



## Phenotypic Heterogeneity of Fulminant COVID-19–Related Myocarditis in Adults

Petra Barhoum, Marc Pineton de Chambrun, Karim Dorgham, Mathieu Kerneis, Sonia Burrel, Paul Quentric, Christophe Parizot, Juliette Chommeloux, Nicolas Bréchet, Quentin Moyon, et al.

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**TITLE:** Phenotypic Heterogeneity of Fulminant COVID-19-Related Myocarditis in Adults

**BRIEF TITLE:** Types of Myocarditis Related to COVID-19

**AUTHORS:** Petra Barhoum<sup>a§</sup>, MD; Marc Pineton de Chambrun<sup>a,b,c,d§</sup>, MD, MSc; Karim Dorgham<sup>d</sup>, PhD; Mathieu Kerneis<sup>b,e</sup>, MD, PhD; Sonia Burrel<sup>f</sup>, PharmD, PhD; Paul Quentric<sup>c,d</sup>, MD, MSc; Christophe Parizot<sup>d</sup>, PhD; Juliette Chommeloux<sup>a</sup>, MD; Nicolas Bréchet<sup>a</sup>, MD, PhD; Quentin Moyon<sup>a,c</sup>, MD; Guillaume Lebreton<sup>g</sup>, MD, PhD; Samia Boussouar<sup>h</sup>, MD; Matthieu Schmidt<sup>a,b</sup>, MD, PhD; Hans Yssel<sup>d</sup>, PhD; Lucie Lefevre<sup>a</sup>, MD; Makoto Miyara<sup>d</sup>, MD, PhD; Jean-Luc Charuel<sup>d</sup>, PharmD; Stéphane Marot<sup>f</sup>, MD; Anne-Geneviève Marcelin<sup>f</sup>, MD, PhD; Charles-Edouard Luyt<sup>a,b</sup>, MD, PhD ; Pascal Leprince<sup>g</sup>, MD, PhD; Zahir Amoura<sup>c</sup>, MD, MSc; Gilles Montalescot<sup>b,e</sup>, MD, PhD; Alban Redheuil<sup>h</sup>, MD, PhD; Alain Combes<sup>a,b</sup>, MD, PhD; Guy Gorochov<sup>d‡</sup>, MD, PhD; Guillaume Hékimian<sup>a‡</sup>, MD.

<sup>a</sup>Sorbonne Université, Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital La Pitié-Salpêtrière, Service de Médecine Intensive Réanimation, Paris, France

<sup>b</sup>Sorbonne Université, Inserm, UMRs\_1166-ICAN, Institut de Cardiométabolisme et Nutrition (ICAN), Paris, France

<sup>c</sup>Sorbonne Université, AP-HP, Hôpital La Pitié-Salpêtrière, Institut E3M, Service de Médecine Interne 2; Centre de Référence National Lupus Systémique, Syndrome des Anticorps Anti-phospholipides et Autres Maladies Auto-Immunes Systémiques Rares, Paris, France

<sup>d</sup>Sorbonne Université, Inserm, Centre d'Immunologie et des Maladies Infectieuses (CIMI-Paris), and AP-HP, Hôpital La Pitié-Salpêtrière, Département d'Immunologie, 47-83, boulevard de l'Hôpital, Paris, France

<sup>e</sup>Sorbonne Université, AP-HP, Hôpital La Pitié-Salpêtrière, ACTION Study Group,  
Département de Cardiologie, Paris, France  
<sup>f</sup>Sorbonne Université, Institut Pierre Louis d'Epidémiologie et de Santé Publique (IPLESP),  
INSERM U1136 and AP-HP, Hôpital La Pitié-Salpêtrière, Service de Virologie, Paris, France  
<sup>g</sup>Sorbonne Université, AP-HP, Hôpital La Pitié-Salpêtrière, Service de Chirurgie Cardio-  
Thoracique, Paris, France  
<sup>h</sup>Sorbonne Université, APHP, Hôpital La Pitié-Salpêtrière, Unité d'Imagerie Cardiovasculaire  
et Thoracique (ICT), Paris, France

§These authors contributed equally: PB and MPdC

‡These authors jointly coordinated the study: GH and GG

### Correspondance to:

- Pr Guy Gorochov, Inserm, Centre d'Immunologie et des Maladies Infectieuses (CIMI-Paris), and AP-HP, Hôpital La Pitié-Salpêtrière, Département d'Immunologie, 47-83, boulevard de l'Hôpital, Paris, FranceE-mail: [guy.gorochov@sorbonne-universite.fr](mailto:guy.gorochov@sorbonne-universite.fr)
- or to Dr. Guillaume Hékimian, Service de Médecine Intensive Réanimation, Institute of Cardiology, Groupe Hospitalier La Pitié-Salpêtrière, 47-83 bd de l'Hôpital, 75651 Paris Cedex 13, France. Tel: + 33 1 42 16 38 28. [guillaume.hekimian@aphp.fr](mailto:guillaume.hekimian@aphp.fr)

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## 1    **ABSTRACT**

2    **BACKGROUND:** Adults who have been infected with SARS-CoV-2 can develop a  
3    multisystem inflammatory syndrome (MIS-A), including fulminant myocarditis. Yet, several  
4    patients fail to meet MIS-A criteria, suggesting the existence of distinct phenotypes in  
5    fulminant COVID-19-related myocarditis.

6    **OBJECTIVE:** To compare the characteristics and clinical outcome between patients with  
7    fulminant COVID-19-related myocarditis fulfilling MIS-A criteria (MIS-A<sup>+</sup>) or not (MIS-A<sup>-</sup>).

8    **METHODS:** A monocentric retrospective analysis of consecutive fulminant COVID-19-  
9    related myocarditis in a 26-bed ICU.

10    **RESULTS:** Between March 2020 and June 2021, 38 patients required ICU admission (male:  
11    66%; mean age: 32±15 years) for suspected fulminant COVID-19-related myocarditis. In-ICU  
12    treatment for organ failures included dobutamine 79%, norepinephrine 60%, mechanical  
13    ventilation 50%, VA-ECMO 42% and renal replacement therapy 29%. In-hospital mortality  
14    was 13%. Twenty-five (66%) patients met the MIS-A criteria. MIS-A<sup>-</sup> compared to MIS-A<sup>+</sup>  
15    patients were characterized by a shorter delay between COVID-19 symptoms onset and  
16    myocarditis, a lower left ventricle ejection fraction, a higher rate of in-ICU organ failure, and  
17    were more likely to require mechanical circulatory support with VA-ECMO (92% versus  
18    16%,  $p<0.0001$ ). In-hospital mortality was higher in MIS-A<sup>-</sup> patients (31% vs. 4%). MIS-A<sup>+</sup>  
19    had higher circulating levels of IL-22, IL-17 and TNF- $\alpha$  whereas MIS-A<sup>-</sup> had higher IFN- $\alpha$ 2  
20    and IL-8 levels. RNA-polymerase-III autoantibodies were present in 7/13 (54%) of MIS-A<sup>-</sup>  
21    but in none MIS-A<sup>+</sup> patients.

22    **CONCLUSION:** MIS-A<sup>+</sup> and MIS-A<sup>-</sup> fulminant COVID-19-related myocarditis patients  
23    have 2 distinct phenotypes with different clinical presentations, prognosis and immunological  
24    profiles. Differentiating these 2 phenotypes is relevant for patients' management and further  
25    understanding of their pathophysiology.

**CONDENSED ABSTRACT:**

The multisystem inflammatory syndrome accounts for a large proportion of COVID-19-related myocarditis in adults (MIS-A). We compared the characteristics of fulminant COVID-19-related myocarditis (n=38) between patients fulfilling MIS-A criteria (MIS-A<sup>+</sup>) or not (MIS-A<sup>-</sup>). As compared to MIS-A<sup>+</sup> (n=25), MIS-A<sup>-</sup> patients (n=13) had a shorter delay between first COVID-19 symptoms and myocarditis, a lower LVEF, higher in-ICU organ failure, need for mechanical circulatory support and in-hospital mortality. Immunological profiles were different in the 2 groups: MIS-A<sup>+</sup> had high levels of IL-22, IL-17 and TNF- $\alpha$ , while MIS-A<sup>-</sup> high IFN- $\alpha$ 2 and IL-8 levels and a high frequency of RNA-polymerase-III autoantibodies (54%).

**KEY WORDS:** COVID-19; SARS-CoV-2; Fulminant Myocarditis; VA-ECMO; Multisystem Inflammatory Syndrome; RNA polymerase III autoantibodies; Cytokines

1    **ABBREVIATIONS:**

2    COVID-19: Coronavirus Disease 2019

3    Ig: immunoglobulin

4    IFN: Interferon

5    IL: Interleukin

6    MIS: multisystem inflammatory syndrome

7    N: SARS-CoV-2 nucleocapsid protein

8    S: SARS-CoV-2 spike protein

9    RBD: Receptor Binding Domain

10   SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

11   TNF- $\alpha$ : Tumor Necrosis Factor- $\alpha$

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## INTRODUCTION

COVID-19-related myocarditis has been reported since the beginning of SARS-CoV-2 outbreak(1–6). Fulminant myocarditis is a rare but life-threatening form of myocarditis leading to significant morbidity and mortality especially in young patients(7). Firstly described in children(8) and subsequently in adults(9), the multisystem inflammatory syndrome (MIS-C or MIS-A, respectively) accounts for a large proportion of COVID-19-related myocarditis. The United States (US) Center for Disease Control and Prevention has developed case definition criteria to standardize its diagnosis(10). Yet, some patients do not meet these criteria suggesting the existence of distinct phenotypes in COVID-19-related myocarditis. We conducted a study to compare the clinical, biological and immunological characteristics of patients with fulminant COVID-19-related myocarditis meeting or not meeting MIS-A criteria.

## METHODS

### Patients and controls

We retrospectively reviewed the database of our 26-bed ICU between March 2020 and June 2021, and included all patients admitted for clinically suspected myocarditis with proven SARS-CoV-2 infection. Clinically suspected myocarditis was then adjudicated as definite or probable myocarditis according to the definition by Bonaca *et al.*(11) (see **Supplementary Materials**) following clinical investigations. Proven SARS-CoV-2 infection was confirmed by positive RT-PCR in either nasopharyngeal aspirate, lower airway respiratory samples or serum and/or positive serology showing the presence of circulating anti-N or anti-S-RBD antibodies, in patients not vaccinated against COVID-19. All laboratory analyses are performed as the standard of care in the myocarditis work-up of our institution. In addition,

1 healthy SARS-CoV-2-negative individuals (n=10) were included as controls for cytokine  
2 measurements.

## 5 **Data collection**

6 The following information was collected on standardized forms: epidemiologic  
7 parameters; severity of underlying condition according to the McCabe & Jackson criteria;  
8 medical history; COVID-19 infection history, manifestations and complications; MIS-A  
9 criteria (**see Supplementary Materials**); day-0 Sequential Organ Failure Assessment (SOFA)  
10 score and Simplified Acute Physiology Score (SAPS) II; day-0 and in-ICU clinical and  
11 biological parameters; day-0 and in-ICU organ-failure support treatment; day-0, in-ICU and  
12 last-follow-up echocardiography parameters; in-ICU cytokine profiling; in-ICU SARS-CoV-2  
13 and myocarditis-specific treatment; in-ICU and follow-up CT-scan and cardiac magnetic  
14 resonance imaging (CMR); complications; vital status at ICU and hospital discharge, as well  
15 as at last follow-up.

## 17 **SARS-CoV-2 RT-PCR and serological analyses**

18 Detection of SARS-CoV-2 was carried out by RT-PCR in clinical specimens, using  
19 Cobas®6800 SARS-CoV-2 Test-Roche Diagnostics (Roche Diagnostic, Meylan, France) and  
20 serological detection of IgG anti-N and IgG anti-S SARS-CoV-2 was performed by ELISA on  
21 the Abbott platform (Abbott Diagnostics, Rungis, France) in accordance with the  
22 manufacturer's specifications.

## 24 **Cytokine measurement**

Whole blood was collected in anticoagulant-free tubes, serum was separated by centrifugation and stored at -80°C. Serum concentrations of IL-1 $\beta$ , IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-22, IFN- $\gamma$ , and TNF- $\alpha$  were measured on a Quanterix® SP-X™ imaging and analysis platform using the Human CorPlex Cytokine Panel Array kit (Quanterix, Lexington, MA, USA). Single-plex bead-based ultrasensitive immunodetection of IL-17A and IFN- $\alpha$  was performed by digital ELISA using the Simoa™ (single molecule array) HD-1 analyzer (Quanterix), according to the manufacturer's instructions. Serum IFN- $\beta$  levels were quantified using a highly sensitive ELISA kit (PBL Assay Science, Piscataway, NJ, USA), according to the manufacturer's instructions. Serum cytokine concentrations were interpolated from the correspondent calibration curve taking into account the dilution factor. All cytokine concentrations were expressed in pg/mL. Samples with non-detectable values or those above the detection range were replaced by the limit of detection value (LOD) and the upper limit of quantification (ULOQ), respectively.

#### **Anti-IFN- $\alpha$ and RNA-polymerase-III autoantibodies**

Auto-antibodies against IFN- $\alpha$  were quantified using the anti-IFN- $\alpha$  Antibody Human ELISA Kit (Thermo Fisher, Invitrogen), according to the manufacturer's instructions. Calibrators were run in duplicate and fit with a 4-parameter logistic (4PL) regression. The concentration of anti-IFN- $\alpha$  antibodies in samples was interpolated from the calibration curve by multiplying the obtained values with the dilution factor. The positivity threshold was 15 ng/mL. For RNA-polymerase-III autoantibodies screening, an indirect immunofluorescence assay was run on HEp-2000 cells (Immuno Concepts, Sacramento, CA, USA). When positive ( $\geq 1/80$ ) and when the immunofluorescence labeling pattern was evocative of RNA-polymerase III autoantibodies (fine-speckled nuclear-labeling pattern with small dots), a confirmatory immunodot assay (Euroline Systemic Sclerosis Test, Bio Advance, Bussy Saint-

Martin, France) was carried out.

### **Statistical analyses**

Continuous variables are expressed as median [interquartile range] and compared with Wilcoxon's signed-rank tests. Categorical variables are expressed as number (percent) and compared with  $\chi^2$  tests or Fisher's exact tests. Cumulative probabilities of survival were calculated using Kaplan-Meier method and compared with Log-Rank tests. A two-tailed p-value  $< 0.05$  was considered statistically significant. Analyses were computed with StatView v5.0 (SAS Institute, Cary, NC) and IBM SPSS Statistics v22.0 software (IBM Corp, Armonk, NY). Unsupervised principal component analysis (PCA) was performed using R v3.6.2 with the FactoExtra and FactoMineR functions, on z-scaled log10-transformed cytokine concentrations. Samples with missing data were excluded from the PCA analysis for one MIS-A<sup>+</sup> and two MIS-A<sup>-</sup> patients).

### **Ethical considerations**

This study was conducted in accordance with the declaration of Helsinki using the database registered at the Commission Nationale de l'Informatique et des Libertés (CNIL, registration no. 1950673). In agreement with the ethical standards of our hospital's institutional review board, the Committee for the Protection of Human Subjects and French law, written informed consent was not needed for demographic, physiological and hospital-outcome data analysis, because this observational study does not modify existing diagnostic or therapeutic strategies; however, patients and/or their relatives were informed of their anonymous inclusion in the study.

## **RESULTS**

## General Patients characteristics

Between March 2020 and June 2021, 38 patients requiring ICU admission for clinically suspected fulminant COVID-19-related myocarditis were included in this study. They were mostly men (66%) of young age (median [IQR25-75] age, 27.5 [19-37] years) with few comorbidities. Their baseline characteristics are reported in **Table 1** and **Supplemental Table 1**. All had positive SARS-CoV-2 RT-PCR (37%) or serology (68%) with a median delay of five days between COVID-19 symptoms onset and the first manifestation of myocarditis. None had previously received any COVID-19 vaccine. Most frequent symptoms were fever (95%), abdominal pain or nausea (60%), chest pain (47%) and dyspnea (42%).

At admission, patients had severely impaired left ventricle function (LVEF 20% [14-37], LVOT-VTI 11 cm [6-15]), an increased high-sensitivity T-troponin (median 1,300 [IQR = 486-4,750] ng/mL) and 79% presented with cardiogenic shock. When performed (n=10), coronary angiography was normal. COVID-19 pneumonia was noted on CT scan examination in 29% of cases. In the 26 patients who had CMR evaluation, myocardial edema and late gadolinium enhancement were reported in 73% and 54% respectively.

Three patients without recovery of cardiac function underwent myocardial biopsy. None were MRI-guided, as all were taken under mechanical circulatory support. Two surgical biopsies were taken in patients during venoarterial-extracorporeal membrane oxygenation (VA-ECMO) centralization. The first one was an apical surgical biopsy in a patient cannulated under cardiopulmonary resuscitation which was nonconclusive. The second surgical biopsy highlighted myocarditis with an inflammatory infiltrate associated with myocyte dystrophy and oedema. SARS-CoV-2 RT-PCR was negative and electron microscopy analysis failed to identify viral particles in cardiomyocytes despite active myocarditis lesions on the evaluated sample. Last biopsy was endomyocardial and disclosed a mild lympho-histiocytic myocarditis with no oedema but severe necrosis. SARS-CoV-2 RT-

PCR was negative. Twenty-nine (76%) patients met the Bonaca classification criteria for definite myocarditis while the others had probable myocarditis (**Table 2**).

#### **In-ICU evolution and Outcomes**

Median length of stay in ICU was 6 days. Seventy nine percent of the patients received dobutamine, 60% norepinephrine, 50% mechanical ventilation and 29% renal replacement therapy (**Table 3**). Four patients had a large pericardial effusion requiring drainage. Sixteen (42%) patients required mechanical circulatory support with VA-ECMO 1 [0-1] day following ICU admission, for a median duration of 7 days. Twenty-eight (74%) were treated with corticosteroids and 27 (74%) with intravenous immunoglobulins. In-hospital mortality was 13%. None of the survivors required cardiac transplantation or long-term ventricular assist device. Among the five deceased patients, all had multiorgan failure before VA-ECMO implantation, including three cannulations during cardiopulmonary resuscitation. None could be weaned from VA-ECMO because of severe cardiac dysfunction. Median LVEF at ICU and hospital discharge was 42% [30-54] and 60% [50-64], respectively. Twenty one survivors (64%) received betablockers at discharge and 25 (76%) were treated with angiotensin-converting-enzyme inhibitors, until distant evaluation with a cardiologist. At the last follow-up (median [IQR] 235 [155–359] days), 32 patients were alive, all but one had normal LVEF. One patient was lost of follow-up.

#### **Comparison between MIS-A<sup>+</sup> and MIS-A<sup>-</sup> patients**

Twenty-five (66%) patients met the MIS-A criteria (**Table 1**). By definition, MIS-A<sup>+</sup> patients had more frequent fever, skin rash, enanthema, pharyngitis and conjunctivitis, as compared to MIS-A<sup>-</sup> patients (**Table 1**). In addition, they had, as expected by the MIS-A

definition, higher levels of systemic inflammation markers, including circulating leucocytes, procalcitonin, C-reactive protein and fibrinogen (**Table 4**).

The median delay between COVID-19 symptoms onset and occurrence of myocarditis was shorter in MIS-A<sup>-</sup> patients: 3 vs. 8 days. Noteworthy, the delay between first COVID-19 symptoms and myocarditis was 32 [25-44] days among the 12 MIS-A<sup>+</sup> patients with prior proven symptomatic SARS-CoV-2 infection. The rate of positive serology was lower in MIS-A<sup>-</sup> patients (15% vs. 96%) and their titer was also much lower than in MIS-A<sup>+</sup> patients ( $p < 0.0001$ ). Conversely, positive nasopharyngeal RT-PCR at the time of myocarditis was infrequent in MIS-A<sup>+</sup> (16%), as compared to MIS-A<sup>-</sup> (85%) patients.

MIS-A<sup>-</sup> patients had swifter ICU admission after myocarditis onset (1 vs. 4 days) with a more severe presentation (day-0 SOFA score of 11 versus 6 in MIS-A<sup>+</sup> patients). They had a lower left ventricular ejection fraction (LVEF 10 vs. 30% and LVOT-VTI 5 vs. 13 cm) (**Table 2**) and were more likely to receive norepinephrine, mechanical ventilation and renal replacement therapy. Large pericardial effusions were also more frequently observed in MIS-A<sup>-</sup> patients. The median lactate level was 5.5 versus 2.1 mmol/L in MIS-A<sup>-</sup> and MIS-A<sup>+</sup> patients, respectively. Finally, MIS-A<sup>-</sup> patients were more likely to require VA-ECMO than MIS-A<sup>+</sup> patients (92% vs. 16%), and had a higher in-ICU mortality (31% vs. 4%,  $p = 0.04$ , **Table 3**). The 3-month cumulative probabilities of survival  $\pm$  standard errors for MIS-A<sup>-</sup> and MIS-A<sup>+</sup> patients were respectively:  $68 \pm 13\%$  and  $96 \pm 4\%$ , Log-Rank test  $p = 0.01$ .

Cytokine profiling highlighted the presence of two distinct cytokine production profiles (**Figure 1 and Table 4**): MIS-A<sup>+</sup> had higher IL-22 (9.93 vs. 1.5 pg/mL,  $p < 0.0001$ ), IL-17 (3.2 vs. 0.15 pg/mL,  $p < 0.0001$ ) and TNF- $\alpha$  (21.1 vs. 8.0 pg/mL,  $p = 0.05$ ) levels, as compared to MIS-A<sup>-</sup> patients, while the latter had higher IFN- $\alpha 2$  (2.4 vs. 0.013 pg/mL,  $p = 0.001$ ) and IL-8 (158.7 vs. 65.7 pg/mL,  $p = 0.02$ ), respectively. Moreover, RNA-polymerase III autoantibodies were found in seven (54%) MIS-A<sup>-</sup> patients, five of them being female

1 **(Table 4).**

2 Finally, to elucidate the relative importance of the various bio-clinical parameters  
3 listed above with the clinical profile of MIS-A<sup>+</sup> or MIS-A<sup>-</sup> patients, we performed non-  
4 supervised PCA using study parameters contributing, in a statistically significant manner, to  
5 inter-patient variation (**Tables 1-3**). The results from PCA underlined important overall  
6 differences between MIS-A<sup>+</sup> and MIS-A<sup>-</sup> patients, (**Figure 2**). The data also further highlight  
7 parameters most contributing to either clinical status *i.e.*: fibrinogen ( $p<0.0001$ ), CRP  
8 ( $p<0.0001$ ), IL-17 ( $p<0.0001$ ), IL-22 ( $p<0.0001$ ), IFN- $\alpha$ 2 ( $p=0.001$ ) levels, SARS-CoV-2  
9 serology ( $p<0.0001$ ) and SARS-CoV-2 RT-PCR ( $p<0.0001$ ), LVEF ( $p=0.01$ ) values on  
10 admission and the presence of RNA polymerase III autoantibodies ( $p=0.001$ ).

## 12 **DISCUSSION**

13 In this retrospective monocenter cohort of fulminant COVID-19-related myocarditis,  
14 we applied the MIS-A criteria case definition and we identified two subsets of patients with  
15 very different clinical/biological presentations, outcomes, and immunological profiles. This  
16 phenotypic heterogeneity being likely explained by important differences in  
17 pathophysiological mechanisms.

18 The patients in this cohort were mostly young men with severely impaired cardiac  
19 function, frequently requiring VA-ECMO, with infrequent concomitant COVID-19-associated  
20 pneumonia. All survivors recovered a near normal cardiac function at distant follow-up. To  
21 our knowledge, this study is the largest cohort of COVID-19-related fulminant myocarditis  
22 and extends prior reports of COVID-19-related myocarditis(9, 12–15) and fulminant non-  
23 COVID-19 myocarditis(7, 16–19).

24 This analysis underscores the major clinical and immunological differences between  
25 patients with fulminant COVID-19-related myocarditis fulfilling or not MIS-A criteria. The



original description of MIS-C was reported in May 2020(8) and MIS-A a few months afterwards(9, 20, 21). This somewhat delayed description, together with the rarity of the disease may have participated to an under-recognition of MIS in the adult population. Furthermore, whereas MIS-C is now well defined with classification criteria established by the World Health Organization and the Center for Disease Control and Prevention(22, 23), only the latter has adapted its criteria to the adult population(10).

The main differences between the phenotypes of MIS-A<sup>+</sup> and MIS-A<sup>-</sup> patients are summarized in the **Central Illustration**. MIS-A<sup>+</sup> COVID-19 related myocarditis appears to be a post-infectious complication of SARS-CoV-2 infection, as suggested by the higher delay between COVID-19 symptoms and myocarditis, as well as by frequently positive serology and negative (or slightly positive) RT-PCR. Mucocutaneous manifestations are frequent in addition to laboratory evidence of severe systemic inflammation. Heart failure is more progressive, leading to fewer accounts of refractory cardiogenic shock, and is associated with a lower mortality rate. Conversely, MIS-A<sup>-</sup> fulminant COVID-19-related myocarditis occurred at the early phase of SARS-CoV-2 infection (negative or slightly positive serology and positive RT-PCR) with an explosive and refractory cardiogenic shock in nearly all patients leading to high morbidity and mortality.

Interestingly, these different clinical phenotypes are supported by immunological findings. The frequency of RNA-polymerase III autoantibodies is high in MIS-A<sup>-</sup>, while absent in MIS-A<sup>+</sup> patients. The presence of these rare autoantibodies, usually associated with severe systemic sclerosis, has been previously reported by Pineton de Chambrun M *et al.* in patients with severe recurrent myocarditis and/or pericarditis, especially related to influenza virus(24). Their role in the susceptibility to viral myocarditis is not yet elucidated. They might reflect altered immune defenses toward viral infections or alternatively exaggerated antiviral responses leading to organ damage. Another patient with recurrent viral myocarditis,

1 including COVID-19-related myocarditis, has been recently reported(25).

2       The cytokine profiles of these patients were also found definitely different in the two  
3 clinical phenotypes (**Figure 2**). In MIS-A<sup>-</sup> patients, high levels of systemic circulating anti-  
4 viral IFN- $\alpha$ 2 likely arise from the ongoing viral infection, in relation to detectable viral  
5 replication and yet undetectable anti-SARS-CoV-2 IgG humoral responses. Levels of IL-8, a  
6 proinflammatory cytokine, were also more elevated in MIS-A<sup>-</sup>, as compared to MIS-A<sup>+</sup>  
7 patients, further underlining the dominance of an innate type of immune response in the  
8 former group. Conversely, elevated IL-17 and IL-22 levels were found particularly associated  
9 with the MIS-A<sup>+</sup> phenotype, in agreement with the mucocutaneous manifestations observed in  
10 these patients. IL-17 and IL-22 shape innate defenses at mucosal and epithelial surfaces, IL-  
11 17 being pro-inflammatory and involved in the pathogenesis of several autoimmune diseases,  
12 while the latter cytokine is playing an important role in tissue regeneration(26). Of note, the  
13 extremely high serum IL-10 levels observed both in MIS-A<sup>-</sup> and MIS-A<sup>+</sup> patients have been  
14 previously associated with severe myocardial injury(27), and increased risk of death in severe  
15 COVID-19 patients(28).

16       MIS pathogenesis is not fully understood, but the delay between SARS-CoV-2  
17 infection and disease onset, and over-expression of mucosal T cell cytokines (IL-22 and IL-  
18 17) suggest a role for the adaptive immune response in MIS-A<sup>+</sup> patients. Conversely, in MIS-  
19 A<sup>-</sup> cases, innate anti-viral immunity and/or direct toxicity of the virus are more likely involved  
20 in heart tissue injury. In our series, SARS-CoV-2 RT-PCR on 2 MIS-A<sup>-</sup> endomyocardial  
21 biopsies (EMBs) were negative. This is in line with previous cases reports(2, 29), even if  
22 positive SARS-CoV-2 RT-PCR in myocardial samples have also been sporadically  
23 reported(30, 31). To the best of our knowledge, only one study demonstrated the presence of  
24 viral particles in cardiomyocytes by electronical microscopy(32), with only mild interstitial  
25 inflammatory infiltrate and no necrosis or microthrombosis, thereby suggesting that the

underlying mechanism of myocarditis development was mainly related to a virus-mediated immune response. The EMBs published results from fulminant COVID-19 related myocarditis often reported important myocardial edema with no or mild inflammatory infiltrate or necrosis(2, 33), a finding which is also consistent with cardiac MRI observations(2, 5).

The phenotypic clustering of patients with fulminant COVID-19-related myocarditis seems relevant for their management. Indeed, MIS-A<sup>-</sup> cases, owing to the high risk of evolution towards refractory cardiogenic shock should be urgently referred to a center with VA-ECMO capability and closely monitored to avoid a too late cannulation, especially under cardiopulmonary resuscitation, known to be associated with poor outcome(34). The five patients who died in our series had late VA-ECMO implantation, while having multiple organ failure or under resuscitation. Conversely, the risk of evolution towards refractory cardiogenic shock is lower in MIS-A<sup>+</sup> cases. Our results are consistent with those of a large series of 186 MIS-C from the United States, where only eight patients required VA-ECMO and four died(35). MIS-A<sup>+</sup> patient identification is all the more important given that numerous data support the efficacy of corticosteroids and/or intravenous immunoglobulins in MIS-C(36). The best treatment regimen is yet to be determined because conflicting results have been reported with standalone or combination treatment(37, 38). However, one should take with caution the results of non-randomized/non-blinded therapeutical intervention in a disease where spontaneous recovery occurs in most patients in a few days.

This study has some limitations that deserve mentioning. First, the external validity is limited by its monocentric and retrospective nature. Notably, as an ECMO center, there might be a selection bias towards the inclusion of the most severe patients. Thus, MIS-A<sup>-</sup> patients could have been better identified and transferred earlier in our center. Second, while being the largest series of fulminant COVID-19 related myocarditis, the sample size remains small,

1 limiting the power of the study. Lastly, EMBs were performed in only 3 patients, while expert  
2 consensus and guidelines recommend to consider EMB in fulminant presentation, for its  
3 diagnostic and therapeutic implications(39–41). However, coagulation disorders are frequent  
4 in COVID-19-related myocarditis and VA-ECMO patients. The benefit/risk ratio was  
5 evaluated against EMB in all but three cases, especially given the known diagnosis of SARS-  
6 CoV-2 infection. It is nevertheless unfortunate that we cannot provide a more extensive  
7 characterization of COVID-19-related myocarditis histopathological findings in MIS-A<sup>+</sup> and  
8 MIS-A<sup>-</sup> patients.

## 10 **CONCLUSION**

11 We identified two phenotypes of fulminant COVID-19-related myocarditis harboring  
12 distinct clinical and laboratory manifestations, evolutions and outcomes. Differentiating these  
13 patients seems relevant for their management and for further pathophysiological studies. The  
14 role of RNA-polymerase-III autoantibodies in fulminant myocarditis requires further  
15 investigation.

## **PERSPECTIVE**

**Competency in Patient Care and Procedural Skills:** Multisystem inflammatory syndrome

A (MIS-A) criteria distinguish phenotypes of patients who develop fulminant myocarditis

related to COVID-19, with different clinical presentations, immunological profiles and

outcomes. Those with MIS-A criteria more often have elevated serum levels of the

interleukins IL-17 and IL-22, and RNA polymerase III autoantibodies, and more often require

extracorporeal membrane oxygenator (ECMO) support.

**Translational Outlook:** Further investigation is needed to characterize the role of RNA-

polymerase-III antibodies in the pathophysiology of MIS-A COVID-19-related fulminant

myocarditis.

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**FIGURE LEGENDS:**

**Central Illustration.** Main phenotypic differences between MIS-A<sup>+</sup> and MIS-A<sup>-</sup> fulminant COVID-19-related myocarditis.

**Caption:** MIS-A<sup>+</sup> and MIS-A<sup>-</sup> fulminant COVID-19-related myocarditis patients have 2 distinct phenotypes with different clinical presentations, prognosis and immunological profiles.

For box and whisker plots: the centrer line denotes the median value (50th percentile), while the box contains the 25th to 75th percentiles of dataset. The whiskers mark the 5th and 95th percentiles.

Abbreviations: COVID-19, Coronavirus Disease 2019; ICU, Intensive Care Unit; US-CDC, United States Center for Disease Control; MIS-A, Multisystem Inflammatory Syndrome in Adults; RT-PCR, Reverse Transcription-Polymerase Chain Reaction.

**Figure 1.**

**Title:** Circulating cytokines levels in fulminant COVID-19-related myocarditis.

**Caption:** Comparison of 6 circulating serum cytokines levels (IL-8,10,17,20, IFN- $\alpha$ 2 and TNF- $\alpha$ ) in patients with MIS-A<sup>+</sup>/MIS-A<sup>-</sup> and healthy controls. MIS-A<sup>+</sup> had higher IL-22, IL-17 and TNF- $\alpha$ , while MIS-A<sup>-</sup> had higher IFN-  $\alpha$ 2 and IL-8. Methods: serum concentrations of IL-8, IL-10, IL-22 and TNF- $\alpha$  were measured on a Quanterix® SP-X™ imaging and analysis platform using the Human CorPlex Cytokine Panel Array kit (Quanterix, Lexington, MA, USA). Single-plex bead-based ultrasensitive immunodetection of IL-17A and IFN- $\alpha$  was performed by digital ELISA using the Simoa™ (single molecule array) HD-1 analyzer (Quanterix), according to the manufacturer's instructions.

For box and whisker plots: the center line denotes the median value (50th percentile), while the box contains the 25th to 75th percentiles of dataset. The whiskers mark the 5th and 95th percentiles.

Abbreviations: COVID-19, Coronavirus Disease 2019; IFN, Interferon; IL, Interleukin; TNF, Tumor Necrosis Factor; MIS-A, Multisystem Inflammatory Syndrome in Adults; RT-PCR, Reverse Transcription-Polymerase Chain Reaction.

## **Figure 2.**

**Title:** Principal component analyses (PCA): of cytokines measured in fulminant COVID-19-related myocarditis and combining clinical and biological features in fulminant COVID-19-related myocarditis.

**Methods:** unsupervised PCA was performed using R v3.6.2 with the FactoExtra and FactoMineR functions, on z-scaled log10-transformed cytokine concentrations. Samples with missing data were excluded from the PCA analysis for one MIS-A<sup>+</sup> and two MIS-A<sup>-</sup> patients). Ellipses with 66 % confidence interval are drawn for each group.

**Panel A: Caption:** the principal component analysis of 10 circulating serum cytokines.

**Panel B: Caption:** the principal component analysis including clinical findings, laboratory findings and immunological profiles highlights the main features of MIS-A<sup>-</sup> and MIS-A<sup>+</sup> fulminant COVID-19 related myocarditis phenotypes.

Abbreviations: SOFA, Sequential Organ-Failure Assessment; CRP, C-reactive protein; LVEF, Left Ventricle Ejection Fraction; IFN, Interferon; IL, Interleukin; MIS-A, Multisystem Inflammatory Syndrome in Adults; TNF, Tumor Necrosis Factor; RT-PCR, Reverse Transcription Polymerase Chain Reaction.

**Table 1. COVID-19-related MIS-A Criteria and SARS-CoV-2 Tests Results**

<b>Variables</b>	<b>All patients n=38</b>	<b>MIS-A<sup>-</sup> n=13</b>	<b>MIS-A<sup>+</sup> n=25</b>	<b>p-value</b>
Age, years	27.5 [19.0-37.0]	33.0 [21.5-38.5]	25.0 [18.5-35.5]	0.3
Women	13 (34)	6 (46)	7 (28)	0.3
BMI, kg/m <sup>2</sup>	24.8 [22.4-28.6]	25.9 [23.3-28.9]	24.7 [21.7-28.2]	0.7
Time from first COVID-19 symptoms to myocarditis, days	5 [0-26]	3 [0-5]	8 [2-38]	0.04
Time from myocarditis symptoms onset to ICU, days	3 [0-5]	1 [0-3]	4 [1-6]	0.009
ICU admission SOFA score	8 [5-11]	11 [8-13]	6 [4-10]	0.002
ICU admission SAPS-II score	30 [20-40]	33 [25-49]	25 [15-35]	0.04
<b>MIS-A criteria<sup>1</sup></b>				
Fever	36 (95)	9 (69)	25 (100)	0.01
Primary clinical criteria				
Myocardial involvement	38 (100)	13 (100)	25 (100)	na
Skin involvement	14 (37)	1 (8)	13 (52)	0.01
Secondary clinical criteria				
Neurological involvement	14 (37)	4 (31)	10 (40)	0.5
Shock or hypotension	36 (95)	13 (100)	23 (92)	0.1
Abdominal involvement	22 (58)	7 (54)	15 (60)	0.7
Platelets <150 G/L	10 (26)	5 (38)	5 (20)	0.09
Laboratory evidence				
Inflammation <sup>2</sup>	29 (76)	2 (15)	25 (100)	<0.0001
Positive SARS-CoV-2 test <sup>3</sup>	38 (100)	13 (100)	25 (100)	na
<b>SARS-CoV-2 tests</b>				
Positive nasopharyngeal RT-PCR	14 (37)	11 (85)	4 (16)	<0.0001
CT value	26 [18-32]	24.0 [18.0-28.0]	32.5 [21.5-34.5]	0.2
Positive serology	26 (68)	2 (15)	24 (96)	<0.0001
IgG anti-S	25 (67)	2 (15)	23/24 (96)	<0.0001

Titer, UA/mL	660 [156-1,440]	2.3 [0.1-59]	854 [528-2,575]	<0.0001
IgG anti-N	26 (68)	2 (15)	24 (96)	<0.0001
Index, value	2.2 [0.1-5.3]	0.4 [0.03-0.8]	4.8 [2.2-5.5]	<0.0001

Abbreviations: COVID-19, the coronavirus disease 2019; MIS-A, Multisystem Inflammatory Syndrome in Adults; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus-2; BMI, body-mass index; ICU, Intensive Care Unit; SOFA, Sequential Organ-Failure Assessment; SAPS-II, Simplified Acute Physiology Score-II; RT-PCR, Reverse transcription Polymerase-Chain Reaction; CT, Cycle Threshold; S, spike protein; N, nucleocapsid protein.

Continuous variables are expressed as median [interquartile range] and compared with Wilcoxon's rank test; categorical variables are expressed as n (%) and compared with Fischer's exact test.

<sup>1</sup>According the Center for Disease Control and Prevention (<https://www.cdc.gov/mis/mis-a/hcp.html>)

<sup>2</sup>Elevated levels of at least two of the following biomarkers including: C-reactive protein >10mg/L, procalcitonin >1ng/mL, fibrinogen >5g/L.

<sup>3</sup>Positive RT-PCR, serology or antigen for SARS-CoV-2

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**Table 2. Fulminant COVID-19-related myocarditis findings**

Variables	n <sup>†</sup>	All patients n=38	MIS-A <sup>-</sup> n=13	MIS-A <sup>+</sup> n=25	p-value
<b>Clinical symptoms</b>					
Chest pain		18 (47)	9 (69)	9 (36)	0.09
Faintness		4 (10)	4 (31)	0 (0)	0.01
Syncope		2 (5)	1 (8)	1 (4)	1.0
Sudden death		1 (3)	1 (8)	0 (0)	0.3
<b>Laboratory findings</b>					
Troponin, ng/mL		526 [224-1,227]	441 [177-1,089]	712 [217-2,025]	0.2
Highest value in ICU		1,300 [486-4,750]	2,836 [450-9,634]	1,000 [471-3,036]	0.2
NT-pro-BNP, ng/L	1	9,931 [2,367-23,934]	2,755 [1,044-8,271]	12,525 [7,000-32,500]	0.007
Creatin Phosphokinase, UI/L		312 [131-1,150]	586 [388-1,802]	190 [115-435]	0.003
<b>Electrocardiogram findings</b>					
Normal electrocardiogram		14 (37)	5 (38)	9 (36)	1.0
Sinus rhythm		36 (95)	12 (92)	24 (96)	1.0
Atrial fibrillation		2 (5)	1 (8)	1 (4)	1.0
ST segment elevation		10 (26)	6 (46)	4 (16)	0.06
ST segment depression		6 (16)	2 (15)	4 (16)	1.0
Negative T wave		10 (26)	1 (8)	9 (36)	0.1
Complete heart block		1 (3)	0 (0)	1 (4)	1.0
Bundle branch block		5 (13)	1 (8)	4 (16)	0.6
Ventricular rhythm disorders		3 (8)	3 (23)	0 (0)	0.03
<b>Echocardiography findings</b>					
LVEF, %					
First evaluation		30 [20-45]	30 [15-45]	30 [25-42]	0.5
On ICU admission		20 [14-37]	10 [5-30]	30 [15-45]	0.01
Lowest value in ICU		20 [10-30]	10 [5-25]	20 [15-30]	0.02
ICU discharge		42 [30-54]	35 [17-57]	45 [35-52]	0.1
Last follow-up	10	60 [50-64]	59 [44-60]	60 [50-65]	0.5

LVOT VTI, cm					
First evaluation		12 [8-16]	8 [7-17]	12 [9-15]	0.2
On ICU admission		11 [6-15]	5 [2-9]	13 [10-17]	<0.0001
ICU discharge		17 [12-18]	12 [7-18]	17 [15-19]	0.08
Ventricular hypertrophy		16 (42)	8 (62)	8 (32)	0.1
Ventricular dilation		5 (13)	2 (15)	3 (12)	1.0
LVEDD, mm		50 [47-56]	48 [46-55]	50 [47-56]	0.7
Right ventricle involvement		15 (39)	7 (54)	8 (32)	0.3
TAPSE, mm	20	14 [12-17]	12 [8-16]	14 [12-17]	0.2
S wave, cm/sec	21	9 [7-11]	6 [1-11]	10 [8-11]	0.1
Mitral valve regurgitation		9 (24)	3 (23)	6 (24)	1.0
Aortic valve regurgitation		3 (8)	0 (0)	1 (4)	1.0
Tricuspid valve regurgitation		3 (8)	0 (0)	3 (12)	0.5
Pericardial effusion		15 (39)	8 (62)	7 (28)	0.08
Pericardiocentesis		4 (10)	4 (31)	0 (0)	0.01
CMR findings					
In-ICU/hospital		26 (68)	5 (38)	21 (84)	
Time from symptoms to CMR, days		7 [4-18]	16 [9-33]	5 [4-10]	
Myocardial edema		19/26 (73)	3/5 (60)	16/21 (76)	0.6
Late gadolinium enhancement		14/26 (54)	4/5 (80)	10/21 (48)	0.3
Myocarditis classification <sup>2</sup>					
Definite myocarditis		29 (76)	9 (69)	20 (80)	
Probable myocarditis		9 (24)	4 (31)	5 (20)	
Pathology		2 (5)	2 (15)	1 (4)	
Imaging					
Cardiac magnetic resonance		22 (58)	4 (31)	18 (72)	
Echocardiography WMA		32 (84)	13 (100)	24 (96)	
Coronary angiography performed and normal		10 (26)	6 (46)	4 (16)	



Electrocardiogram	23 (60)	8 (61)	15 (60)
Syndrome	38 (100)	13 (100)	25 (100)
Biomarkers	38 (100)	13 (100)	25 (100)

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Abbreviations: MIS-A, Multisystem Inflammatory Syndrome in Adults; ICU, Intensive Care Unit; LVEF, Left Ventricle Ejection Fraction; LVOT VTI, Left Ventricular Outflow Tract Velocity-Time Integral; LVEDD, Left Ventricle End Diastolic Diameter; TAPSE, Tricuspid Annular Plane Systolic Excursion; CMR, Cardiac Magnetic Resonance; RVEF, Right Ventricle Ejection Fraction; WMA, Wall Motion Abnormality.

Continuous variables are expressed as median [interquartile range] and compared with Wilcoxon’s rank test; categorical variables are expressed as n (%) and compared with Fischer’s exact test.

<sup>1</sup>Number of missing values.

<sup>2</sup>According the myocarditis classification proposed by Bonaca et al.(11)

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**Table 3. Organ failure support, myocarditis treatment, complications and outcome in ICU**

<b>Variables</b>	<b>All patients n=38</b>	<b>MIS-A<sup>-</sup> n=13</b>	<b>MIS-A<sup>+</sup> n=25</b>	<b>p-value</b>
Time in ICU, days	6 [4-16]	12 [7-30]	5 [2-6]	<0.0001
Cardiac arrest before ICU	1 (3)	1 (8)	0 (0)	0.3
<b>Organ failure support in ICU</b>				
Dobutamine	30 (79)	12 (92)	18 (72)	0.2
Norepinephrine	23 (60)	12 (92)	11 (44)	0.005
Mechanical ventilation	19 (50)	11 (85)	8 (32)	0.005
Time on mechanical ventilation, days	15 [6-28]	15 [8-35]	11 [4.25-25.5]	0.3
Renal replacement therapy	11 (29)	8 (61)	3 (12)	0.003
VA-ECMO	16 (42)	12 (92)	4 (16)	<0.0001
VA-ECMO under CPR	4 (11)	4 (31)	0 (0)	0.02
Time on VA-ECMO, days	7 [5-12]	8 [6-12]	5 [4-12]	0.1
Time from admission to VA-ECMO, days	1 [0-1]	0 [0-1]	1 [0-5]	0.4
VV-ECMO	4 (10)	2 (15)	2 (8)	0.6
Time on VV-ECMO, days	18 [14-29]	24 [16-24]	17 [14-17]	0.4
<b>Myocarditis treatment in ICU</b>				
Corticosteroids	28 (74)	7 (54)	21 (84)	0.06
Intravenous immunoglobulins	27 (71)	8 (61)	19 (76)	0.5
Tocilizumab	0 (0)	0 (0)	0 (0)	Na
<b>Outcome</b>				
In-ICU mortality	5 (13)	4 (31)	1 (4)	0.04
In-hospital mortality	5 (13)	4 (31)	1 (4)	0.04
3-month probability of survival*	86±6%	68±13%	96±4%	0.01

Abbreviations: MIS-A, Multisystem Inflammatory Syndrome in Adults; ICU, Intensive Care Unit; VA-ECMO, Veno-Arterial Extracorporeal Membrane Oxygenation; CPR, CardioPulmonary Resuscitation; VV-ECMO, Veno-Venous Extracorporeal Membrane Oxygenation.

Continuous variables are expressed as median [interquartile range] and compared with Wilcoxon's rank test; categorical variables are expressed as n (%) and compared with Fischer's exact test.

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\*Probability of survival were calculated using Kaplan-Meier method and compared with Log-Rank tests.

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**Table 4. Laboratory findings and cytokine profiling in ICU**

Variables	n <sup>†</sup>	All patients n=38	MIS-A <sup>-</sup> n=13	MIS-A <sup>+</sup> n=25	p-value
<b>Day-0 laboratory findings</b>					
<b>Hemogram and haemostasis</b>					
Leukocytes G/L		12.6 [9.2-19.7]	8.7 [5.7-11.4]	18.5 [11.7-21.0]	<0.001
Lymphocytes, G/L		0.8 [0.5-1.5]	1.2 [0.6-2.3]	0.8 [0.5-1.2]	0.08
Polymorphonuclear cells, G/L		10.7 [5.8-18.0]	5.8 [3.4-8.1]	15.6 [10.3-19.0]	<0.001
Haemoglobin, g/dL		12.1 [11.1-13.5]	12.5 [10.4-16.0]	12 [11.6-13.3]	0.8
Platelets G/L		192 [152-247]	192 [92-258]	206 [160-243]	0.7
Prothrombin time, %		72 [64-81]	65 [56-90]	72 [69-77]	0.4
D-dimers, µg/L	3	3,860 [1,290-6,700]	2,500 [396-20,000]	4,217 [1,602-6,035]	0.6
<b>Inflammatory parameters</b>					
C-reactive protein, mg/L	5	257 [110-329]	5 [4-72]	277 [226-376]	<0.0001
Procalcitonin, ng/mL		7.4 [0.5-46]	0.2 [0.1-1.1]	12.8 [3.7-65]	<0.0001
Fibrinogen, g/L		6.8 [4.2-8.5]	3.2 [2.2-4.3]	7.9 [6.8-9.2]	<0.0001
<b>Biochemical findings</b>					
Serum creatinine, µmol/L		105 [69-156]	85 [60-105]	134 [71-265]	0.038
LDH, IU/L	2	419 [315-634]	619 [320-973]	385 [307-526]	0.2
AST, IU/L		83 [46-139]	70 [42-168]	94 [46-129]	0.9
ALT, IU/L		50 [32-101]	39 [26-110]	60 [37-101]	0.4
Serum total bilirubin, µmol/L		11 [8-19]	6 [4-14]	12 [10-21]	0.006
pH	1	7.43 [7.30-7.46]	7.31 [7.15-7.42]	7.44 [7.41-7.47]	0.004
pO <sub>2</sub> , mmHg	1	90 [70-120]	106 [80-235]	81 [69-99]	0.06
pCO <sub>2</sub> , mmHg	1	30 [24-36]	29 [20-46]	30 [27-36]	0.7
Serum bicarbonates, mmol/L	2	19 [15-23]	16 [10.4-19.4]	21 [17-24]	0.005
Arterial lactate, mmol/L	2	2.5 [1.7-3.9]	5.5 [1.8-8.2]	2.1 [1.5-2.7]	0.009
Highest value in ICU	2	3.1 [2.4-7.1]	7.5 [5.2-15.5]	2.7 [1.7-3.4]	<0.0001
Serum protein, g/L		61 [52-68]	51 [40-57]	65 [58-70]	<0.0001
Serum albumin, g/L		25 [22-28]	27 [23-33]	25 [20-27]	0.1

Triglycerides, mmol/L	15	2 [1.7-3]	2.0 [1.1-3.0]	2.3 [1.8-3.2]	0.4
<b>Immunological findings</b>					
RNA polymerase 3 autoantibodies	7 (18)		7 (54)	0 (0)	0.001
Serum cytokine levels in ICU					
IL-12p70, pg/mL	3	0.03 [0.01-0.4]	0.03 [0.01-0.1]	0.03 [0.01-0.4]	0.3
IL-1 $\beta$ , pg/mL	3	0.2 [0.02-0.4]	0.3 [0.01-0.9]	0.2 [0.02-0.3]	0.5
IL-4, pg/mL	3	0.4 [0.2-1.1]	0.3 [0.3-0.5]	0.6 [0.2-2.1]	0.3
IL-5, pg/mL	3	0.1 [0.01-0.5]	0.04 [0.01-0.6]	0.3 [0.06-0.6]	0.1
IFN- $\gamma$ , pg/mL	3	0.4 [0.2-2.2]	0.4 [0.09-2.0]	1.2 [0.2-2.6]	0.2
IL-6, pg/mL	3	55.2 [25.1-207.6]	39.6 [16.6-225.4]	57.8 [26.9-198.9]	0.7
IL-8, pg/mL	3	82.7 [58.2-166.4]	158.7 [74.9-784.2]	65.7 [55.7-118.3]	0.02
IL-22, pg/mL	3	6.4 [2.3-15.7]	1.5 [0.7-2.9]	9.93 [5.28-28.99]	<0.0001
TNF- $\alpha$ , pg/mL	3	14.2 [8.9-38.1]	8.0 [4.9-34.0]	21.1 [9.9-41.9]	0.05
IL-10, pg/mL	3	50.3 [15.9-76.6]	67.8 [20.1-143.1]	44.2 [12.8-68.4]	0.3
IL-17A, pg/mL	3	1.6 [0.2-5.2]	0.15 [0.08-0.3]	3.2 [0.8-6.2]	<0.0001
IFN- $\alpha$ 2, pg/mL	3	0.02 [0.005-1.3]	2.4 [0.2-15.0]	0.013 [0.002-0.04]	0.001
IFN- $\beta$ , pg/mL	4	0.6 [0.6-0.6]	0.6 [0.6-1.8]	0.6 [0.6-0.6]	0.2
Anti-IFN $\alpha$ autoantibodies	4	5 (15%)	1 (10%)	4 (17%)	1

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Abbreviations: MIS-A, Multisystem Inflammatory Syndrome in Adults; ICU, Intensive Care Unit;

APTT, Activated Partial Thromboplastin Time; LDH, Lactate Dehydrogenase; AST, Aspartate Aminotransferase;

ALT, Alanine Aminotransferase; ALP, Alkaline Phosphatase; GGT,  $\gamma$ -Glutamyl Transpeptidase; IL, interleukin;

TNF, tumor necrosis factor; IFN, interferon.

Continuous variables are expressed as median [interquartile range] and compared with Wilcoxon's rank test;

categorical variables are expressed as n (%) and compared with Fischer's exact test.

<sup>1</sup>Number of missing values.