# Detection of faecal SARS-CoV-2 RNA in a prospective cohort of children with multisystem inflammatory syndrome (MIS-C)

## Determinazione di SARS-CoV-2 in campioni fecali di bambini affetti da sindrome infiammatoria multisistemica (MIS-C)

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### WHAT IS ALREADY KNOWN

- **MIS-C is a systemic multi-inflammatory syndrome temporally associated with SARS-CoV-2 infection.**
- **The virus has been detected in 30%-40% of faecal samples from children with acute COVID-19, but the residual infectivity of the virus, transmitted by faeces, is not clear. The SARS-CoV-2 presence in the wastewater is used to track the infection diffusion.**
- **No data are currently available regarding the presence of SARS-CoV-2 in stools of paediatric patients with MIS-C.**

### WHAT THIS STUDY ADDS

- **SARS-CoV-2 is detectable in more than 10% of children with MIS-C up to >21 days from the presumed contact with the virus.**
- **Confirmation of the presence of SARS-CoV-2 in larger samples of patients with MIS-C and assessment of the contagiousness of the virus in the same samples could help define the appropriate intrahospital isolation measures to be taken for patients with MIS-C, as there is no specific guidance yet.**

### ABSTRACT

**BACKGROUND:** Multisystem Inflammatory Syndrome in Children (MIS-C) is a rare but severe illness associated with SARS-CoV-2 infection. A dysregulated immune response is recognized as the main pathogenic mechanism. Previous studies demonstrated the presence of SARS-CoV-2 RNA in faeces of almost one-third of patients with COVID-19, while data are currently missing about MIS-C.

**OBJECTIVES:** to evaluate faecal sample positivity to SARS-CoV-2 in MIS-C and to compare the positivity rate between MIS-C and COVID-19 hospitalised children.

**DESIGN:** observational descriptive study with prospective patient enrollment.

**SETTING AND PARTICIPANTS:** the SARS-CoV-2 positivity was evaluated in stool samples obtained in a prospective series of 63 paediatric patients admitted to Regina Margherita Children's Hospital (Azienda Ospedaliero Universitaria – Città della Salute e della Scienza, Turin, Northern Italy) with diagnosis of MIS-C (N. 31) or COVID-19 (N. 32), during the first year of pandemic emergency. The real-time reverse transcription polymerase chain reaction (real-time RT-PCR), was performed using a validated kit measuring 3 target SARS-CoV-2 genes: *E* gene, *N* gene, and *ORF1ab* gene

**MAIN OUTCOME MEASURES:** SARS-CoV-2 stool positivity and concomitant gastrointestinal symptoms.

**RESULTS:** overall, 16/63 (25%) stool samples revealed the presence of SARS-CoV-2 mRNA. In patients with COVID-19, faecal samples were collected 8 days as median (IQR 7) after the presumed viral exposure and were positive in 12/31 (39%; 95%CI 23.2-56.2); among children with MIS-C, stools were collected 27.5 days as median (IQR 26.25) after presumed contact and the positivity rate was 12.5% (95%CI 4.4-27.0) (4/32). More than 80% of the children with MIS-C presented gastrointestinal symptoms, but the frequency of gastrointestinal symptoms in patients with positive stools for SARS-CoV-2 RNA is not higher than patients tested negative (p=0.092).

**CONCLUSIONS:** MIS-C patients frequently experienced gastrointestinal symptoms, confirming the intestinal involvement in MIS-C already described in the literature. The presence of SARS-CoV-2 mRNA in faecal samples is confirmed in more than 10% of MIS-C patients and stool positivity was also detected many days after presumed first contact with the virus. This data suggests the possibility of tracing SARS-COV-2 also in faeces for a better description of its circulation and spread in the environment.

**Keywords:** SARS-CoV-2, stools, COVID-19, MIS-C, SARS-CoV-2 infection, children

### RIASSUNTO

**INTRODUZIONE:** la sindrome infiammatoria multisistemica in età pediatrica (MIS-C) correlata a SARS-CoV-2 è una manifestazione rara, ma grave e potenzialmente fatale, che è stata descritta in letteratura a partire dall'aprile 2020. La patogenesi non è ancora del tutto chiarita. La tempistica di esordio, a 2-4 settimane dall'infezione acuta da SARS-CoV-2, suggerisce che siano coinvolti meccanismi di tipo immuno-mediato post-infettivo, piuttosto che un meccanismo virale diretto. Numerosi studi precedenti hanno dimostrato la presenza di mRNA di SARS-CoV-2 in un terzo delle feci di bambini affetti da COVID-19, mentre non sono presenti dati su pazienti affetti da MIS-C.

**OBIETTIVI:** valutare la positività di SARS-CoV-2 su campioni fecali di bambini con diagnosi di MIS-C a confronto con casi pediatrici di COVID-19.
**DISEGNO:** studio osservazionale descrittivo con arruolamento prospettico dei pazienti.
**SETTING E PARTECIPANTI:** la ricerca diretta di SARS-CoV-2 su campioni di feci è stata effettuata in una serie di 63 pazienti pediatrici ricoverati presso l'Ospedale infantile Regina Margherita dell'Azienda ospedaliero-universitaria Città della Salute e della Scienza di Torino con diagnosi di MIS-C (n. 31) o COVID-19 (n. 32) durante il primo anno di emergenza pandemica. La positività a SARS-CoV-2 è stata valutata con test molecolare real-time (*Reverse Transcription and Polymerase Chain Reaction, RT-PCR*) utilizzando un kit validato e valutando la presenza di tre geni target di SARS-CoV-2: gene *E*, gene *N* e gene *ORF1ab*.
**PRINCIPALI MISURE DI OUTCOME:** positività delle feci a mRNA di SARS-CoV-2 e presenza di sintomi gastrointestinali (dolore addominale, diarrea, nausea e/o vomito).
**RISULTATI:** la ricerca dei geni di SARS-CoV-2 nelle feci con RT-PCR ha messo in evidenza positività in 16/63 (25%) campioni raccolti. Nei pazienti con COVID-19, i campioni fecali sono stati raccolti 8 giorni (mediana, IQR 7) dopo la presunta esposizione al virus e sono risultati positivi nel 39% (IC95% 23.2-56.2) dei casi (12/31); nei pazienti con MIS-C, le feci sono state raccolte 27,5 giorni (mediana, IQR 26,25) dopo il presunto contatto e il tasso di positività è stato del 12,5% (IC95% 4,4-27,0) (4/32). La presenza di sintomi gastrointestinali è stata osservata in più dell'80% dei pazienti affetti da MIS-C, ma non risulta più frequente nei pazienti con feci positive per SARS-CoV-2 rispetto ai pazienti con feci negative (p=0,092).
**CONCLUSIONI:** i pazienti con diagnosi di MIS-C hanno sofferto con elevata frequenza di sintomi gastrointestinali, confermando il coinvolgimento intestinale già descritto in letteratura. È confermata la presenza di mRNA di SARS-CoV-2 in più del 10% dei campioni fecali di pazienti affetti da MIS-C, anche dopo numerosi giorni dal presunto contatto con il virus. Questi dati sottolineano l'opportunità di tracciare la presenza del virus anche nelle feci al fine di descriverne meglio la circolazione e la diffusione nell'ambiente.

**Parole chiave:** SARS-CoV-2, MIS-C, feci, COVID-19, età pediatrica

## BACKGROUND AND OBJECTIVES

Multisystem inflammatory syndrome in children (MIS-C) is a rare but severe illness associated with SARS-CoV-2 infection firstly described in April 2020, approximately one month after the first COVID-19 outbreak.[1,2] The incidence in COVID-19 hotspot area has been estimated at 2 per 100,000 persons aged <21 years.[3] MIS-C usually develops after the infection rather than during the acute stage of the disease, suggesting a dysregulated immune response (i.e., continuous activation of adaptive immune responses driven by persisting antigen presentation) as the primary pathogenic mechanism.[3,4] Only one-third of reported cases presents a positive reverse transcription polymerase chain reaction (RT-PCR) for SARS-CoV-2 on nasal swab; on the other hand, serology for SARS-CoV-2 results positive in the majority of cases.[5,6] MIS-C clinical presentation, characterized by multi-organ involvement, commonly involves gastrointestinal signs and symptoms.[7-9] To the best of the Authors' knowledge, few data are present in literature regarding the detection of SARS-CoV-2 mRNA in faecal samples of patients with MIS-C,[10] even if biospecimen collection methods for children were recently recommended.[11]
This is an observational descriptive study that aims to detect SARS-CoV-2 mRNA in faecal samples of prospectively enrolled patients who matched Centre for Disease Control and Prevention (CDC) case definition criteria for MIS-C.[12]

## MATERIALS AND METHODS

Patients aged below 15 years, with no comorbidities, providing a faecal sample for SARS-CoV-2 assessment, admitted to the major tertiary care paediatric hospital in Piedmont Region (Northern Italy), Regina Margherita Children's Hospital (Azienda Ospedaliera Universitaria - Città della Salute e della Scienza, Turin), during the first year of pandemic (from 15 April 2020 to 29 February 2021) with a diagnosis of MIS-C (according to CDC criteria)[12] were enrolled. As comparison group, patients aged below 15 years and admitted in the same period in the same hospital or followed-up in the outpatient department for a diagnosis of COVID-19 (positive nasopharyngeal swab) and without comorbidities were enrolled.
Written informed consent was obtained from the parents of patients and an official approval was obtained from the Institutional Ethical Committee (Città della Salute e della Scienza di Torino, protocol number 00564/2020).
For each patient enrolled in the study, SARS-CoV-2 stool positivity was evaluated. Moreover, demographic data, clinical presentation (with a specific focus on gastrointestinal symptoms recorded during the acute phase of both COVID-19 and MIS-C diseases), and time from SARS-CoV-2 infection to faecal sample collection were registered (Table 1A). The time from the presumptive contact with the virus was defined as follow:

| | | | COVID-19 (N. 31) | | MIS-C (N. 32) | | p-value |
|---|---|---|---|---|---|---|---|
| A | **Gender** | Female | 14 | 45.2 | 14 | 43.8 | **0.910** |
| | | Male | 17 | 54.8 | 18 | 56.2 | |
| | **Ethnicity** | Africans | 4 | 12.9 | 4 | 12.5 | **0.394** |
| | | Caucasian | 26 | 83.9 | 26 | 81.25 | |
| | | Chinese | 1 | 3.2 | 0 | 0 | |
| | | Hispanic | 0 | 0 | 2 | 6.25 | |
| | **Age** | years | 5.61 | ±5.03 | 7.94 | ±3.93 | **0.037** |
| | **Antibodies against SARS-CoV-2*** | IgG (+) | NA | NA | 27 | 96 | |
| | | IgG (–) | | NA | 1 | 4 | |
| | **Nasopharyngeal swab** | Pos (+) | 31 | 100 | 10 | 31.2 | **<0.001** |
| | | Neg (–) | 0 | 0% | 22 | 68.7 | |
| | **Estimated time from the SARS-CoV-2 contact** | days | 14.3 | ±15.9 | 29.4 (±21.0) | | **<0.001** |
| B | **Extracted RNA from stool** | µg/µL | 120.3 | ±159.0 | 56.0 | ±78.9 | **0.021** |
| | **Gastrointestinal symptoms** | No | 22 | 71 | 6 (18.75) | 18.75 | **<0.001** |
| | | Yes | 9 | 29 | 26 (81.25) | 81.25 | |
| | **SARS-CoV-2 RNA in stool** | Neg (-) | 19 | 61.3 | 28 (87.5) | 87.5 | **0.017** |
| | | Pos (+) | 12 | 38.7 | 4 (12.5) | 12.5 | |

**Table 1.** **(A)** Demographic characteristics, **(B)** gastrointestinal symptoms, and SARS-CoV-2 collected data of the two cohorts of patients. Continuous variables are expressed as mean and standard deviation (SD); the categorical variables are expressed as absolute numbers and percentages.
**Tabella 1.** Dati raccolti dalle due coorti di pazienti e riferiti a **(A)** caratteristiche demografiche, **(B)** sintomi gastrointestinali e positività per SARS-CoV-2. Le variabili continue sono espresse come media e deviazione standard (SD); le variabili categoriche sono espresse come numeri assoluti e percentuali.

* data available only for 28 patients with MIS-C (serological tests by enzyme-linked immunosorbent assays, ELISA) / *dati disponibili solo per 28 panzienti con MIS-C (test sierologici condotti con metodo ELISA)*

- when available, the date of the first positive SARS-CoV-2 nasopharyngeal swab with the addiction of 5 days as typical incubation period was considered;[13]
- for patients with MIS-C without previous positive swab and ascertained intra-familial exposure to SARS-CoV-2, the date of the first positive SARS-CoV-2 swab of familial contacts was considered;
- for patients with MIS-C without positive swab and untested intra-familial exposure, 15 days as the minimum time to produce specific antibodies from exposure were considered.

Faecal samples were obtained at the first stool emission after hospital admission when possible (for 7 COVID-19 patients, faecal sample was collected at the first control visit) and after having obtained written informed consent by the caregiver. They were stored at -80°C immediately after collection, until the extraction and analysis were performed in the lab (Hygiene Section, Public Health and Paediatric Department, University of Turin). Extraction of total mRNA from faecal samples was performed with a commercialized kit (Power Microbiome RNA kit, QIAGEN). The extracted mRNA was quantified using a NanoQuant Plate (TECAN Trading AG, Switzerland), which allows quantification using a spectrophotometer read at 260 nm. The spectrophotometer used was the TECAN Infinite 200 PRO, and the software was i-Control (version 1.11.10). The RNA quality was tested both by spectrophotometer analysis (mean 260/280 ratio: 1.93±0.20) and by gel electrophoresis to control no excessive nucleic acids fragmentation. Extracted nucleic acids were stored at –80°C until molecular analysis was performed.

The real-time RT-PCR was performed using the Novel Coronavirus (2019-nCoV) Real-Time Multiplex RT-PCR kit from LifeRiver Ltd., and CFX Instruments (Bio-Rad, Hercules, CA, USA) following the suggested thermal protocol included in the kit instructions and according to literature data.[14] The assay measures simultaneously 3 target genes: SARS-CoV-2 *E* gene, *N* gene, *ORF1ab* gene according to international validated testing protocols.[14,15]

## DATA ELABORATION AND STATISTICAL ANALYSES

The statistical analysis was performed using IBM SPSS statistics package 27.0. A descriptive analysis of the variables was conducted. The data were reported as absolute numbers and percentages for categorical variables, as means and standard deviations for continuous variables. Moreover, the subjects were divided into two groups based on the diagnosis: COVID-19 or MIS-C. Differences between COVID-19 and MIS-C children were assessed using the chi2 test with Fisher's correction for categorical variables and Mann-Whitney test for continuous variables. The Spearman rank-order correlation coefficient was also determined to assess the relationships between variables.


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## RESULTS

Overall, 28 females and 35 males, aged 1 month to 15 years, were prospectively enrolled in the study (Table 1A). Among 31 patients with COVID-19, 24 were enrolled during the acute stage of the disease and 7 at the follow-up in outpatients' clinics after hospitalization. Demographic and clinical characteristics of patients are reported in Table 1A. The age of the COVID-19 included patients is lower than MIS-C, as reported in the literature.[3]

Data regarding IgG against SARS-CoV-2 were available for 28/32 patients with MIS-C, as 4 children were enrolled before the development of validated methods (i.e., serological tests by enzyme-linked immunosorbent assays, ELISA); 27/28 (96%) children with MIS-C had a positive serology, confirming past infection.

Gastrointestinal symptoms (i.e., at least one among abdominal pain, nausea, vomiting or diarrhoea) were reported during hospitalization for the acute phase of the diseases in 9/31 (29%) patients with COVID-19 and 26/32 (81%) children with MIS-C (p <0.001). Overall, 16/63 (25%) faecal samples revealed the presence of SARS-CoV-2 RNA. The extracted RNA concentration was meanly 17.6 ± 25.6 µg/mg stool and varied in a wide range of quantity (from 3 to >790 ng/µg faeces).

Considering the whole sample (N. 63), time from presumptive viral exposure was 14 days (median, IQR 26) in patients with negative stools and 8 days (median, IQR 16.50) in patients with positive stools (p=0.110). Moreover, cycle threshold values (Ct)) observed for each SARS-CoV-2 gene correlated with the estimated days from the contact (Spearman's rho 0.309 and p=0.014 for *ORF1ab*; 0.269 and p=0.033 for *N* gene; 0.301 and p=0.016 for *E* gene), showing a presumably lower concentration of the virus when the time from the contact is longer. Indeed, a significant difference is observed between the Ct in MIS-C (2 cycles higher) and the COVID-19 (35.2 ± 2.6 vs 32.9 ± 3.9 p= 0.022 for *ORF1ab*; 34.6 ± 3.0 vs 32.5 ± 4.2, p=0.015 for *N* gene; 35.4 ± 4.4 vs 33.2 ± 5.5, p=0.021 for *E* gene).

The positivity rate of stools was comparable in patients with or without gastrointestinal clinical involvement, 37.5% (6/35) vs 35% (10/28), p=0.092.

In patients with COVID-19, faecal samples were collected 8 days as median (IQR 7) after the presumed viral exposure and were positive in 12/31 (39%, 95%CI 23.2-56.2); among children with MIS-C, stools were collected 27.5 days as median (IQR 26.25) after presumed contact and the positivity rate was 12.5% (4/31) (95%CI 4.4-27.0) (Table 1B). Positive stools of patients with MIS-C were collected at 27, 36, 43, and 72 days from presumptive primary infection (Figure 1); in the latter, concomitant serology for SARS-CoV-2 resulted negative, but the exposure to a confirmed COVID-19 case was ascertained.

## DISCUSSION

Multisystem inflammatory syndrome in children is a severe emerging illness affecting paediatric patients, temporally related to SARS-CoV-2 infection.[1,2] The SARS-

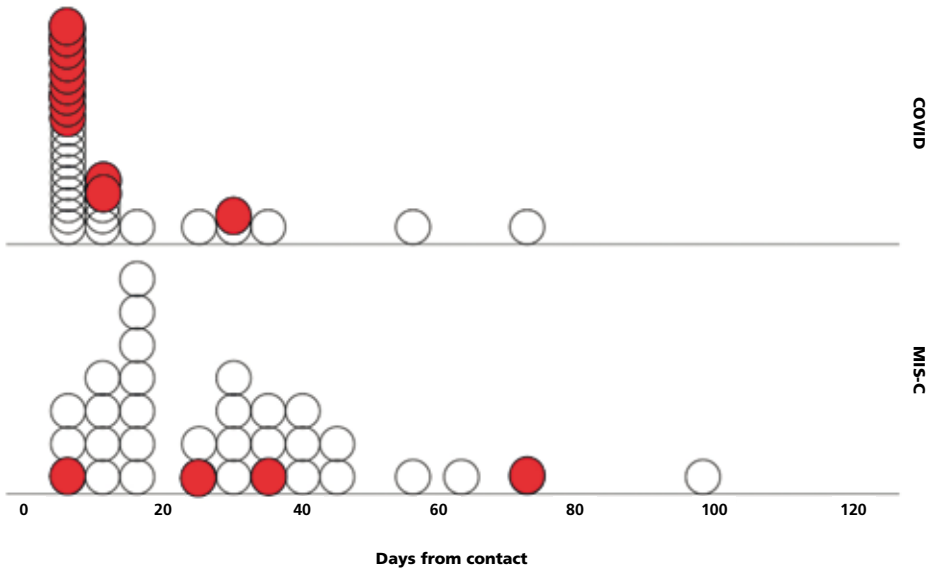


**Figure 1.** Distribution of the samples concerning the estimated time from the SARS-CoV-2 contact causing the following COVID-19 or MIS-C and stool positivity (red).
**Figura 1.** Distribuzione dei campioni sul tempo stimato dal contatto col virus SARS-CoV-2 che ha causato la malattia COVID-19 oppure MIS-C e campione di feci positivo (rosso).


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CoV-2 attachment site, i.e., the angiotensin-converting enzyme 2 (ACE-2 receptor), is widely expressed also in the gut.[16-20] Previous studies have already demonstrated the presence of SARS-CoV-2 mRNA in faeces of almost one third of symptomatic patients with COVID-19,[17,21-25] both adults and children. Prolonged persistence of SARS-CoV-2 mRNA in stools have been already described.[16,26-28] The contagiousness of the virus in the stool and the possible faecal-oral route of transmission remain unproven,[21,29-33] even if in the 35% of positive samples the virus is vital.[34] The presence of the virus in the faeces can be used to track the spreading of the infection.[35] Such principle constitutes the basis of the waste-water epidemiology and of the SARS-CoV-2 waste-water surveillance today active in Italy and in the European Community,[35] as in other countries.[36]

Children, compared to adults, have a higher ACE-2 expression in the gut[17] and present a higher ACE expression inside the intestine than in the respiratory tract;[25] these data may explain both the higher proportion of gastrointestinal symptoms and the higher percentage of positive faeces in the paediatric than in the adult form of COVID-19.[17]

MIS-C clinical presentation, characterized by multi-organ involvement, commonly includes gastrointestinal signs and symptoms. According to literature,[7-9] in the cohort considered in the present study, diarrhoea, nausea, vomiting or abdominal pain were more frequent in patients with MIS-C compared to children with COVID-19 ($p< 0.001$).

However, to the best of the Authors' knowledge, data regarding the presence of the virus in the gut of patients with MIS-C is lacking. This study would like to fill such knowledge gap, although it has several limitations, including

- the conventional but arbitrary estimated method applied to define the time from exposure to SARS-CoV-2;
- not perfectly comparability between the two groups of subjects (for example considering age);
- a limited real-time faeces sampling for some patients, due both to the slow intestinal transit in MIS-C subjects and to the limited length of stay for COVID-19 patients, although the study was designed to obtain stool sampling at the first emission after admission;
- finally, the small sample size and the significant difference in collection time from exposure between the two groups could have a potential impact on the results and on their interpretation.

Nevertheless, in the present study, more than 10% of MIS-C patients tested positive for SARS-CoV-2 mRNA in faeces, while the rate of patients with acute COVID-19 and faecal SARS-CoV-2 positivity was consistent with previously reported data,[25] confirming the reliability of the methods used in this study.

## CONCLUSIONS

In the considered cohort, SARS-CoV-2 was detectable in more than 10% of children with MIS-C up to >21 days from the presumed contact with the virus (21 days is the time limit for isolation of SARS-CoV-2 infected subjects with persistently positive molecular testing and no residual symptoms, introduced by the Italian Ministry of Health). If confirmed on larger case series, these data could add additional knowledge regarding the presence and duration of positive faecal samples in children with MIS-C. Regarding the intra-hospital setting, the confirmation of the presence of SARS-CoV-2 in larger sampling of MIS-C patients and the concomitant evaluation of virus viability and contagiousness could help to define appropriate intra-hospital isolation measures. These strategies, which have not been applied so far, include personal equipment and infection control procedures to be adopted for this type of patients during the in-hospital care.

**Conflicts of interest:** none declared.

**Ethical approval and consent to participate:** the study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Città della Salute e della Scienza di Torino, protocol number 00564/2020. Informed written consent was obtained from all legal guardians of the subjects to participate in the study

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**Authorship:** EP and DT and UR gave substantial contribution to conception and design, drafted the article, reviewed and critically revised the manuscript. DT and EF made stool sample processing and extraction and bio-molecular analysis. DT made statistical analysis and bioinformatics. AC, EF, GP, FP and MD contributed to conception and design, collected data, and revised the manuscript. All the authors approved the final version of the manuscript.

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