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ORIGINAL ARTICLE

Spectrum of Cardiovascular Diseases in Children During High Peak Coronavirus Disease 2019 Period Infection in Northern Italy: Is There a Link?

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Background. Children with coronavirus disease-2019 (COVID-19) have a milder clinical course than adults. We describe the spectrum of cardiovascular manifestations during a COVID-19 outbreak in Emilia-Romagna, Italy.

Methods. A cross-sectional multicenter study was performed, including all patients diagnosed with Kawasaki disease (KD), myocarditis, and multisystem inflammatory syndrome in children (MIS-C) from February to April 2020. KD patients were compared with those diagnosed before the epidemic.

Results. KD: 8 patients (6/8 boys, all negative for severe acute respiratory syndrome coronavirus-2 [SARS-CoV-2]): complete presentation in 5/8, 7/8 immunoglobulin (IVIG) responders, and 3/8 showed transient coronary lesions (CALs). Myocarditis: one 5-year-old girl negative for SARS-CoV-2 and positive for parvovirus B19. She responded to IVIG. MIS-C: 4 SARS-CoV-2-positive boys (3 patients with positive swab and serology and 1 patient with negative swab and positive serology): 3 presented myocardial dysfunction and pericardial effusion, and 1 developed multicoronary aneurysms and hyperinflammation; all responded to treatment. The fourth boy had mitral and aortic regurgitation that rapidly regressed after steroids.

Conclusions. KD, myocarditis, and MIS-C were distinguishable cardiovascular manifestations. KD did not show a more aggressive form compared with previous years: coronary involvement was frequent but always transient. MIS-C and myocarditis rapidly responded to treatment without cardiac sequelae despite high markers of myocardial injury at the onset, suggesting a myocardial depression due to systemic inflammation rather than focal necrosis. Evidence of actual or previous SARS-CoV-2 infection was documented only in patients with MIS-C.

Key words. children; COVID-19; Kawasaki disease; KDSS; multisystem inflammatory syndrome; myocarditis.

Coronavirus disease 19 (COVID-19) is an infectious disease caused due to the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has rapidly become a major global health issue due to the current pandemic outbreak this year. Italy is among the most severely affected countries. Data from the Italian National Health Institute (*Istituto Superiore di Sanità*), updated on May 7, indicate that Emilia-Romagna is the third most affected Italian region with a cumulative incidence of 591.53 per 100 000 people [1], a total of 26 379 recorded cases accounting for a 12.3% of total Italian cases, and 632 (2.4%) younger than 20 years of age.

Clinical manifestations of COVID-19 in adult patients include primarily respiratory symptoms and signs ranging from a dry cough to severe acute respiratory syndrome, which can lead to severe complications and death [2]. On the contrary, children diagnosed with COVID-19 disease seem to have a milder clinical course, with a benign respiratory involvement, rare complications, and favorable outcomes [3, 4].

Even though the symptoms affecting the respiratory system are the most noticeable, SARS-CoV-2 is responsible for a systemic inflammation with cytokine release that can result in multiorgan dysfunction.

SARS-CoV-2 enters the cells through the angiotensin-converting enzyme 2 (ACE2) receptor. ACE2 receptor is widely distributed over organs, accounting for the systemic nature of the disease [5, 6]. A wide range of cardiovascular manifestations has been described, including myocardial infarction, myocardial injury, myocarditis, arrhythmias, and venous thromboembolism, usually associated with pulmonary lesions [7].

Growing data are being published on cardiovascular manifestations of COVID-19 in children, particularly about

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Kawasaki disease (KD)-like and multisystem inflammatory syndrome in children (MIS-C); however, many questions are open to the possible link between these entities and the virus. Recently, a high number of KD-like cases were reported in areas with a high rate of SARS-CoV-2 infection in countries, such as Italy, the UK, and France [4, 8–10]. The subjects were older than typical KD patients, with a globally severe clinical presentation, ventricular dysfunction, shock, and tendency to hyperinflammation. Verdoni et al [9] described *coronary* dilations in 2 of the 10 children, and Riphagen et al [11] recorded the *presence* of brightness of coronaries in most of the children and the development of giant aneurysm in 1 of the 8 patients. While Italian researchers found positive nasopharyngeal swabs for SARS-CoV-2 in 2 of the 10 patients and positive serology in 8 of the 10 patients, English researchers found that all patients negative for SARS-CoV-2 on bronchoalveolar lavage or nasopharyngeal aspirates, but 2 of the 8 patients were positive after discharge.

This paper describes the spectrum of cardiovascular manifestations in children at the peak of COVID-19 outbreak in the Italian region of Emilia-Romagna to provide an early characterization of the manifestations, clinical course, echocardiographic features, treatment, and outcomes.

METHODS

We performed a cross-sectional multicenter study, including patients aged from 0 to 17 years diagnosed with KD, myocarditis, and MIS-C from February 2020 to April 2020. KD was diagnosed in 4 pediatric departments located in Emilia-Romagna (Bologna, Rimini, Modena, and Piacenza); myocarditis and MIS-C were diagnosed in Bologna Hospital, a tertiary referral Hospital.

KD diagnoses were made according to the 2017 American Heart Association (AHA) guidelines [1], distinguishing between complete and incomplete/atypical forms of clinical presentation. The onset of illness was defined as the first day of fever.

All patients were given the standard treatment (immunoglobulins [IVIG] at 2 g/Kg in a single infusion within the tenth day with aspirin at 30-50 mg/Kg/day, subsequently switched to 3-5 mg/Kg/day, once the patient became afebrile for at least 48 hours).

IVIG resistance was defined as persistent/recrudescent fever for at least 36 hours but not longer than 7 days after the completion of the first IVIG infusion. In the case of IVIG unresponsiveness, a second dose of IVIG was administered, as recommended [12]. Intravenous 2 mg/Kg/day methylprednisolone was administered in children with persistent fever at least 36 hours after the completion of the second IVIG dose, according to the RAISE study [13].

Myocarditis was diagnosed according to the European Society of Cardiology (ESC) criteria [14], if more than one clinical and more than one diagnostic criteria were met. Clinical findings included: acute chest pain; new onset or worsening of dyspnea and/or fatigue; palpitation, unexplained arrhythmia symptoms, syncope, aborted sudden cardiac death; and unexplained cardiogenic shock. Diagnostic criteria included laboratory and instrumental noninvasive investigations (myocardial injury markers, ECG/Holter/stress tests, echocardiogram, coronary angiography, and magnetic resonance imaging [MRI]). Children diagnosed with myocarditis were given 2 g/kg IVIG and vasoactive agents, when necessary.

MIS-C was defined according to the WHO criteria, including clinical, laboratory, and microbiological features, in patients with evidence of SARS-CoV-2 infection or likely in contact with confirmed cases [15].

We divided patients into 3 groups: group 1, diagnosed with KD; group 2, diagnosed with myocarditis; and group 3, diagnosed with MIS-C.

For each patient, demographic and clinical features, laboratory values, microbiological analysis, and radiological examinations were recorded.

Laboratory data included complete blood cell count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), interleukin (IL)-6, ferritin, fibrinogen, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), troponin-I (cTnI), and B-type natriuretic peptide (BNP).

Patients diagnosed with KD in Bologna were tested for immunoglobulin levels (IgG, IgA, and IgM) using the turbidimetric method and lymphocyte immunophenotyping: $CD3^+$ (PAN-T), $CD3^+CD4^+$ (T-helper cells), $CD3^+CD8^+$ (T-cytotoxic cells), $CD19^+$ (PAN-B), and $CD16^+56^+$ (NK cells) using multiparametric flow cytometry, Tumor Necrosis Factor (TNF) alfa, IL-8, IL-12 p70, IL-10, and IL-6. Immunological analyses and main laboratory tests from patients diagnosed with KD in 2020 in Bologna were compared with those from patients diagnosed with KD between 2016 and 2019. Demographic data, IVIG responsiveness, and incidence of coronary lesions (CALs) were compared with KD patients of our historic cohort described elsewhere [16].

Lymphocyte subsets of MIS-C patients were compared with patients diagnosed with KD between 2016 and 2019.

A nasopharyngeal swab specimen was collected from each patient. Swabs were placed in 3 mL viral transport media and then transported to the laboratory.

Common respiratory viruses (adenovirus, influenza virus A/B, parainfluenza virus, parvovirus B19 [PB19], and respiratory syncytial virus) and atypical bacteria (*Mycoplasma pneumoniae* and *Chlamydia pneumoniae*) were tested by Polymerase chain reaction (PCR) or real-time PCR (RT-PCR).

Until February 29, 2020, when the first case of COVID-19 was recorded in Emilia-Romagna, samples were not routinely tested for SARS-CoV-2 if other microorganisms were detected.

Since then, a real-time PCR assay is performed on nasopharyngeal samples to detect SARS-CoV-2 nucleic acid.

Ten patients were tested with serological assays for SARS-CoV-2. Commercially available chemiluminescent-immunoassays for the detection of SARS-CoV-2-specific IgG and IgM antibodies (iFlash-SARS-CoV-2 IgG and IgM, Yhlo Biotech, Shenzhen, China) were performed on a fully automated iFlash Immunoassay Analyzer (Yhlo Biotech, Shenzhen, China). The assays were performed according to the manufacturer's protocols. The IgG and IgM titers were automatically calculated as arbitrary units (AU/mL), and the cutoff value for a positive test was 10 AU/mL.

All patients received a transthoracic echocardiogram to evaluate systolic function measured by ejection fraction (EF), mitral and aortic valve function, and the presence of pericardial effusion.

The diameters of coronary arteries were measured and indexed to body surface area and subsequently recorded as *z*-score. We classified coronary involvement according to the 2017 AHA criteria [12]: *z*-score < 2, normal; *z*-score between 2 and 2.5, dilation; small aneurysm when ≥2.5 and <5, medium aneurysm when ≥5 and <10, and absolute dimension <8 mm, large/giant aneurysm when ≥10, or absolute dimension ≥8 mm.

Statistical Analysis

Continuous data are presented as mean ± standard deviation (SD).

We tested the normality for each variable using the Kolmogorov-Smirnov test.

For categorical variables, the percentage of patients in each category was calculated and compared with chi-square or Fisher's exact test, when appropriate.

The 2 groups were compared running a 2-tailed independent-samples *t*-test. Levene's test was used to assess the equality of variances for the considered variables. $P < .05$ was considered statistically significant. The study analysis was performed using SPSS V26 for Macintosh.

Declarations

There was no funding source. The study was approved by the local Ethics Committee (approval numbers 340/2017/O/Oss and 98/2016/O/sper). Declaration of Helsinki was fulfilled. Informed consent was collected from each patient. The authors declare that there is no conflict of interest regarding the publication of this article.

RESULTS

Demographic, clinical, laboratory and imaging, echocardiographic data, and therapy of all patients are presented in Table 1. All patients were previously healthy. Eight patients were diagnosed with KD (group 1), 1 with myocarditis (group 2), and 4 with MIS-C (group 3).

Group 1

Eight patients (patients 1 to 8 in Table 1) were diagnosed with KD: 5 of the 8 (62.5%) children showed complete presentation. Exanthema and erythema of oral mucosa and lips were the most common clinical manifestations in incomplete forms (3/4 patients, 75%).

All children were given standard treatment. One patient (Pt 3) was IVIG nonresponder (1/8, 12.5%).

In the cohort CRP, ESR, ferritin, and fibrinogen values were high (mean + SD, respectively: 17.16 ± 11.95, normal <0.5 mg/dL; 66 ± 31.94, normal <11 mm/h; 142 ± 72, normal 24–336 ng/mL; and 574.57 ± 159.49, normal 150–400 mg/dL). IL-6 was elevated when tested (Pts 1, 2, 3, and 4; 193.9 ± 206 pg/mL normal <5.9 pg/mL).

All children tested for SARS-CoV-2 and resulted negative. Chest imaging was performed on 2 patients: Pt 3 showed findings consistent with pneumonia and Pt 5 with bronchitis.

Coronary aneurysms were detected in 3 of the 8 patients (Pts 1, 3, and 4; 37.5%). All CALs regressed by the third week after the onset. These data were confirmed at 6-weeks follow-up echocardiography. ECG showed no abnormalities in 8 of the 8 patients (100%).

Compared with our historic regional cohort of KD, age (48.08 ± 34.28 vs 32.8 ± 27.3 months, $P > .05$), the percentages of IVIG responders and CALs were not significantly different (respectively, 87.5% vs 72.9% and 37.5% vs 22.57%, $P > .05$). Comparing laboratory, cytokines, and immunological features of the KD patients diagnosed in Bologna in 2020 with those diagnosed in Bologna from 2016 to 2019 (Table 2), significantly lower ESR and higher $CD3^+CD8^+$ percentage were observed in the KD group diagnosed in 2020, while cytokines were comparable.

Group 2

Myocarditis was diagnosed in a 5-year-old girl (Pt 9), admitted for repeated syncope and abdominal pain, presenting with mild arterial hypotension. She required inotropic support.

BNP and cTnI were elevated. Echocardiography findings are presented in Table 1.

Microbiological analysis revealed: positive PB19 DNA PCR (18 378 DNA copies/mL), positive PCR analysis for influenza A and *M. pneumoniae* on nasopharyngeal swab, and IgM and IgG for *M. pneumoniae*.

ECG showed low QRS voltage, nonspecific ST segment-T wave abnormalities. After therapy (Table 1), cTnI levels rapidly normalized dropping from 1057.60 ng/L on day 4 from the onset of symptoms to 23.40 on day 13. Cardiac MRI 14 days after the onset of symptoms showed normal biventricular diameters, volumes, and systolic function.T2-mapping sequences showed mild myocardial interstitial transmural edema. T1 mapping showed increased values, which confirmed the presence of edema.

Table 1. Demographic, Clinical Data, Treatment, Laboratory and Microbiological Data, Chest Imaging Features, and Echocardiographic Findings of All Patients.

Patient No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Sex	F	M	M	F	M	M	F	M	F	M	M	M	M
Age (m)	93	54	43	83	14	41	12	72	68	106	78	77	134
Diagnosis	Incomplete KD	Incomplete KD	Complete KD	Complete KD	Complete KD	Incomplete KD	Complete KD	Complete KD	Myocarditis	Multisystem inflammatory syndrome	Multisystem inflammatory syndrome	Multisystem inflammatory syndrome	Multisystem inflammatory syndrome
IVIG administration (+ days from the onset of fever)	10	10	9	8	4	9	7	5	5	7	4	6	–
IVIG responder	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	–	Yes	–	–	–
Therapy	IVIG, ASA	IVIG, ASA	IGIV, ASA, steroids	IVIG, ASA	IVIG, ASA	IVIG, ASA	IVIG, ASA	IVIG, vasopressors, diuretics, ASA, steroids	IVIG, vasopressors, diuretics, ibuprofen, colchicine	IGIV, ASA, steroids	IVIG, vasopressors, diuretics, ASA, enoxaparin	IVIG, vasopressors, diuretics, steroids, ASA, hydroxychloroquine, enoxaparin	Steroids
Chest imaging	Not performed	Not performed	Left consolidation	Not performed	Bronchitis	Not performed	Not performed	Not performed	Mild right pleural effusion	Bilateral consolidations, ground-glass opacities, and pleural effusion	Bilateral consolidations and mild bilateral pleural effusion	Bilateral consolidations and pleural effusion	Bronchitis
WBC (×10^9/L)	5.42	11.26	23.64	11.58	40.71	10.97	16.15	16.26	6.58	6.8	13.19	4.21	12.76
N (%)	54.8	60.8	90.2	83.7	42.1	56.8	52.2	81	63.1	90	79.8	69.6	81
L (%)	36.2	32.6	7.2	11.7	47.2	29.4	36.4	8	30.2	5	10.3	22.3	10.6
Hb (g/dL)	10.8	10	11.1	11.5	11.2	9.9	9.7	11.2	12.4	10.90	10.1	9.8	10.3
PLT (×10^9/L)	134.0	459	390	194	607	140	1,015	354	186	183	343	108	527
CRP (mg/dL)	4.18	12.72	24.84	39.40	22.00	3.40	19.7	11	0.56	23	19.79	23.52	8.5
ESR (mm/h)	33	50	38	74	–	103	50	114	–	–	59	–	58
BNP (pg/mL)	17	96	711	383	–	–	–	–	370	11 800	1170	18	104
IL-6 (pg/mL)	22.3	37.3	259	457	–	–	–	–	–	–	130	358	20
Ferritin (ng/mL)	197	–	169	–	60	–	–	–	167	940	148	1515	470
Fibrinogen (mg/dL)	484	495	468	657	398	–	660	860	–	630	621	289	520
Triglycerides (mg/dL)	196	143	113	127	–	–	–	140	–	231	150	295	233
ALT (U/L)	10	12	10	83	12	5	19	–	10	24	45	371	36
AST (U/L)	24	25	38	72	34	31	29	68	38	17	13	492	26
LMCA, *z*-score	3.01	<2	2.50	<2	<2	<2	<2	<2	<2	<2	<2	2.38	<2
LAD, *z*-score	<2	<2	2.50	<2	<2	<2	<2	<2	<2	2.26	<2	3.90	<2
Cx, *z*-score	2.2	<2	2.30	<2	<2	<2	<2	<2	<2	<2	<2	3	<2
RAD, *z*-score	2.9	<2	<2	2.50	<2	<2	<2	<2	<2	<2	<2	3.80	<2
Pericardial effusion	No	No	Yes	No	No	No	No	No	Yes	No	No	Yes	<2
Valve regurgitation	No	Yes	Yes	No	No	No	No	No	No	No	No	No	Yes
FE (%)	>55	>55	>55	>55	>55	>55	>55	>55	54	40	>52	45	>55
COVID-19	Negative serology	Negative serology	Negative swab and serology	Negative serology	Negative serology	Negative swab	Not tested	Negative swab	Negative serology	Positive swab and serology	Positive swab and serology	Positive swab and serology	Negative swab, positive serology
Coinfections	No	No	No	Adenovirus	No	No	No	No	Parvovirus B19, influenza A, *Mycoplasma Pneumoniae*	No	Adenovirus	Parvovirus B19, parainfluenza virus 3, rhinovirus, enterovirus	No

Abbreviations: ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; BNP, B-type natriuretic peptide; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FE, ejection fraction; IL-6, interleukin-6; IVIG, immunoglobulins; KD, Kawasaki disease; LAD: left anterior descending artery; LMCA, left main coronary artery; RAD, right coronary artery; WBC, white blood cells.

Table 2. Comparison of Laboratory Values Including Cytokines and Immunological Workup in Patients Diagnosed With KD In Sant'orsola Hospital in 2020 and 2016–2019

	Normal Values	2020 Patients	2016-2019 Patients	*P*-value[a] (2020 vs 2016-2019)
Number of patients		5	21	
AST (U/L)	<60	38.6 ± 19.59	42.38 ± 23.26	n.s.
ALT (U/L)	<45	25.40 ± 32.21	41.38 ± 45.20	n.s.
Albumin (g/L)	35-50	35.60 ± 4.46	35.42 ± 4.63	n.s.
Na^+ (mmol/L)	136-145	136.00 ± 2.35	134.52 ± 3.52	n.s.
CRP (mg/dL)	<0.5	17.76 ± 13.80	11.53 ± 7.76	n.s.
ESR (mm/h)	<11	48.00 ± 15.92	85.90 ± 34.47	**0.038**
WBC (/mmc)	4.8-12	17.26 ± 12.95	15.35 ± 5.36	n.s.
N (%)	33-74[a]	64.73 ± 18.43	72.08 ± 11.59	n.s.
L (%)	22-51	27.38 ± 15.20	19.44 ± 8.32	n.s.
Hb (g/dL)	11.2-14.6	10.75 ± 0.66	10.83 ± 1.23	n.s.
PLT (/mmc)	180-415	320.67 ± 194.66	401.48 ± 141.47	n.s.
IL 1beta (pg/mL)	<6.7 (adults)	7.50 ± 13.67	55.21 ± 107.72	n.s.
TNF alpha (pg/mL)	<8.1 (adults)	4.00 ± 8	11.53 ± 22.29	n.s.
IL-6 (pg/mL)	<5.9	193.90 ± 206.1	427.64 ± 893.95	n.s.
IL-8 (pg/mL)	<70 (adults)	3271.75 ± 6510.17	8217.21 ± 11 971.61	n.s.
IL-12 p70 (pg/mL)	<4.7 (adults)	0.50 ± 0.58	4.79 ± 15.63	n.s.
IL-10 (pg/mL)	<5.3 (adults)	3.75 ± 2.75	11.74 ± 14.28	n.s.
Lymphocytes (×10^9/L)	1.4-5.5[a]	3.40 ± 1.59	2.70 ± 1.29	n.s.
$CD3^+$ PAN T (%)	56-86[a]	63.60 ± 6.69	56.83 ± 12.51	n.s.
$CD3^+$ PAN T (×10^9/L)	0.85-4.30[a]	1890 ± 1.09	1.32 ± 1.04	n.s.
$CD3^+$ $CD4^+$ (%)	31-58[a]	37.80 ± 7.22	34.83 ± 11.08	n.s.
$CD3^+$ $CD4^+$ (×10^9/L)	0.50-2.70[a]	1.12 ± 0.73	0.84 ± 0.69	n.s.
$CD3^+$ $CD8^+$ (%)	13-39[a]	24.00 ± 5.87	18.61 ± 3.99	**0.025**
$CD3^+$ $CD8^+$ (×10^9/L)	0.2-1.8[a]	0.69 ± 0.34	0.42 ± 0.33	n.s.
$CD4^+/CD8^+$ (%)	1-2.7[a]	1.66 ± 0.46	1.95 ± 0.81	n.s.
$CD56^+$ $CD16^+$ $CD3^-$ (NK) (%)	5-26[a]	7.40 ± 4.22	9.28 ± 4.44	n.s.
$CD56^+$ $CD16^+$ $CD3^-$ (NK) (×10^9/L)	0.061-0.51[a]	0.27 ± 0.27	0.19 ± 0.14	n.s.
$CD19^+$ (PAN B) (%)	5-20[a]	27.81 ± 7.1	32.61 ± 12.21	n.s.
$CD19^+$ (PAN B) (×10^9/L)	0.18-1.30[a]	0.78 ± 0.39	0.67 ± 0.53	n.s.
IgG (mg/dL)	528-1959[a]	960.20 ± 105.65	880.20 ± 333.10	n.s.
IgA (mg/dL)	37-257[a]	127.20 ± 50.97	107.27 ± 78.60	n.s.
IgM (mg/dL)	49-292[a]	112.20 ± 44.35	104.90 ± 44.60	n.s.

Continuous values are expressed as % or mean +– standard deviation. Bold values indicate the values that reach the statistical significance for being less than 0.05.
Abbreviations: ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; BNP, B-type natriuretic peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IgG, IgA, IgM, IVIG, immunoglobulins; IL, interleukin; KD, Kawasaki disease; WBC, white blood cells.
[a]age dependent; n.s., not significant.

Group 3

MIS-C was diagnosed in 3 boys (2 Caucasian, Pt 10 and Pt 11, and 1 African, Pt 12) with nasopharyngeal swab positive for SARS-CoV2 and in 1 Caucasian boy (Pt 13) with positive IgG and negative nasopharyngeal swab for SARS-CoV-2.

Pt 10 presented with oral mucositis, non-exudative conjunctivitis and exanthema, profuse diarrhea, and shock. His chest imaging was consistent with bilateral consolidations, ground-glass opacities, and pleural effusion. Echocardiogram showed a mildly reduced Left Ventricle (LV) function that normalized after 48 hours. He developed CALs that regressed by the third week from the onset. He showed an increase in cardiac markers and D-dimer (BNP 11 800 pg/mL, troponin 59 ng/L, and D-dimer 25 mg/L FEU).

Pt 11 presented with high-peak fever and cutaneous rash in the trunk and proximal limbs. ECG documented nonspecific ST segment-T wave abnormalities and T wave inversion. Aspirin was started because of thrombocytosis on the sixth day after onset. Adenovirus was detected on the nasopharyngeal swab. On day 4 from the onset of symptoms, his troponin level was 3351.50 ng/L and rapidly decreased during the following days (356.9 ng/L on day 7, 68.4 ng/L on day 10, and 19.4 ng/L on day 13). LV ejection function (EF) was 52% at admission and 65% at 48 hours later.

Pt 12 presented with high-peak fever and sore throat. In addition to myocardial dysfunction and shock and pericardial effusion, he developed multi-coronary aneurysms, bilateral pneumonia with pleural effusion, and laboratory test consistent with hyperinflammation. Microbiological analyses showed rhinovirus, enterovirus, human parainfluenza virus 3 on nasopharyngeal swab, and PB19 DNA PCR in serum (14 298 couples/mL on admission). On day 4 from the onset of symptoms, his

troponin level was 98.30 ng/L and dropped to 28.2 ng/L on day 10 and 16.3 ng/L on day 13. Therapy is reported in Table 1. Acetylsalicylic acid (ASA) was added when CALs were documented. Echographic monitoring showed normalization of ventricular function on day 7, as well as CALs.

Cardiac MRI 3 weeks after the onset of fever showed normal biventricular volumes and systolic function. T2-mapping sequences showed mild/trivial myocardial interstitial edema. Phase-sensitive inversion recovery sequences showed the absence of late gadolinium.

Pt 13 presented with high-peak fever, cutaneous macular rash on extremities and bilateral conjunctivitis, abdominal pain, and diarrhea. Antistreptolysin-O titer (509 U/mL, normal <200 U/mL) and D-dimer (5.37 mg/L FEU, normal <0.55) were elevated, and BNP was normal. Echocardiography documented moderate mitral-aortic regurgitation and normal biventricular function without pericardial effusion. Microbiological tests for other agents were negative. The patient was started on antibiotics, and methylprednisolone and valvular injuries normalized after 3 days. At the 4-week cardiological follow-up, he did not show any valvular sequelae.

Comparison of lymphocyte immunophenotyping of MIS-C patients with KD patients showed no significant difference (Table 3).

At the 4 months cardiological follow-up, all patients showed normal EF, valve function, and coronary arteries.

DISCUSSION

In the current COVID-19 scenario, a wide variability of manifestations has been reported, probably due to the systemic nature of SARS-CoV-2 infection and its physiopathological mechanisms.

We report a spectrum of cardiovascular manifestations in children during the high peak period of the outbreak of the COVID-19 from February 1 to April 30 in the Italian region of Emilia-Romagna, which has been deeply affected by SARS-CoV-2.

In our experience, KD and MIS-C, despite presenting overlapping features and manifestations, were distinguishable.

In adults, cardiovascular manifestations increase COVID-19 mortality when associated with pneumonia [17]. Despite the fact that children and adolescents seem to be less affected by COVID-19 and usually with milder disease severity compared with adults, both in China and in the United States [18], a recent new alert has risen concerning an outbreak of severe KD-like disease [9], hyperinflammatory shock syndrome [11], and KD [4, 10], describing clusters of children and adolescents presenting with fever and clinical manifestations KD-like and shock, requiring intensive care support [19]. However, despite an increase of reported cases diagnosed as KD, it is likely that many of them might be experiencing MIS-C, given the outbreak of COVID-19 ongoing at the time.

The wide spectrum of COVID-19 manifestations can be explained by the distribution of SARS-CoV-2 site of entry, ACE2 [6]. ACE2 is highly expressed not only in type-2 lung alveolar cells [20] but also in the intestinal epithelium, kidneys, skin, and immune organs [6]. The myocardial damage may be secondary to 2 mechanisms: direct cardiotoxicity since ACE2 is present in more than 7.5% of myocardiocytes [21] and indirect injury through a cytokine storm and the subsequent release of proinflammatory cytokines [22] that depress myocardial function. ACE2 is also expressed on the vascular endothelium of veins and arteries such as coronary arteries [6] explaining vasoplegia and coronary involvement.

Since February 1, 2020, 8 cases of KD in a 3-month period were reported in the Italian region of Emilia-Romagna, while, previously, about 14–15 new cases of KD per year were reported. Although the method of collecting data is retrospective, thus data may be missing, it seems that during the first months of the current year more diagnoses have been made compared with previous years during the same time period. However, the "temporal clusters" and seasonality of KD make it difficult to compare the incidence of KD in the COVID-19 period with previous years. In our experience, the disease did not present

Table 3. Comparison of Lymphocyte Subsets in Patients Diagnosed With MIS-C Compared With Patients Diagnosed With KD in Sant'orsola Hospital in 2020 and 2016–2019

	Normal Values	MIS-C	2016-2019 KD Patients	*P*-value[a] (2020 vs 2016-2019)
Number of patients		4	21	
White blood cells (×10^9/L)	4.8-12	10.88 ± 4.12	15.34 ± 5.35	n.s.
Lymphocytes (×10^9/L)	1.4-5.5[a]	1.51 ± 1.12	2.70 ± 1.29	n.s.
$CD3^+$ PAN T (%)	56-86[a]	57.33 ± 5.51	56.83 ± 12.51	n.s.
$CD3^+$ $CD4^+$ (%)	31-58[a]	32.93 ± 5.51	34.83 ± 11.08	n.s.
$CD3^+$ $CD8^+$ (%)	13-39[a]	20.78 ± 4.04	18.61 ± 3.99	n.s.
$CD4^+/CD8^+$ (%)	1-2.7[a]	1.61 ± 0.43	1.95 ± 0.81	n.s.
$CD56^+$ $CD16^+$ $CD3^-$ (NK) (%)	5-26[a]	5.86 ± 2.08	9.28 ± 4.44	n.s.
$CD19^+$ (PAN B) (%)	5-20[a]	39.12 ± 6.97	32.61 ± 12.21	n.s.

Continuous values are expressed as % or mean +– standard deviation.
Abbreviations: KD, Kawasaki disease; MIS-C, multisystem inflammatory syndrome in children.
[a]age dependent; n.s., not significant.

a peculiar and more aggressive form: at the onset, classical features of KD with complete presentation were present in more than half of the children.

Compared with our historic cohort [23], KD patients were older but not statistically significant, and similarly, they presented a male prevalence (2:1 vs 1.4:1) and were mostly IVIG responders (8/9, 88.9% vs 214/257, 83.3%, $P > .05$). Although coronary abnormalities were frequent (4/9, 44.4%), they were not severe (3 mild aneurysms and 1 dilation) and all regressed 3 weeks from the onset. In addition, the incidence was not different compared with previous years ($P > .05$).

Comparing children diagnosed with KD in Bologna in 2020 to those diagnosed in 2016–2019 (Table 2), white blood cells (WBC), neutrophils and lymphocytes, Platelets (PLT), CRP, AST, ALT, and albumin and sodium levels were not different, while ESR was significantly lower in the 2020-diagnosed KDs. Regarding the cytokines panel in the KD patients diagnosed during the SARS-CoV-2 pandemic, all including IL-6 were comparable with those of the previous years.

Moreover, an increased percentage of $CD3^+CD8^+$ cells was observed in 2020 KDs compared with 2016–2019 KD. The differences in ESR and $CD3^+CD8^+$ cells may be due to the small number of 2020 KD patients and, therefore, needs to be taken carefully and, hopefully, to be confirmed in further studies. In addition, oligoclonal IgA B-lymphocytes were identified driving the hypothesis that the immune system activation could be triggered by an intracellular respiratory pathogen. Furthermore, a higher proportion of $CD8^+$ T cells activation correlates with a worse response to IVIG treatment [16]; thus, the ratio of $CD8^+$ HLA-DR^+ T cells/$CD8^+$ $CD69^+$ T cells may be used as a predictor of IVIG sensitivity/severe course disease.

Many reports suggest SARS-CoV-2 as a cause of myocarditis in adult patients. Huang et al [2] first reported that 12% of adult patients with COVID-19 suffered from an acute myocardial injury with an elevation of cTnI. Sala et al [24] performed an endomyocardial biopsy in a COVID-19 patient, revealing diffuse T-lymphocytic infiltrates, interstitial edema, and limited focal necrosis without evidence of SARS-CoV-2 genome.

At the time of writing, since the beginning of 2020, the reported case of the infective myocarditis is the only one diagnosed in our center. Notably, our patient was positive for PB19 and negative for SARS-CoV-2. PB19 represents a well-described etiology of viral myocarditis in children, usually characterized by a fulminant course with high rates of both morbidity and mortality [25]. On the contrary, our patient was admitted with a very mild systolic dysfunction but shock and abdominal pain and rapidly responded to IVIG, fluid restoration, and mild vasoactive support.

The cases with MIS-C were all SARS-CoV-2 positive. The 3 patients with myocarditis and systemic shock dramatically responded to treatment. One of them received hydroxychloroquine as targeted therapy for viral infection, and both received IVIG and enoxaparin. Notably, ventricular function, CALs, and pulmonary lesions normalized without sequelae. It is worthy to note that troponin rapidly decreased according to the clinical and echographic improvement, potentially suggesting for the myocardial damage, a mechanism of stunning due to cytokine storm rather than direct virus-related damage.

The lack of necrosis at the cardiac MRI and the rapid improvement of ventricular function and cardiac enzymes after IVIG infusion could support the global inflammatory nature of the myocarditis rather than severe direct myocardial insult. The patient presenting with valvulitis responded to steroids within 72 hours with complete regression of valvular injuries, confirmed at 1-month follow-up.

Consistently with the normalization of cardiac injuries during the hospitalization, the cardiac features were normal in all MIS-C patients at the 4-months follow-up.

Previous works [8–10, 26, 27] differently report the demonstration of SARS-Cov-2 infection in patients with MIS-C and KD-like forms.

We documented current or prior SARS-Cov-2 infection in all children with MIS-C (3 of which with a positive swab), potentially supporting a potential physiopathogenetic link with the infection.

Notably, coinfections were frequent: 4 of the 13 children tested positive for at least 1 virus. The child with MIS-C and positive PB19 PCR had a more pronounced ventricular dysfunction that rapidly resolved without necrosis on MRI: the concomitant infection by PB19 and SARS-CoV-19 might have maintained myocardial inflammation and consequently the myocardial depression.

Conflictual data are reported about coinfections in COVID-19 children: Chinese researchers report coinfections with common respiratory agents in 46% of screened SARS-CoV-2 children [28], UK researchers isolated adenovirus and enterovirus in 1 of the 8 children [8], and none had coinfections when tested in the French study [10]. All considering, we support the need for SARS-CoV-2 screening during the peak season for respiratory infections.

To date, there is not a standard treatment for MIS-C, and conflictual data are reported for myocarditis. IVIG has an anti-inflammatory effect in different ways, such as acting on macrophages and adhesion molecules to vascular endothelium, containing antibodies neutralizing cytokines and activated complement proteins, and influencing T-regulatory cells [29]. They represent the standard treatment in KD, while current evidence still does not support their routine use in myocarditis [14], despite a recent meta-analysis suggested a superiority of IVIG therapy to conventional treatment in reducing in-hospital mortality [30]. Regarding MIS-C, reported cases of children and adolescents treated with IVIG have shown a positive outcome. Although in our cohort IVIG was administered to all but one patient, a proper diagnosis

should be done to help clinicians to choose the optimal therapeutic regimen (beyond IVIG) and proper timing, to handle the potential complications of the disease, and to assess the prognosis of the patient.

All children should have a cardiological follow-up since little is known about the cardio-vascular mid- and long-term complications of COVID-19.

CONCLUSIONS

In Emilia-Romagna, one of the most affected areas by SARS-CoV-2 in Italy, KD, myocarditis, and MIS-C were cardiovascular manifestations in the COVID scenario. In our experience, KD presented classical manifestations, either complete or incomplete. Despite an outbreak of KD, our patients had negative SARS-CoV-2 serology. KD patients did not show a more aggressive form of the disease compared with KD diagnosed before the pandemic: coronary involvement was frequent but always transient. MIS-C and myocarditis, as well, responded rapidly to treatment despite the critical clinical onset, cardiac involvement, and high markers of myocardial injury. The myocardial injury could be related to systemic inflammation rather than direct cardiotoxicity since cardiac sequelae were not detected on the short-term echocardiogram and MRI. In our experience, KD and MIS-C were distinguishable entities showing, as expected, comparable laboratory tests since both are systemic inflammatory syndromes. All children should have a cardiological follow-up. The small number of cases and the limited follow-up period represent the limitations of our study. Collaborative multicenter and long-term studies are needed.

Note

Potential conflicts of interest. The authors declare that there is no conflict of interest regarding the publication of this article. All authors have submitted the ICMJE Form for Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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