



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Letter to the Editor

No detection of SARS-CoV-2 in cystic fibrosis patients at the Regional (Lazio) Reference Center for CF in Italy**1. Letter**

The newly emergent human coronavirus (HCoV), known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a worldwide threat and the most severe public health-care concern. The impact of SARS-CoV-2 on cystic fibrosis (CF) remains poorly understood, still there are serious concerns over its potential effect due to deficiencies in respiratory tract physiology of CF patients that prevent efficient clearance of pathogens from the airways. SARS-CoV-2 infections have been observed in few CF patients and were associated to a not severe infection [1]. Moreover, an asymptomatic case of COVID-19 in an infant with CF has been recently reported [2].

Hence, we retrospectively investigated the incidence of SARS-CoV-2 in a cohort of patients with CF ($n = 265$) who attended clinic visits at the Regional (Lazio) CF Reference Center, either with respiratory disease that required medical attention or in stable conditions, from November 2019 to March 2020, before and after the Italian government restrictions for COVID-19. Three groups of patients were investigated, where a residual amount of respiratory sample was sufficient for respiratory viruses detection: young children ($n = 43 \leq 10$ years of age), adolescents ($n = 39$ 11–18 years), and adults ($n = 183 > 18$ years, Table 1). The local ethics committee approved the study protocol (Sapienza University of Rome, Policlinico Umberto I Hospital); all adults patients and parents/guardians gave informed consent to participate in the study and patients' data were anonymized. Respiratory tract samples were analyzed for common and emerging microorganisms by using appropriate media (BD BBLTM Stacker Plates, Heidelberg, Germany). A panel of reverse transcriptase-polymerase chain reaction methods have been used for the detection of Respiratory syncytial virus (RSV A/B), Influenza A virus (FluA), Rhinovirus (HRV), the HCoVs OC43, 229E, NL-63, and HUK1, and Metapneumovirus. Real-Time PCR reactions targeting the RdRp and the E- genes of SARS-CoV-2 were developed in-house following published protocols [3]. No SARS-CoV-2 positive result was identified in the respiratory samples analyzed; we retain that testing all samples with two primer-probe sets [3] warranted a low rate of false-negative results. By contrast, 95/265 samples (35.8%) were positive for one or more non-SARS-CoV-2 viruses (Table 1). Among virus positive samples, 8.4% ($n = 8/95$) were simultaneously infected by 2 viruses. HRV was the most frequently detected viruses ($n = 56$; 58.9%), followed by RSV ($n = 11$; 11.6%), and FluA ($n = 10$; 10.5%). Less frequently detected respiratory viruses were HCoV-229E ($n = 7$; 7.3%) and HCoV-HKU1 ($n = 3$; 3.2%). A coinfection between HRV and other respiratory viruses (RSV A/B, FluA and HCoV-HKU1) was found in 6 respiratory samples (6.3%); moreover, 2 samples were positive to both RSV A and RSV B (2.1%). The rates of HRV and RSV infections were significantly higher in children compared to the other groups

($p < 0.001$, $p = 0.048$). The few HCoVs infections, with the exception of HCoV-229E, indicated a marginal role of HCoVs in CF. By contrast, as expected, HRV, RSV and FluA are the most frequently detected viruses especially in young CF population. Overall, most of the viral respiratory tract infections in CF individuals were of mild severity or without any symptoms; notably, 3/11 FluA cases were hospitalized.

Evidences across several countries from the early pandemic period suggested that CF condition does not represent a higher risk of SARS-CoV-2 infection and that disease course does not seem to differ from the general population [1, 2, 4].

From this study data, it is difficult to identify specific factor(s) that could have contributed to the apparent low spread of SARS-CoV-2 in CF. The implementation of social distancing and preventive measures might have mitigated the diffusion of the SARS-CoV-2 in CF individuals. In the Italian COVID-19 decree effective since March 10, the general population was requested to stay home, unless strictly necessary; as a consequence, routine clinic appointments at the Regional (Lazio) CF Reference Center were canceled and only visits for emergency were performed. Beside the lower number of CF patients attending the Center in March, the virus positivity rate was also the lowest of the study period, because the decrease in social interactions may have caused a reduction in respiratory disease incidence. However, virus positivity distribution in the study period, lower in November and March and peaking in January, may be consistent also with the seasonal circulation of many respiratory viruses. As regards SARS-CoV-2 circulation in March, on 32,846 molecular tests performed in Lazio Region, 2914 (8.9%) tested positive for SARS-CoV-2 (from http://www.salute.gov.it/imgs/C_17_notizie_4362_0_file.pdf). Taken together, these figures would suggest that SARS-CoV-2 circulated in Lazio Region and that CF patients had a chance to be infected. Of course, we cannot exclude that asymptomatic or mild SARS-CoV-2 infections in CF individuals eventually happened but did not induce them to attend the CF Reference Center for care, given the Government restriction measures.

In conclusion, this study, conducted in an early pandemic period in Italy, showed that SARS-CoV-2 was not present in CF patients of different ages in stable conditions or with respiratory signs and symptoms, differently from common respiratory viruses. These findings encourage further studies to determine the prevalence of SARS-CoV2 in CF patients across a broad range of age, not just among individuals seeking medical attention, due to the possible severe disease course, to understand more fully the burden of this novel pathogen and to develop adequate preventive measures against this infection.

Funding

This work was supported by a grant to A.G. (Antonelli Guido) from Fondazione Ricerca Fibrosi Cistica-Onlus (FFC#14/2018) and

Table 1

Demographic, genetic, clinical characteristics and microbiological status of cystic fibrosis patients ($n = 265$) and virus positive patients ($n = 95$) divided by three age groups (≤ 10 years, 11–18 years, > 18 years).

Items	Patients Total $n = 265$	Children $n = 43$	Adolescents $n = 39$	Adults $n = 183$
Male, n (%)	142 (53.6%)	21 (48.8%)	15 (38.5%)	106 (57.9%)
$\Delta F508$ homozygous/ heterozygous/others, n	89/85/91	17/11/15	13/14/12	59/60/64
FEV1% predicted, mean (SD)	N.A.	N.A.	N.A.	62.4 (25.2)
BMI, mean (SD)	20.0 (3.9)	16.9 (3.5)	19.8 (3.8)	20.9 (3.6)
Inhaled antibiotics, n (%)	151 (57.0%)	7 (16.3%)	14 (35.9%)	130 (71.0%)
IV antibiotics, n (%)	14 (5.3%)	1 (2.3%)	1 (2.6%)	12 (6.5%)
<i>P. aeruginosa</i> chronic/ intermittent colonization, n	109/47	0/9	4/11	105/27
<i>S. aureus</i> colonization, n	139	31	34	74
Nasopharyngeal aspirate samples/sputum, n	82/183	43/0	39/0	0/183
Number of respiratory samples per month, n (%)				
November 2019	25 (9.4%)	3 (7.0%)	2 (5.1%)	20 (10.9%)
December 2019	55 (20.8%)	5 (11.6%)	7 (17.9%)	43 (23.5%)
January 2020	87 (32.8%)	23 (53.5%)	17 (43.6%)	47 (25.7%)
February 2020	74 (27.9%)	7 (16.3%)	8 (20.5%)	59 (32.2%)
March 2020	24 (9.1%)	5 (11.6%)	5 (12.9%)	14 (7.7%)
Detection of one or more respiratory virus, n (%)	95/265 (35.8%)	25/43 (58.1%)	13/39 (33.3%)	57/183 (31.1%)([▲])
SARS-CoV-2, n (%)	0/95 (0%)	0/25 (0%)	0/13 (0%)	0/57 (0%)
HRV, n (%)	56/95 (58.9%)	17/25 (68%)	8/13 (61.5%)	31/57 (54.4%)([■])
RSV A/B, n (%)	11/95 (11.6%)	0/25 (0%)	3/13 (23.1%)	8/57 (14%)([●])
FluA, n (%)	10/95 (10.5%)	2/25 (8%)	2/13 (15.4%)	6/57 (10.5%)
HMPV, n (%)	0/95 (0%)	0/25 (0%)	0/13 (0%)	0/57 (0%)
HCoV-229E, n (%)	7/95 (7.5%)	0/25 (0%)	0/13 (0%)	7/57 (12.4%)
HCoV-HKU1, n (%)	3/95 (3.2%)	0/25 (0%)	0/13 (0%)	3/57 (5.3%)
HCoV-OC43, n (%)	0/95 (0%)	0/25 (0%)	0/13 (0%)	0/57 (0%)
HCoV-NL63, n (%)	0/95 (0%)	0/25 (0%)	0/13 (0%)	0/57 (0%)
Coinfection, n (%)	8/95 (8.4%)	6/25 (24%)	0/13 (0%)	2/57 (3.5%)
Exacerbated patients, n (%)	14/95 (14.7%)	2/25 (8%)	2/13 (15.4%)	10/57 (17.5%)
Hospitalized patients, n (%) ([●])	9/95 (9.5%)	2/25 (8%)	1/13 (7.7%)	6/57 (10.5%)
Mild symptoms, n (%)	24/95 (25.3%)	8/25 (32%)	3/13 (23.1%)	13/57 (22.9%)
No symptoms, n (%)	56/95 (58.9%)	15/25 (60%)	7/13 (53.8%)	34/57 (59.6%)

Data are presented as number or mean (SD). N.A.: not applicable. ([▲])= $p < 0.01$ by Chi-Squared test (viral detection higher in children) ([■])= $p < 0.001$ by Chi-Squared test (HRV detection higher in children) ([●])= $p < 0.05$ by Chi-Squared test (RSV detection higher in children) ([●]) FluA $n = 3/11$; HRV $n = 5/62$; FluA+HRV $n = 1/95$; RSV $n = 0/17$; HCoVs $n = 0/11$.

from Sapienza University (Fondi Progetti di ricerca universitaria 2018). Funding sources had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of this manuscript; and in the decision to submit the article for publication.

Declaration of Competing Interest

The authors declare that they have no conflicts of interests.

References

- [1] Cosgriff R, Ahern S, Bell SC, et al. A multinational report to characterise SARS-CoV-2 infection in people with cystic fibrosis. *J Cyst Fibros* 2020 press. doi:10.1016/j.jcf.2020.04.012.
- [2] Poli P, Timpano S, Goffredo M, et al. Asymptomatic case of Covid-19 in an infant with cystic fibrosis. *J Cyst Fibros* 2020 press. doi:10.1016/j.jcf.2020.03.017.
- [3] Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* 2020;25:2000045. doi:10.2807/1560-7917.ES.2020.25.3.2.000045.
- [4] Colombo C, Burgel PR, Gartner S, et al. Impact of COVID-19 on people with cystic fibrosis. *Lancet Respir Med* 2020;8:e35–6. doi:10.1016/S2213-2600(20)30177-6.

Corrado De Vito, Maria Trancassini
Department of Public Health and Infectious Diseases, Sapienza
University of Rome, Rome, Italy

Fabio Midulla
Department of Maternal Science, Sapienza University of Rome, Rome
00185, Italy

Giuseppe Cimino
Lazio Reference Center for Cystic Fibrosis, Policlinico Umberto I
University Hospital, Rome 00185, Italy

Alessandra Pierangeli
Laboratory of Virology, Department of Molecular Medicine, Sapienza
University of Rome, Istituto Pasteur Italia, Rome 00185, Italy

Guido Antonelli
Laboratory of Virology, Department of Molecular Medicine, Sapienza
University of Rome, Istituto Pasteur Italia, Rome 00185, Italy
Microbiology and Virology Unit, Hospital "Policlinico Umberto I",
Sapienza University, Rome 00185, Italy

*Corresponding author.

E-mail address: carolina.scagnolari@uniroma1.it (C. Scagnolari)

Revised 19 June 2020

Carolina Scagnolari*, Camilla Bitossi, Federica Frasca,
Agnese Viscido, Giuseppe Oliveto, Mirko Scordio
Laboratory of Virology, Department of Molecular Medicine, Sapienza
University of Rome, Istituto Pasteur Italia, Rome 00185, Italy