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RESEARCH ARTICLE

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# The comprehensive clinic, laboratory, and instrumental evaluation of children with COVID-19: A 6-months prospective study

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## Abstract

**Objectives:** To perform a comprehensive clinic, laboratory, and instrumental evaluation of children affected by coronavirus disease (COVID-19).

**Methods:** Children with a positive result of nasopharyngeal swab for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) underwent laboratory tests, anal and conjunctival swab, electrocardiography, lung, abdomen, and cardiac ultrasound. Twenty-four-hour ambulatory blood pressure monitoring was performed if abnormal basal blood pressure. Patients were followed-up for 6 months.

**Results:** Three hundred and sixteen children were evaluated; 15 were finally included. Confirmed family member SARS-CoV-2 infection was present in all. Twenty-seven percent were asymptomatic. Anal and conjunctival swabs tests resulted negative in all. Patients with lower body mass index (BMI) presented significantly higher viral loads. Main laboratory abnormalities were: lactate dehydrogenase increasing (73%), low vitamin D levels (87%), hematuria (33%), proteinuria (26%), renal hyperfiltration (33%), and hypofiltration (13%). Two of the patients with hyperfiltration exhibited high blood pressure levels at diagnosis, and persistence of prehypertension at 6-month follow-up. No abnormalities were seen at ultrasound, excepting for one patient who exhibited B-lines at lung sonography. Immunoglobulin G seroconversion was observed in all at 1-month.

**Conclusions:** Our study confirm that intra-family transmission is important. The significant higher viral loads recorded among patients with lower BMI, together with low vitamin D levels, support the impact of nutritional status on immune system. Renal involvement is frequent even among children with mild COVID-19, therefore prompt evaluation and identification of patients with reduced renal function reserve would allow a better stratification and management of patients. Seroconversion occurs also in asymptomatic children, with no differences in antibodies titer according to age, sex and clinical manifestations.

  

KEYWORDS
COVID-19, pediatric, renal, serology, ultrasound, vitamin D

## 1 | INTRODUCTION

In adults, clinical manifestation of 2019 novel coronavirus disease (COVID-19) is nonspecific, ranging from completely asymptomatic to severe pneumonia, acute respiratory distress syndrome, and death.[1] Common manifestations include fever, cough, fatigue, and dyspnea. Conversely, children generally exhibit milder symptoms and better prognosis compared to adult patients,[2] although respiratory failure has been described among them[3] with mortality rates of less than 1%.[4,5]

Reported frequency of asymptomatic infection in children is largely variable among centers, ranging from 13% to 64.9%; however, due to the absence of systematic testing and longitudinal studies, this could be an underestimation.[6] Extra-pulmonary involvement, and in particular renal impairment, is frequent among patients,[7] although data on clinic and radiologic evaluation are scarce in pediatrics. Moreover, the antibody responses against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remain poorly understood and characterization and perdurance of seroconversion is unclear.[8] Aim of this prospective study was to perform a comprehensive clinic, laboratory and instrumental evaluation, including pulmonary, intestinal, renal and cardiac ultrasound (US) evaluation of children affected by COVID-19.

## 2 | PATIENTS AND METHODS

This is a prospective cohort study on pediatric patients (<18 years) with COVID-19 referred to the Pediatric Unit of Santa Maria Goretti Hospital, Latina—Sapienza University of Rome (Polo Pontino) for SARS-CoV-2 infection. All children admitted to our emergency department from April 6, 2020 to June 5, 2020 were evaluated for study inclusion. As per hospital protocol, to limit the contagion, every child was tested for SARS-CoV-2 infection at the admission, by using a nasopharyngeal swab for SARS-CoV-2 RNA. Children who resulted positive to the test and consented to the study protocol, were consecutively enrolled in the study.

### 2.1 | Study design

At diagnosis, children underwent extensive laboratory tests, including serological test for COVID-19. A urine sample was collected for urine analysis. Twenty-four-hour urine collection was performed to evaluate kidney function. In addition, a fecal sample was collected for calprotectin level assessment. Patients underwent anal and conjunctival swab tests for SARS-CoV-2 nucleic acid assessment. Moreover, 12-lead electrocardiogram (ECG), lung, abdomen and cardiac US was performed at baseline. Vital signs (oxygen saturation, blood pressure, pulse rate, and respiration rate) were monitored. Twenty-four-hour ambulatory blood pressure monitoring (ABPM) was performed in patients with abnormal blood pressure. Prehypertension and hypertension were defined according to recent recommendations for the standard assessment of ABPM in children and adolescents.[9] Data on demographics, clinical history, comorbidities, family history of diseases, and SARS-CoV-2 infection were collected on an electronic database. Patients were followed-up for 6 months: visits were scheduled at 1 and 6 month. Urine analysis were repeated on a weekly basis, if abnormalities were seen. Similarly, nasopharyngeal swab test for SARS-CoV-2 was repeated on a weekly basis during the follow-up period until they were negativized. Hematological, kidney function test, and instrumental tests were repeated at 1 month if abnormal; further investigations or earlier repetition of abnormal exams were planned according to medical decision. Serology for COVID-19 was repeated at 1 and 6 months. Written informed consent was obtained from parents of children or their legal surrogates before the enrollment. The study was explicitly approved by the institutional review board of Santa Maria Goretti Hospital. The work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

### 2.2 | Swab-based SARS-CoV-2 detection

The presence of SARS-CoV-2 nucleic acid was detected on nasopharyngeal, anal, and conjunctival swab tests by real-time reverse-transcription polymerase chain reaction (rRT-PCR) targeting three genes: envelope protein (E), RNA-dependent RNA polymerase (RdRp), and nucleocapsid protein (N).

The STARMag 96 × 4 Universal Cartridge Kit (Seegene Inc) was used to extract total RNA, and gene fragments were detected by Allplex 2019 n-CoV assay (Seegene Inc). The cycle threshold ($C_t$) values of rRT-PCR were used as indicators of the copy number of SARS-CoV-2 RNA. According to the manufacturer's instructions, samples with a $C_t$ value of <40 were regarded as SARS-CoV-2 detected, and a $C_t$ value <40 for only one of the three targets was considered positive.

### 2.3 | Laboratory tests and serology for COVID-19

Laboratory tests performed included: full blood count, clotting test (PT, aPTT, and D-dimers, fibrinogen, antithrombin III), blood gas analysis, immunoglobulins (IgA, IgM, and IgG), glucose, amylase, lipase, aspartate transaminase (AST), alanine transaminase, gamma-glutamyltransferase, bilirubin, electrolytes, troponin, creatine phosphokinase, creatine kinase MB, lactate dehydrogenase (LDH), procalcitonin, serum ferritin, c-reactive protein (CRP), erythrocyte sedimentation rate (ESR), vitamin D, total proteins, albumin, urea,

creatinine, lupus anticoagulant (LAC), antinuclear antibodies (ANA), extractable nuclear antigen (ENA), complement $C_3$ and $C_4$, perinuclear antineutrophil cytoplasmic antibodies (p-ANCA), anti-neutrophil cytoplasmic antibodies (c-ANCA), anticardiolipin, anti-beta2 glycoprotein I (GPI) IgG and IgM, fecal calprotectin, urine analysis and 24 h urine collection for kidney function test, such as glomerular filtration rate (GFR), tubular phosphate reabsorption, proteinuria/creatininuria ratio (Pr/Cr), calcium/creatinine ratio (Ca/Cr), 24-h urine protein excretion (Prot/24 h). Vitamin D deficiency was defined if serum hydroxyvitamin D levels were less than 20 ng/ml, while insufficiency if between 20 and 30 ng/ml.[10]

The iFlash-SARS-CoV-2 IgG and IgM assays (YHLO Biotech Co., Ltd) were used to evaluate the antibody response to the virus by chemiluminescence immunoassay, according to the manufacturer's instructions. The antibody levels were expressed as arbitrary unit per ml (AU/ml). The results ≥10 AU/ml was considered positive, and the results <10 AU/ml negative.

## 2.4 | Instrumental studies

Patients underwent 12-lead ECG evaluation and a comprehensive US assessment, including lungs, heart, and abdomen, with a particular attention for kidneys and gut wall. To minimize interobserver variation, US were performed by operator experts in their field, specialized in acquiring and interpreting images for specific parts of the body, and in particular lung US were all performed by S.B., abdominal US by A. B., intestinal US by S. M., and cardiac US by M. R. Chest sonography was carried out using a Samsung RS80A US scanner with Prestige equipment (Samsung Medison Co. Ltd.), with a 12 MHz linear transducer. Abdominal US was carried out after 6 h fasting, by using MyLab 70 XVision system (Esaote SpA), first by means of a 3–6 MHz convex probe for a panoramic view of the abdomen and organs and then with a high frequency 7.5–13 MHz linear-probe, for a better evaluation of the bowel wall (BW). Transthoracic echocardiographic examination was performed by using MyLab 70 XVision system (Esaote SpA), equipped with 2–4-MHz transducer. Lung evaluation was performed according to Copetti et al. technique;[11] assessed variables were: separate B-line, fuse B-lines, fixed B-lines, shining on-off band, irregular pleural line, consolidations, air bronchogram.[12] Abdominal US variables were selected based on a literature search and included: BW thickness, BW stratification, vascularity, mesenteric lymph nodes, mesenteric hypertrophy, abdominal free fluid collections evaluation.[13] Renal US included artery Doppler and evaluation of Doppler-derived renal resistive index. Standard echocardiography imaging protocol included M-mode based measurement of left atrial diameter, left ventricular end-diastolic and systolic diameter, wall thickness, and left ventricular mass index calculation.[14]

## 2.5 | Statistical analysis

Statistical analyses were executed using GraphPad Prism version 6 (GraphPad Software Inc.). Continuous variables were expressed as a median (range), median (interquartile range [IQR]) and mean ± SD and categorical variables as frequencies (%).

We analyzed the differences between the groups using the $\chi^2$ test for nominal variables. For continuous variables we tested the approximation to normal of the distribution of the population by Kolmogorov–Smirnov one-sample test and statistics for kurtosis and symmetry. As results were asymmetrically distributed, nonparametric tests were used. To analyze the potential association between the continuous variables (i.e., age, laboratory parameters) and nasopharyngeal swab positivity levels ($C_t$) a regression analysis was performed. A $p$ value below 0.05 was regarded as statistically significant.

# 3 | RESULTS

During the study period, a total of 316 children were referred to our emergency department. A total of 311/316 patients (98.4%) were tested for SARS-CoV-2 infection by using a nasopharyngeal swab for SARS-CoV-2 RNA. A total of 17/311 children (5.5%) resulted positive to the test, thus they were enrolled in the study. However, 2/311 children (0.6%) were subsequently excluded due to scarce compliance to the study protocol. A total of 15/311 (4.8%) patients were finally analyzed. Patients' demographics and clinical features are summarized in Table 1.

## 3.1 | Epidemiological characteristics

Confirmed family member SARS-CoV-2 infection was present in all the children included, and in particular 12 (80%) had both parents infected, and 3 (20%) only one, whose symptoms developed earlier. A total of 12 siblings were present in 11 families of the patients enrolled. The remaining four, were single child families. Siblings were all infected in 9/11 families (81.8%), with a total of 9/12 siblings (75%), and a negative result of rRT-PCR on nasopharyngeal swabs was described in 3/12 siblings (25%) from 2/11 (18.1%) different clusters. Patients comorbidities were allergic rhinitis in 2 (13.3%), allergic asthma in 1 (6.7%) and coeliac disease in 1 (6.7%).

## 3.2 | Clinical manifestations

Characteristics of symptoms are described in Table 1. Mean time from symptom onset and diagnosis was 2.45 days (±1.2 *SD*), with a median of 2 days (range = 1–5; interquantile range [IQR] = 1.5). Median temperature recorded among febrile patients was 38°C (mean = 37.7 ± 1.53; IQR = 1.5), with median fever duration of 3.6 days (mean = 1.9 ± 3.65; IQR = 2). Physical examination revealed hyperemia of the pharynx in 8 (53.3%), abdominal swelling, tender to the touch in 5 (33.3%), active conjunctival injection with no discharge in 1 (6.7%). No abnormal breath sounds, or other signs were found. Vital signs were all within normal limits for age, excepting for 2 (13.3%) who exhibited a sisto-diastolic blood pressure greater than 90th percentile for age, sex, and height.

**TABLE 1** Patients' characteristics and clinical features

| Patients ($n$ = 15) | |
|---|---|
| Age, median (range), years | 12.2 (4.8–17.8) |
| Gender (F/M) | 7/8 |
| Caucasians, $n$ (%) | 15 (100) |
| BMI, mean ± $SD$, (median; range) | 19.48 ± 3.2 (20.1; 13.4 to 24.1) |
| BMI $Z$ score, mean ± $SD$, (median; range) | 0.57 ± 1.08 (0.4; −2.07 to 2.48) |
| Family cluster, $n$ (%) | 15 (100) |
| Comorbidities, $n$ (%) | 4 (26.7) |
| Symptoms, $n$ (%) | 11 (73.3) |
| Fever (bt > 37.5°C), $n$ (%) | 8 (53.3) |
| Respiratory symptoms, $n$ (%) | 3 (20) |
| Dry cough, $n$ (%) | 3 (20) |
| Sore throat, $n$ (%) | 1 (6.7) |
| Rhinitis, $n$ (%) | 1 (6.7) |
| Gastrointestinal symptoms, $n$ (%) | 5 (33.3) |
| Diarrhea, $n$ (%) | 4 (26.7) |
| Abdominal pain, $n$ (%) | 2 (13.3) |
| Inappetence, $n$ (%) | 2 (13.3) |
| Nausea, $n$ (%) | 1 (6.7) |
| Generalized urticaria, $n$ (%) | 1 (6.7) |
| Myalgia, $n$ (%) | 3 (20) |
| Ageusia, $n$ (%) | 3 (20) |
| Anosmia, $n$ (%) | 2 (13.3) |
| Headache, $n$ (%) | 2 (13.3) |
| Fatigue, $n$ (%) | 1 (6.7) |
| Arthralgia, $n$ (%) | 1 (6.7) |

Abbreviations: BMI, body mass index; bt, body temperature; $n$, number; y, years.

### 3.3 | Nasopharyngeal, anal, and conjunctival swab test results

Characterization of genes detection of nasopharyngeal swab with mean $C_t$ values ± $SD$ and 95% confidence interval (CI) are shown in Table 2. The $C_t$ values were not influenced by the patients' age ($p$ = .48), sex ($p$ = .35), comorbidities ($p$ = .56), and time from symptoms onset ($p$ = .62). Analyzing the possible predisposing factors to a high viral load, we found a direct correlation between BMI and $C_t$ values ($r$ = .78; 95% CI = 0.45–0.92; $R^2$ = 0.61; $p$ = .0006) (Figure 1A). No significant correlation was found between presence and severity of symptoms, excepting for fever, and in particular we found an inverse correlation between the patient's highest body temperature recorded and the $C_t$ values ($r$ = −0.73; 95% CI = −0.9 to −0.3; $R^2$ = 0.53; $p$ = .002) (Figure 1B). No significant correlation was found with laboratory parameters. Anal and conjunctival swab for viral RNA were performed in all patients at diagnosis, however, they showed a negative result for all three viral genes tested (E, N, and RdRp) in all patients.

### 3.4 | Laboratory findings

Main laboratory results are all summarized in Table 3, while abnormal findings only are shown in Table 4. No statistically significant differences in laboratory findings were observed among genders, different age groups, symptomatic and asymptomatic patients, and symptoms characteristics.

### 3.5 | Instrumental evaluation

ECG was performed in all and showed no pathological signs. All patients underwent lung US, that was normal in all the patient

| | Swab no 1 | Swab no 2 | Swab no 3 | Swab no 4 |
|---|---|---|---|---|
| Gene E, *n* (%) | 8 (53.3) | 0 (0) | 0 (0) | 0 (0) |
| $C_t$, mean ± *SD* (95% CI) | 26.72 ± 6.55 (4.85) | – | – | – |
| Gene RdRp, *n* (%) | 9 (60) | 3 (20) | 1 (6.7) | 0 (0) |
| $C_t$, mean ± *SD* (95% CI) | 27.21 ± 6.46 (4.22) | 35.07 ± 2.42 (2.73) | 37.44 | – |
| Gene N, *n* (%) | 13 (86.7) | 4 (26.7) | 2 (13.3) | 1 (6.7) |
| $C_t$, mean ± *SD* (95% CI) | 32.16 ± 5.72 (3.1) | 34.26 ± 5.36 (5.25) | 36.19 ± 0.44 (0.6) | 37.64 |

Abbreviations: $C_t$, cycle threshold; CI, confidence interval; *n*, number.

**TABLE 2** Nasopharyngeal swabs result at diagnosis and during follow-up period, with characterization of gene detection among patients and $C_t$ values of rRT-PCR

but one, who exhibited separate B-lines, even if not numerically significant. Renal US with renal artery doppler, gastrointestinal US and echocardiogram was performed in all; however, no abnormalities were noticed. The two patients who exhibited abnormal blood pressure levels at diagnosis, underwent to a 24-h ABPM in the next few days, showing a nocturnal diastolic prehypertension in one, and diurnal and nocturnal systolic hypertension in the other.

### 3.6 | Serology testing for COVID-19

Quantitative IgG and IgM test for COVID-19 was negative in all the children at baseline. An increasing of IgG-levels for COVID-19 were recorded in all the patients at 1 month, conversely, IgM-levels were negative in all, and in particular: mean IgG levels were 84.9 ± 24.7 *SD*, median = 89.4, IQR = 32.3; mean IgM levels were 4.2 ± 2.8 *SD*, median = 4.6, IQR = 4.81. No significant correlation was found neither between patient's characteristics, symptoms and serological response at 1-month, nor between $C_t$ values at diagnosis and serological response at 1-month ($p > .05$). None of the patients accepted to repeat serology testing at 6-months follow-up.

### 3.7 | Follow-up

After the initial evaluation, no patient presented sign or symptoms requiring hospitalization; therefore, they were managed with outpatient care with supportive therapy and home isolation instructions. Regular exercise and low sodium diet were prescribed to those exhibiting elevated blood pressure levels. Ten out of eleven symptomatic patients (90.9%) reported symptoms resolution within a week of being diagnosed; 1/11 (9.1%) within 14 days. During follow-up, nasopharyngeal swabs were repeated on average every 7 days (mean 6.81 ± 3.7 days; range = 4–10). Table 2 shows nasopharyngeal swabs results at diagnosis and during follow-up period, with mean $C_t$ ± *SD* and 95% CI. The mean time length of negativization was 13.5 days (±4.8 *SD*), with a median of 12.5 days (IQR = 6.7). No differences in time length of negativization was observed according to age, sex, presence of symptoms and $C_t$ values at baseline ($p > .05$). Microscopic hematuria and proteinuria resolved in all patients after 1–2 weeks after presentation (mean 8.3 ± 3.4 days; range = 5–14). At 1-month follow-up visit patients were all asymptomatic and report complete recovery from initial disease. No significant hematologic alterations were recorded at this point. Twenty-four hour ABPM was repeated at 1-month in the patient with diurnal and nocturnal systolic hypertension and showed a nocturnal systolic prehypertension. At 6-months follow-up visit, all

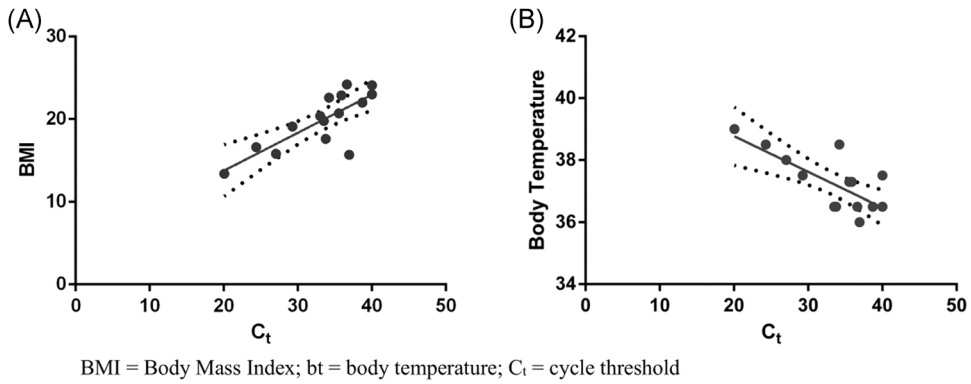


**FIGURE 1** (A) Correlation of patients' BMI values and nasopharyngeal swabs rRT-PCR cycle threshold values and (B) correlation of patients' highest body temperature recorded and nasopharyngeal swabs rRT-PCR cycle threshold values. BMI, body mass index; rRT-PCR, real-time reverse-transcription polymerase chain reaction

**TABLE 3** Laboratory results in children with COVID-19

| Main laboratory parameters | Value (*n*) mean ± *SD* | Median; range | IQR | Reference value |
|---|---|---|---|---|
| Leukocytes | 6.9 ± 1.5 | 7.5; 4.4–10.4 | 1.9 | 4.5–13.5 × $10^3$/ml |
| Neutrophils | 3.2 ± 0.9 | 2.9; 1.8–5.1 | 0.8 | 1.5–8.5 × $10^3$/ml |
| Lymphocytes | 2.9 ± 1 | 2.8; 1.4–5.2 | 1.2 | 1.5–6.5 × $10^3$/ml |
| Platelets | 284.5 ± 39.9 | 273; 217–364 | 45.5 | 150–350 × $10^3$/ml |
| Hemoglobin | 14.1 ± 1.2 | 13.6; 12.8–16.9 | 1.6 | 11.5–13.5 g/dl |
| APTT | 26.7 ± 2.2 | 27; 22–31 | 2.5 | 31.8–46.1 s |
| PT | 11.5 ± 0.6 | 11.3; 10.9–13 | 0.7 | 11.7–16.1 s |
| INR | 1.1 ± 0.06 | 1.1; 1–1.24 | 0.07 | 0.87–1.3 |
| AT III | 104.7 ± 9.4 | 105.5; 88.8– 126.6 | 10.2 | 90%–132% |
| Fibrinogen | 265 ± 60 | 246; 198–400 | 77 | 199–433 mg/dl |
| D-Dimer | 0.3 ± 0.2 | 0.2; 0.18–0.9 | 0.09 | 0.10–0.39 |
| Albumin | 4.8 ± 0.3 | 4.7; 4–5.3 | 0.3 | 3.6–5.2 g/dl |
| IgA | 1.3 ± 0.5 | 1.2; 0.3–2.5 | 0.24 | 0.21–2.91 g/L |
| IgG | 10.9 ± 2.8 | 11.9; 4.4–15 | 3.11 | 5.40–18.22 g/L |
| IgM | 1.2 ± 0.3 | 1.2; 0.5–1.7 | 0.4 | 0.41–1.83 g/L |
| Proteins | 7.3 ± 0.5 | 7.4; 6.2–8.6 | 0.35 | 6–8 g/dL |
| AST | 23 ± 6.6 | 21; 16–37 | 11.5 | 13–40 UI/L |
| ALT | 14.9 ± 4.2 | 15; 8–23 | 5 | 10–35 UI/L |
| GGT | 14.2 ± 10.4 | 11; 9–51 | 4 | 5–28 UI/L |
| Total bilirubin | 0.5 ± 0.2 | 0.4; 0.3–0.9 | 0.3 | <1.5 mg/dl |
| Amilase | 57.8 ± 15 | 59; 22–87 | 15.5 | 25–101 UI |
| Lipase | 19.8 ± 10.2 | 18; 8–47 | 12 | 3–32 UI/L |
| Urea | 28 ± 6.5 | 28; 20–39 | 9.5 | 17–50 mg/dl |
| Creatinine | 0.62 ± 0.1 | 0.58; 0.48–0.87 | 0.18 | 0.4–0.9 mg/dl |
| Sodium | 140.1 ± 1.35 | 140; 138–144 | 1 | 135–147mEq/L |
| Potassium | 4.1 ± 0.4 | 4; 3.5–5 | 0.5 | 3.4–4.7 mEq/L |
| Chlorine | 106.1 ± 2.3 | 106; 101–110 | 2.5 | 97–107 mg/dl |
| Calcium | 9.7 ± 0.4 | 9.7; 8.8–10.5 | 0.5 | 8.5–10.5 mg/dl |
| Magnesium | 1.9 ± 0.1 | 1.9; 1.8–2.2 | 0.1 | 1.6–2.4 mg/dl |
| Phosphorus | 4.4 ± 0.6 | 4.4; 3.5–5.9 | 0.7 | 3.3–5.4 mg/dl |
| Troponin | 0 ± 0 | 0; 0–0.01 | 0 | 0–0.016 ng/ml |
| CPK | 99.1 ± 30.4 | 86; 63–154 | 50 | 20–180 UI/L |
| CK-MB | 2.1 ± 3.5 | 1.1; 0.3–14.4 | 0.9 | 0–3.1 ng/ml |
| LDH | 231 ± 41.4 | 236; 171–320 | 62.5 | 100–190 UI |
| Glucose | 83.9 ± 8.8 | 84; 66–104 | 7.5 | 60–100 mg/dl |
| Procalcitonin | 0.04 ± 0.04 | 0.03; 0–0.18 | 0.02 | <0.1 ng/ml |
| ESR | 9.8 ± 6.4 | 8; 2–27 | 7 | 0–10 mm/h |
| Serum ferritin | 49.8 ± 40.7 | 33; 18–146 | 36.5 | 7–140 ng/ml |
| CRP | 0.13 ± 0.25 | 0.04; 0.01– 0.87 | 0.05 | 0–0.5 mg/dl |

(Continues)

| Main laboratory parameters | Value (*n*) mean ± *SD* | Median; range | IQR | Reference value |
|---|---|---|---|---|
| Vit. D | 25.8 ± 8.4 | 25; 17–51 | 9 | 16–65 pg/ml |
| $C_3$ | 1.04 ± 0.1 | 1.05; 0.8–1.3 | 0.13 | 0.88–1.95 g/L |
| $C_4$ | 0.23 ± 0.07 | 0.23; 0.12–0.42 | 0.07 | 0.12–0.40 g/L |
| P-ANCA | 0 ± 0 | 0; 0–0 | 0 | <10 UA/ml |
| C-ANCA | 0 ± 0 | 0, 0–15 | 0 | <10 UA/ml |
| Anti-cardiolipine IgM | 1.2 ± 3.1 | 0; 0–9 | 0 | <10 U. MPL/ml |
| Anti-cardiolipine IgG | 0 ± 0 | 0; 0–0 | 0 | <10 GPL/ml |
| Anti-beta2 GPI IgM | 0.7 ± 1.7 | 0; 0–5.7 | 0 | <10 UA/ml |
| Anti-beta2 GPI IgG | 0.03 ± 0.12 | 0; 0–0.5 | 0 | <10 UA/ml |
| LAC | 39.1 ± 4.03 | 38.2; 34.4–50.9 | 4.05 | 30–45 s |
| ANA | 0 ± 0 | 0; 0–1 | 0 | Absent (1:160) |
| ENA | 0.36 ± 0.4 | 0.25; 0–1.5 | 0.47 | <1 |
| Fecal calprotectin | 48.7 ± 50.2 | 22.5; 19–189 | 47.5 | <50 mcg/g |
| Gas | | | | |
| Ph | 7.3 ± 0.03 | 7.34; 7.28–7.4 | 0.06 | 7.35–7.45 |
| Hco3- | 21.5 ± 1.8 | 21.6; 19–25.2 | 2.45 | 22–26 mEq/L |
| BE (ecf) | −2.4 ± 2.5 | −2.2; −6.4 to 1.9 | 3.95 | −2 to +2 mmol/L |
| Anion gap | 15.9 ± 1.8 | 16.2; 12.3–19.6 | 2 | 10–20 mmol/L |
| Urine analysis | | | | |
| Red blood cells | 5.15 ± 6 | 4; 0–20 | 9 | 0–5 n°/μl |
| Leukocytes | 10.8 ± 23.7 | 2; 0–87 | 5 | 0–18 n°/μl |
| Ph | 6.05 ± 0.28 | 6; 5.5–6.5 | 0 | 5–8 |
| Albumin | 11 ± 11.4 | 6.5; 0–45 | 6.5 | 2–20 mg/dl |
| FENa | 0 ± 0 | 0; 0.003–0.02 | 0 | <1% |
| GFR | 175 ± 85.4 | 137; 67–286 | 148 | 90–150 ml/min/1.73 $m^2$ |
| TPR | 90.07 ± 2.81 | 90; 86–96 | 3.7 | >85% |
| Ca/Cr | 0.11 ± 0.01 | 0.09; 0.04–0.26 | 0.01 | 0.05–0.33 |
| Prot/24 h | 116 ± 72.7 | 90; 30–224 | 120 | 0–150 mg/dl |
| Pr/Cr | 0.12 ± 0.004 | 0.11; 0.05–0.26 | 0.007 | <0.2 |

Abbreviations: ALT, alanine transaminase; ANA, antinuclear antibodies; AST, Aspartate transaminase; BE (ecf), base excess in extracellular fluid; Ca/Cr, calcium/creatinine ratio; c-ANCA, antineutrophil cytoplasmic antibodies; CK-MB, creatine kinase MB; CPK, creatine phosphokinase; CRP, c-reactive protein; ENA, extractable nuclear antigen; ESR, erythrocyte sedimentation rate; FENa, fractional excretion of sodium; GFR, glomerular filtration rate; GGT, gamma-glutamyltransferase; GPI, glycoprotein I; LAC, lupus anticoagulant; LDH, lactate dehydrogenase; p-ANCA, perinuclear anti-neutrophil cytoplasmic antibodies; Pr/Cr, proteinuria/creatininuria ratio; Prot/24 h, 24-hour urine protein excretion; TPR, tubular phosphate reabsorption.

patients were asymptomatic. Both the patients with abnormal blood pressure levels at baseline underwent 24-h ABPM at 6-month, showing the persistence of the nocturnal prehypertension in both. No other laboratory or instrumental re-evaluation was performed. Reactivation of COVID-19 infection was not observed among patients.

## 4 | DISCUSSION

Very few studies have been published in children to understand the characteristics, the epidemiology, the management and outcomes in this cohort.[2,5,15]

**TABLE 4** Abnormal laboratory findings in children with COVID-19

| Parameters | Trend, % Pts | Mean ± *SD* | Range | Reference value |
|---|---|---|---|---|
| LDH | ↑ 73.3 | 247 ± 32 | 203–320 | 100–190 mU/ml |
| Vitamin D | ↓ insufficiency 60 | 25.3 ± 3.4 | 20–28 | 30–100 ng/ml |
| | ↓ deficiency 26.6 | 18.2 ± 0.9 | 17–19 | |
| GFR | ↑ 33.3 | 260 ± 26.1 | 224–286 | 90–150 ml/min/1.73 $m^2$ |
| | ↓ 13.3 | 73.5 ± 9.1 | 67–80 | |
| ESR | ↑ 40 | 16 ± 5.7 | 12–27 | 0–10 mm/h |
| Microhematuria | ↑ 33.3 | 11.2 ± 5.3 | 6–20 | <5 RBC/HPF |
| Proteinuria | ↑ 26.6 | 188.7 ± 28.9 | 160–224 | 0–150 mg/dl/24 h |
| CRP | ↑ 13.3 | 0.74 ± 0.18 | 0.61–0.87 | 0–0.5 mg/dl |
| D-Dimer | ↑ 13.3 | 0.66 ± 0.33 | 0.43–0.9 | 0.1–0.39 mcg/ml |
| Fecal Calprotectin | ↑ 13.3 | 149.5 ± 57.2 | 109–190 | 0–100 mg/kg |
| ENA | ↑ 13.3 | 1.4 ± 0.14 | 1.3–1.5 | <1 |
| GGT | ↑ 6.7 | 51 | 51 | 5–28 UI/L |
| Procalcitonin | ↑ 6.7 | 0.2 | 0.2 | <0.1 ng/ml |
| CK-MB | ↑ 6.7 | 14.4 | 14.4 | 0–3.1 ng/ml |
| C-ANCA | ↑ 6.7 | 15 | 15 | <10 IU/ml |

Abbreviations: c-ANCA, antineutrophil cytoplasmic antibodies; CK-MB, creatine kinase MB; CRP, c-reactive protein; ENA, extractable nuclear antigen; ESR, erythrocyte sedimentation rate; GFR, glomerular filtration rate; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase.

Our study confirm that children are infected mainly inside familial clusters,[16] as 100% of our cohort presented at least one affected family member whose symptoms developed earlier. To better interpret our data, it is worth to point out that restrictive social distancing was implemented throughout the study period, including school closures, community events cancellation, dining rooms closure at restaurants and bars. Early reports postulate that lower proportion of cases recorded among children could be attributed to a low susceptibility to infection, possibly due to an immune cross-protection from other coronaviruses, a more robust innate immune response, or from nonspecific protection resulting from recent exposure to other respiratory viruses.[17,18] However, recent evidence suggest that children present similar rates of household secondary attack,[19] even though they exhibit milder symptoms; in effects, according to literature data, asymptomatic, mild and moderate infections comprise over 90% of all infected ones.[15]

The most common clinical manifestation in our cohort, although small, was fever, and that was in line with other pediatric reports. [15] Differently from literature data, we reported lower rates of respiratory symptoms, such as cough, that was present in 20% only of our cohort, compared to 40% in other reports.[20] Despite the large presence of gastrointestinal symptoms (33.3%), the investigation of extrapulmonary localization of the virus trough anal swab failed to identify the SARS-CoV-2 in the digestive tract in all the children tested. The detection of SARS-CoV-2 RNA in stool samples has been largely demonstrated, both in adults and in children even after viral clearance in the respiratory tract.[21,22] Considering the median duration time between the onset symptoms and the first positive rRT-PCR test result for viral RNA in the feces previously described,[23] a too early assessment of the virus at anal swab test in our study, could probably explain the negative result found in our cohort. Similarly, ocular swab for SARS-CoV-2 nucleic acid showed a negative result in all. To our knowledge, this is the first study reporting conjunctival swabs evaluation for viral RNA in children. Negative result for ocular swab was in line with other adult reports, as positive conjunctival swab rates varies between 0% and 5% among adult studies.[24] This finding certainly contributes to raise awareness on ocular transmission, and it is an encouraging fact for ophthalmologists, with implications on patient's management.

Interestingly, analyzing the viral load in the samples obtained from the nasopharyngeal swab at disease presentation, we noticed that patients with lower BMI exhibited lower $C_t$ values ($r = .78$), hence higher viral loads. Scarce data are available regarding the association between viral loads of SARS-CoV-2 and children characteristics as well as clinical manifestation and disease course. A study conducted on 145 children with mild or moderate COVID-19, reported lower median $C_t$ values among children <5 years compared to those 5–17 years or adults,[25] however nutritional status was not evaluated. A recent meta-analysis conducted in adults,[26] showed that higher BMI represented an important risk factor for complication, but with higher infection rates among patients with BMI < 25 kg/$m^2$. Study on children have reached mixed conclusions as to whether higher viral load is associated to severity of symptoms.[27,28] Considering that our patients were all affected by a mild form of

COVID-19, we could not evaluate this aspect; however, we found a significant correlation with viral load and body temperature, thus the viral load detected could be correlated to the body response to battling the virus. Conversely, no correlation between laboratory parameter and $C_t$ values was found, therefore no potential infection biomarker was identified.

This study provides an extensive hematologic evaluation of children with COVID-19. In adult patients, it is common to see abnormalities of full blood count, especially lymphopenia, which have not been confirmed in pediatrics. De Souza et al.[29] recently reviewed the main laboratory findings in children, reporting that the most common hematologic abnormality is a decreased neutrophil count, followed by an increased LDH, procalcitonin and CRP, noted in 38%, 28%, 26%, 18%, respectively. Interestingly, we observed that over two-thirds of our cases showed an increasing in LDH levels. The latter is a common indicator of tissue damage, and some authors have postulated that it can be associated to the lung damage that takes place in COVID-19 patients.[30,31] An inverse association has been previously reported between $C_t$ values in salivary rRT-PCR analysis and LDH levels in children with severe COVID-19.[31] Interestingly, most of our patients (86.6%) presented low vitamin D levels, but no correlation was found with symptoms/signs experienced. To our knowledge, only one pediatric study is available investigating the prevalence of vitamin D deficiency among 40 children with COVID-19,[32] and it reports statistically significant low vitamin D levels in 72.5% compared to 24.3% of healthy matched control subjects; moreover, the authors reported a negative correlation between fever symptom and vitamin D level. It is known that vitamin D has an immunomodulation role, inducing the secretion of antiviral peptides which improve the innate immunity mucosal defense,[33] and vitamin D insufficiency has been associated to an increased risk of acute respiratory tract infections in clinical studies.[34] Some retrospective adult studies showed a correlation between vitamin D level and COVID-19 cases and outcomes.[35] One small cohort study reported the beneficial effect of combined vitamin D, Mg, and vitamin B12 against clinical deterioration of COVID-19.[36] Interventional trials evaluating the role of vitamin D supplementation in preventing COVID-19 are ongoing but have not yet reported their findings.

Another relevant laboratory finding of our study was the high rate of renal impairment in children, with microscopic hematuria in 33% and proteinuria in 26.6%, and this was not associated to worse symptoms presentation nor to other patient's characteristics. Moreover, the evaluation of renal function based on 24-h urine collection demonstrated an abnormal GFR in about 46.6% of our patients, and in particular renal hyperfiltration in 33.3% and renal hypofiltration in 13.3%. During follow-up, we noticed a normalization of the urinalysis in 1 to 2 weeks after presentation; however, two of the patients with hyperfiltration exhibited high blood pressure levels at diagnosis, with persistence of a prehypertension at 6-month follow-up. Renal involvement is frequent in adults, especially in those with critical illness,[37] counting about 75% of patients, and presenting more frequently with proteinuria, and secondly with hematuria, although, the rate of acute kidney injury (AKI) is low.[38] AKI has been reported in three critically ill children with COVID-19 needing intensive care unit.[39] No renal complications have been described in children with mild COVID-19, excepting for one report.[40] Autopsy data on COVID-19 patients have found the presence of relevant renal microvascular damage also in those without clinically detected AKI.[41] We believe that in children a normal renal function reserve allow the kidneys to increase GFR in response to stress, and it depends mainly on nephron mass; therefore a reduced renal function reserve in elderly patients or in patients with comorbidities could represent a risk factor of the development of AKI.

No abnormalities were seen at kidneys US in all patients, nor in other body district evaluated, such as lungs, bowel, and heart, excepting for one patient who exhibited B-lines at lung sonography. The presence alterations of the pleural line at lungs US, the B-lines, white lung areas, and consolidations have largely been described in COVID-19 patients,[42] together with kidneys abnormalities, such as increased renal cortical echogenicity, loss of corticomedullary differentiation, and diminished color Doppler flow to the parenchyma.[43] Conversely, there is a paucity of literature on US abdominal imaging features.[13] Cardiac involvement may occur even without other symptoms in patients with COVID-19[44] and can appear at echocardiography as dilated cardiomyopathy, decrease in ventricular systolic function, pericardial effusion, or as localized wall motion abnormalities or global ventricular depression.[12] Most of these ultrasonography findings are generally characteristic of critically ill patients.

Serology testing for COVID-19 demonstrated IgG seroconversion in all of our patients at 1-month follow-up also in asymptomatic children, while no antibody response, IgG and IgM, was noted at diagnosis. Unfortunately, none of these patients accepted to repeat serology at 6-month follow-up. In most studies, mean time for seroconversion is 10–12 days for IgM, and 12–14 days for IgG.[8,45,46] Considering the clinical significance and social implications it would be interesting to evaluate the longevity of antibody response, that is still unknown in children.

The major limitation of this study is the small sample size. Furthermore, the population analyzed in this study is composed of individuals affected by mild form of COVID-19 who spontaneously referred to our hospital, then the number of asymptomatic children could be larger. Moreover, the quantitative viral load was not available, as $C_t$ values highlights a trend in viral load but does not allow a quantification of the viral copies per milliliter. In addition, although nasopharyngeal swabs were all performed by specialized pediatric nurses, the collection technique could also affect the $C_t$ values. The strengths of this study include the combination of comprehensive epidemiological, clinical, laboratory, and instrumental analysis together with outcomes of a homogeneous cohort of young patients with COVID-19, as well as the assessment of a patient population outside of East Asia.

In conclusion, our study confirm that intra-family transmission is important; even though children exhibit milder symptoms, they should be considered in analyses of transmission and control. Higher viral loads have been recorded among patients with lower BMI,

supporting the impact of nutritional status on immune system. Furthermore, low vitamin D levels have been found among infected patients, rising need for randomized controlled trials and large-scale cohort studies evaluating the association between vitamin D level and COVID-19 infection and severity. Renal involvement is frequent even among asymptomatic or mild COVID-19 pediatric patients, therefore prompt evaluation and identification of patients with reduced renal function reserve would allow a better stratification and management of patients. Even though we agree with Copetti, who defined the lung US "the stethoscope of the new millennium," [47] we did not find any significant abnormalities at lung, abdomen and cardiac sonography in children with mild disease. Lastly, we demonstrate that seroconversion occurs also in asymptomatic children, and no differences in antibodies titer was found between age, sex, and clinical manifestations. Larger study including more severe children with COVID-19 are needed to confirm and better interpret our findings.

## CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## AUTHORS' CONTRIBUTIONS

Dr. Sara Isoldi conceptualized and designed this study, coordinated and supervised data collection, carried out the initial analyses, drafted the initial version of this paper, and reviewed and revised this paper. Formal analysis and investigations were performed by Dr. Saverio Mallardo, Dr. Alessia Marcellino, Dr. Silvia Bloise, Dr. Anna Dilillo, Dr. Emanuela Del Giudice, Dr. Alessia Testa, Dr. Antonio Barberi and Dr. Massimo Raponi. Dr. Donatella Iorfida, Dr. Vanessa Martucci and Dr. Mariateresa Sanseviero designed the data collection instruments, collected data, and carried out the initial analyses. Prof. Flavia Ventriglia and Prof. Riccardo Lubrano critically reviewed this paper for important intellectual content.

## PEER REVIEW

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