Original Article
Detection of SARS-CoV-2 infection in a pediatric population from south Italy without symptoms of Coronavirus Disease 2019

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Abstract: Background: SARS-CoV-2 has had dramatic consequences on the world population in morbidity and mortality and socially. Clinical manifestations range from common cold-like to more severe disease such as bronchitis, pneumonia, severe acute respiratory distress syndrome, multi-organ failure and even death. The pediatric population may be infected with SARS-CoV-2, but is less likely to be symptomatic or develop severe symptoms. Methods: We analyzed a cohort of pediatric subjects from Campania Region, south Italy, without symptoms of SARS-CoV2, to evaluate the distribution of infection in relation to gender and age. Detection on nasopharyngeal swabs was performed with two different RT-PCR methods, a qualitative rapid test (VitaPCR™ SARS-CoV-2 assay) and a quantitative test (SARS-CoV-2 ELITe MGB® assay). Results: Positive subjects were 52.63% male and 47.36% female. Regarding age distribution, we described a consistent increase of detection rate (82.45%) in 0-2 year-old patients. Conclusion: The importance of children in transmitting the virus remains uncertain; however our analysis of the distribution of the infection in these subjects may help monitor SARS-CoV2 spread in the general population.

Keywords: SARS-CoV-2, pediatric population, asymptomatic

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for Coronavirus Disease 2019 (COVID-19) has spread rapidly, causing countless deaths and creating serious economic problems. Many patients present with atypical symptoms or absence of symptoms, complicating early detection and timely isolation of the source. Although clear epidemiologic data are still lacking, children of any age may be infected with SARS-CoV-2, at reduced frequency and severity compared to adults [1-5].

Most of the data relating to the pediatric population come from screening studies carried out following hospital admission for causes independent of the classic symptoms of SARS-CoV-2 infection. However, targeted studies to analyze the prevalence of infection even in asymptomatic children could be useful because it is closely related to the incidence of COVID-19 in the general population [4, 6].

To evaluate the distribution of infection, in this study we selected 1343 pediatric subjects without symptoms of COVID-19 but enrolled for molecular tests for detection of SARS-CoV-2 following the indications of the Ministry of Health on tracking viral spread.

Materials and methods

Case series

From July 1, 2020 to October 31, 2020, 1343 pediatric subjects were admitted to the Santobono hospital in Naples for different pathologic conditions and screened, following the indications of the Ministry of Health on tracking viral spread, for detection of SARS-CoV-2 on nasopharyngeal swabs. In some cases, children had symptoms that overlapped with symptoms of
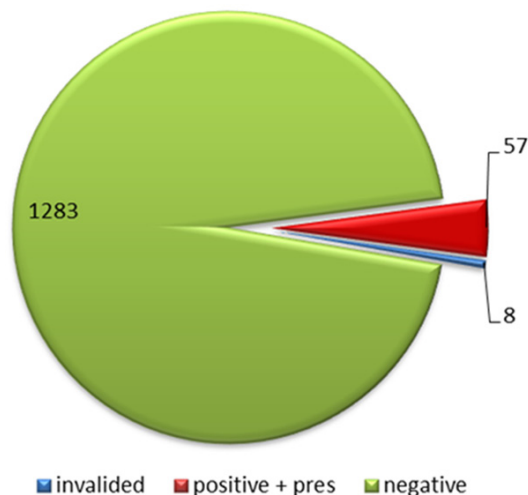
Figure 1. SARS-CoV-2 RNA presence in the pediatric cohort. Invalidate: invalid RT-PCR results; Positive+pres: positive and presumptive (pres) positive for virus infection; Negative: negative for virus infection.

COVID-19, but testing was not done out of suspicion of SARS-CoV-2 as the primary cause of illness.

Before admission, the patients' parents signed a specific consent for data treatment, approved by Santobono hospital.

SARS-CoV-2 detection

We used a rapid test VitaPCR™ SARS-CoV-2 assay (Menarini Diagnostics, Firenze, Italy) that includes three detection systems: (1) one targeting the human β-globin gene, to check the quality of DNA extracts; (2) a second targeting a specific sequence on the nucleocapside N-encoding gene; (3) a third targeting a conserved sequence common to SARS-CoV-2, SARS-CoV, and SARS-like coronavirus, also located on the N-encoding gene [7].

Positive samples for N gene analysis were tested also for the SARS-CoV-2 ELITe MGB® quantitative test (ELITechGroup, Puteaux, France). The kit targets RdRp gene and ORF 8, a specific region of SARS-related coronaviruses that allows specific detection and typing of SARS-CoV-2 wild type species and ORF 8 mutants. The test consists in processing of a swab sample that is collected from the nasopharyngeal tract. Inside the ELITe InGenius®, the sample is automatically mixed with reagents that break

up the virus and release the viral RNA. SARS-CoV-2 RNA is then used to set up a detection reaction by RT-PCR specific probes.

Statistical analysis

Non-parametric tests were used to compare independent groups. Differences in SARS-CoV-2 distribution according to gender groups were analyzed using the Mann-Whitney U-test, and differences in the age groups were detected using Kruskal-Wallis rank test. The level of significance was set at $P<0.05$. All the statistical analyses were carried out using SPSS statistics 20.

Results

Rapid SARS-CoV-2 assay in pediatric cohort

A nucleic acid test (NAT) for SARS-CoV-2 in respiratory specimens is the primary method for diagnosing COVID-19. The gold standard for the laboratory diagnosis of SARS CoV-2 is real-time reverse-transcription polymerase chain reaction panel (rRT-PCR) recommended by FDA [8].

RT-PCR methods are generally designed to amplify S, E, N, RdRp and ORF1a/b genes; however, the N gene RT-PCR is recommended as a screening assay, while ORF1a/b and RdRp provide a quantitative confirmatory test [9].

Our pediatric cohort had 606 females (45.12%) and 737 males (54.87%) with a median age of 52.48 months. SARS-CoV-2 RNA was detected with rapid test VitaPCR™ SARS-CoV-2 assay in nasopharyngeal samples from 60 children (4.46%). The other 1283 children (95.53%) were negative for virus infection. A small percentage of samples had invalid RT-PCR results (8 of 1343; 0.59%) as shown in **Figure 1**.

Quantitative SARS-CoV-2 assay in positive children

Although N gene RT-qPCR can provide quantitative results using C_T values and a standard curve, it is considered a qualitative method for COVID-19 detection [10]. Since both qualitative and quantitative detection of nucleic acids of SARS-CoV-2 play a significant role in COVID-19 diagnosis, surveillance, prevention, and control, we compared qualitative and quantitative
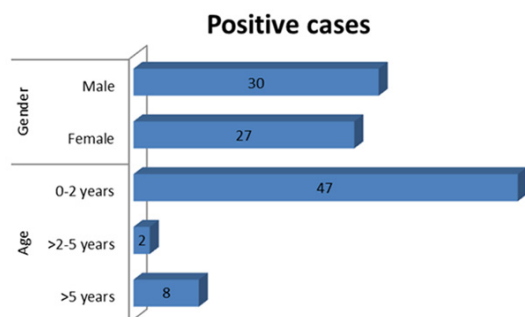
Figure 2. Distribution by gender and age of SARS-CoV-2 RNA-positive children.

analyses to evaluate the diagnostic performance. 60 positive samples from N gene analysis were tested also for ORF and RdRp genes with the SARS-CoV-2 ELITe MGB® test.

We found that 57 of 60 children (95%) were positive for COVID 19 by both methods. Positive subjects were aged between 20 days and 13 years with similar percentages of male and female patients as reported in **Figure 2** (30 males and 27 females; 52.63% and 47.36% respectively).

Regarding age distribution of SARS-Cov2 we divided the 57 patients into three age groups: early childhood, 0-2 years; preschool age, 2-5 years; third childhood: ≥5 years. The detection rate was lowest in 2-5 years patients (3.5%), median in ≥5 year-old patients (14.03%), while a consistent increase in the detection rate in 0-2 year-old patients has been highlighted (82.45%). The distribution by age group is shown in **Figure 2**.

Laboratory investigations in pediatric cohort

Adults with COVID-19 usually show a significant or progressive decrease in the absolute number of peripheral blood lymphocytes at the early stage of the disease. The unfavorable clinical course may be associated with lymphopenia, thombocytopenia, neutrophilia, increased concentration of biomarkers of cardiac injury (i.e., cardiac troponins), C reactive protein and other inflammatory cytokines, liver and kidney function tests, as well as D-dimer and procalcitonin [11]. Since little information related to children is present in the literature, we also considered these markers in COV2 SARS-positive subjects (Supplementary Table 1). We

did not find a significant association. The neutrophil and lymphocyte percentage was below the normal range in a few patients. Procalcitonin level was elevated in only one case and D-dimer and ferritin level were significantly increased in 3 patients (Supplementary Table 1).

Discussion

Although children infected with SARS-CoV-2 tend to have milder symptoms with significantly lower mortality than adults [12], they can spread infection and carry virus into their household [13]. For this reason children may be a source of contagion in the SARS-CoV-2 pandemic despite having milder disease or a lack of symptoms [14].

Our data showed that in the context of a pediatric sample population without apparent symptoms of infection with SARS-Cov2, in a defined time period, the incidence is very low. This is in line with the data from the literature, including the hypothesis that children have a reduced incidence of COVID-19 because angiotensin-converting enzyme 2 (ACE2) expression in the nasopharynx increases with age [15, 16].

In our cohort, the interesting data are represented by the greater distribution in the male sex, particularly in pre-school children (0-2 years old), as evidenced also in the adult population [17]. A large multicentre cohort study involving 82 participating health-care institutions across 25 European countries, considered 585 cases of children and adolescents with SARS-CoV-2 infection hospitalized for severe symptoms. Also in this case the trend was in favor of the male sex, and, in the most extreme condition, hospitalization in intensive care occurred in 69% of males and 31% of females [18].

The latter result requires greater attention, because children under age 1, who can become infected during childbirth, appear to be at higher risk of severe illness with COVID-19 than older children [17-20]. This is likely due to their immature immune systems and smaller airways, which make them more likely to develop breathing issues with respiratory virus infections.

The high transmissibility of SARS-CoV-2 before and shortly after the onset of symptoms sug-

gests that merely diagnosing and isolating symptomatic patients may not be sufficient to stop the spread of infection.

Although the spread of coronavirus in the pediatric population is lower, since most children are asymptomatic, analyzing the prevalence of the infection in these subjects can be important to monitoring its spread in the general population [6]. Obviously, the pediatric population is often more varied than the adult one and, above all, demographic factors can strongly influence analysis of the results, making them heterogeneous. However, the collection of these data may be useful for monitoring the spread of COVID-19 in certain geographic areas.

Disclosure of conflict of interest

None.

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Supplementary Table 1. Laboratory values: peripheral blood lymphocyte count, liver and renal function markers, inflammatory markers

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Complete blood count																									
Leukocytes (10^3/µλ) (normal range 6.0-17.0)	19,31	6,55	10,39	4,24	7,76	5,55	5,73	13,42	4,23	6,19	8,33		6,4	6,49	7,16	3,4	6,74	4,97	8,07		4,25	11,44	25,83	12,63	14,35
Neutrophils (%) (normal range 30.00-50.00)	29,9	46,1	87,4	13,5	68,6	60,2	48,8	37,2	56,9	32,4	37,6		10,3	11,8	59,3	33,5	74,2	50,5	15,7	11,2	33,2	24,4	51,9	65,1	11,2
Lymphocytes (%) (normal range 50.00-55.00)	59,6	39,2	8,2	75,7	21,3	20,5	28,6	51	28,4	49,1	48,9		77,2	80,7	30	59,4	11,9	33,8	71,4	78	47,8	60,8	39,2	30,8	78
Lymphocytes (abs 10^3/µλ) (normal range 2.50-16.50)	11,51	2,57	0,85	3,21	1,65	1,14	1,64	6,85	1,2	3,04	4,07		4,94	5,24	2,15	2,02	0,8	1,68	5,76	11,19	2,03	6,96	10,12	3,89	11,19
Hemoglobin (g/dL) (normal range 10.50-13.50)	12,7	13,2	12,1	11,1	11,9	15,7	9,9	12	rs	10,8	10,7		10,4	12,3	13	14,6	12,7	13,5	13,7	12,1	13,5	12	13,8	12,7	12,1
Platelets (10^3/µλ) (normal range 140.00-440.00)	325	162	230	224	218	378	304	265	411	293	537		373	253	212	166	114	212	527	447	416	213	681	472	447
Liver and renal function																									
Creatinine (mg/dL) (normal range 0.20-0.45)	0,34	0,26	0,54	0,34	0,25	0,21	0,23	0,25	0,22	0,32	0,29	0,2	0,2	0,38	0,27	2,2	12,07	0,27	0,17	0,22	0,21	0,22	0,18	0,25	0,22
Aspartate aminotransferase (U/L) (normal range 5-58)	40	36	27		41	41	32	52	54	29	41		51	46	28	24	7	30	16	35	37	62	34	30	35
Alanine aminotransferase (U/L) (normal range 8-40)	16	11	11		22	20	29	52	38	18	27		28	18	15	15	6	17	14	14	29	65	20	9	14
Urea (mg/dL) (normal range 10-38)	16	15	20	28	21	15	8	16	11	26	16	18	18	27	18		160	16	ins	16	22	19	14	22	16
Inflammatory markers																									
C-reactive protein (mg/L) (normal range 0.00-5.00)	0,14	6,63	42,97	1,72	1,15	12,24	3,56	12,75	5,08	0,9	3,24	5,38	0,79	1,18	16,07		0,47	6,03	0,33	0,39	0,87	8,28	13,24	18,73	0,39
D-dimer (mg/L) (normal range (0.00-270.00)								3528	239				588		345						173	197	385	1117	173
Procalcitonin (ng/mL) (normal range 0.00-0.50)		0,04		0,12		0,13	0,14	0,15	0,14	0,2	0,12	0,14					0,51	0,14	0,07	0,05	0,1	0,18	0,02	0,18	0,05
Creatine kinase (U/L) (normal range 24-170)	148	256	356	149	193	110	87	100	145	242	149	59	305	201	141	58	78	135	60	121	145	144	61	37	121
Fibrinogen (mg/dL) (normal range 180.00-400.00)	187							165	217				192							223	235	217	363	245	223
Ferritin (ng/mL) (normal range 5-130)								254,7	440	438,4		161,8						322,5			85,5	255	119,6	43,3	
CK-MB (ng/ml) (normal range 0.10-6.20)	7,01							4,73	3,98			3,41									5,89			1,49	
Troponin (ng/L) (normal range 1-14)	5							27	36												23			5	