SD, IQR)**	98.6 (55.5,75.0)	79.4 (52.5,84.0)	126 (49.3, 48.0)	103.6 (71.2, 118.0)	**0.022**[a]	0.985[a]
	Median (min–max)	108 (11–204)	78 (11–180)	114 (60–204)	90 (2–241)		
Gender, *n* (%)							
Girl		18 (52.9)	9 (45)	9 (64.3)	16 (47.1)	0.447[b]	0.880[b]
Boy		16 (47.1)	11 (55)	5 (35.7)	18 (52.9)		
BMI (kg/m^2)		18 (13–41,5)	18.5 (13–27)	18 (13.4–41.5)		0.514	
Vitamin D level (ng/mL), median (min–max)		9 (2–18)	9 (5–18)	7.5 (2–17)	19 (10–43)	**0.024**	**<0.001**[a]
Vitamin D status, *n* (%)							
Vitamin D deficiency (<12 ng/mL)		27 (79.4)	14 (70.0)	13 (92.9)	4 (11.8)	**<0.001**[c]	
Vitamin D insufficiency (12–20 ng/mL)		7 (20.6)	6 (30.0)	1 (7.1)	16 (47.1)		
Normal vitamin D (>20 ng/mL)		0 (0.0)	0 (0.0)	0 (0.0)	14 (41.2)		

Abbreviations: IQR, interquartile range; MIS-C, multisystem inflammatory syndrome in children.
Note: Values in bold indicate statistical significance ($p < 0.05$).
[a]Mann Whitney U test.
[b]Yates continuity correction.
[c]Chi-square test.

presenting symptoms overall, while those with severe versus moderate MIS-C presented less commonly with rash (14.3 vs. 75.0%, $p = 0.002$) and more commonly with dyspnea (50.0 vs. 10.0%, $p = 0.017$) (►**Table 2**).

Severe versus moderate MIS-C was associated with significantly lower lymphocyte count [705 (190–2030) vs. 955 (540–5200) cells/μL, $p = 0.023$], whereas with significantly higher levels of procalcitonin [7.6 (0.9–82) vs. 1.7 (0.2–42) - ng/mL, $p = 0.030$], BUN [11.1 (7.4–50.5) vs. 9.4 (5.6–14.7) mg/dL, $p = 0.042$], urea [24 (16–108 vs. 20 (12–32) mg/dL, $p = 0.040$], and troponin [211 (4.8–4545) vs. 14.2 (2.4–3065) ng/L, $p = 0.008$] (►**Table 3**).

Echocardiography findings were abnormal in 91.2% of patients with MIS-C, including tricuspid insufficiency (51.6% total, 35.0% in moderate form, and 64.3% in severe form) and mitral insufficiency (77.4, 55.0, and 92.9%, respectively) in most of patients, being more common and more advanced in patients with severe MIS-C (►**Table 4**).

Overall reduced ejection fraction was noted in 32.4% of patients. Ejection fraction was significantly lower [50.8 (7.4) vs. 62.8 (10.8) %, $p = 0.003$] and percentage of patients with reduced ejection fraction was significantly higher (75.0 vs. 15.4%, $p = 0.004$) in patients with severe versus moderate MIS-C (►**Table 4**).

Overall, IMV and NIMV were required in 2.9 and 50.0% of patients with MIS-C, while the need for NIMV was more marked in those with severe versus moderate MIS-C (100.0 vs. 15.0%, $p < 0.001$) (►**Table 4**).

Patients with severe versus moderate MIS-C had significantly longer PICU stay (median 5 vs. 4 days, $p < 0.001$), higher rate of hypotension (92.9 vs. 45.0%, $p = 0.009$), higher PIM2 [8.2 (1–100) vs. 1 (0.8–4.6), $p < 0.001$], PRISM [14.5 (12–30) vs. 11 (8–14), $p < 0.001$], and PELOD [20 (11–32) vs. 10 (10–20), $p < 0.001$] scores and higher VIS [15 (10–55) vs. 5 (5–10), $p < 0.001$] (►**Table 5**).

Discussion

Our findings revealed that serum vit D levels were lower in the MIS-C group versus non-MIS-C group of pediatric COVID-19 patients and in those who were more severely affected by MIS-C. Older patient's age, presenting with dyspnea rather than rash, lower lymphocyte counts, and higher serum levels of procalcitonin and troponin were associated with an increase in the severity of MIS-C. Cardiac dysfunction (advanced tricuspid and mitral insufficiency and reduced ejection fraction) was also more remarkable in patients with severe versus moderate MIS-C along with an increase in the need for NIMV, longer PICU stays, higher rate of hypotension, and higher VIS and prognostic (PIM2, PRISM, and PELOD) scores in these patients.

Cardiovascular dysfunction is considered to be the most frequently described physiological abnormality in patients with MIS-C which includes an increase in cardiac biomarkers (NT-pro-BNP and troponin levels), symptomatic myocarditis (40–80%), left ventricle dysfunction (63.3%), tachycardia (82%), hypotension (61.0%), a depressed ejection fraction (>45.0%), and coronary artery abnormalities (9–24%), while arrhythmia, valvular regurgitation, and conduction abnormalities have also been detected in some cases of MIS-C.[8,16,18,19]

Our findings support that MIS-C has distinct epidemiological and clinical features such as older age and low rates of co-morbidity and respiratory symptoms but a high rate of gastrointestinal symptoms and significant cardiovascular dysfunction commonly resulting in hypotension and echocardiographic abnormalities when compared with features of acute severe COVID-19 infection in children.[16,20] Nonetheless, despite its rarity, MIS-C is considered to be of significant concern due to the severity of the illness and cardiovascular dysfunction which necessitate intensive care (80%) and mechanical ventilation (20%) support.[16,21]

Table 2 Presenting characteristics in patients with moderate vs. severe MIS-C

	MIS-C			
	Total ($n = 34$)	Moderate ($n = 20$)	Severe ($n = 14$)	*p*-Value
Presenting characteristics, *n* (%)				
Fever	34 (100.0)	20 (100.0)	14 (100.0)	–
Weakness	34 (100.0)	20 (100.0)	14 (100.0)	–
Abdominal pain	26 (76.5)	14 (70.0)	12 (85.7)	0.422[a]
Rash	17 (50.0)	15 (75.0)	2 (14.3)	**0.002**[b]
Nausea/vomiting	15 (44.1)	7 (35.0)	8 (57.1)	0.353[b]
Diarrhea	10 (29.4)	4 (20.0)	6 (42.9)	0.252[a]
Dyspnea	9 (26.5)	2 (10.0)	7 (50.0)	**0.017**[a]
Mucocutaneous involvement	11 (32.4)	7 (35.0)	4 (28.6)	1.00[a]
Neurological involvement	6 (17.6)	2 (10.0)	4 (28.6)	0.202[a]
Cardiovascular involvement	31 (91.2)	18 (90.0)	13 (92.9)	1.00[a]

Abbreviation: MIS-C, multisystem inflammatory syndrome in children.
Note: Values in bold indicate statistical significance ($p < 0.05$).
[a]Fisher's Exact test.
[b]Yates continuity correction.

Table 3 Laboratory findings in patients with moderate versus severe MIS-C

	MIS-C			*p*-Value
Median (min–max)	**Total ($n = 34$)**	**Moderate ($n = 20$)**	**Severe ($n = 14$)**	
WBC (x10^9/L)	8,225 (910–21,810)	8,690 (3,780–15,500)	7,605 (910–21,810)	0.916
Lymphocyte count (cells/μL)	815 (190–5,200)	955 (540–5,200)	705 (190–2,030)	**0.023**
Neutrophil count (cells/mm^3)	6,480 (700–19,500)	6,690 (3,050–13,760)	6,280 (700–19,500)	0.529
Hemoglobin (g/dL)	10.8 (6.7–13.7)	10.5 (6.7–13.7)	11 (9.13)	0.220
Platelet (cells/mm^3)	163,500 (49,000–491,000)	184,500 (58,000–491,000)	140,500 (49,000–366,000)	0.208
CRP (mg/L)	18.5 (2.9–62.3)	16.7 (6.5–31.2)	20.2 (2.9–62.3)	0.649
ESR (mm/h)	40 (9–104)	40 (21–94)	39.5 (9–104)	0.676
PCT (ng/mL)	3.4 (0.2–82)	1.7 (0.2–42)	7.6 (0.9–82)	**0.030**
Fibrinogen (mg/dL)	616 (184–914)	585.5 (258–749)	616 (184–914)	0.861
D-dimer (ng/mL)	798 (154–3,872)	778.5 (201–3,496)	959.5 (154–3,872)	0.363
Ferritin (mg/L)	276 (52.6–1,500)	276 (52.6–630)	321.5 (101–1,500)	0.558
Na (mmol/L)	132 (125–142)	132 (125–136)	131.5 (125–142)	0.619
Albumin (g/dL)	2.8 (2.2–4)	3 (2.2–3.8)	2.8 (2.2–4)	0.352
BUN (mg/dL)	9.7 (5.6–50.5)	9.4 (5.6–14.7)	11.1 (7.4–50.5)	**0.042**
Urea (mg/dL)	21 (12–108)	20 (12–32)	24 (16–108)	**0.040**
Creatinine (mg/dL)	0.4 (0.2–3.3)	0.4 (0.2–1.2)	0.5 (0.2–3.3)	0.172
AST (U/L)	42.5 (14–168)	39.5 (14–138)	52.5 (15–168)	0.624
ALT (U/L)	32.5 (7.139)	31 (11–131)	34 (7–139)	0.624
Troponin (ng/L)	44.6 (2.4–4,545)	14.2 (2.4–3,065)	211 (4.8–4545)	**0.008**
Lactate	1.6 (1–4)	1.6 (1–2.8)	2 (1.3–4)	0.077
TSH (mIU/L)	1.2 (0.3–5.6)	1.1 (0.3–5.6)	30.6 (20–52)	0.919
FT3 (pg/mL)	2.4 (1.6–4.3)	2.7 (1.7–4.3)	2.3 (1.6–2.7)	0.214
FT4 (μg/dL)	1.1 (0.6–2)	1.1 (0.7–2)	1 (0.6–1.7)	0.984

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; MIS-C, multisystem inflammatory syndrome in children; PCT, procalcitonin; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone; WBC, white blood cell.
Note: Mann Whitney U test; Values in bold indicate statistical significance ($p < 0.05$).

Notably, the degree of elevation in cardiac (troponin, NT pro-B-type natriuretic peptide) and biochemical parameters (C-reactive protein, serum ferritin, procalcitonin, interleukin-6 level, and D-dimers) was reported to be correlated with the need for intensive care support in patients with MIS-C.[19] Similarly, our findings revealed a more advanced cardiac dysfunction (higher degree tricuspid and mitral insufficiency and reduced ejection fraction), higher troponin levels, increased need for NIMV, a longer ICU stay, higher rate of hypotension, and higher VIS and prognostic (PIM2, PRISM, and PELOD) scores in patients with severe versus moderate MIS-C.

In addition, supporting the association of older age and a more advanced cardiovascular dysfunction with increased severity of MIS-C in the present study, the severe disease requiring intensive care due to myocarditis was reported in approximately 50% of patients with MIS-C and the risk was considered to be higher in older children.[22] Also, the higher likelihood of reduced ejection fraction in patients with severe versus moderate MIS-C in the current study (75.0 vs. 15.4%) supports the findings from a past study in MIS-C patients that indicated a higher rate of abnormal left ventricular ejection fraction in PICU versus non-PICU group (100 vs. 50%) of MIS-C patients.[15]

Lymphopenia and increased procalcitonin and troponin levels were associated with an increase in the severity of MIS-C in the current study. Likewise, neutrophilia and lymphopenia were reported among the frequent findings in MIS-C[16,23] and the degree of separation between the two cell lines is considered likely to be associated with the severity of inflammation.[23] Also, the elevation in inflammatory markers such as procalcitonin and troponin was also considered potential predictors of MIS-C outcomes.[23,24] Notably, very high procalcitonin levels, exceeding the levels in bacterial septic shock, in MIS-C patients are considered to emphasize the level of pronounced systemic inflammation

Table 4 Echocardiography findings and ventilator support in moderate vs. severe MIS-C

		MIS-C			
		Total ($n = 34$)	**Moderate ($n = 20$)**	**Severe ($n = 14$)**	***p*-Value[a]**
Echocardiography findings, *n*(%)					
Overall					
Abnormal		31 (91.2)	18 (90.0)	13 (92.9)	1.00
Normal		3 (8.8)	2 (10.0)	1 (7.1)	
Tricuspid insufficiency		16 (51.6)	7 (35.0)	9 (64.3)	
First degree		8 (50.0)	4 (57.1)	4 (44.4)	–
Second degree		4 (25.0)	3 (42.9)	1 (11.1)	
Third degree		4 (25.0)	0 (0.0)	4 (44.4)	
Mitral insufficiency		24 (77.4)	11 (55.0)	13 (92.9)	
First degree		9 (37.5)	5 (45.5)	4 (30.8)	0.131
Second degree		11 (45.8)	6 (54.5)	5 (38.5)	
Third degree		4 (16.7)	0 (0.0)	4 (30.8)	
Aortic insufficiency		2 (5.9)	1 (5.0)	1 (7.1)	
First degree		2 (100.0)	1 (100.0)	1 (100.0)	–
Coronary involvement					
Yes		7 (23.3)	7 (41.2)	0 (0.0)	**0.010**
No		23 (76.7)	10 (58.8)	13 (100)	
Pericardial effusion					
Yes		5 (16.1)	2 (11.1)	3 (23.1)	0.625
No		26 (83.9)	16 (88.9)	10 (76.9)	
Ejection fraction ($n = 25$)	**%, mean(SD)**	57.0 (11.0)	62.8 (10.8)	50.8 (7.4)	**0.003**[b]
	reduced, *n* (%)	11 (32.4)	2 (15.4)	9 (75.0)	**0.004**
Ventilator support, *n* (%)					
IMV					
Yes		1 (2.9)	0 (0.0)	1 (7.1)	0.412
No		33 (97.1)	20 (100.0)	13 (92.9)	
NIMV					
Yes		17 (50.0)	3 (15.0)	14 (100.0)	**< 0.001**
No		17 (50.0)	17 (85.0)	0 (0.0)	

Abbreviations: IMV, Invasive mechanical ventilation; MIS-C, Multisystem inflammatory syndrome in children; NIMV, Non-invasive mechanical ventilation.
Note: Values in bold indicate statistical significance ($p < 0.05$).
[a]Yates continuity correction.
[b]Student's *t*-test.

seen in MIS-C cases.[23] In a systematic review on the comparison of the characteristics between MIS-C and pediatric confirmed COVID-19 cases, the authors reported a higher level of inflammation to be experienced in MIS-C than in COVID-19.[23]

In the current study, more patients with versus without MIS-C (79.4 vs. 11.8%), as well as more patients with severe versus moderate MIS-C (92.9 vs. 70.0%), had vit D deficiency (levels <12 ng/mL). Overall, 41.2% of patients without MIS-C but none of patients in the MIS-C group had normal vit D levels (>20 ng/mL). Likewise, in a past study that included 18 children with MIS-C, 72% of the overall cohort and 83% of patients admitted to PICU were reported to be vit D deficient, along with a non-significant tendency for lower serum vit D levels in the PICU versus a non-PICU group of MIS-C patients.[15]

In fact, a greater need for active vit D in advanced inflammatory process and thus increased consumption of vit D by cells involved in immunomodulation are considered to result in reduced vit D levels in severe disease.[10] Nonetheless, the higher likelihood of vit D deficiency in patients with versus without MIS-C as well as in those with severe versus moderate MIS-C in the current study seems to emphasize the value of initial measurement of vit D level at the time of

Table 5 PICU stay, hypotension and prognostic scores in moderate vs. severe MIS-C

		MIS-C			
		Total ($n = 34$)	Moderate ($n = 20$)	Severe ($n = 14$)	*p*-Value
Length of PICU stay (day), median (min–max)		4 (2–18)	4 (2–4)	5 (4–18)	< **0.001**
Length of ward stay (day), median (min–max)		5 (4–16)	5 (4–9)	6 (4–16)	0.108
Hypotension, *n* (%)	Yes	22 (64.7)	9 (45.0)	13 (92.9)	**0.009**
	No	12 (35.3)	11 (55)	1 (7.1)	
PIM2, median (min–max)		1.7 (0.8–100)	1 (0.8–4.6)	8.2 (1–100)	< **0.001**
PRISM, median (min–max)		12 (8–30)	11 (8–14)	14.5 (12–30)	< **0.001**
PELOD, median (min–max)		13 (10–32)	10 (10–20)	20 (11–32)	< **0.001**
VIS, median (min–max)		12.5 (5–55)	5 (5–10)	15 (10–55)	< **0.001**

Abbreviations: MIS-C, multisystem inflammatory syndrome in children; PELOD, pediatric logistic organ dysfunction; PICU, pediatric intensive care unit; PIM 2, pediatric index of mortality 2; PRISM 2, pediatric risk of mortality 2; VIS, vasoactive inotropic score.
Note: Mann Whitney U test. Values in bold indicate statistical significance ($p < 0.05$).

diagnosis and then serial measurements during the course of MIS-C to monitor the disease progression as well as for correction of low levels.[10,25]

In fact, the levels of several cytokines proposed as useful markers to monitor treatment response in MIS-C patients have also been reported to be decreased by vit D.[14] Moreover, pediatric COVID-19 patients with deficient/insufficient versus normal vit D levels were reported to have significantly higher fever symptom (34.5 vs. 0.0%), as suggested to be secondary to vit D-mediated reduction in interleukin 6 and interferon-gamma inflammatory reactions, both of which are also potent predictors of worse clinical outcome in severe COVID-19.[12,26] Notably, a meta-analysis study indicated a higher risk (adjusted OR: 1.77, non-adjusted OR: 1.75) and higher severity (adjusted OR: 2.57, non-adjusted OR: 10.61) of SARS-CoV-2 infection in the vit D deficient group.[27]

Indeed, growing evidence on the role of amplified inflammatory responses to SARS-CoV-2 in the development of MIS-C and the regulatory actions of vit D on pro-inflammatory cytokine signaling is considered to potentiate the possible role of vit D in MIS-C.[15] Moreover, low vit D levels were reported to be associated with an increased likelihood of developing Kawasaki disease (KD) as well as the risk of coronary outcomes in KD, which seems notable given that MIS-C shares considerable overlap with KD.[28,29]

Vit D is considered to have important immunomodulatory and anti-inflammatory effects such as reduction in the plasma concentrations of pro-inflammatory cytokines produced as part of the cytokine storm in viral infections such as COVID-19, increase in concentrations of anti-inflammatory markers, regulation of adaptive immune response, and improvement of cellular immunity.[27]The association of vit D deficiency with an increased risk of acute viral respiratory infections and the potential protective effects of supplementation has been extensively reported.[11] However, currently there remains insufficient evidence to recommend routine supplementation to prevent acute respiratory tract infections or COVID-19.[15,30] Hence, our findings seem to emphasize that vit D levels could be valuable in predicting severe forms of MIS-C and correction of abnormal levels may potentially have a favorable effect in reducing the severity of MIS-C in certain circumstances.[10] Nonetheless, while the evidence on the link between vit D status and SARS-CoV-2 continues to emerge, with a suggestion of an inverse relationship between circulating 25OHD levels and SARS-CoV-2 positivity,[31] the relevance of vit D as a modifiable risk factor affecting the unregulated cytokine response in severe MIS-C requires further consideration.[15]

Our findings support the consideration of hyper-inflammatory shock as a common element in MIS-C with a need for vasopressor support and/or fluid resuscitation and ICU stay in most children.[23] Nonetheless, similar to our findings, although children with MIS-C are considered critically ill, most respond to prompt administration of anti-inflammatory agents including intravenous immunoglobulin and corticosteroids.[18,23]

Certain limitations to this study should be considered. First, the potential lack of generalizability is an important limitation due to a single-center study design with a relatively small sample size. Second, given that all pediatric COVID-19 patients admitted to our hospital within the study period had mild COVID disease, we could not evaluate the impact of severity of initial COVID-19 on the likelihood of later development of MIS-C. Third, given the non-uniform time to evaluate the level of vit D, especially in the control group, it seems not possible to conclude that vit D predicts MISC occurrence. Third, the lack of data on long-term post-discharge outcomes and the potential risk factors in non-survivors is another limitation that otherwise would extend the knowledge achieved in the current study.

In conclusion, our findings indicate the higher prevalence of vit D deficiency in pediatric COVID-19 patients with versus without MIS-C, as well as in those with severe versus moderate MIS-C. Hence, our findings emphasize the value of serial measurements of vit D level on admission and

during the course of MIS-C to monitor the disease progression. Apart from a decrease in vit D levels, older age, higher troponin and procalcitonin levels and dyspnea and lower lymphocyte counts at presentation seem to be the risk factors for developing severe MIS-C and thus an experience of more pronounced cardiac dysfunction, increased need for NIMV, longer ICU stays, higher rate of hypotension and higher VIS and prognostic scores by these patients. Further real-life data in MIS-C patients are needed to clarify whether vit D deficiency contributes to, or is a consequence of MIS-C, and thus these real life data are needed to determine the utility of vit D levels in early identification of children with higher susceptibility to develop MIS-C and those at high risk of severe disease course.

Conflict of Interest
None declared.

Authors' Contributions
N.Z. contributed to the study conception and design. Material preparation, data collection and analysis were performed by A.B., T.A.G., S.S.B., F.A., and S.A. The first draft of the manuscript was written by N.Z. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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