Journal of Multidisciplinary Healthcare

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Prognostic Value of Caspase-3 and Cardiac Troponin I in Assessing Cardiovascular Risk in Pediatric COVID-19 and Multisystem Inflammatory Syndrome

Kateryna Kozak[1], Halyna Pavlyshyn[1], Sandor George Vari[2]

[1]Department of Pediatrics, No.2, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine; [2]International Research and Innovation in Medicine Program, Cedars–Sinai Medical Center, Los Angeles, CA, USA

Correspondence: Kateryna Kozak, Email kozakk@tdmu.edu.ua

Purpose: The studies of COVID-19-related cardiovascular events in children are not widespread. Criteria for cardiovascular dysfunction have been explicitly proposed for multisystem inflammatory syndrome in children (MIS-C), while for other COVID-19 manifestations, it remains unclear. Although instrumental confirmation of cardiovascular injury is well-known, laboratory markers for such injuries have not been thoroughly studied. Our study aimed to identify prognostic cut-off values for caspase-3 and cardiac troponin I (cTI) to define cardiovascular injury in pediatric COVID-19 patients during acute infection and MIS-C.

Patients and Methods: A cross-sectional study was conducted in Ternopil, Ukraine, involving two hundred sixty children aged one month to 17 years who had not previously been vaccinated against SARS-CoV-2. The research focused on different severities of COVID-19: mild (n=87); moderate (n=66); severe COVID-19 (n=22); MIS-C (n=40) and 45 non-SARS-CoV-2 infected persons. ELISA tests were used to measure caspase-3 and cardiac troponin I levels.

Results: Caspase-3 and cTI levels were significantly higher in patients with severe COVID-19 and MIS-C compared to non-infected individuals. Furthermore, COVID-19 and MIS-C patients with cardiac abnormalities had substantially higher levels of caspase-3 and cTI compared to those without structural changes. The study also revealed a positive correlation between caspase-3 and cardiac troponin I levels in both the COVID-19 group ($r = 0.41$; $p < 0.05$) and the MIS-C group ($r = 0.55$; $p < 0.05$). The study has identified specific cut-off values for caspase-3 and cTI that can be used to predict cardiovascular structural changes in pediatric patients with COVID-19 and MIS-C. These values are caspase-3 $\geq$ 5.22 ng/mL, cTI$\geq$ 1.34 ng/mL for COVID-19, and caspase-3 $\geq$ 6.52 ng/mL and cTI $\geq$ 0.39 ng/mL for MIS-C.

Conclusion: Current research has demonstrated that children with severe COVID-19, as well as patients with MIS-C, must undergo careful screening for cardiovascular events that could include biomarkers.

Keywords: caspase-3, cardiac troponin I, COVID-19, MIS-C, children

Introduction

The clinical manifestations of COVID-19 in pediatric patients can range from asymptomatic courses to severe forms and the development of multisystem inflammatory syndrome in children (MIS-C). MIS-C is characterized by involvement of the cardiovascular system, such as hypotension or shock, cardiac dysfunction, pericarditis, valvulitis, or coronary abnormalities (including echocardiographic findings or elevated troponin/BNP), arrhythmias and fulminant myocarditis.[1,2] However, due to the SARS-CoV-2 virus`s affinity for the cardiovascular system, experts believe cardiovascular involvement in COVID-19 may often not present with obvious clinical symptoms. Instead, there may be underlying pathophysiological changes, which can be reversible or irreversible. Notably, pathomorphological studies (autopsy) have confirmed a high viral load in myocardial tissue in cases of COVID-19, which can have significant health consequences.[2]

The incidence of cardiovascular events associated with SARS-CoV-2 infection varies among countries and age groups. According to a study by Shu et al, variability in the occurrence of acute myocardial injury in adults ranges from

Received: 29 December 2024
Accepted: 22 May 2025
Published: 30 May 2025

6.9% to 36%.[3] A significant majority of patients with MIS-C (80–100%) experience cardiovascular involvement, with 40% exhibiting myocarditis, 25% experiencing pericarditis, and 32% showing pericardial effusion.[4] It is worth noting that most COVID-19 patients, including those with cardiovascular abnormalities, can make a full recovery. However, the underlying pathophysiology of this recovery remains unclear.

According to the literature, cardiac troponin I (cTI) is a sensitive indicator of mortality in adult COVID-19 patients. Still, its validity in children has not been confirmed.[3,5] Both cardiac troponin I and T are biomarkers of myocyte injury, as well as markers for various cardiovascular conditions in both adults and children.[2,6] Following ischemic injury, cytosolic cTI is released monophasically into the bloodstream, allowing for early identification of myocardial damage.[7] Later, cTI elevation of cTI is based on the release of structurally bound cTI from myofibrils.[7] Recent research has raised doubts about the reliability of cTI as a marker for acute cardiovascular conditions.[8] Studies have shown that cTI levels can be elevated in cases of tachycardia, which can lead to reduced coronary perfusion.[8,9] It must be highlighted that infection by itself could be related to the non-cardiac conditions for cardiac troponin release.[10] This is because of cardiomyocyte necrosis due to direct inflammatory influence.[11] Some experts have suggested that high levels of troponin I may not only indicate myocardial injury, but also cell death.[8] However, other studies have found that high cTI levels may be caused by reversible membrane damage and may not necessarily be linked to cell death.[9] Therefore, further investigation into apoptosis in cases of cardiovascular involvement is essential.

Recent research has revealed a potential link between SARS-CoV-2 and cell death, specifically through various processes such as apoptosis, pyroptosis, necroptosis, ferroptosis, and netosis.[12] While apoptosis typically serves as a protective mechanism against viral replication and spreading, uncontrolled apoptosis can lead to severe illness or even death.[12,13] Ischaemia is considered to be a crucial factor in cardiomyocyte apoptosis.[14] Notably, in inflammatory conditions, the balance between vascular smooth muscle cell (VSMC) proliferation and apoptosis is altered, with apoptosis dominating.[14] This state is commonly seen in cases of aneurysms.[14] Additionally, in vitro studies have demonstrated that high levels of TNF-α, which is associated with COVID-19 and MIS-C, can induce apoptosis.[15] Among the known caspases, the executioner caspase-3 is a critical apoptotic protein. Importantly, oxidative stress in the case of severe disease course can stimulate the intrinsic mitochondria-mediated apoptotic pathway and initiate cell death, even in the cardiovascular system.[16]

Taking into account the considerations mentioned, the objective of our study was to identify prognostic cut-off values for both caspase-3 and cardiac troponin I to define cardiovascular injury in pediatric COVID-19 patients during acute infection and MIS-C. This is intended to facilitate early-stage diagnosis of cardiovascular events for subsequent medical follow-up and management.

Materials and Methods

A cross-sectional study was done in Ternopil, Ukraine, in pediatric hospital settings − Ternopil Municipal Children's Hospital and Ternopil Regional Children's Clinical Hospital during 2021–2023. Two hundred and eighty-five patients who had not previously been vaccinated against SARS-CoV-2 were enrolled in the research: 240 patients with confirmed SARS-CoV-2 infection and 45 healthy children without evidence of SARS-CoV-2 (PCR (polymerase chain reaction) negative and Ig G negative) and without underlying heart disease. COVID-19 was confirmed by a positive PCR or antigen test. The required sample size was calculated using G*Power 3.1.9.7. The input parameters included an effect size of 0.8, an α-error probability of 0.05, a power of 0.95, and an allocation ratio of 0.25 (based on the incidence of cardiovascular injury in cases of COVID-19). The study was fixed as two-tailed.

We defined four patient groups based on the COVID-19 severity:[1,17]

1) Group 1 – mild COVID-19 (n=106) − symptoms of upper respiratory infection in the absence of X-ray or ultrasound local changes and respiratory distress;
2) Group 2 – moderate COVID-19 (n=72); pneumonia confirmed by imaging studies or upper airway symptoms with respiratory distress;
3) Group 3 − severe COVID-19 (n=22) – upper airway symptoms in combination with oxygen saturation <92% on room air, severe respiratory distress or systemic symptoms (drowsiness, lethargy, seizures, dehydration);
4) Group 4 − MIS-C (n=40) − children and adolescents aged 0–19 years with a fever lasting 3 and more days and two of the following symptoms: a rash, bilateral non-purulent conjunctivitis, or signs of mucocutaneous inflammation

(in the mouth, hands, or feet); hypotension or shock; features of myocardial dysfunction, pericarditis, valvulitis [pathological mitral regurgitation and/or aortic regurgitation and morphological changes of valvulitis in the mitral valve and aortic valve on echocardiography], or coronary abnormalities (including ECHO findings or elevated Troponin/NT-proBNP); evidence of coagulopathy (indicated by PT, PTT, or elevated d-Dimers); acute gastrointestinal problems (such as diarrhea, vomiting, or abdominal pain); and elevated markers of inflammation such as ESR, C-reactive protein, or procalcitonin. Additionally, no other obvious microbial cause of inflammation should exist, including bacterial sepsis, staphylococcal or streptococcal shock syndromes. Finally, there should be evidence of COVID-19 (positive RT-PCR, antigen test, or serology) or likely contact with patients who have COVID-19 (World Health Organization criteria).[1,18]

Age stages of children involved in the study were defined according to the National Institute of Child Health and Human Development Pediatric Terminology: infancy − 56 persons (19.56%); toddler – n=26 (9.12%); early childhood – n=67 (23.51%); middle childhood – n=70 (24.56%); early adolescence – n=66 (23.16%).[19]

Cardiovascular events were defined in cases when myocarditis, pericardial effusion, coronary artery aneurysm, coronary artery dilatation, hypotension, or shock were diagnosed. Myocarditis was confirmed using criteria proposed by the American Heart Association.[20] In our study, the diagnosis of myocarditis was based on clinical characteristics at presentation, elevated cardiac biomarkers (cTI), electrocardiography findings (ST-segment changes and T wave abnormalities, low-voltage QRS complexes in the limb leads, atrioventricular conduction delays, ventricular and atrial arrhythmias), and echocardiography features (left ventricular (LV) or right ventricular systolic function, varying degrees of LV enlargement, thickened myocardium, pericardial effusion, and functional valvular regurgitation). Cardiac magnetic resonance imaging or endomyocardial biopsy were not used to confirm myocarditis due to the lack of technical capabilities in our clinical settings.

The Bioethics Committee of I. Horbachevsky Ternopil National Medical University approved the study (Protocol No. 71, October 25, 2022) and ensured compliance with the Declaration of Helsinki. Informed consent was obtained from all the children's caregivers.

ELISA tests were used to measure caspase-3 (Human CASP3 (Caspase 3) ELISA kit, Elabscience, USA) and cardiac troponin I level (Cardiac Troponin I ELISA kit, Monocent, USA).

Statistical analysis was done using IBM SPSS Statistics 21.0 and GraphPad Prism 8.4.3. Complete case analysis was used in the research. The distribution of parameters was assessed in normality tests (histograms, Shapiro–Wilk`s W test). Based on the results obtained, parametric (ANOVA) and nonparametric tests (Kruskal–Wallis ANOVA, Spearman correlation) were used. Frequency tables were analyzed using the Pearson Chi-square test. ROC (Receiver Operating Characteristic) analysis was conducted to determine the proposed test's optimal cut-off point and diagnostic accuracy. The area under the curve (AUC) and its corresponding 95% confidence interval (95% CI AUC) were calculated for each ROC curve. Sensitivity and specificity were also calculated for each classifier threshold. A statistically significant result was assumed at a p-value < 0.05. The research achieved a statistical power of 0.97.

Results

Children with mild and moderate COVID-19 severity were significantly younger than the control group ($p < 0.05$). At the same time, severe COVID-19 cases were more typical for school-aged children, whereas mild COVID-19 for preschoolers ($p < 0.05$) (Table 1). There were no sex differences in the group structures ($p > 0.05$) (Table 1).

Research has shown that the cardiovascular system can be affected in patients with severe COVID-19, not just those with MIS-C (Figure 1). The incidence of myocarditis and coronary artery aneurysm did not show a significant difference between severe COVID-19 and MIS-C ($p > 0.05$). Pericardial effusion and coronary artery dilatation were most commonly seen in patients with MIS-C. Coronary artery dilatation was only observed in patients with MIS-C (22.5%). Coronary artery aneurysms in our study were low, with only 2.5% of MIS-C patients affected and no cases reported in severe COVID-19. No cases of valvulitis were observed in the study. Hypotension or shock was diagnosed in 11 individuals: 5 (45.45%) were children with a severe COVID-19 course, and 6 (54.55%) were patients with MIS-C. Six patients (1 with a severe COVID-19 course and 5 with MIS-C) had an ejection fraction below 55%. Only two patients

Table 1 Baseline Characteristics of COVID-19 Pediatric Patients and Non-COVID-19 Control Patients

Patient`s Group		Age, Years	Sex, n (%)	
			Male	Female
Mild COVID-19, n=106	A	4.36±4.87 [C, D, E]	50 (47.17)	56 (52.83)
Moderate COVID-19, n=72	B	6.12±5.97 [E]	45 (62.50)	27 (37.50)
Severe COVID-19, n=22	C	9.21±6.50	8 (36.36)	14 (63.64)
MIS-C, n=40	D	7.38±4.50	24 (60.00)	16 (40.00)
Control group, n=45	E	9.84±4.23 [A, B]	28 (62.22)	17 (37.78)

Notes: Analysis of sex differences between study groups – χ^2=8.64; p > 0.05. [A, B, C, D, E] – a statistically significant difference (p < 0.05) with the corresponding group.
Abbreviations: MIS-C, multisystem inflammatory syndrome in children; χ^2, Pearson Chi-square test; p, level of its significance.

had underlying cardiovascular abnormalities, specifically congenital heart diseases − one with moderate disease severity and another with a severe COVID-19 course.

Caspase-3 level was significantly higher in patients with severe COVID-19 course and in patients with MIS-C (p < 0.05) (Table 2). Mild and moderate COVID-19 courses did not vary in caspase-3 levels from healthy controls (Table 2). Notably, cTI levels increased with disease severity and were significantly higher in patients with severe COVID-19 and MIS-C compared to non-infected individuals (Table 2).

There was no significant age difference observed for caspase-3 and cTI levels in the COVID-19, MIS-C, and control groups (p > 0.05). However, the levels of caspase-3 and cTI in the MIS-C group were higher than those in the COVID-19 and control groups in early and middle childhood age (Figure 2).

The study also revealed sex differences in caspase-3 levels among MIS-C patients, with boys having significantly higher levels compared to girls (p < 0.05). Additionally, male patients with MIS-C had significantly higher caspase-3 levels compared to males with COVID-19. In both male COVID-19 and MIS-C patients, caspase-3 levels were higher than those in healthy male individuals (p < 0.05). Furthermore, the MIS-C group had significantly higher levels of cardiac troponin I in female and male groups compared to healthy individuals and patients with COVID-19 (Figure 2).

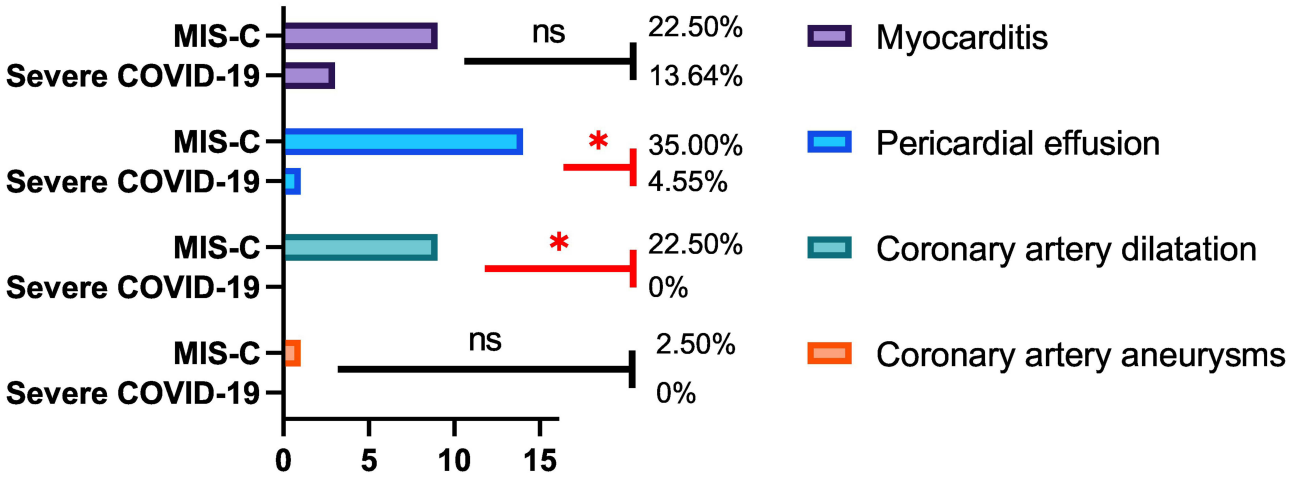

Figure 1 Structural cardiovascular abnormalities in pediatric patients with COVID-19.
Notes: *a statistically significant result (p < 0.05). ns – non-significant result (p > 0.05).

Table 2 Caspase 3 and Cardiac Troponin I Level in Children with Different COVID-19 Courses, MIS-C, and Control Group

Parameter		Mild COVID-19, n=106	Moderate COVID-19, n=72	Severe COVID-19, n=22	MIS-C, n=40	Control group, n=45
		A	B	C	D	E
Caspase 3	ng/mL	1.98 (C, D) (1.67; 2.19)	2.09 (C, D) (1.46; 3.00)	5.26 (A, B, E) (3.65; 6.89)	6.07 (A, B, E) (4.05; 8.93)	1.04 (0.78; 1.86)
	H, p	H=40.07; p < 0.001*				
	p-value	$p_{A-C, A-D, B-C, B-D, C-E, D-E} < 0.05$*; $p_{A-E, B-E, C-D} > 0.05$				
Cardiac Troponin I	ng/mL	0.23 (D) (0.20; 0.25)	0.23 (D) (0.22; 0.27)	0.31 (E) (0.23; 0.68)	0.44 (A, B, E) (0.25; 0.65)	0.15 (0.12; 0.23)
	H, p	H=25.61; p < 0.001*				
	p-value	$p_{A-D, B-D, C-E, D-E} < 0.05$*; $p_{A-B, A-C, B-C, B-E, C-D} > 0.05$				

Note: $p_{A-B, A-C, A-D, A-E, B-C, B-D, B-E, C-D, C-E, D-E}$ – p-values for post-hoc group comparisons. $^{A, B, C, D, E}$ – a statistically significant difference with the corresponding group ($p < 0.05$).* – a statistically significant result ($p < 0.05$).
Abbreviations: MIS-C, multisystem inflammatory syndrome in children; H, Kruskal–Wallis test and p – level of its significance.

Our previous research demonstrated a significantly higher prevalence of the protective TT genotype CASP3 rs113420705 in healthy individuals (46.67%) compared to patients with COVID-19 (10%).[21] Therefore, we analyzed the caspase-3 level depending on the genetic host`s predisposition. Patients with genotype TT rs113420705 had significantly lower caspase-3 levels (0.94 [0.65; 1.78] ng/mL) compared to heterozygous TC (3.04 [1.93; 5.50] ng/mL) and rare homozygous CC (2.67 [1.98; 6.34] ng/mL) (Kruskal–Wallis test H=13.00; $p < 0.05$). The study revealed higher caspase-3 levels in allele C carriers (2.89 [1.93; 6.34] ng/mL) compared to allele T carriers (2.09 [0.94; 4.78] ng/mL) ($p < 0.05$).

The levels of caspase-3 and cardiac troponin I were found to be significantly higher in COVID-19 and MIS-C patients with cardiac abnormalities compared to those without such structural changes (Table 3). It is important to note that there was no significant difference in caspase-3 and cTI levels between COVID-19 and MIS-C patients with myocarditis,

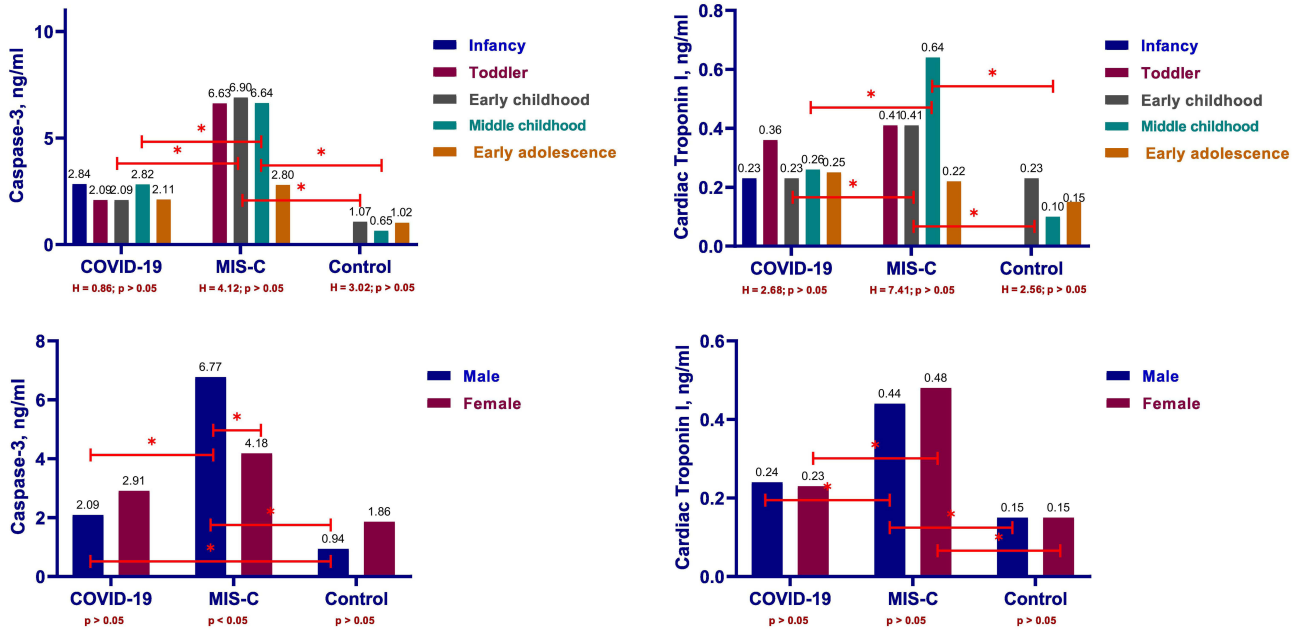

Figure 2 Sex and age differences in caspase-3 and cardiac troponin I levels in children with SARS-CoV-2 infection and non-infected children.
Note: *a statistically significant result ($p < 0.05$).

https://doi.org/10.2147/JMDH.S514776

Table 3 Capase-3 and Cardiac Troponin I Levels in Children with SARS-CoV-2 Infection, Depending on the Structural Cardiovascular Abnormalities

Parameter		Caspase-3, ng/mL		Cardiac Troponin I, ng/mL	
		COVID-19	MIS-C	COVID-19	MIS-C
Myocarditis	Absence	2.03 (1.11; 3.09)	5.50* (3.17; 8.93)	0.23 (0.21; 0.25)	0.32 (0.24; 0.51)
	Presence	5.23 ↕ (3.64; 6.58)	8.06 (4.05; 9.15)	1.42 ↕ (0.36; 1.52)	0.65 ↕ (0.55; 0.87)
Pericardial effusion	Absence	2.04 (1.19; 3.26)	4.85* (2.47; 8.58)	0.23 (0.21; 0.27)	0.44* (0.34; 0.60)
	Presence	6.58	6.77 (4.78; 8.93)	0.36	0.46 (0.24; 1.12)
Coronary abnormalities (dilatation/ aneurism)	Absence	2.04 (1.19; 3.64)	4.78* (3.17; 6.90)	0.23 (0.21; 0.29)	0.34* (0.25; 0.63)
	Presence	–	9.15 ↕ (8.93; 9.34)	–	0.49 (0.33; 0.76)
Structural cardiovascular abnormalities (total)	Absence	2.03 (1.11; 3.09)	4.20 (1.78; 6.90)	0.23 (0.21; 0.25)	0.33 (0.23; 0.34)
	Presence	5.23 ↕ (3.64; 6.58)	8.06 ↕ (4.78; 9.34)	1.42 ↕ (0.36; 1.52)	0.57 ↕ (0.30; 0.87)

Note: *, ↕ a statistically significant result in comparing patients with the absence or presence of structural cardiovascular abnormality.
Abbreviation: MIS-C, multisystem inflammatory syndrome in children.

pericardial effusion, or coronary abnormalities. However, in cases where structural cardiovascular abnormalities were absent, children with MIS-C had higher levels of caspase-3 compared to COVID-19 patients (Table 3).

The study found a significant increase in cardiac troponin I levels as caspase-3 levels rose. This connection was supported by a positive correlation between the two in both the COVID-19 group ($r = 0.41$; $p < 0.05$) and the MIS-C patients` group ($r = 0.55$; $p < 0.05$) (Figure 3).

A study has identified specific cut-off values that can be used to predict cardiovascular structural changes in pediatric patients with COVID-19 and MIS-C. These values are caspase-3 ≥ 5.22 ng/mL and cTI ≥ 1.34 ng/mL for COVID-19; and caspase-3 ≥ 6.52 ng/mL and cTI ≥ 0.39 ng/mL for MIS-C (Table 4, Figure 4).

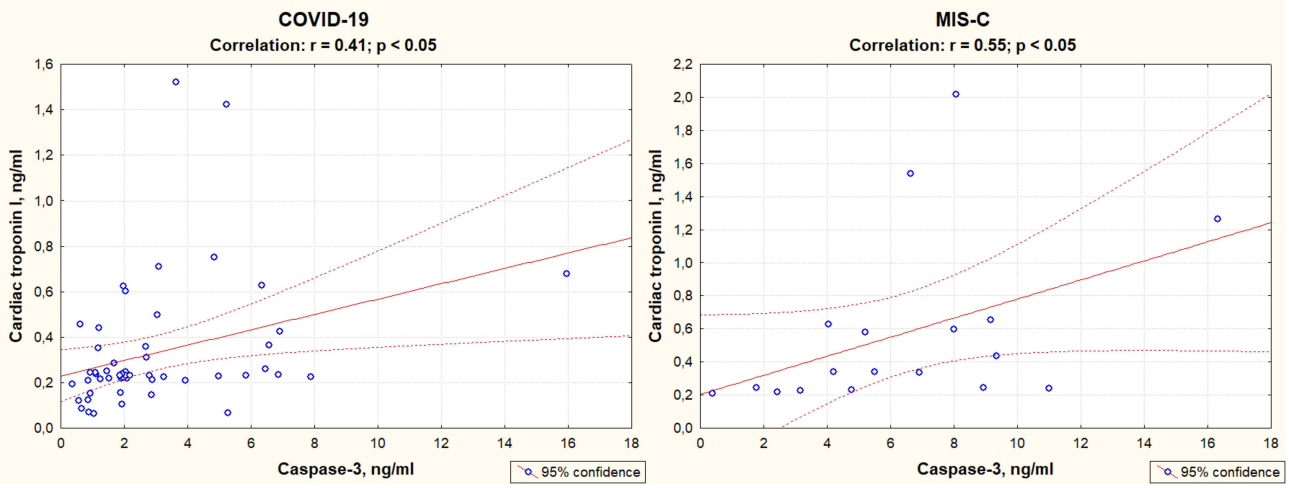

Figure 3 Correlation analysis between cardiac troponin I and caspase-3 levels.

Table 4 Caspase-3 and Cardiac Troponin I Levels as Predictors of Cardiovascular Structural Changes in Pediatric Patients with COVID-19 and MIS-C

Parameter	Caspase-3, ng/mL		Cardiac Troponin I, ng/mL	
Group	COVID-19	MIS-C	COVID-19	MIS-C
AUC	0.729	0.878	0.894	0.763
Standard Error	0.064	0.050	0.069	0.076
95% CI AUC	0.603–0.856	0.781–0.975	0.758–1.000	0.615–0.911
P-value	0.181	<0.001*	0.022*	0.006*
Cut-off value	5.22	6.52	1.34	0.39
Sensitivity	0.67	0.64	0.67	0.64
Specificity	0.74	0.88	0.97	0.79

Note: * – a statistically significant result ($p < 0.05$).
Abbreviations: AUC, area under the curve; 95% CI AUC, 95% confidence interval for the area under the curve.

Discussion

The analysis of COVID-19 severity among hospitalized pediatric patients in the Ternopil region, Ukraine, demonstrated age-related differences. Of the children studied, 62% were under the age of 5, and this age group had the highest proportion of mild COVID-19 cases. In contrast, severe COVID-19 cases were more common among school-aged children. Our findings align with previously published data, indicating that 90% of children with COVID-19 under age 5 experience mild or moderate disease courses.[22]

COVID-19 in children presents with various clinical manifestations. Typically, physicians primarily focus on the respiratory symptoms of SARS-CoV-2, and only in cases of MIS-C do they become more concerned about cardiovascular symptoms. The pathophysiology of heart involvement associated with COVID-19 is still under discussion among scientists. Most often, the primary causes of cardiac damage are oxidative stress and cell death, which are related to severe hypoxia, respiratory failure, direct invasion of SARS-CoV-2 into cardiomyocytes through the ACE2 receptor, and the cytokine storm as a response to the infection.[23,24] A severe course of COVID-19, characterized by respiratory distress manifestations, can lead to a decreased oxygen supply that does not correspond to the demand. As a result, severe

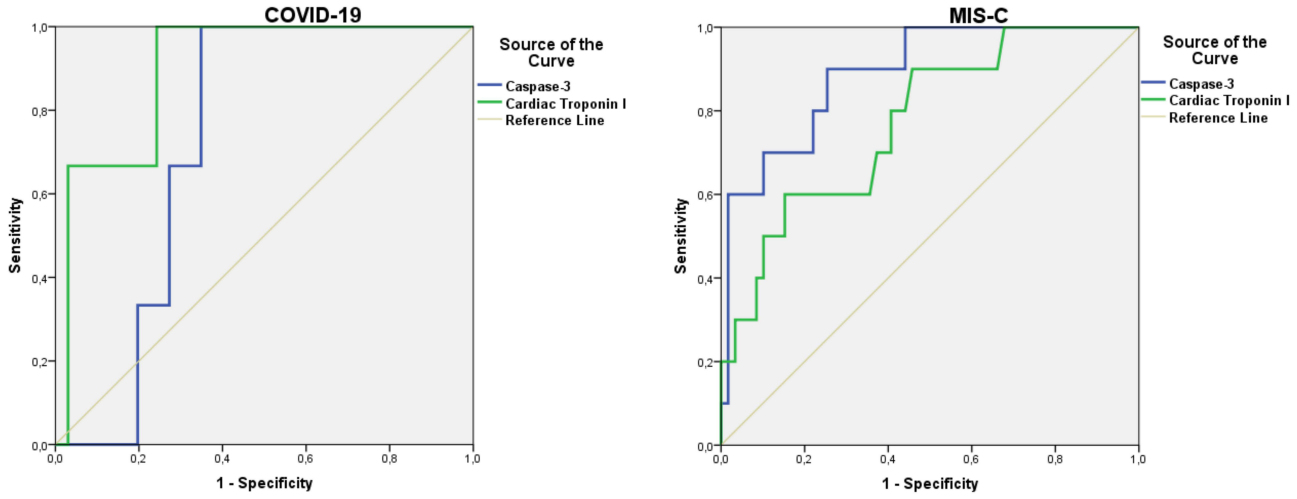

Figure 4 ROC-curves for cardiovascular structural change prediction in pediatric COVID-19 patients.

https://doi.org/10.2147/JMDH.S514776

hypoxia occurs, likely leading to cell death.[25] Systemic inflammation and the release of inflammatory cytokines can also contribute to necrosis and apoptosis.[26]

Our study revealed a remarkably elevated caspase-3 level in patients with COVID-19 and MIS-C compared to healthy children. The executioner caspase-3 is a critical apoptotic protein released through stimulation of both pathways − intrinsic (through the activation of the apoptosome and caspase-9) and extrinsic (through the activation of the death-inducing signaling complex and caspase-8).[27,28]

Previous research has indicated that the spike protein of the SARS-CoV-2 coronavirus stimulates the upregulation of both caspase-3 and caspase-6.[29] Furthermore, experiments conducted on lung cells infected with SARS-CoV-2 demonstrated the activation of caspase-8, caspase-9, and caspase-3.[30]

Emphasizing the critical role of caspase-3 in cardiomyocyte apoptosis is essential. Physiologically, most human cardiomyocytes exhibit a lifespan synchronized with that of the entire organism. Only approximately 0.01% to 0.001% of cardiomyocytes undergo apoptosis influenced by various factors at any specific moment.[31] Caspase-3 plays a pivotal role by cleaving myofibrillar proteins, which can ultimately lead to contractile heart dysfunction.[31] It is not obvious that all caspase-3 activations will manifest with cardiomyocyte death. The literature describes such a pattern as "interrupted apoptosis", in which cells are still in a proapoptotic state before eventual death.[31] This pattern is of great importance in the case of heart damage associated with SARS-CoV-2 because not all cardiovascular abnormalities are revealed through clinical evaluation. At the same time, the elevation of the X-linked inhibitor of apoptosis protein (XIAP) is typical for end-stage heart failure. XIAP inhibits caspase-3, caspase-7, and caspase-9, preventing cell death and the following lethal clinical outcome.[31] This mechanism can be fundamental in MIS-C, characterized by cardiovascular structural abnormalities and elevated apoptotic activity, as indicated by increased caspase-3 levels.

There are three known isoforms of troponin: C (cTC), I (cTI), and T (cTT), with cTI and cTT being more specific to the cardiac system.[6] These isoforms have distinct functions in regulating muscle contraction within the actin-myosin cross-bridge. The 32 cTT subunit links the entire troponin complex to tropomyosin, and the cTC subunit. cTC serves as a calcium ion sensor during the muscle contraction process. In addition, activated cTC binds with cTI. It induces a shift in cTI on the actin filament. This shift exposes binding sites that enable myosin to bind, facilitating muscle contraction.[32]

Significantly, troponins are related to ischemia-related cardiac muscle injuries.[32] An elevated troponin level may be associated with reversible and irreversible myocyte injury. Reversible injury is connected to myocyte turnover, heightened cell wall permeability, and the release of proteolytic enzymes by cells. On the other hand, irreversible injury results from apoptosis and injury induced by hypoxia.[6] Current data suggest that an elevated troponin level is associated with a fivefold higher risk of lethal outcome compared to patients with an average troponin level.[5] We detected a relationship between cTI levels and the incidence of cardiovascular alterations. It is essential to highlight that cTI levels increased in cases of myocarditis. In contrast, in cases of other cardiovascular structural abnormalities, cTI levels did not differ from those in the group without cardiovascular involvement. Current research data suggests that the cause of myocarditis is the direct invasion of the SARS-CoV-2 virus through the ACE2 receptor.[33] Virus entry into the myocardium triggers the secretion of proinflammatory cytokines and activation of T-cell and B-cell immunity. However, immune cells' sustained secretion of interleukins and infiltration of the myocardium ultimately results in cardiomyocyte injury and the release of cTI.[33]

Our study did not reveal any sex or age-related differences in cTnI levels. These findings correspond to the meta-regression data, demonstrating that troponin elevation does not vary with age, sex, or comorbidities.[5] Importantly, cTI was significantly higher in the MIS-C group compared to the COVID-19 group in the corresponding age and sex. This difference indicates that myocardial damage is more typical for patients with MIS-C, which, in fact, is defined as its criterion, in contrast to severe COVID-19.

It is crucial to notice that cTI is a valuable marker for risk stratification in cardiac morbidity, even among healthy subjects.[6] Consequently, this approach can be applied to children for the timely diagnosis of cardiac abnormalities and to provide a differential diagnosis of cTI elevation, which could also be observed in cases of severe inflammation and acute respiratory distress syndrome.[34]

Our study has identified specific cut-off values that can be used to predict cardiovascular structural changes in pediatric patients with COVID-19 and MIS-C. These values are caspase-3 $\geq$ 5.22 ng/mL and cTI $\geq$ 1.34 ng/mL for COVID-19; and caspase-3 $\geq$ 6.52 ng/mL and cTI $\geq$ 0.39 ng/mL for MIS-C. Previously, U.U. Güllü et al determined cut-

off points for developing MIS-C.[35] However, their research primarily focused on MIS-C, while our study encompasses all severities of COVID-19. G. Guner Ozenen et al also suggested that elevated cTI levels can predict cardiac involvement in pediatric COVID-19 patients.[23] This can help define the need for further cardiological assessment and monitoring.

A positive correlation between the apoptotic marker caspase-3 and cardiac troponin I released from cardiac myocytes through processes such as inflammation, apoptosis, or necrosis can be confirmed by our results.[32] The release of cTI can occur due to localized cardiomyocyte apoptosis, ultimately leading to the breakdown of the plasma membrane.[36] It's worth highlighting that the initial stage of cardiomyocyte apoptosis has the potential to evolve into secondary necrosis. This progression occurs due to the interconnected pathways of cell death, wherein one pathway involves cell surface death receptors, and the other involves mitochondria.[35] The literature has shown that the interplay between apoptosis and necroptosis can increase cardiac troponin levels.[37]

The significant correlation between the two examined biomarkers, caspase-3 and cardiac troponin I, supports their incorporation into a comprehensive pathogenetic model. This is particularly applicable in acute cardiovascular injury observed in pediatric patients with COVID-19 and MIS-C.

The results of our study allowed us to identify a group of patients with elevated laboratory markers of cardiovascular injury who, despite clinical recovery, could be scientifically categorized for continued observation and follow-up. Previous studies have demonstrated that patients with MIS-C − even those without overt cardiac involvement during hospitalization − require ongoing monitoring. Follow-up examinations in such cases have revealed abnormalities on cardiac MRI, ambulatory heart rate monitoring, and cardiopulmonary exercise testing.[38] Therefore, our findings are important in supporting the need for structured diagnostic work-up and long-term follow-up in children with COVID-19 and MIS-C.

Limitations

Our study is limited by the inclusion of children from different age groups with varying COVID-19 severity. We compared caspase-3 and cardiac troponin I levels within each age group to minimize this bias. No age-specific differences were observed in our research. Nevertheless, future studies with larger cohorts could help clarify these effects.

Due to the fact that the study began before the outbreak of full-scale war in Ukraine and was conducted during the war years, we were unable to provide further cardiac follow-up of the recommended observation group - children with severe COVID-19 and MIS-C. Therefore, further studies are needed to establish the practical significance of the newly established thresholds for caspase-3 and cTI.

Conclusion

Current research has demonstrated that children with severe COVID-19, as well as patients with MIS-C, must undergo careful screening for cardiovascular events that could include biomarkers. This novel aspect of our study will enable medical professionals to promptly refer patients for instrumental examinations, leading to timely diagnosis and proactive management of cardiovascular pathology. The results showed a significant association between the biomarkers caspase-3 and cTI and cardiovascular events in pediatric COVID-19 and MIS-C patients. These specific cut-off values are caspase-3 $\geq$ 5.22 ng/mL and cTI $\geq$ 1.34 ng/mL for COVID-19, and caspase-3 $\geq$ 6.52 ng/mL and cTI $\geq$ 0.39 ng/mL for MIS-C. By establishing threshold values for these markers, we can also facilitate the ongoing monitoring of children diagnosed with both MIS-C and COVID-19. While current guidelines and recommendations address the follow-up of children after MIS-C, there is currently no established approach for pediatric patients after severe acute respiratory syndrome caused by SARS-CoV-2. Determining laboratory markers could benefit pediatric COVID-19 and MIS-C patients by aiding physicians in improving diagnostic accuracy. This, in turn, will help develop effective management strategies and potentially enhance early and long-term outcomes of SARS-CoV-2 infection in the pediatric population.

Abbreviations

AUC, area under the curve; COVID-19, Coronavirus disease 2019; cTI, cardiac troponin; MIS-C, multisystem inflammatory syndrome in children; 95% CI AUC, 95% confidence interval for the area under the curve.

Acknowledgments

We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program and the Association for Regional Cooperation in the Fields of Health, Science, and Technology (RECOOP HST Association) for their support.

Funding

The study was partially financed by RECOOP HST Association Fusion Grant # 030, «COVID-19 Severity and Gene Polymorphism in Children and Adults».

Disclosure

The authors declare no conflicts of interest in this work.

References

1. Multisystem inflammatory syndrome in children and adolescents temporally related to COVID-19. Available from: https://www.who.int/news-room/commentaries/detail/multisystem-inflammatory-syndrome-in-children-and-adolescents-with-covid-19. Accessed January 2024.
2. Zhao Y, Patel J, Huang Y, Yin L, Tang L. Cardiac markers of multisystem inflammatory syndrome in children (MIS-C) in COVID-19 patients: a meta-analysis. *Am J Emerg Med*. 2021;49:62–70. doi:10.1016/j.ajem.2021.05.044
3. Shu H, Wen Z, Li N, et al. COVID-19 and cardiovascular diseases: from cellular mechanisms to clinical manifestations. *Aging Dis*. 2023;14(6):2071–2088. doi:10.14336/AD.2023.0314
4. Vale Capucho ACMD, Resende PLS, Mascarenhas DA, et al. Cardiac manifestations in pediatric COVID-19. *Clinics*. 2021;76:1–4.
5. Wibowo A, Pranata R, Akbar MR, Purnomowati A, Martha JW. Prognostic performance of troponin in COVID-19: a diagnostic meta-analysis and meta-regression. *Int J Infect Dis*. 2021;105(January):312–318. doi:10.1016/j.ijid.2021.02.113
6. Leite L, Matos P, Leon-Justel A, et al. High sensitivity troponins: a potential biomarkers of cardiovascular risk for primary prevention. *Front Cardiovasc Med*. 2022;9(3):1–10. doi:10.3389/fcvm.2022.1054959
7. Zrinski Topic R, Lenicek Krleza J. Cardiac markers in pediatric laboratory medicine: critical review. *Diagnostics*. 2025;15(2):165. doi:10.3390/diagnostics15020165
8. Wu L, Ji W, Huang Y, et al. Establishment and validation of a prediction model for myocarditis in Chinese children below 14 years old: a protocol for a retrospective cohort study. *BMJ Open*. 2023;13(12):e075453. doi:10.1136/bmjopen-2023-075453
9. Yoldaş T, Örün UA. What is the significance of elevated troponin i in children and adolescents? A diagnostic approach. *Pediatric Cardiology*. 2019;40(8):1638–1644. doi:10.1007/s00246-019-02198-w
10. Senekovič Kojc T, Marčun Varda N. Novel biomarkers of heart failure in pediatrics. *Children*. 2022;9(5):740. doi:10.3390/children9050740
11. Bohn MK, Steele S, Hall A, Poonia J, Jung B, Adeli K. Cardiac biomarkers in pediatrics: an undervalued resource. *Clin Chem*. 2021;67(7):947–958. doi:10.1093/clinchem/hvab063
12. Yuan C, Ma Z, Xie J, et al. The role of cell death in SARS-CoV-2 infection. *Signal Transduct Target Ther*. 2023;8(1):1–17. doi:10.1038/s41392-022-01259-6
13. Majchrzak M, Poręba M. The roles of cellular protease interactions in viral infections and programmed cell death: a lesson learned from the SARS-CoV-2 outbreak and COVID-19 pandemic. *Pharmacol Rep*. 2022;74(6):1149–1165. doi:10.1007/s43440-022-00394-9
14. Bennett MR. Apoptosis in the cardiovascular system. *Heart*. 2002;87(5):480–487. doi:10.1136/heart.87.5.480
15. Teringova E, Tousek P. Apoptosis in ischemic heart disease. *J Transl Med*. 2017;15(1):87. doi:10.1186/s12967-017-1191-y
16. Kim NH, Kang PM. Apoptosis in cardiovascular diseases: mechanism and clinical implications. *Korean Circ J*. 2010;40(7):299–305. doi:10.4070/kcj.2010.40.7.299
17. Venturini E, Montagnani C, Garazzino S, et al. Treatment of children with COVID-19: update of the Italian society of pediatric infectious diseases position paper. *Ital J Pediatr*. 2021;47(1):1–11. doi:10.1186/s13052-021-01132-2
18. Pandian NG, Kim JK, Arias-Godinez JA, et al. Recommendations for the use of echocardiography in the evaluation of rheumatic heart disease: a report from the American society of echocardiography [published correction appears in]. *J Am Soc Echocardiogr*. 2023;36(4):445. doi:10.1016/j.echo.2022.10.009
19. Williams K, Thomson D, Seto I, et al. Standard 6: age groups for pediatric trials. *Pediatrics*. 2012;129(SUPPL. 3):P.153–160. doi:10.1542/peds.2012-0055I
20. Law YM, Lal AK, Chen S, et al. American heart association pediatric heart failure and transplantation committee of the council on lifelong congenital heart disease and heart health in the young and stroke council. diagnosis and management of myocarditis in children: a scientific statement from the American heart association. *Circulation*. 2021;144(6):e123–e135. [Erratum in: Circulation. 2021 Aug 10;144(6):e149]. doi:10.1161/CIR.0000000000001001
21. Kozak K, Pavlyshyn H, Kamyshnyi O, Shevchuk O, Korda M, Vari SG. The relationship between COVID-19 severity in children and immunoregulatory gene polymorphism. *Viruses*. 2023;15(10):1–24. doi:10.3390/v15102093

22. Bhuiyan MU, Stiboy E, Hassan MZ, et al. Epidemiology of COVID-19 infection in young children under five years: a systematic review and meta-analysis. *Vaccine*. 2021;39(4):667–677. doi:10.1016/j.vaccine.2020.11.078
23. Guner Ozenen G, Akaslan Kara A, Kiymet E. The evaluation of troponin i levels and myocarditis in children with COVID-19: a pediatric single-center experience. *Pediatr Cardiol*. 2023;44(4):873–881. doi:10.1007/s00246-022-03017-5
24. Çevik BŞ, Arici Ş, Ergenç Z, Kepenekli E, Günal Ö, Yakut N. How safe are children with COVID-19 from cardiac risks? Pediatric risk assessment; insights from echocardiography and electrocardiography. *Turkish J Med Sci*. 2021;51(3):981–990. doi:10.3906/sag-2010-240
25. Rodriguez-Gonzalez M, Castellano-Martinez A, Cascales-Poyatos HM, Perez-Reviriego AA. Cardiovascular impact of COVID-19 with a focus on children: a systematic review. *World J Clin Cases*. 2020;8(21):5250–5283. doi:10.12998/wjcc.v8.i21.5250
26. Saed Aldien A, Ganesan GS, Wahbeh F, et al. Systemic inflammation may induce cardiac injury in COVID-19 patients including children and adolescents without underlying cardiovascular diseases: a systematic review. *Cardiovasc Revasc Med*. 2022;35(January):169–178. doi:10.1016/j.carrev.2021.04.007
27. Li X, Zhang Z, Wang Z, Gutiérrez-Castrellón P, Shi H. Cell deaths: involvement in the pathogenesis and intervention therapy of COVID-19. *Signal Transduct Target Ther*. 2022;7(1):1–21. doi:10.1038/s41392-021-00710-4
28. Premeaux TA, Yeung ST, Bukhari Z, et al. Emerging insights on caspases in COVID-19 pathogenesis, sequelae, and directed therapies. *Front Immunol*. 2019;13:1–9.
29. Barhoumi T, Alghanem B, Shaibah H, et al. SARS-CoV-2 coronavirus spike protein-induced apoptosis, inflammatory, and oxidative stress responses in THP-1-like-macrophages: potential role of angiotensin-converting enzyme inhibitor (perindopril). *Front Immunol*. 2021;12 (September):1–13. doi:10.3389/fimmu.2021.728896
30. Deshpande R, Zou C. Programmed cell death in SARS-CoV-2 infection: a short review. *J Respir*. 2021;1(4):223–228.
31. Yang B, Dewei Ye YW, Wang Y. Caspase-3 as a therapeutic target for heart failure. *Expert Opin Ther Targets*. 2013;17(3):255–263. doi:10.1517/14728222.2013.745513
32. Giordano S, Estes R, Li W, et al. Troponin structure and function in health and disease. *Am Soc Clin Lab Sci*. 2018;31(4):192–199. doi:10.29074/ascls.2018000679
33. Pannucci P, Jefferson SR, Hampshire J, Cooper SL, Hill SJ, Woolard J. COVID-19-induced myocarditis: pathophysiological roles of ACE2 and toll-like receptors. *Int J Mol Sci*. 2023;24(6):5374. doi:10.3390/ijms24065374
34. Di Filippo P, David D, Attanasi M, Rossi N, Chiarelli F. Case report: increased troponin level in 125 children during COVID-19. *Front Pediatr*. 2023;11:1059685. doi:10.3389/fped.2023.1059685
35. Güllü UU, Güngör Ş, Ipek S, Yurttutan S, Dilber C. Predictive value of cardiac markers in the prognosis of COVID-19 in children. *Am J Emerg Med*. 2021;48:307–311. doi:10.1016/j.ajem.2021.06.075
36. Amgalan D, Pekson R, Kitsis RN. Troponin release following brief myocardial ischemia: apoptosis versus necrosis. *JACC Basic to Transl Sci*. 2017;2(2):118–121. doi:10.1016/j.jacbts.2017.03.008
37. Hammarsten O, Mair J, Möckel M, Lindahl B, Jaffe AS. Possible mechanisms behind cardiac troponin elevations. *Biomarkers*. 2018;23 (8):725–734. doi:10.1080/1354750X.2018.1490969
38. Zimmerman D, Shwayder M, Souza A, et al. Cardiovascular follow-up of patients treated for MIS-C. *Pediatrics*. 2023;152(6):e2023063002. doi:10.1542/peds.2023-063002