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REVIEW ARTICLE

Is the epithelial barrier hypothesis the key to understanding the higher incidence and excess mortality during COVID-19 pandemic? The case of Northern Italy

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Abstract

The high incidence and increased mortality of COVID-19 make Italy among the most impacted countries by SARS-CoV-2 outbreak. In the beginning of the pandemic, Northern regions accounted for 40% of cases and 45% of deaths from COVID-19 in Italy. Several factors have been suggested to explain the higher incidence and excess mortality from COVID-19 in these regions. It is noticed that Northern Italian regions, and particularly the cities in Po Valley, are the areas with the highest air pollution due to commercial vehicle traffic, industry and a stagnant meteorological condition, with one of the highest levels in Italy and Europe of fine particulate matter 2.5 micron or smaller in size (PM2.5). PM2.5, the major environmental pollutant deriving mainly by factory and automobile exhaust emissions and coal combustion, increases the expression of angiotensin-converting enzyme 2, the epithelial cell entry receptor for SARS-CoV-2, and thus increase the susceptibility to this virus. The epithelial barrier hypothesis proposes that many diverse diseases may rise from the disruption of epithelial barrier of skin, respiratory tract and gastrointestinal system, including allergic diseases, metabolic and autoimmune diseases, and chronic neuropsychiatric conditions. There is evidence of a close correlation between air pollution and airway epithelial barrier dysfunction. Air pollution, causing lung epithelial barrier dysfunction, may contribute to local chronic inflammation, microbiome dysbiosis and impaired antiviral immune response against SARS-CoV-2, all of which contribute to the high incidence and excess mortality from COVID-19. In addition, air pollution and epithelial barrier dysfunction contribute also to the higher prevalence of several comorbidities of COVID-19, such as diabetes, chronic obstructive pulmonary disease and obesity, which have been identified as risk factors for mortality of COVID-19. In this article, on the basis of epidemiological and environmental monitoring data in Northern Italy, it is suggested that epithelial barrier hypothesis may help to understand the excess burden and mortality from COVID-19.

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KEYWORDS
air pollution, COVID-19, epithelium

1 | INTRODUCTION

Infection of SARS-CoV-2 has caused the most dramatic epidemics of modern history with over 5 million deaths worldwide by the end of 2021. However, death rates from COVID-19 markedly differ in different countries and even in different regions of the same country, independently from infection rate and number of inhabitants (https://covid19.who.int/). This has been the case for Italy, where the observed excess mortality was mainly confined to Northern regions. Therefore, the question arises whether co-factors may contribute to the higher incidence and excess mortality of COVID-19.

The 'epithelial barrier hypothesis'[1,2] has recently been proposed for explaining the increasing prevalence of many chronic non-communicable diseases. These may be classified into three groups. The first group comprises diseases in which the barriers of the affected tissues have been damaged, as it occurs in asthma, rhinitis, chronic rhinosinusitis, atopic dermatitis, eosinophilic esophagitis, coeliac and inflammatory bowel disease. The second group includes metabolic and autoimmune diseases related to a gut barrier leakiness, such as obesity, diabetes, multiple sclerosis, rheumatoid arthritis, fatty liver, autoimmune hepatitis, liver cirrhosis, systemic lupus erythematosus and ankylosing spondylitis. The third group that requires more causal relationship studies includes chronic neuropsychiatric conditions such as Alzheimer's, Parkinson's disease, autism spectrum disorders, chronic depression and stress-related psychiatric disorders.[1] The importance of the respiratory, gut and skin epithelium in representing a barrier between environmental agents and immune cells has been emphasized for almost two decades.[1] The epithelial barrier hypothesis proposes that a disruption of the epithelial barrier caused by several offending substances, including allergens, particulate matter, diesel exhaust, ozone, nanoparticles and microplastics cause tissue inflammation and microbial dysbiosis and play a role in the development and exacerbation of many chronic non-communicable diseases.[1]

The structure and function of the epithelium differ in the skin, respiratory tract and gastrointestinal system. However, mechanisms protecting the epithelium integrity are quite similar and resemble the barrier wall of a defending football team against a direct kick. The integrity of the epithelial barrier towards offending environmental agents is made possible by tight junctions (TJs), adherens junctions (AJs), and desmosomes that, among other functions, contribute to sealing intercellular spaces.[2] This 'gate and fence' function consists of a complex architecture of polymorphic transmembrane proteins (such as occludins, tricellulins, claudins and junctional adhesion molecules) that through adaptor proteins (ZO-1, 2 and 3) interact with the cell cytoskeleton.[2–4] Exposure to harmful agents such as those produced by industrialization, urbanization and westernized lifestyle leads to a leaky epithelial barrier, microbial dysbiosis, translocation of bacteria and tissue microinflammation.[1,2,5]

In this article, we discuss some evidence produced during the SARS-CoV-2 outbreak that may stand for a role of epithelial dysfunction in influencing the incidence, severity and outcome of COVID-19, and in particular, the excess mortality observed in the Northern regions of Italy.

2 | EPIDEMIOLOGY OF COVID-19 IN Italy

Italy has been among the most impacted by the coronavirus outbreak, with 6,566,947 million cases as of 7 January 2022. Also, the number of deaths due to COVID-19 (138,045 as of 7 January 2022) makes Italy one of the countries with the highest fatality rates from SARS-CoV-2 worldwide (www.salute.gov.it). However, the distribution of cases and the number of deaths recorded in Italy show marked differences among Italian regions. Some Northern Italian regions (Lombardia, Veneto and Emilia Romagna) account for approx. 40% of cases and over 45% of deaths from COVID-19, whereas relatively low mortality was recorded in Southern cities of mainland Italy, Sicily and Sardinia. These higher morbidity and mortality rates are still evident when corrected for the number of inhabitants in the regions (Italian Institute of Statistic, ISTAT).

In the same ISTAT data set, an analysis of the excess mortality during the first months of the SARS-CoV-2 coronavirus outbreak across the 107 Italian provinces revealed a strong geographical pattern with an extremely high incidence in the number of deaths in the Northern regions. Lombardia alone experienced 25.782 excess deaths and two other Northern regions (Veneto and Emilia-Romagna), accounted for 71.0% of all the excess mortality estimated in Italy. This excess mortality was comparatively lower in Central Italy and almost absent in the South Italy and the Islands. Average daily mortality at the outbreak of the pandemic was much higher in the cities of the North as compared to that in Central and South Italy. The Italian mortality surveillance system (SiSMG) showed a higher excess in Northern cities compared to Central and Southern cities (Figure 1).[6]

The study showed differences in the increase in mortality, regardless of gender, in the geographical areas of Italy from the beginning of the pandemic to the 28 April 2020 (+67% in men and +57% in women in the north and +12% in men and +7% in women in the Center-South). When mortality data from COVID-19 were compared to those from influenza in the pre-pandemic years, the same seasonality was observed (with peaks during the winter seasons). However, the number of deaths in 2020–2021 were almost double of the average number observed in 2015–2019.

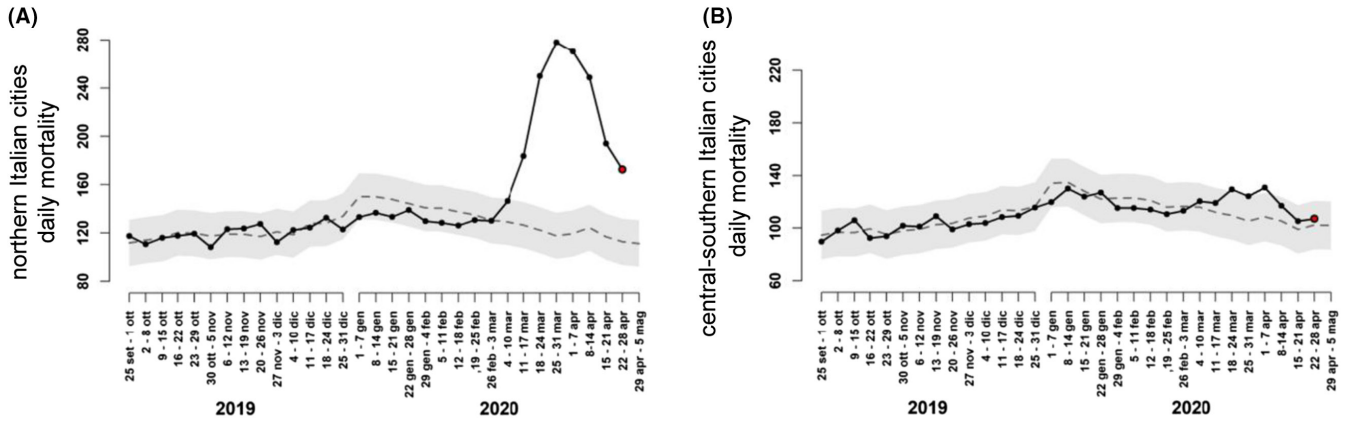

FIGURE 1 (A and B) Average daily mortality (per week) in the northern (A) and Central-Southern (B) Italian cities included in SiSMG from 25 September 2019 to 28 April 2020. The dashed line represents the expected average daily mortality (on a weekly basis) with expected confidence limits (a grey area). Source: Sistema Di Sorveglianza Della Mortalità Giornaliera (SiSMG), Italian Ministry of Health[91]

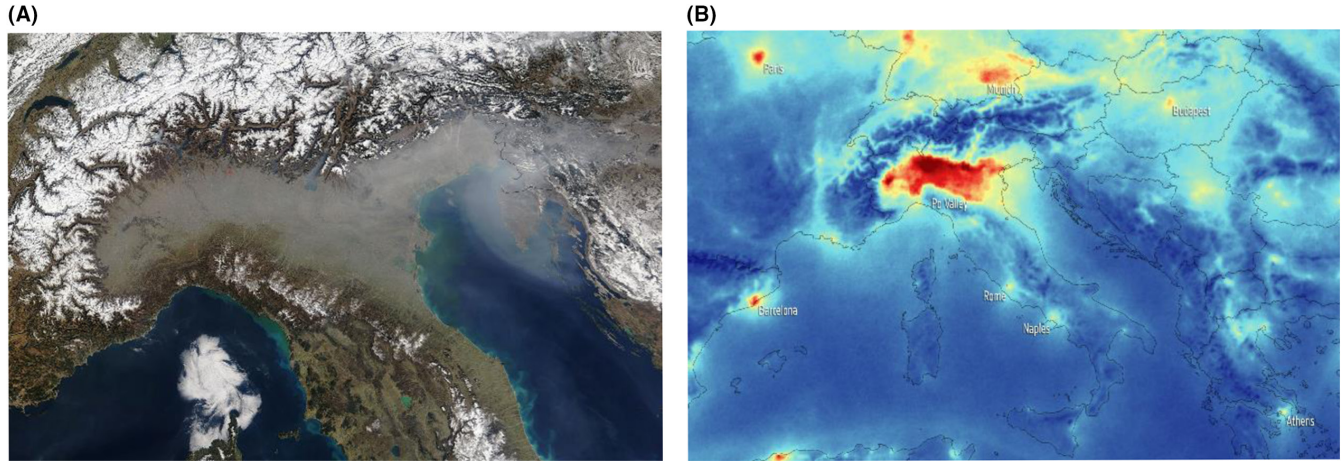

FIGURE 2 (A) Nasa Modis frame showing the Po Valley under a thick layer of ultrafine particles (https://modis.gsfc.nasa.gov/gallery/). (B) Concentration of NO_2 in the Po Valley due to industrial activity and to the barrier created by the Alps between January to April 2019. Since it is denser than air, the gas remains at ground level. From: Copernicus Atmosphere Monitoring Service (CAMS) (https://atmosphere.copernicus.eu/)

Similar data were recorded in the second wave of the COVID-19 pandemics in Italy. The assessment of the mortality rate due to COVID-19 in the Eurostat Nomenclature of Territorial Units for Statistics (NUTS) 2 regions, recorded until 1 June 2021, showed that the regions of Northern Italy were among the top European regions with the highest death toll per 100,000 inhabitants: the first being Valle d'Aosta, Lombardia the third, Friuli Venezia Giulia the fifth and Emilia Romagna the seventh (Sources: JRC ECML, official national government sources and Eurostat. https://covid-statistics.jrc.ec.europa.eu/). While the relationship between deaths and infections (lethality) was 3.5% at the national level, Lombardia experienced the highest value (5.4%), while Campania had the lowest (1.3%).

The high number of cases and excess mortality recorded in Northern Italian regions has prompted us to analyze which co-factors could influence COVID-19 incidence and severity.

3 | AIR POLLUTION AND EXCESS MORTALITY FROM COVID-19

Several factors have been suggested to explain the higher incidence and excess mortality from COVID-19 recorded in Northern Italy. These include the occurrence of mass-gathering events (such as a Championship football match in Milan at the beginning of the pandemics) that boosted local clusters of infections, the demographic and socio-economic features of the population, and the mismanagement occurred in some residential care homes for old people.[7] There are also differences in the standard of care among Italian regions. In fact, the health care system is less organized in the South of Italy with less general practitioners, hospital facilities, beds in intensive care units and private structures supporting the public health sector (The most recent official report published by the Ministry of Health-http://www.salute.gov.it/imgs/C_17_pubblicazioni_2245_allegato.pdf.).[8,9]

Differences in lifestyle and dietary habits might also be relevant, due to the lower average economic standard in the South of Italy, where large families often live in a confined space and sometimes in poor hygienic conditions. However, due to the above reported differences in socio-economic conditions and standard of care, a higher impact of the virus in the South of Italy as compared to its impact in the North should have been expected. Conversely, the more industrialized and rich regions in Northern Italy were those who suffered more from the SARS-CoV-2 pandemic. Similarly, studies in Spain showed that the incidence and mortality of COVID-19 were higher in industrialized/urban areas than in the agricultural/rural zones.[10] These data encouraged us to consider that pollution could have been a relevant co-factor for the dramatic outbreak of COVID-19 in Northern Italy,[11] as suggested for England by Konstantinoudis G et al.[12] The Po Valley is the Italian area with the highest air pollution, mainly due to commercial vehicle traffic and industry, associated with a stagnant meteorological condition. It is worth noting that the regions (Lombardy, Piemonte, Veneto, Emilia Romagna) where there has been the highest mortality rate from COVID-19 are the same regions with the highest number of premature deaths from particulate matter (PM) and NO_2 pollution (Figure 2).

Since the beginning of the pandemic, many studies have reported a close correlation between air pollution and increased mortality from COVID-19. Fine particulate matter (PM2.5) and other environmental pollutants increased the risk of death from COVID-19 in the USA.[13] Preliminary studies addressed the influence of air pollution on COVID-19 worldwide.[14] In China, the incidence of COVID-19 was found to be significantly enhanced by PM2.5 air concentration, while a correlation between ambient PM2.5 and the mortality rate was also demonstrated.[15] A nationwide observational study pointed to a significant positive relationship between COVID-19 incidence rates and PM2.5 and NO_2 levels in Italy.[16] It was hypothesized that chronic exposure to PM2.5 induced overexpression of angiotensin-converting enzyme 2 (ACE2), the receptor for SARS-CoV-2, had an influence to increase the viral load in patients exposed to pollutants.[17]

A study using an artificial intelligence model showed a strong association between prolonged exposure to air pollution, particularly high levels of PM2.5, and SARS-CoV-2 infection and mortality in northern Italian regions. The same analysis revealed that air pollution plays a much more important role than other health and socio-economic factors in influencing COVID-19 outcome.[18] A report based on Medicare data for >60 million people and nationwide air quality measurements showed that in the USA, the PM2.5 exposure was associated with the worse COVID-19 outcome.[19] Data were collected for 98% of the population in 3087 of the total number of 3142 countries, of which 42% had reported increased COVID-19 deaths up to the third week of April 2020[a]. The role of the chronic exposure to air pollution levels in the COVID-19 outbreak risk has also been highlighted by others.[20,21]

The 2019 Lancet Report on health and climate change highlighted that Italy is taking the first place in Europe and eleventh in the world for the premature deaths from exposure to particulate matter (PM2.5 fine and ultrafine dust).[22] Italy has the highest EU value of premature deaths for nitrogen dioxide (NO_2) (214,600), ozone (O_3) (3000) and the second for fine particulate matter PM2.5 (58,600) and is in the group of those countries, which systematically exceed the legal limits for the main air pollutants[b]. The highest PM2.5 mortality burden was estimated for the cities in the Po Valley. Among these cities, those with the highest burden in Italy were Brescia, Bergamo, Vicenza and Saronno[l].[23]

4 | AIR POLLUTION, LUNG EPITHELIAL BARRIER, LOCAL INFLAMMATION AND COVID-19

When a virus enters the human body through the respiratory tract, the local tissue antiviral responses, such as type I and III interferons and the immune-competent cells of the respiratory mucosa, could clear the infection by limiting viral spread. If the viral neutralization does not timely take place, excessive inflammation and tissue destruction by cytotoxic cells and consequent development of immunopathological damage may happen. The epithelial cells have a fundamental role in balancing these two, either physiological or pathological reactions.[24] The upper respiratory tract represents the first line of defence against airborne inhaled toxicants and, when attacked by harmful substances, activates a complex of pro- and anti-inflammatory signalling pathways to recruit neutrophils, monocytes and macrophages to protect the respiratory system. Population density, dust events, wind speed are factors all of which influence the spread of COVID-19.[25]

In fact, histological analysis on biopsies carried out in early/moderate phase of the COVID-19 interstitial pneumonia and post-mortem autopsies showed that the most observed pattern is diffuse alveolar damage with alveolar-epithelial type-II cells (AECII) hyperplasia, hyaline membranes and frequent thromboembolic disease.[26] Epithelial cells have been found to be the principal target of SARS-CoV-2 infection in both the upper respiratory system and the distal lung.[27–29] There are two key host proteins utilized by the virus to gain entry and replicate within cells: ACE2 and the cell surface transmembrane protease serine 2 (TMPRSS2).[30] AECII are physiologically committed to produce IL-6 when stimulated by different stimuli, and IL-6 RNA expression was evidenced in cells corresponding, for morphology and distribution, to SARS-CoV-2 infected AECII.[31] These findings suggest that ambient air pollution-derived particles and chemicals, activating AECII, induce a local production and triggers a further overproduction of cytokines in case a viral infection occurs within the pulmonary

[a]Coronavirus Resource Center. Johns Hopkins University and Medicine, Baltimore, MD, USA, 2020; https://coronavirus.jhu.edu

[b]https://www.eea.europa.eu/publications/air-quality-in-europe-2019

microenvironment.[32] Numerous studies showed that air pollution increases the risk of respiratory tract infections in both paediatric and adult populations,[33,34] and reported a close correlation between the higher incidence of respiratory viral infections and environmental pollution that can be considered an important risk factor for allergic, respiratory and cardiovascular diseases.[35] Cytokine-mediated inflammation appears to be the hallmark of air pollution exposure and it has been hypothesized that air pollution can alter the innate immune system's response to infection.[36]

A study of diesel exhaust exposure's effect on human respiratory epithelial cells observed an upregulation of the interferon (IFN) gene production via the Toll-like receptor pathway,[37] indicating a downstream effect of particulate air pollution on the immune response to viral infection.[38] A recent study performed *in vitro* demonstrated that airborne particulate matter (PM10) modulates the innate immune system associated pathway upon viral infection and significantly enhances the viral replication of the RNA viruses via down-regulation of innate immune responses and upregulation of several metabolic pathways-related genes, which collectively facilitates the infectivity of the virus to cause enhanced respiratory illness.[39] It has been hypothesized that the clinical manifestations of the COVID-19 syndrome are a direct consequence of the involvement of AECII through the existence of a vicious cycle by which once alveolar damage starts in AECII+ cells the inflammatory state is supported by the polarization of the macrophages to the pro-inflammatory subset (M1), release of the cytokines and the activation of the NF-κB pathway (Figure 3).[40] Immunologic responses to pollutant exposure may impact host defence through weakened antiviral mechanisms, impaired mucus production and weakened tight junctions essential for the epithelial airway barrier. The specific immunopathological alterations at mucosal and submucosal levels of the airway epithelial barrier and ultimately in the adaptive immune system increase susceptibility to infection due to a ROS-mediated direct effect of the pollutants on host immune cells and epithelium.[41]

The airway inflammatory roles of PM, O_3 and diesel exhaust have been previously reported, and there are numerous examples of the activation of pathogenic immune cells in mucosal tissues with disrupted barriers exposed to air pollution.[15,42,43] There was a substantial increase in multiple sclerosis patients in January in Stockholm, which was linked to exposure to airborne PM10 associated with increased disease activity of multiple sclerosis.[42] A significant correlation between mean PM10 levels and expression of CCR6 CD4+ T cells with migratory properties that can allow them to pass through the blood–brain barrier were demonstrated. In addition, increased numbers of myeloid dendritic cells that express -cytokines such as IL-1β, IL-6 and IL-23, which stimulate the development of T_H17 cells, were identified in these patients.[42] PM induces direct epithelial barrier disruption via occludin reduction at the plasma membrane and ZO-1 dissociation.[15] Supporting these findings, a significantly weak expression of claudin-1, occludin and ZO-1 in biopsies of patients during a peak inner-city air pollution time in winter compared to summer and PM2.5 *in vitro* caused an epithelial barrier leakiness.[43] In a mouse model of lung inflammation, a single dose ozone exposure caused an immediate lung epithelial barrier disruption followed by myeloid cell infiltration under the control of IL-33/ST2 axis.[44] The tight junction barrier is located at the apical side of interepithelial contacts. Upon their disruption, viruses and aforementioned pollutants can penetrate between the epithelial cells and can directly contact the proinflammatory receptors and virus-binding receptors (ACE2) that are less or not expressed at the apical surface but rather accumulated at the basolateral areas of the cells. Exposure to PM has been demonstrated to increase gut permeability both *in vitro* and *in vivo*. Similar to the effects of PM on alveolar-epithelial cells, treatment of gut epithelial cells with PM caused increased production of mitochondrial reactive oxygen species (ROS), the release of inflammatory cytokines, and induced apoptosis of colonocytes resulting in the increase of gut epithelial permeability.[45,46]

All these data support the hypothesis that a chronic inflammatory response to airborne toxic pollutants could lead to a stronger viral attack to the respiratory epithelial barrier in long term exposed subjects and corroborate the observation of an increased incidence of SARS-CoV-2 infection in the most polluted area.

5 | AIR POLLUTION, MICROBIOME AND COVID-19

The majority of data about air pollution, epithelial barrier, microbiome and COVID-19 refer to the gut. However, emerging evidence showed complex associations between SARS-CoV-2 infection and respiratory tract microbiome. Several studies in both animals and humans showed that air pollutants, such as particulate matter, nitrogen oxides and ozone, also have the potential to alter the gut microbiota,[47] which can further contribute to altered gut permeability by inducing inflammation.[46,48,49] Thus, the intestinal epithelial injury induced by PM may affect the barrier function of the gut.[50] The GI tract, through its resident microbiota, plays a fundamental role in modulating host immune responses.[51] There is more and more evidence that gut microorganisms are linked with inflammatory diseases within and beyond the gut and that gut microorganisms are likely involved in the modulation of host inflammatory responses in COVID-19.[51,52] Numerous studies report the significant involvement of the gastrointestinal tract (GI) in the SARS-CoV-2 infection. The ability of SARS-CoV-2 to infect and replicate in human small intestine enterocytes,[53] the detection of virus RNA in faecal samples[54,55] and the altered gut microbiota composition in SARS-CoV-2 infected subjects have been demonstrated.[56,57] Furthermore, it has been shown that gut microbiota composition of patients with COVID-19 during hospitalization is correlated with plasma concentrations of several cytokines, chemokines and inflammation markers, suggesting that the gut microbiota could influence disease severity and outcomes.[58]

Microbiota is present in the human respiratory tract since birth, representing a primary factor in influencing the maturation of the immune system. The composition of the human microbiota is influenced by many factors including diet, smoking, infections, local inflammation, antibiotics, vaccinations and particularly epithelial

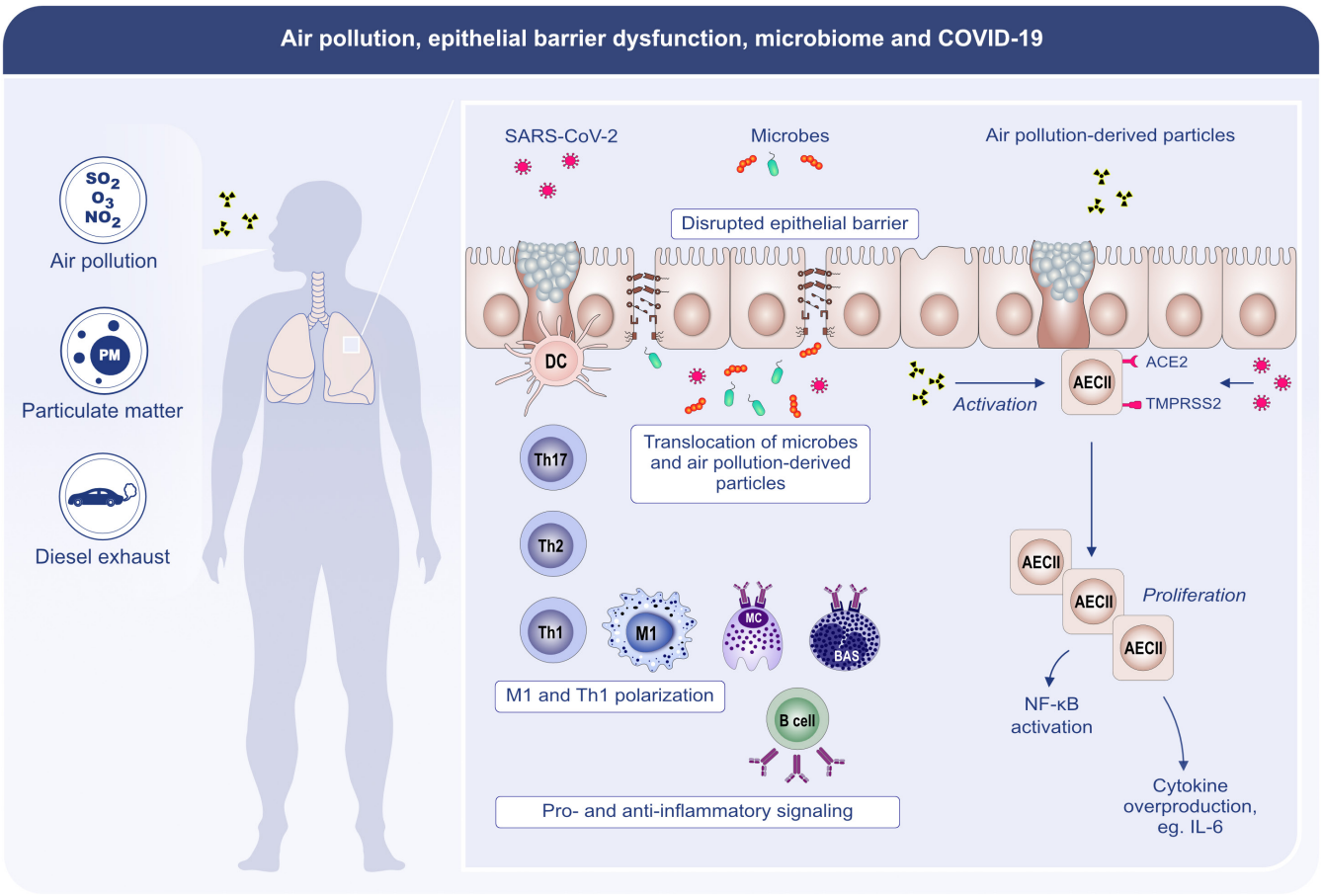

FIGURE 3 Air pollution, epithelial barrier dysfunction, microbiome and COVID-19. Air pollution-derived particles including SO_2, O_3, NO_2, particulate matter and diesel exhaust affect the epithelial cells and lead to the development of leaky barriers and translocation of microbes and these particles to inter- and sub-epithelial areas, pro- and anti-inflammatory signalling, and M1 and Th1 polarization. Air pollution-derived particles activate AECII, which expresses ACE2 and TMPRSS2, and induce cytokine overproduction, e.g. IL-6, and NF-κB activation in case a viral infection. ACE2, angiotensin-converting enzyme 2; AECII, alveolar-epithelial type-II cells; BAS, basophil; DC, dendritic cell; IL, interleukin; M1, macrophage; MC, mast cell; NO_2, nitrogen dioxide; O_3, ozone; SO_2, sulphur dioxide; Th, T helper cell; TMPRSS2, transmembrane protease serine 2

barrier damage in the tissue of interest during life. These factors influence the population dynamics, airways colonization, the existence of opportunistic pathogens, immune response to microbiome, and the relative balance among bacteria genera and microbiome species. The microbiome's composition differs in the upper and lower respiratory tracts depending on the individual anatomical regions. However, there is a bi-directional communication and interaction not only between microbiota from upper and lower respiratory mucosa, but also between microbiota from the respiratory tract and gut.[59]

A balanced symbiotic microbiota is essential for good health. Local changes that cause dysbiosis favour the colonization of pathogens and diseases. In general terms, a more stable balance provides infection resistance and resilience, while an altered balance is responsible for infection and inflammation susceptibility.[60]

It was recently suggested that gut microbiome-mediated priming of the intensity of host inflammatory responses may cause differential susceptibility to SARS-CoV-2 may potentially contribute to the severity of COVID-19.[61]

Interestingly enough, the characterization of the human upper respiratory microbiome using 16S ribosomal RNA sequencing showed marked differences in diversity and abundance of bacterial taxa between subjects with confirmed COVID-19 and non-infected controls, and in relation to the viral load in SARS-CoV-2 infected subjects.[62] The observed species index was significantly higher in SARS-CoV-2-infected adults than in uninfected adults. In differential abundance testing, 9 amplicon sequence variants were significantly different, with *Peptoniphilus lacrimalis*, *Campylobacter hominis*, *Prevotella 9 copri* and an Anaerococcus unclassified amplicon sequence variant being more abundant in those with SARS-CoV-2 infection and in those with high viral load during COVID-19, whereas Corynebacterium unclassified, *Staphylococcus haemolyticus*, *Prevotella disiens* and 2 Corynebacterium_1 unclassified amplicon sequence variants were more abundant in those without SARS-CoV-2 infection and in those with low viral load during COVID-19.[57]

These findings suggest complex associations between SARS-CoV-2 infection and the human microbiome, prompting further

studies to evaluate whether these interactions can impact the progression, severity and recovery of COVID-19.

6 | THE FINAL FACTOR: THE IMMUNE RESPONSE

Numerous studies showed a link between severe viral respiratory diseases and air pollution.[21,63–66] Air pollution, epithelial damage and microbiome diversity and abundance may have profound effects on the susceptibility and immune response to the SARS-CoV-2 infection. We suggest that one of the main mechanisms underlying the highest morbidity and mortality from COVID-19 in those regions where there is an excess of premature deaths from air pollution is likely to be related to the effects of the virus on a functionally defective antiviral immune response in people exposed for a long time to high concentrations of airborne pollutants.

During acute viral infections, early innate and adaptive immune responses lead to viral suppression, followed by the development of adaptive immunity. An intact T cell-mediated adaptive immune response is essential for clearing and maintaining long-term suppression of viral infections.

Innate and adaptive immune responses elicited by SARS-CoV-2 infection and the immunological pathways that likely contribute to disease severity and death have been extensively dissected.[67,68] Single-cell RNA sequencing on nasopharyngeal and bronchial samples from patients with moderate or severe COVID-19 showed that the overexpression of $ACE2^+$ cells correlates with IFN signals and renders these patients more vulnerable to SARS-CoV-2 infection.[69] Infection may affect primarily T lymphocytes, particularly $CD4^+$ and $CD8^+$ T cells, resulting in a decrease in numbers as well as in IFN-γ production by $CD4^+$ T cells.[70,71] This failure of host protective immunity, with consequent viral dissemination and organ injury, may precede and have the same relevance in disease progression and mortality as the subsequent hyper-inflammatory cytokine storm. Circulating T cell subsets are found to be profoundly reduced in COVID-19 patients, and stimulated blood mononuclear cells of the same subjects produce less than 40%–50% of the IFN-γ and TNF-α as compared to patients with sepsis and to severe ill non-septic patients.[72] These findings are in line with other reports indicating that a dysregulated innate immune response contributes to the clinical presentation of patients with severe COVID-19 infections.[73] A marked and protracted lymphopenia is almost the hallmark in patients with severe COVID-19[74] and correlates with morbidity and mortality. $CD4^+$ T cells, $CD8^+$ T cells and natural killer (NK) cells, which play important antiviral roles, have been shown to be depleted in spleen and other organs in post-mortem investigations of COVID-19 dead subjects.[75] A study performed in COVID-19 patients showed that the total number of NK and $CD8^+$ T cells was decreased markedly.[76] The functional exhaustion of NK and $CD8^+$ T cells and an increased expression of NKG2A, an inhibitory receptor that induces NK cell exhaustion in chronic viral infections, were observed in COVID-19 patients as compared to healthy controls.

Ultrafine particles (UFPs), particulate matter of nanoscale size less than 100 nm in diameter, and NO_2, have toxic and pro-inflammatory effects on bronchopulmonary tissue. Besides these well-known effects, UFPs have been demonstrated to exert both *in vitro* and *in vivo*, a derangement of some immune-competent cells with consequent failure of antiviral immune responses.[77] The exposure of human T lymphocytes to diesel exhaust nanoparticles (DEPs) *in vitro* has been shown to have a strong impact on their phenotype and function, inducing a decrease of IL-2 and IFN-γ production, leading to defective immune surveillance and an abnormal persistence of activated T cells.[78] In association with the recent observation that DEPs decrease markers of cytotoxic natural killer cells and functionally suppress cell-mediated cytotoxicity,[79] these findings strongly support the hypothesis that chronic DEP exposure increases the susceptibility to viral infections.

The chronic inflammatory effect on lung and gut epithelial barriers, resulting in impaired immune responses, together with the direct impact on the immune system induced by long-lasting exposure to air pollution, might favour the aggressiveness of the virus and be a critical determinant of outcomes in subjects living in polluted regions. It has been observed that the virus can be carried and spread even at great distances by polluting atmospheric nanoparticles after binding to their surface. The atmospheric particulate constitutes a substrate that can allow the virus to remain in the air in vital conditions for hours or days. This is one additional element to explain the high incidence of the viral infection in the regions of the Po valley where the stagnant meteorological conditions (the Po Valley extend at the foot of the mountain ranges of the Alps to the north and the Apennines to the South), prevent a rapid dispersion of polluting substances, favouring their persistence *in situ*.

6.1 | Comorbidities that increase the severity of COVID-19 are associated with epithelial barrier leakiness

Since the first report on human-to-human contact, old age, obesity and diabetes represent major comorbidities related to increased severity.[80] Interestingly, all of these conditions have been linked to leaky epithelial barriers.[1] Several studies have investigated whether a leaky gut or a disrupted respiratory barrier is the initiator or a consequence of disease development. Recent studies in type 1 diabetes point to a leaky gut as the initiator because intestinal barrier dysfunction has been determined in individuals before the clinical onset of disease.[81] Similarly, spontaneous type 1 diabetes development in a rat model shows increased gastric and small intestinal permeability that has taken place before insulitis and clinical diabetes.[82] Interestingly, epithelial barriers are regulated by high tissue glucose concentrations that negatively affect the tightness of epithelial barriers.[83,84] High glucose levels can lead to the down-regulation of TJ proteins by decreasing connexin 43 expression in airway epithelial cell cultures,[85] demonstrating the continuation of dysregulated gut

barrier in diabetes through a positive feedback mechanism. Obesity and type II diabetes commonly occur together, dysbiosis of the intestinal microbiota together with persistent low-grade inflammatory response in the gut and fat tissue is observed in obesity.[86,87] Age-dependent increase in intestinal permeability has been demonstrated in humans, which may be associated with decreased expression of barrier proteins such as ZO-1 and occludin. However, little is known about the changes of airway epithelial barrier integrity with age.[88]

Comorbid chronic obstructive pulmonary disease (COPD) was identified as a risk factor for mortality in COVID-19 patients admitted in ICU with an HR of 1.68 (95% CI: 1.28–2.19) in multivariate analysis in Lombardy, Italy.[89] A recent study in human lung tissues showed that E-cadherin, occludin and zonula occludens-1 (ZO-1) expression were lower in the frequently exacerbated COPD patients than in the infrequently exacerbated COPD group.[90] Overall, these results suggest a mechanism in COVID-19 severity namely increased systemic inflammation due to the continuous epithelial barrier disruption.

7 | CONCLUSIONS

The evidence presented appears to be the pieces of a puzzle still waiting to be combined into a well-defined picture. However, they strongly suggest that the epithelial barrier hypothesis may represent a reading key for linking exposure to environmental pollutants, epithelial damage, microbiome changes and altered immune response with increased susceptibility to SARS-CoV-2 infection as well as to a progression and less favourable outcome of the disease. All of these factors are affected by air pollution and they are linked to each other as well as the epithelial barrier hypothesis. The inflammation in the gut and respiratory barrier by external and internal exposome may represent an important target to decrease the burden of the COVID-19 pandemic.

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CONFLICT OF INTEREST

SB is consultant for Lallemand Pharma and DSMB member for some COVID-19 vaccines and monoclonal antibodies. CA has received research grants from the Swiss National Science Foundation, European Union (EU CURE), Novartis Research Institutes (Basel, Switzerland), Stanford University (Redwood City, Calif) and SciBase (Stockholm, Sweden); he is the Co-Chair for EAACI Guidelines on Environmental Science in Allergic diseases and Asthma, and serves on the Advisory Boards of Sanofi/Regeneron, Novartis, GlaxoSmithKline, and SciBase, and is the Editor-in-Chief of Allergy. SF, MS, YG and IO have no interests to disclose.

AUTHOR CONTRIBUTIONS

SF, SB and CA made substantial contributions to the conception and design, analysis and interpretation of data; they have been equally involved in drafting the manuscript, writing and revising it critically for the intellectual content. MS, YG and IO contributed equally to the elaboration of the graphic part, to the bibliographic research and to the revision of the manuscript.

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