

SARS-CoV-2 Infection in patients with cystic fibrosis. An overview

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Abstract. The novel coronavirus SARS-CoV-2 was first identified in China in December 2019 and has since spread worldwide. People with Cystic Fibrosis (CF) have reduced survival mainly because of respiratory failure due to chronic pulmonary infections. Therefore, CF patients should be considered to have an increased risk of developing severe manifestations in case of SARS-CoV-2 infection. Surprisingly, the results of recent studies concerning SARS-CoV-2 infection in patients with CF show that in these patients the infection rate was lower than that of the general population. Various factors have been considered to explain a possible protective effect of CF against SARS-CoV-2 infection.

Key words: SARS-CoV-2, Cystic Fibrosis, Respiratory Viruses

Introduction

In December 2019 a new severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) were identified in Wuhan, the capital of Hubei, China, causing numerous cases of severe pneumonia often followed by acute respiratory distress syndrome (ARDS) (1). Since then SARS-CoV-2 disease (termed COVID-19) has taken alarming proportions worldwide. By 24 March 2020, SARS-CoV-2 had infected more than 381,000 people across 195 countries and killed more than 16,000: a pandemic as declared by the World Health Organization (2).

Cystic fibrosis (CF) is a inherited, autosomal recessive disorder caused by mutations in the gene encoding an anion channel termed Cystic Fibrosis Transmembrane conductance Regulator (CFTR). People with CF have reduced survival mainly because of respiratory failure due to chronic pulmonary infections. Therefore, CF patients should be considered to have an increased risk of developing severe manifestations in case of SARS-CoV-2 infection.

In this review we reported the results of recent studies concerning SARS-CoV-2 infection in patients with CF.

SARS-CoV-2 Infection

The 2019 novel CoV (SARS-CoV-2) belongs to human CoVs (HCoVs) that also include 229E, OC43, HKU1, NL63, SARS-CoV, and Middle East respiratory syndrome (MERS). CoV. 229E and NL63 are members of the Betacoronavirus, whereas others of the Alphacoronavirus.

All HCoVs are enveloped RNA viruses and have a zoonotic origin from bats, rodents, or domestic animals (3). Among these, four HCoVs (229E, NL63, OC43 and HKU1) typically infect only the upper respiratory tract and cause relatively minor symptoms (4). However, there are three coronaviruses (SARS-CoV, MERS-CoV and SARS-CoV-2) that can spread in the lower respiratory tract and cause pneumonia, which can be fatal (4).

Cell entry of SARS-CoV-2 is mediated by the spike (S) protein expressed on the surface of the virus particles, giving the characteristic 'crown' appearance. The S protein comprises two subunits: S1 and S2. Firstly, S1 binds to a cellular receptor, the angiotensin-converting enzyme 2 (ACE2) which is present on the plasma membrane of airway epithelial cells, goblet secretory cells and type II pneumocytes. S1 binding to ACE2 triggers endocytosis of the SARS-CoV-2 virion. Within the endosome, the S1 subunit is cleaved away by cellular proteases, transmembrane serine protease 2 (TMPRSS2), and the S2 subunit drives the fusion of viral and cellular membranes, releasing the viral package into the host cytoplasm (5).

It has been recently proposed that SARS-CoV-2 uses a dual strategy: its spike protein could also interact with sialic acid receptors of the cells in the upper airways, in addition to the known interaction with ACE-2 in the lower airways. This mechanism may explain the high diffusion speed of this virus (6). The loss of pulmonary ACE2 function induced by SARS-CoV2 infection could result in a dysfunction of the renin-angiotensin system (RAS), which influences blood pressure and fluid/electrolyte balance, enhancing inflammation and vascular permeability in the airways (7).

Once SARS-CoVs have infected the host cells, and viral genome is released into the cell cytoplasm, several signaling cascades take place inducing a huge pro-inflammatory response. Viral infection in airway epithelial cells could cause high levels of virus-linked pyroptosis, a form of programmed cell death that is a trigger for the subsequent inflammatory response, involving increased secretion of the pro-inflammatory cytokines and chemokines such as IL-6, IFN γ , MCP1 and IP-10.

Severe cases of SARS-CoV-2 infection progress to acute respiratory distress syndrome, a condition that is characterized by the secretion of such cytokines and chemokines attracting immune cells, notably monocytes, macrophages, and T lymphocytes, but not neutrophils, from the blood into the infected lungs (7).

In most individuals, recruited cells clear the infection in the lung, the immune response recedes and patients recover. However, in some patients, a dysfunctional immune response occurs, which triggers a cytokine storm and symptoms of sepsis that determines

multi-organ damage leading to organ failure, especially of the cardiac, hepatic and renal systems (8,9).

Although the strong containment measures imposed by Italian government, Italy was dramatically affected by SARS COV2 infection, particularly in the northern regions (Lombardia, Emilia-Romagna, Veneto, Piemonte) with a very high overall Italian case fatality rate (CFR) of 7.5% (range 3.1– 16.7%) compared with European countries with a similar population (10).

It is known that COVID-19 affects elderly population 3–4 times more than average with men being more likely to be infected than women. Therefore, Italian mortality may be attributable to a large proportion of elderly persons in the Italian population, at greater risk of being affected by the virus and of dying, because of comorbidities like diabetes, cardiovascular diseases, or cancer.

It has been recently reported that also pediatric patients can develop COVID-19-disease. An Italian multicentre study reported that at 10 April 2020 168 children aged 1 day to 17 years has been infected by COVID 19 and thirty-three children (20%) had underlying chronic diseases such as chronic lung disease and congenital malformations or complex genetic syndromes (12).

Cystic Fibrosis and Respiratory Viruses

CF is a lethal, inherited, autosomal recessive disorder that affects approximately 80,000 people worldwide, most common in Caucasians, and is caused by CFTR gene mutations.

The gene was identified in 1989 and to date, more than 2000 CFTR mutations have been classified with over 300 known to be disease causing. The commonest and most well characterised genetic abnormality is a deletion of three base pairs encoding a phenylalanine residue at position 508 on chromosome 7 (Phe508; Δ 508) (13).

People with CF have reduced survival mainly because of respiratory failure due to chronic pulmonary infections. Moreover, CF is a multiorgan disease that leads to exocrine pancreatic insufficiency resulting in malabsorption, malnutrition and impaired growth. Other classic disease manifestations are excessive salt

loss via sweat, male infertility, diabetes, and sinusitis (13, 14). In a recent study, the median survival in UK was estimated to be 46 years in males and 41 years in females for babies born with CF now who are F508del homozygous (15).

Mutations in CFTR reduce the availability of functional CFTR channels in the apical cell membrane of airway epithelial cells leading to a dramatic reduction in chloride and bicarbonate secretion, a decrease in the volume and pH of the airway surface liquid and hypersecretion of sticky mucus. Fluid depletion together with abnormally adherent mucus leads to decreased mucociliary clearance and poor bacterial clearance from the lungs (13).

The intense inflammatory reaction to this airway infection consists of chemokine and cytokine expression (IL-8) and tumor necrosis factor (TNF) and mucin secretion as well as polymorphonuclear leukocytes (PMNs) accumulation and the associated release of serine proteases, which are themselves proinflammatory stimulants (13).

In addition, mutations in CFTR also results in abnormal innate immunity and dysregulated inflammation. In fact, the dysfunctional CFTR negatively affects the epithelial innate immune function through a constitutive activation of the nuclear factor κ light-chain enhancer of activated B cells (NF- κ B) pathway with increased IL-8 production and neutrophil accumulation; moreover, in cystic fibrosis epithelial cells, the surface expression of toll-like receptor -4 (TLR4) is reduced, resulting in decreased in type I IFN regulation. This defect contributes in turn to the diminished activation of pulmonary dendritic cells (DCs), critical for immune surveillance and for orchestrating appropriate T-cell signaling (16-19).

Thus, in CF the airways become susceptible to dramatic inflammation and chronic infection, in particular by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In this environment, pulmonary exacerbations (PEX) caused by acquisition of new infectious agents, are associated with an acute decline in lung function and adverse impact on patients' quality of life, and despite very intensive treatment may does not completely recover (20).

Respiratory viruses are detected approximately in 28 to 48% of CF patients with PEX; hence, viruses may be important triggers of exacerbation in CF. Wat et al.

demonstrated that influenza A and B and Rhinovirus are major viruses causing PEX in CF (21). Moreover, Viviani et al (22). in a multicenter study worldwide showed that H1N1 pandemic infection in 2009 had a significant impact on CF patients (110 infected by A/H1N1): 53 % required intravenous antibiotic therapy and 48 % were hospitalized. Six patients required intensive care unit and 3 died during the course of the infection.

Previous studies have shown that respiratory syncytial virus (RSV) were also important in CF exacerbations, with the highest incidence in young children (23). It has been postulated that bacterial infection-induced changes in host mucosa may modulate the innate immune responses to viral infection given that the infection of CF airway epithelia cells with *P. aeruginosa* reduced the interferon response to Rhinovirus and increased the viral load (24). Conversely, Ramsey et al. (25) were unable to demonstrate any significant adverse effect of viral infections on lung functions in patients with CF. This study also suggested that viral infections may possibly protect against *Pseudomonas* acquisition. Consistently with this hypothesis, Smith et al. (26) comparing PEX with and without rhinovirus concomitant infection, found that those children in whom a non-rhinovirus was identified had a significantly greater decline in FEV1, whereas patients with rhinovirus infection had fewer declines.

Cystic Fibrosis and SARS-COV2 Infection

Since viral infections play a key role on the course of CF with pulmonary disease, CF patients should be considered to have an increased risk of developing severe manifestations in case of SARS-CoV-2 infection.

A recent multinational report (27) described 40 CF patients (median age 33 years) with SARS-COV2 infection across 8 countries (Australia, Canada, France, Ireland, Netherlands, New Zealand, UK and US). 31 out 40 (78%) were symptomatic, 33% required oxygen, 10% were admitted to an Intensive Care Unit (ICU) and one patient required invasive ventilatory support. Twenty-eight out 40 (70%) recovered from SARS-CoV-2 infection, with no deaths.

The median age of this cohort was 33 years, higher than the general CF population. This adult

predominance in CF may reflect the picture for SARS-CoV-2 in the general population (28). Moreover, only 43% of this cohort are male, unlike current reports of 60–75% of male in the general population admitted in hospital (29).

The Italian situation is quite similar to the international one as shown by The Italian Cystic Fibrosis Registry Questionnaire, reporting 13 CF patients resulted SARS-CoV-2 positive in the February-May 2020 period (30). All SARS-CoV-2 positive CF patients were resident in endemic areas in Northern Italy regions and acquired infection from family members. CF patients had generally mild respiratory symptoms: 61,5% of patients (N=8) were treated at home; 38,5% (N=5) were hospitalised. No patient needed ICU. Eleven patients (85%) had recovered at the date of the answer to the questionnaire (May 15th 2020). Two patients were still COVID-19 positive.

These observations, as well as data from other European countries (five patients with CF in France have been reported to have SARS-CoV-2 infection, seven in the UK, five in Germany and three in Spain) (28) suggest that unexpectedly, the incidence of SARS-CoV-2 amongst the CF population (0.07%) appears to be lower than the average incidence in the general populations (0.15%) (27). The apparent lower rate in CF may be partly attributable to the lower average age of the CF population.

A variety of factors has been advocated as protective toward SARS COV2 in CF patients, for example use of long-term antibiotic therapy such as azithromycin. It has been demonstrated that azithromycin has an anti-inflammatory effect (31) as well as an antiviral effects in a model of COPD (32). Moreover, it has been suggested that mutations in the CFTR gene may alter the protein abundance of ACE2 and TM-PRSS2 in such a way to mitigate the effects of SARS-CoV-2 infection on lung damage (33).

It is also worth noting that the low rate of SARS COV2 infection in CF patients may be maight also be due to the fact that people with CF usually adopt shielding and protective self-isolation to avoid the risk of cross infection.

Since the start of the pandemic in Italy, the Cystic Fibrosis Centre in Parma strongly recommended self-isolation for CF patients. Recommendations for

preventive measures that were already established in this population, such as wearing face masks, practising careful hand hygiene, and segregation between patients were reinforced. CF adult patients were encouraged to perform home working and to avoid group activities like gym or dance. Moreover, the routine CF clinic was cancelled to avoid viral spread among CF patients. Only urgent visits were performed; in addition, respiratory function testing were discontinued. Phone calls and email contacts were used to monitor the clinical condition of patients and to provide therapeutic plan.

On other hand cancelling routine CF clinics may have a negative impact on the course of the disease over time. Anecdotally, we reported three case of CF adolescents, males, who had severe worsening in pulmonary function and kyphoscoliosis due to a prolonged immobility during lockdown period.

Conclusions

CF has been susceptible to various respiratory virus, almost with severe consequences. However, the role of viral infections in CF pulmonary decline may be controversial.

Worldwide few patients with CF, mainly adults, are becoming infected with SARS-CoV-19, without apparent effect on cystic fibrosis disease severity. These findings suggest that CF patients may be somehow protected by SARS-CoV-2-dependent severe lung disease. However, the medium and long-term impact of SARS-CoV-2 amongst infected patients is still not known. Further studies on a larger population of CF patients are needed to determine the real impact of SARS-CoV-2 on CF lung disease.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Guan W et al. Clinical Characteristics of Coronavirus Disease 2019 in China *N Engl J Med* 2020; 382:1708–1720.
2. World Health Organization. WHO Director- General's opening remarks at the media briefing on COVID-19 -11 March

2020. *WHO* <https://www.who.int/dg/speeches/detail/who-director-general-s-openingremarks-at-the-media-briefing-on-covid-19---11-march-2020>
3. Fung SY et al. A tug-of-war between severe acute respiratory syndrome coronavirus 2 and host antiviral defence: lessons from other pathogenic viruses, *Emerging Microbes & Infections* 2020, 9, 558–570
 4. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. *Methods. Mol. Biol.* 2015; 1282, 1–23).
 5. Hoffmann et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*, 2020, 181, 271–280).
 6. Milanetti E, Miotto M, Di Rienzo L, Monti M, Gosti G, Ruocco G. In-Silico evidence for two receptors based strategy of SARS-CoV-2 bioRxiv preprint doi: <https://doi.org/10.1101/2020.03.24.006197>
 7. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. *Nature Reviews* 2020, 20, 363–374.
 8. Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet*. 2020;395(10229):1033–1034.
 9. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395(10229):1054–1062).
 10. Immovilli et al. COVID-19 mortality and ICU admission: the Italian experience *Critical Care* 2020, 24:228
 11. Remuzzi A, Remuzzi G. COVID-19 and Italy: what next? *Lancet* 2020, 395: 1225–28
 12. Garozzino S et al, Multicentre Italian study of SARS-CoV-2 infection in children and adolescents, preliminary data as at 10 April 2020. *Euro Surveill.* 2020 ; 25(18).
 13. Elborn JS. Cystic fibrosis. *Lancet*. 2016; 388:2519–2531.
 14. Ratjen F, Bell SC, Rowe SM, Goss CH, Quittner AL, and Bush A. Cystic fibrosis. *Nat Rev Dis Primers* 1: 15010, 2015
 15. Keogh RH, Szczesniak R, Taylor-Robinson D, Bilton D. Up-to-date and projected estimates of survival for people with cystic fibrosis using baseline characteristics: A longitudinal study using UK patient registry data. *Journal of Cystic Fibrosis* 2018; 17:218–227.
 16. Cohen TS, Prince A. Cystic fibrosis: a mucosal immunodeficiency syndrome. *Nat Med* 2012, 18(4), 510–519
 17. Bruscia EM and Bonfield TL. Cystic Fibrosis Lung Immunity: The Role of the Macrophage *J Innate Immun* 2016; 8: 550–563
 18. Skopelja S, Hamilton BJ, Jones JD, Yang ML, Mamula M, Ashare A, Gifford AH, and Rigby WF. The role for neutrophil extracellular traps in cystic fibrosis autoimmunity. *JCI Insight* 2016 1(17): e88912)
 19. Cantin AM, Dominik Hartl D, Michael W. Konstan MW, James F. Chmiel JF. Inflammation in cystic fibrosis lung disease: Pathogenesis and therapy. *Journal of Cystic Fibrosis* 2015; 14:419–430.
 20. Stenbit AE, Flume PA. Pulmonary exacerbations in cystic fibrosis *Curr opin Pulm Med* 2011, 17(6), 442–447.
 21. Wat D, Gelder C, Hibbitts S, Cafferty F, Bowler I, Pierpoint M, Evans R, Doull I. The role of respiratory viruses in cystic fibrosis *Journal of Cystic Fibrosis* 2008; 7:320–328.
 22. Viviani L, Assael BM, Kerem E. Impact of the A (H1N1) pandemic influenza (season 2009–2010) on patients with cystic fibrosis. *J Cyst Fibros.* 2011; 10:370–6
 23. Somayaji R, Goss CH, Khan U, Neradilek M, Neuzil KM, and Ortiz JR. Cystic Fibrosis Pulmonary Exacerbations Attributable to Respiratory Syncytial Virus and Influenza: A Population-Based Study. *Clin Infect Dis* 2017; 64:1760–1767.
 24. Chattoraj SS, Ganesan S, Faris A, Comstock A, Lee WM, and Sajjan US. *Pseudomonas aeruginosa* suppresses interferon response to rhinovirus infection in cystic fibrosis but not in normal bronchial epithelial cells. *Infect Immun* 2011; 79:4131–4145
 25. Ramsey BW, Gore EJ, Smith AL, Cooney MK, Redding GJ, Foy H. The effect of respiratory viral infections on patients with cystic fibrosis. *Am J Dis Child* 1989; 143:662–8
 26. Smyth AR, Smyth RL, Tong CY, Hart CA, Heaf DP. Effect of respiratory virus infections including rhinovirus on clinical status in cystic fibrosis. *Arch Dis Child* 1995;73(2):117–20.
 27. Cosgriff R, Ahern S, Bell SC, et al. A multinational report to characterise SARS-CoV-2 infection in people with cystic fibrosis. *Journal of Cystic Fibrosis* 2020; 19: 355–358
 28. Colombo C, Burgel P-R, Gartner S, et al. Impact of COVID-19 on people with cystic fibrosis. *Lancet Respir Med* 2020; 15 S2213–2600(20)30177–6
 29. Yang X, Xu J, Shu H, Xia J, Liu H, Wu Y, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centred, retrospective, observational study. *Lancet Respir Med* 2020 Feb 21 S2213–2600(20)30079–5.
 30. <http://registroitalianofibrosicistica.it>
 31. Cigana C, Nicolis E, Pasetto M, Assael BM, Melotti P. Anti-inflammatory effects of azithromycin in cystic fibrosis airway epithelial cells. *Biochemical and Biophysical Research Communications* 2006; 350: 977–982
 32. Menzel M, Akbarshahi H, Bjermer L, and Uller L. Azithromycin induces anti-viral effects in cultured bronchial epithelial cells from COPD patients. *Sci Rep* 2016; 6: 28698
 33. Voisin G, Bouvet GF, Legendre P, Dagenais A, Masse C, and Berthiaume Y. Oxidative stress modulates the expression of genes involved in cell survival in DeltaF508 cystic fibrosis airway epithelial cells. *Physiol Genomics* 2014; 46: 634–646

Received: 3 August 2020

Accepted: 3 August 2020

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