In-depth cardiovascular and pulmonary assessments in children with multisystem inflammatory syndrome after SARS-CoV-2 infection: a case series study

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Abstract

Objective

To perform an in-depth assessment of cardiovascular and pulmonary outcomes in a series of 5 post discharged multisystem inflammatory syndrome (MIS-C) survivors.

Methods

Data were collected ≈1.9 month after hospital discharge at a tertiary hospital in São Paulo, Brazil. All patients (7-18 years; 3 females) fulfilled the MIS-C diagnosis according to CDC. The battery of tests included: 13 N-ammonia PET-CT imaging, standard echocardiography, brachial flow-mediated dilation using a Doppler ultrasound, cardiopulmonary exercise test, and blood markers.

Results

Upon PET-CT scans, two patients exhibited severe perfusion defect developed in the left ventricular cavity suggesting extensive myocardial ischemia, and one patient showed persistent mild pericardial effusion. Other two patients had endothelial dysfunction. All patients exhibited abnormal cardiopulmonary reserve during exercise (e.g., low VO$_{2peak}$). Three patients had abnormal values for D-dimer and fibrinogen.

Conclusion

This study reveals novel pathological findings in MIS-C patients, which may help optimize treatment protocols in this condition.

New & Noteworthy

The pathophysiology of MIS-C and its natural course remains to be elucidated. In this manuscript, we report on a broad, in-depth assessment of cardiovascular and pulmonary outcomes in a series of post discharged MIS-C survivors, using a battery of tests including PET-CT imaging, brachial flow-mediated dilation and maximal cardiopulmonary exercise assessment. Our findings reveal novel pathophysiological findings in MIS-C patients, which advances the knowledge on this newly described condition and may help tailor better treatments for these patients.

Introduction

Multisystem inflammatory syndrome in children (MIS-C) is a hyperinflammatory response that commonly develops within 2-6 weeks following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, requiring hospitalization in the acute phase (8). While multiple cohorts have shown that MIS-C
patients may present with heterogenous signs that include hemodynamic instability, tachycardia, left ventricular dysfunction, and respiratory distress, possibly primary or caused by cardiac dysfunction, (7, 13) the pathophysiology of MIS-C and its natural course are yet to be fully elucidated.

Herein we report on a broad, in-depth assessment of cardiovascular and pulmonary outcomes in a series of post discharged MIS-C survivors, which unravels novel pathological features associated with this syndrome.

**Methods**

**Study design and patients**

This case series is part of a prospective cohort study aimed at exploring the spectrum of the long-term effects of COVID-19 and MIS-C in the pediatric population (clinicaltrials.gov NCT04659486). Data of the patients’ acute phase were retrospectively assessed through medical record. The post discharge data were collected prospectively in a dedicated, multidisciplinary, outpatient clinic for COVID-19 and MIS-C at Children’ and Adolescents’ Institute of the Clinical Hospital of the University of Sao Paulo, between October 2020 to July 2021. All patients (median age: 9, range: 7-18 years; 3 females) fulfilled the MIS-C diagnosis according to the Center for Disease Control (CDC) criteria (4). Four patients had positive serologic tests (IgG) and 1 was exposed to a confirmed COVID-19 case within 4 weeks prior to the onset of symptoms. None of the patients had any preexisting pediatric chronic conditions. Patients’ main characteristics at hospital admission (during acute phase) are shown in Table 1. Four out of 5 patients were admitted to pediatric intensive care unit. Two patients required respiratory support and oxygen therapy, and 3 had vasodilatory shock. The median length stay was 12 (range: 3-18) days. Median time elapsed from discharge to the follow-up visit was 1.9 (range: 1.3-6.2) months. At the follow-up visit, we conducted a battery of assessments as follows: 13N-ammonia PET-CT imaging, standard echocardiography, brachial flow-mediated dilation (FMD) using a Doppler ultrasound, maximal cardiopulmonary exercise test, and blood markers (C-reactive protein, D-dimer, fibrinogen, and troponin T). This study was approved by Comitê de Ética e Pesquisa do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (CAPPesq), number 37460620.8.0000.0068 and are registered in the ClinicalTrials.gov (https://clinicaltrials.gov) number 37460620.8.0000.0068. Patients and guardians signed an informed consent to participate in the study.

**13N-ammonia PET-CT imaging protocol**

The 13N-ammonia was produced by means of an on-site cyclotron installed at our institution (PETtrace™ 880, GE Healthcare), by $^{16}\text{O}(p,\alpha)$ 13N. In this procedure, 13N-ammonia is synthesised directly in the target water (in-target production) by adding ethanol 5mmol as a free radical scavenger to prevent the formation of the oxo anions. The radiochemical purity was >99.9% within 60 min from the end of bombardment. Subjects were maintained in a fasting state for at least 6 hours before the study and were told not to consume methylxanthine-containing foods or beverages (coffee, chocolates, soft drinks and tea) for at least 24 hours before the PET scan.
For measurement of myocardial blood flow (MBF) at rest and at pharmacological stress (adenosine-induced hyperemia), 13N-Ammonia was administered intravenously (0.286 mCi/kg) over a 10-sec period, the intravenous line was flushed with additional saline over a 10-sec interval and standardized imaging protocols were performed according to the American Society of Nuclear Cardiology guidelines (2). Stress imaging was performed identically, after adenosine infusion over 6 minutes (0.142 mg/min/kg). The myocardial perfusion radiopharmaceutical was injected about halfway into the adenosine infusion (at 3 minutes), when maximal vasodilatation and myocardial hyperemia were assumed to occur. MBF was expressed as mL/g/min.

The quantitative PET datasets were fused with CT using commercially available software (CardIQ Fusion, GE Healthcare). Quantitative MBF and myocardial flow reserve (MFR) was determined using the PMOD™ software package, version 3.4002 (PMOD Technologies LLC; Zurich, Switzerland). Myocardial and blood-pool time-activity curves (TAC) were obtained from dynamic frames corrected for radioisotope decay. Segmental MBF was measured in each phase (rest and stress adenosine) by the model fitting of the blood pool and myocardial TACs, corrected for spill-over and partial volume. MFR was calculated as the ratio of stress MBF over the rest MBF (the 17-segment model according to the American Society of Nuclear Cardiology recommendations). For each left ventricle (LV) segment (see figure 2), MFR at right coronary artery (RCA), left circumflex artery (LCX), left anterior descending (LAD) and MFR global were considered abnormal when < 2, in gray zone when between 2 and 2.5 and normal when > 2.5 (5) (see Figure 1 and figure 2 for illustrative exams).

**Standard echocardiography**

Standard transthoracic echocardiography was performed according to the recommendations of the American Society of Echocardiography (12). Cardiac chamber dimensions were obtained using two-dimensional mode and left ventricle ejection fraction (LVEF) was calculated by Simpson's method (normal LV EF ≥ 55%) (12). The z-score values of cardiac chambers were calculated according to Lopez et al (normal values between 2 and +2). The equipment used was a Philips Affiniti 70 (Andover, MA 01810 USA), with multifrequency transducers (S 5-1 and S 8-3 MHz).

**Brachial flow-mediated dilation**

FMD was evaluated according to current guidelines (15) using a high-resolution Doppler ultrasound machine (LOGIQ e PRO – GE Healthcare, Chicago, IL, US) equipped with a 4.0–12.0 MHz linear transducer. Initially, participants were positioned in the supine position with their right arm extended at an angle of ~80° from the torso. Longitudinal images of the brachial artery diameter were taken using the B-mode ultrasound, and simultaneous pulse-waved Doppler blood flow velocity was obtained using a 60° intonation angle with the sample volume placed in mid-artery and aligned with the blood flow. Initially, a 1-min baseline recording of the brachial artery diameter and blood flow velocity was performed. Then, the ischemic stimulus was performed by inflating a cuff placed in the forearm to 60 mmHg above the patient's resting systolic pressure for 5 minutes. Recordings were resumed 30 seconds before cuff deflation and continued for 3 minutes thereafter. Brachial artery diameter and shear rate (4 x mean blood
velocity/internal diameter) were analyzed by a blinded evaluator using a semi-automatic edge-detection and wall-tracking software (Cardiovascular Suite, Quipu®, Pisa, Italy). FMD was calculated as the percentage change of the brachial artery diameter after cuff release in relation to baseline brachial artery diameter \[ \text{FMD} = \left( \frac{\text{baseline diameter} - \text{peak diameter}}{\text{baseline diameter}} \right) \times 100 \]. To describe the relevant shear rate stimulus for FMD, we also calculated the area-under-the-curve of the shear rate up to the peak diameter (SRAUC). FMD lower than the age- and sex-specific 25th percentile(10) was considered as suggestive of endothelial dysfunction.

**Cardiopulmonary exercise test**

A symptom-limited maximal cardiopulmonary exercise test was carried out on treadmill (Centurion model 300; Micromed, Brazil) using a ramp protocol test at a controlled room temperature (21-23°C). Peak oxygen consumption (VO$_{2peak}$), oxygen consumption at ventilatory anaerobic threshold (VO$_{2VAT}$), oxygen uptake efficiency slope (OUES), heart rate-oxygen consumption relationship (HR/VO$_2$ slope), oxygen pulse at peak of exercise (O$_2$ pulse peak), V$_E$/VCO$_2$ slope were measured breath-by-breath through a computerized system (MetaLyzer 3B; Cortex, Germany). One patient (P4) was prohibited to perform the test by the cardiologist because she had lower % LVEF and persistent discrete pericardial effusion. Reference values from healthy children sorted by age and sex were used for identifying abnormal exercise capacity (3, 11).

**Results**

The main findings can be seen in Table 1. P1 and P4 exhibited homogeneous rest but heterogeneous stress perfusion with perfusion defects developed in the slightly dilated left ventricular cavity, suggesting stress-induced myocardial ischemia associated with MFR lower than 2.0 (LAD: 1.2; RCA: 2.0; and LCX: 2.1 and LAD: 1.9; RCA: 1.4; and LCX: 2.0) respectively, see P4 in Figure 1B and Figure 2B.

All patients showed signs suggesting normal coronary arteries (all score-z ≥2.5) (12) by standard echocardiogram at post discharge, except for one patient (P4).

FMD assessment was not completed in one participant (P2) who experienced significant discomfort during the procedure. Of the remaining 4 participants, mean (SD) FMD% was 6.38±3.41. Participants P1 and P4 presented with preserved FMD, while participants P3 and P5 had reduced FMD suggestive of endothelial dysfunction.

Mean (SD) VO$_{2peak}$ was 26.3±8.4 mL/kg/min. All patients showed abnormal VO$_{2peak}$, with lower predicted values (range: 35.2 to 64.5%). Similarly, all patients had lower predicted values for VO$_{2VAT}$ (range: 15.6 to 38.2%), OUES (range: 1.0 to 1.3 L/min) and O$_2$ Pulse (range: 4 to 7 ml/beat). A ventilatory inefficiency was also identified, considering the mean (SD) value of V$_E$/VCO$_2$ Slope 34.8±4.8 units (11). Collectively, these findings indicate an impairment in cardiorespiratory and oxidative metabolism during physical exercise.
P1, P2 and P4 had abnormal values for D-dimer and fibrinogen, respectively. The other parameters were within normal range.

**Discussion**

This study reveals novel pathological findings in MIS-C patients, which may help optimize treatment protocols in this condition. P1 and P4 exhibited impaired MFR, whereas P3 and P5 showed reduced endothelial function. All patients showed dysfunctional cardiorespiratory responses to a maximal exercise test.

To our knowledge, this is the first study to investigate myocardial perfusion and blood flow by PET imaging in a case series of MIS-C. This robust technique has been considered useful in clinical decision making for patients with suspected coronary artery disease, as it can detect multivessel ischemia that could otherwise appear as normal on stress imaging if ischemia is global and balanced among all coronary territories (14). The ratio of MBF at stress over rest is labelled myocardial flow reserve (MFR). It is primarily controlled by the release of local metabolites such as adenosine or nitric oxide. As the heart has minimal ability to increase oxygen extraction and to rely on anaerobic metabolism, increased metabolic demands of the heart are met primarily via increases in coronary blood flow. In the absence of obstructive epicardial coronary artery disease, as it was the case of our patients presumably, coronary blood flow is primarily controlled by changes in resistance in the small arteries and arterioles (i.e., microvasculature), which play an important role in myocardial perfusion in general in regional and transmural distribution. Herein 2 patients showed abnormal MFR, which could be a consequence of coronary microvascular dysfunction, resulting from vasomotor dysregulation or endothelial dysfunction of the small coronary arterioles. In fact, our data add to post-mortem evidence suggesting that coronary microvascular involvement appears to comprise COVID-19/MIS-C pathophysiology (6). Of relevance, we also observed brachial endothelial dysfunction (as assessed by FMD) in 2 other patients different from those with abnormal findings upon PET imaging, suggesting that vascular involvement is not restricted to microvasculature in MIS-C. Collectively, the present results may be of clinical relevance since vascular dysfunction is a potentially reversible condition that is associated with future cardiovascular events (9).

Another striking finding was the abnormal cardiorespiratory response during exercise. Some metrics of impaired oxidative metabolism (e.g., lower VO$_{2\text{VAT}}$ and OUES) and ventilatory inefficiency (e.g., higher VEVO$_{2}$ slope) were below normal values for all patients. Also, all patients showed lower VO$_{2\text{peak}}$, which is an independent risk factor associated with poor prognosis in several diseases and all-cause mortality in general population (1). Rehabilitating cardiopulmonary capacity emerges as a potential therapeutic goal in MIS-C to prevent any cardiac events, improve patients’ fitness and restore performance in daily living activities.

This study has limitations. First, given the paucity of 13N-ammonia PET/CT studies and large normal database in children, the arbitrary threshold limit (i.e., 2.5 mL/g/min) used to separate normal from abnormal MBF has not been yet validated in the pediatric population. Second, the low number of patients
enrolled, and the lack of a control group without MIS-C and the longitudinal assessments preclude any causative inferences and insights on natural course of the syndrome. Therefore, studies assessing the frequency, predictors, clinical repercussion, and mechanisms of the cardiovascular and pulmonary findings described herein are warranted.

In conclusion, we reported on novel pathophysiological cardiovascular and pulmonary findings in MIS-C patients, which advances the knowledge on this newly described condition and may help tailor better treatments for these patients. In-depth investigation using 13N-ammonia PET-CT imaging, brachial FMD, and cardiopulmonary exercise testing provides supplementary information that might be helpful in clinical decision making in MIS-C care.

Declarations

Conflict of interest

All authors declare no conflict of interest.

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**Table 1**
| Patient's characteristics | Mean (SD), median (range) or N (%) | P1 | P2 | P3 | P4 | P5 |
|--------------------------|-----------------------------------|----|----|----|----|----|
| Sex (female)             | 3 (60)                            | Female | Male | Female | Female | Male |
| Age (years)              | 10.2 (3.56)                       | 16 | 7 | 9 | 8 | 11 |
| Previously medical history| 0 (0)                             | None | None | None | None | None |
| BMI (kg·m⁻²)             | 20.1 (3.51)                       | 24.7 | 21.2 | 18.3 | 21.1 | 15.3 |
| Height (cm)              | 140 (0.11)                        | 156 | 126 | 145 | 135 | 138 |
| Weight (kg)              | 40.0 (11.9)                       | 60.3 | 33.7 | 38.6 | 38.4 | 29.3 |
| Signs and symptoms at admission |             |     |     |     |     |     |
| Fever (days)             | 7.60 (4.72)                       | Yes (12) | Yes (8) | Yes (12) | Yes (1) | Yes (5) |
| Conjutivitis             | 3 (60)                            | Yes | Yes | No | No | Yes |
| Hypotension              | 4 (80)                            | Yes | Yes | Yes | No | Yes |
| Shock                    | 3 (60)                            | No | Yes | Yes | No | Yes |
| Abdominal pain           | 5 (100)                           | Yes | Yes | Yes | Yes | Yes |
| Diarrhea                 | 3 (60)                            | Yes | No | No | Yes | Yes |
| Treatment                |                                   |     |     |     |     |     |
| ICU admission            | 4 (80)                            | No | Yes | Yes | Yes | Yes |
| Length of stay at hospital (days) | 10.4 (6.26)         | 3 | 14 | 18 | 5 | 12 |
| Respiratory support /Oxygen therapy | 2 (40)                      | No/No | Yes/Yes | No/No | No/No | Yes/Yes |
| Anti-inflammatory treatment | 2 (40)                        | No | Yes (mPRED) | Yes (mPRED) | No | No |
| Immunoglobulin treatment | 5 (100)                           | First dose 2g/kg | First dose and | First dose 2g/kg | First dose 2g/kg | First dose 2g/kg |
|                           | second dose 2g/kg | 2g/kg |
|--------------------------|------------------|-------|
| **13 N-ammonia PET-CT**  |                  |       |
| Global MFR (abnormal when < <2), gray zone 2-2.5 and normal > 2.5 | 2 (40) | 1.6 | 3.7 | 3.2 | 1.8 | 2.5 |

**Echo parameters**

| Normal echocardiogram at follow-up | 4 (80) | normal | normal | normal | abnormal* | normal |
|------------------------------------|--------|--------|--------|--------|-----------|--------|
| LVDD z-score                        | -0.13 (1.01) | 0.81 | -0.74 | 0.1 | -1.57 | 0.74 |
| LVSD z-score                        | -0.66 (0.32) | -0.52 | -0.83 | -0.39 | -0.43 | -1.17 |
| Septum z-score                      | 0.91 (0.38) | 0.55 | 1.3 | 0.56 | 0.84 | 1.33 |
| LVPW z-score                        | 0.62 (0.37) | 0.21 | 1 | 0.31 | 0.58 | 1 |
| LA z-score                          | -0.72 (1.07) | 0.68 | 0 | -0.78 | -1.82 | -1.7 |
| LVEF (%) (abnormal £ 55)            | 1 (20) | 75 | 70 | 70 | 54 | 79 |

**Doppler ultrasound of the brachial artery**

| FMD (%)                             | 6.38 (3.41) | 9.36 | - | 3.92 | 10.07 | 2.14 |
| FMD reference value (25th percentile [9]) | - | 5.92 | - | 6.19 | 6.23 | 5.36 |
| Endothelial dysfunction (i.e., below the FMD 25th percentile [9]) | 2 (50) | No | - | Yes | No | Yes |

**Cardiopulmonary exercise test**

| VO_{2peak} (mL·kg^{-1}·min^{-1})     | 26.3 (8.35) | 22.5 | 17.4 | 28.6 | - | 36.8 |
| Predicted VO_{2peak} (%) (<80 abnormal) | 50.2 (12.8) | 48.7 | 35.2 | 64.5 | - | 52.7 |
| VO_{2VAT} (mL·kg^{-1}·min^{-1})     | 13.7 (4.64) | 7.2 | 12.4 | 17.0 | - | 16.9 |
| % from expected value               | -54.0 (14.5) | -70.4 | -60.8 | -37.3 | - | -47.6 |
| VO_{2VAT} (%)/Predicted             | 27.8 | 15.6 | 25.1 | 38.2 | - | 32.3 |
| Parameter                        | Value          |
|---------------------------------|----------------|
| VO_{2}\text{peak} (<40 abnormal) | (9.73)         |
| OUES (L·min^{-1})               | 1.20 (0.14)    |
|                                 | 1.2            |
|                                 | 1.0            |
|                                 | 1.3            |
|                                 | -              |
|                                 | 1.3            |
| OUES/kg                         | 31.7 (10.1)    |
|                                 | 20.0           |
|                                 | 30.3           |
|                                 | 31.7           |
|                                 | -              |
|                                 | 44.8           |
| VE/VCO_{2} slope (units) (>31 abnormal) | 34.8 (4.77)   |
|                                 | 41.7           |
|                                 | 31.8           |
|                                 | 34.4           |
|                                 | -              |
|                                 | 31.4           |
| % from expected value           | 12.6 (9.45)    |
|                                 | 25.6           |
|                                 | 3.0            |
|                                 | 10.0           |
|                                 | 12.0           |
| PetCO_{2} rest (mmHg) (<35 abnormal) | 29.7 (4.27)   |
|                                 | 24             |
|                                 | 33             |
|                                 | 33             |
|                                 | 29             |
| % from expected value           | -15.1 (12.0)   |
|                                 | -31.4          |
|                                 | -6.0           |
|                                 | -6.0           |
|                                 | -17.1          |
| O_{2} Pulse peak (mL/beat) (<14 abnormal) | 6.25 (1.50)   |
|                                 | 7              |
|                                 | 4              |
|                                 | 7              |
|                                 | -              |
|                                 | 7              |
| % from expected value           | -55.2 (10.5)   |
|                                 | -50.0          |
|                                 | -71.0          |
|                                 | -50.0          |
|                                 | -50.0          |

**Laboratory data**

| Parameter                        | Value          |
|---------------------------------|----------------|
| C-reactive protein (0.3-10 mg/L) | 1.29 (2.00)    |
|                                 | <0.30          |
|                                 | 0.42           |
|                                 | <0.30          |
|                                 | 4.85           |
|                                 | 0.57           |
| D-dimer (≤ 500 ng/ml)            | 623.5 (349-97592) |
|                                 | 794            |
|                                 | 97572          |
|                                 | -              |
|                                 | 453            |
|                                 | 349            |
| Fibrinogen (200-400 mg/dL)      | 289.2 (108.5)  |
|                                 | 311            |
|                                 | 190            |
|                                 | 217            |
|                                 | 465            |
|                                 | 263            |
| Troponin (<0.004 ng/ml)         | 0.004 (0.001)  |
|                                 | 0.003          |
|                                 | 0.004          |
|                                 | 0.004          |
|                                 | 0.004          |
|                                 | 0.007          |

*Patient 4 exhibited discrete pericardial effusion at. Categorical data were reported as percentages and continuous data as mean ± standard deviation (SD) or median (range). Abbreviation: BMI: body mass index; ICU: intensive care unit; MFR: myocardial flow reserve; LVDD: left ventricle diastolic diameter; LVSD: left ventricle systolic diameter; Septum: interventricular septum thickness; LVPW: left ventricle posterior wall thickness; LA: left atrium diastolic diameter; LVEF: left ventricle ejection fraction; FMD: flow mediated dilatation; VO_{2}\text{peak}: peak oxygen consumption; VO_{2}\text{VAT}: oxygen consumption at ventilatory anaerobic threshold; OUES: oxygen uptake efficiency slope; HR/VO_{2}: heart rate-oxygen consumption relationship; V_E: pulmonary ventilation.
Figure 1

Representative data of 13N-ammonia PET distribution - illustrative case: A) Female patient, 9 years old, showed normal MFR. B) Female patient, 8 years, showed abnormal values for MFR, showing a transient perfusion defect in the anteroapical, inferoapical e inferolateral territories.
13N-ammonia PET data. Polar maps of MBF values with the table on a 17 American Heart Association (AHA) segments. A) Female patient, 9 years old, showed normal values for MFR. B) Female patient, 8 years old, showed abnormal values for MFR. In the bullseye illustrations, white-to-purple means normal values of MFR and blue-to-black means abnormal or decreased flow reserve.