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.

Journal of Infection 81 (2020) 255–259

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Severe air pollution links to higher mortality in COVID-19 patients: The “double-hit” hypothesis.

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ARTICLE INFO

Article history:
Accepted 6 May 2020
Available online 21 May 2020

SUMMARY

Objectives: In areas of SARS-CoV-2 outbreak worldwide mean air pollutants concentrations vastly exceed the maximum limits. Chronic exposure to air pollutants have been associated with lung ACE-2 overexpression which is known to be the main receptor for SARS-CoV-2. The aim of this study was to analyse the relationship between air pollutants concentration (PM 2.5 and NO2) and COVID-19 outbreak, in terms of transmission, number of patients, severity of presentation and number of deaths.
Methods: COVID-19 cases, ICU admissions and mortality rate were correlated with severity of air pollution in the Italian regions.
Results: The highest number of COVID-19 cases were recorded in the most polluted regions with patients presenting with more severe forms of the disease requiring ICU admission. In these regions, mortality was two-fold higher than the other regions.
Conclusions: From the data available we propose a “double-hit hypothesis”: chronic exposure to PM 2.5 causes alveolar ACE-2 receptor overexpression. This may increase viral load in patients exposed to pollutants in turn depleting ACE-2 receptors and impairing host defences. High atmospheric NO2 may provide a second hit causing a severe form of SARS-CoV-2 in ACE-2 depleted lungs resulting in a worse outcome.

Background and work hypothesis

The potential correlation between air pollution and outbreaks of COVID-19 have been described in the recent literature.[1–4]

While SARS-CoV-2 diffusion has been documented in every country and a pandemic has been declared,[5] there is still debate about the death rate and the severity of pneumonia encountered in specific countries, like Italy and China. In Italy the majority of severe cases and deaths occurred in the Po Valley, accounting for three quarters of the entire Italian caseload, pointing to a specific characteristic of the territory that may favour spread and severity of SARS-CoV-2 virus. The worst COVID-19 outbreaks have been reported in the Po valley, in the cities of Lodi, Cremona, Bergamo and Brescia which are known to be the four Italian cities with highest pollution levels[6]. Here we propose a hypothesis linking PM 2.5 and NO2 concentrations and the severity of SARS-CoV-2 which could have important implications in the prevention and management of the pandemic.

Methods

We collected population data provided by the Italian Civil Protection Agency and data concerning the emissions of air pollutants registered in every Italian region (air pollution data have been collected from Air-matters App and website – https://www.air-matter.com). Pearson correlation analysis was used to examine relationship between COVID-19 cases and PM 2.5 levels. Cut-off level for significance was set at $P < 0.05$. A two-way graph was created showing the simple association between PM 2.5 and number of ICU admission per cumulative days censored. Each Italian region population has been weighted for number of individuals over 65 years old. Patient data are official government data from Italian Civil Protection website (https://github.com/pcm-dpc/COVID-19). Population data were collected from the Italian Statistical Agency (https://dati.istat.it/Index.aspx?QueryId=42869). All analyses were performed using STATA 16 software.

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https://doi.org/10.1016/j.jinf.2020.05.031
0163-4453/

Table 1
Distribution of SARS-CoV-2 cases on March 31th 2020 (total number of cases, ICU admitted patients, deaths) according to each Italian region and the mean value of PM 2.5 registered in each region during February 2020, the month before the beginning of the outbreak in Italy. (Air pollution data have been collected from Air-matters app which include daily measurements from air quality measurement stations all over Italian territory. Data from P.A. Bolzano, Sardegna, Valle d'Aosta, Molise and Basilicata were unavailable at the time of writing).

Italian regions	Mean PM 2.5 in February 2020 (μg/m3)	Total number of cases (n)	DEATHS (n)	Total number of hospitalized patients (n)	ICU admissions (n)
Lombardia	35	43,208	7199	13,207	1324
Emilia-Romagna	30	14,074	1644	4118	353
Piemonte	39	9301	854	3626	452
Veneto	35	9155	477	2036	356
Toscana	12	4608	244	1413	293
Marche	5	3825	452	1115	169
Liguria	5	3416	428	1332	179
Lazio	12	3095	162	1300	173
Campania	12	2092	133	634	133
Puglia	11	1803	110	714	105
P.A. Trento	11	1746	164	434	80
Sicilia	7	1647	81	575	72
Friuli Venezia Giulia	23	1593	113	275	60
Abruzzo	8	1401	115	408	73
P.A. Bolzano	n/a	1371	76	311	62
Umbria	7	1078	37	219	43
Sardegna	n/a	722	31	141	28
Calabria	8	659	36	149	17
Valle d'Aosta	n/a	628	56	117	26
Basilicata	n/a	226	7	54	17
Molise	n/a	144	9	37	8

Table 2
Correlations between mean PM 2.5 registered in February 2020 and COVID-19 cases in terms of total number, hospitalized patients, ICU admissions per cumulative days and deaths (data updated to 31st March 2020).

Correlations	Pearson's coefficient (r-value)	Significance (p-value)
Mean PM 2.5 – Total number cases	0.64	0.0074
Mean PM 2.5 – ICU admissions per day	0.65	0.0051
Mean PM 2.5 – Deaths	0.53	0.032
Mean PM 2.5 – Hospitalized *cases*	0.62	0.0089

Results

A clear association is apparent between PM 2.5 levels and COVID-19 outbreak distribution (see Table 1 and Table 2).

Our data show a significant relationship between the mean PM 2.5 concentration during February 2020, one month before the beginning of the outbreak, and the number of COVID-19 cases per region (updated to March 31st), confirming how more polluted areas are the ones where the contagion is more widespread. More significantly patients in polluted areas present with more severe forms of the disease requiring ICU. Mortality is two-fold higher than the other regions despite similar rates of ICU admission (crude death rate 14 vs 7%) (see Fig. 1).

One explanation for the geographical disparity in the number of cases, is that the high level of pollutants may favour the transmission of viral illnesses and increase their persistence in the community. A recent study indicates that the virus may remain viable in aerosol for some hours.[7] The high pollutant levels and specific climate in the Po Valley may enhance aerosol stability of the virus, explaining the ongoing outbreaks and the associated high rate of contagion.

However, this does not explain the high fatality rate in the aforementioned areas. Atmospheric pollution may have a more fundamental role by increasing the susceptibility to the infection and mining pulmonary defence mechanisms favouring the establishment of more severe forms of the disease.

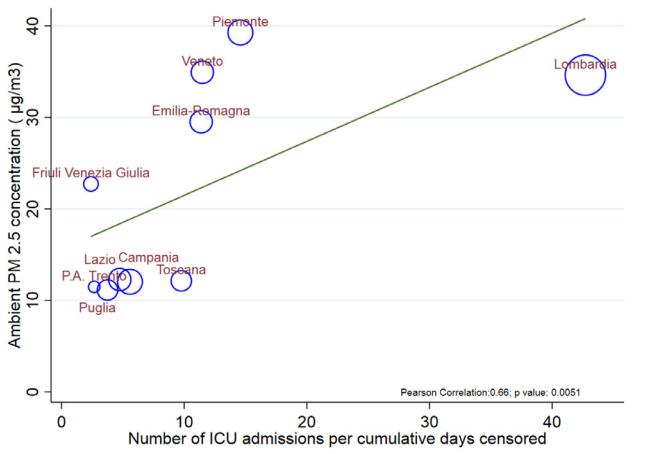

Fig. 1. Scatter plot showing the correlation of the mean concentration of PM 2.5 during February 2020 with the number of ICU admissions for severe COVID-19 infection per cumulative days (patient data updated at 31st March 2020). Regions which presented with the highest level of air pollution during the month of February 2020 are the ones presenting with more severe patients requiring ICU treatments). The size of the circles represents the proportion of population over 65 years of age.

PM 2.5 local concentrations as the mainstem of viral susceptibility

PM 2.5 penetrates deep into the peripheral air spaces[8] and may facilitate viral infection through the interaction with the lung renin-angiotensin system (RAS).

Pulmonary RAS consists of two axes participating in local inflammatory responses with opposing functions[9]: ACE/AngII/AT1R axis is involved in release of pro-inflammatory cytokines (such as IL-6 and TNF-alfa); ACE-2/Ang1-7/Mas axis culminates in Mas activation that represses STAT3 and ERK exerting an anti-inflammatory effect.

ACE-2 cleaves Ang II into the cardioprotective Ang 1–7, which acts through Mas receptors to counterbalance the detrimental effects of Ang II signalling. Therefore, ACE-2 protects against RAS-induced injuries through two processes: 1) degrading Ang I and Ang II to limit substrate availability in the adverse ACE/Ang II/AT1 receptor axis; 2) generating Ang 1–7 to increase substrate availability in the protective ACE-2/Ang 1–7/Mas receptor axis.[9]

ACE-2 knock-out mice are more prone to develop lung injury after exposure to PM 2.5 and pulmonary repair in this context is attenuated relative to controls. This suggests a crucial role for ACE-2 in lung protection from air pollutants.[10]

Chronic exposure to PM 2.5 in mice causes upregulation of pulmonary ACE expression and activity[10,11] which may be a protective response to a chronic deleterious insult. Despite having normal lung structure and function, ACE-2 knockout mice exhibit very severe pathology of acute respiratory distress syndrome (ARDS) compared with wild-type control mice.[10,12]

The spike (S) protein of coronaviruses facilitates viral entry into target cells by engaging ACE-2 receptors.[13] ACE-2 is expressed mainly at alveolar level, explaining the viral tropism for lower airways. Binding and entry of both SARS-CoV and SARS-CoV-2 into human cells is facilitated by the interaction between receptor-binding domain (RBD) of the S1 subunit on viral spike glycoproteins with the ectodomain of ACE- 2.[14] SARS-CoV infection and challenge with recombinant SARS-Spike protein trigger a marked downregulation of ACE-2 expression in lungs and in cell culture causing more severe acute lung injury.[15] Viral depletion of ACE-2 appears to be crucial in mediating lung injury.[16,17]

We postulate that patients chronically exposed to high levels of PM 2.5 overexpress ACE-2 facilitating viral penetration and subsequent depletion of ACE-2 leads to more severe forms of the disease.

This may explain the low incidence of severe pneumonia in children most of whom are asymptomatic. The limited exposure to PM 2.5 due to their young age may exempt them from pulmonary ACE-2 receptor overexpression. In China out of 72.314 infected patients <1% of the cases were children younger than 10 years old[18] and developed milder disease.[19] Similarly, chronic upregulation of ACE-2 in a PM 2.5 concentration-dependant manner could explain the high variability in clinical presentation ranging from asymptomatic patients to patients presenting with mild, moderate or severe form of the disease.[20,21]

Early in March 2020, the Italian village of Vo' Euganeo near Padua, an area with relatively low atmospheric levels of pollution (yearly means PM 2.5 and NO2 respectively 19 and 14 $\mu g/m^3$)[22] experienced one of the first circumscribed outbreak of SARS-CoV-2 in Italy. Swabs were performed in the entire population of Vo'Euganeo, accounting for 3.275 inhabitants; SARS-CoV-2 had a prevalence of 2.6% in the overall population. Interestingly, it was found that 43% of patients positive to SARS-CoV-2 were completely asymptomatic,[23] shedding insights in the incidence of asymptomatic infection and the possible role of asymptomatic carrier in spreading the disease.

Mean viral loads of severe cases of SARS-CoV2 have been reported to be 60 times higher than mild cases.[24] In this case patients who had low exposure to PM 2.5 would have low pulmonary expression of ACE-2 with subsequent low viral load and mild symptoms.

Putative role of NO2 toxicity in the context of SARS-CoV-2 infection

Numerous sources of NO2 exist, making it one of the most common and widespread air pollutants[25] with a geographical distribution overlapping that of PM 2.5.

Acute inhalation of high concentrations of NO2 occurring in conditions such as Silo filler's disease causes increased permeability pulmonary oedema.[26] Long-term low- to moderate-level air pollutant exposure, including NO2, is associated with a greater risk of developing ARDS after severe trauma.[27] Being a free radical, NO2 acts as a potent oxidant depleting anti-oxidant stores leading to impaired tissue defences, increased inflammatory response and cellular damage.

Two days after exposure to moderate levels of NO2 in an ice arena, exposed individuals experienced dry cough (97%), headache (45%), haemoptysis (35%) and dyspnoea (65%).[28] The acute intoxication was secondary to the malfunctioning of ice resurfacer engine associated with poor ventilation. Interestingly, anosmia is frequently present also in the context of NO2 intoxication.[29]

Environmental concentrations of NO2 increase susceptibility to pneumonia by Klebsiella pneumoniae[30] and Streptococcus pneumoniae[31] in experimental murine models. The increased susceptibility seems to be linked to an impairment of pulmonary defence mechanisms, especially phagocytic activity at progressively higher concentrations[32,33] and is associated with high mortality rates.[34]

Rose et al. reported that mice exposed to NO2 prior to cytomegalovirus infection required a viral load 100-fold lower than in control mice and re-infection from viral sources was more common.[35,36] In this model, mice exposed to 5 ppm of NO2 were readily developing viral replication in lower respiratory tract soon after exposure evolving toward clinical form of acute lung injury as confirmed by histological samples showing pneumonitis. Inhalation of the same concentration of NO2 without viral exposure did not result in tissue damage. *Re*-inoculation of the virus after 30 days produced re-infection only in previously exposed mice.

These observations suggest a putative role of NO2 in worsening pulmonary damage in COVID-19 patients. Recent exposure to moderately high levels of nitric dioxide may have caused a worsening of the disease with exacerbation of the symptoms and of the respiratory distress. This would cause an overlap between the COVID-19 presentation and NO2-induced alveolar damage.

"Double-hit" hypothesis

The recent outbreak of COVID-19 is straining healthcare systems due to the high infectiousness and large number of patients with severe pneumonia requiring ICU treatment. Some geographical locations, such as the province of Hubei in China and Po Valley in Italy, have suffered the highest number of cases and deaths. This geographical distribution of the outbreaks shows a remarkable overlap with the local levels of air pollution.

From this initial observation, we formulated our working hypothesis according to which a linkage exists between the air pollution levels and COVID-19 outbreak, in terms of transmission, number of patients, severity of presentation and number of deaths. Air pollutants, (such as PM 2.5 and NO2) plus SARS-CoV-2 give a "double-hit" to the lungs leading to acute lung injury by attenuating tissue remodelling and influencing local inflammatory response.

In Italy, the areas with the highest incidence of cases and deaths are the ones with levels of PM 2.5 and NO2 that are chronically high or with recent increases in the 2 months prior to the outbreak. Chronic exposure of the lungs to high levels of PM 2.5 causes upregulation of its protective mechanisms, such as ACE-2.

SARS-CoV-2 has shown a specific affinity for ACE-2 receptors and overexpression in patients subjected to chronic pollutants could represent a trojan horse for viral infection. Moreover, the SARS-CoV-2 binding to ACE-2 may induce deficient anti-inflammatory action leading to acute lung injury, attenuating local tissue repair. It is therefore likely that patients who present overexpression are the ones more readily infected and the ones with

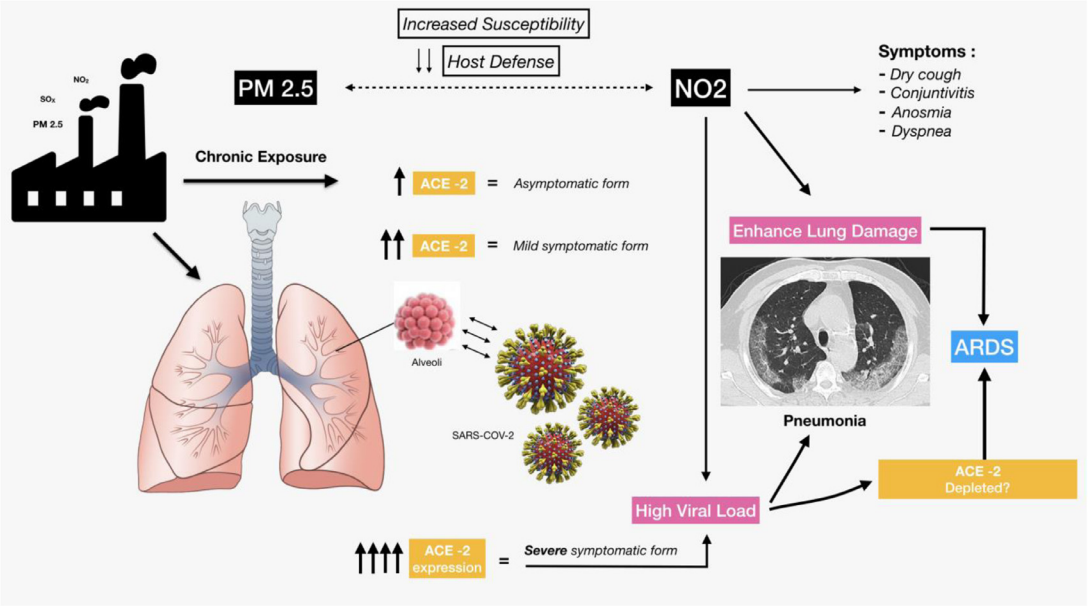

Fig. 2. Hypothesis of the SARS-CoV-2 infection mechanisms and severe lung disease induced by the combined effect of PM 2.5 and NO2. ACE-2 plays a bifunctional role as sort of "double-edged sword": it turns off the RAS system and leads to beneficial effects but also mediates unique susceptibility to lung and CV disease in COVID-19 patients by serving as the SARS-CoV-2 receptor.

more severe presentations. Chronic lung exposure to NO2 may favour viral injury due to local damage induced by oxidative stress and due to local reduction of macrophage function and adaptive immune responses.

In addition, a putative role of NO2 in worsening pulmonary damage can be hypothesized in these patients. Many of the symptoms and signs of COVID-19 infection resemble that of moderate NO2 poisoning, including anosmia. It is possible that ACE-2 depletion following COVID-19 infection increases tissue vulnerability to NO2 toxicity that eventually contributes to the acute lung injury observed in patients with pneumonia-induced ARDS (Fig. 2).

Undoubtedly, many other factors such as age, transmission patterns, population density and co-morbilities have an important impact on both the number and severity of COVID-19. A more detailed epidemiological analysis is necessary to provide a comprehensive understanding of the differences in severity of SARS-CoV-2 in different areas. Likewise, our observations are limited as detailed geographic measurements of pollutants, such as NO2 in the last few months have not yet been published. Nevertheless, we find the available evidence showing a direct link between atmospheric pollution and SARS-CoV-2 linked by ACE-2 receptor expression compelling. ACE-2 receptor expression can be obtained from swabs and if linked to alveolar expression from ex-vivo tissue samples may provide a method for testing this hypothesis.

Conclusions

A link between SARS-CoV-2 infection and air pollution is plausible and this may have a strong impact on the high rate of infection and mortality. If confirmed, our hypothesis has both immediate and long-term implications. In the short term it may help in identifying areas where serious cases are most likely to occur allowing timely allocation of limited and precious resources. It may also have prophylactic and treatment implications in the modulation of ACE-2 receptors before and after infection. In the medium and long-term our hypothesis should lead to an increased awareness that in specific environments defined by high density of population and high industrialization, together with certain climatic characteristics, influencing urban planning to bring about changes such as delocalization of factories, in order to avoid recreating the conditions associated with worse outcomes after viral infections. Weather forecasting and seasonal prediction systems may also contribute in fighting the virus spread.

Declaration of Competing Interest

The authors declare no conflict of interest and no financial support for this study.

Acknowledgements

We wish to thank all the people who assisted with this research, in particular: Rosalba Lembo (MSc, Anaesthesia and Intensive Care, San Raffaele Scientific Institute, Milan, Italy) for the graph and statistics.

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