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Instant review

Lung microbiota in the acute respiratory disease: from coronavirus to metabolomics

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Abstract

Healthy lungs are not sterile. In the last decade, it was demonstrated that the healthy lung has its specific microbiota. It is much smaller in numerical terms, compared to the gut microbiota, but it is a unique microbiota that can affect the health and the diseases. With an estimated number of 10-100 bacteria for 1.000 human cells, the lower respiratory tract is one of the less populated surfaces by the bacteria of the whole human body. Even human fetal lungs host a "signature" of the microbiota. The composition of the lung microbiota depends on several factors, including the so-called "microbial immigration" from micro-aspiration and inhalation of microorganisms. The connection between the lung and the gastrointestinal tract is not entirely understood. Patients with respiratory infections generally have gut dysfunctions complications, which are related to a more severe clinical course, thus indicating gut-lung crosstalk. In this review we analyse the lung microbiota in newborns, infants and adults with respiratory disease. In acute pulmonary diseases such as sepsis, trauma, and acute respiratory distress syndrome (ARDS), the lung microbiota becomes rich in gut bacteria, such as *Bacteroidetes* and *Enterobacteriaceae*. This phenomenon is also called "more gut in the lung". In acute situations, the gut becomes hyper-permeable (leaky gut), and the bacteria can translocate through the colon wall and reach the lung affecting the inflammation, the infection, and the acute pulmonary damage. The increased gut permeability is associated with an increased alveolus-capillary hyper-permeability as well.

There are tight correlations between the lung microbiota and the admission in intensive care. In particular, the modifications of the lung microbiota can help in predicting in which way the patients in critical condition will respond to the treatments. It has been investigated if the different incidence depending on age and the different courses between adults and children for Novel COronaVIrus Disease 2019 (COVID-19) could be due to the different concentrations and/

or activation of angiotensin-converting enzyme 2 (ACE2) at the intestinal and pulmonary level. ACE2 is mainly localized on the luminal surface of the intestinal epithelial cells and it has been hypothesized that gut microbiota influences the action of ACE2. Thus, a close relationship between COVID-19 and the microbiota can be hypothesized (it has been studied in cats). Potential interventions for COVID-19 are: nutritional, antiviral, anti-coronavirus, and miscellanea. Other options could include also probiotics, especially *Bifidobacteria* and *Lactobacilli*, namely *L. gasseri*. In the next future, metabolomics could be applied in the study of COVID-19, deciphering the secret languages between viruses, bacteria and the organism.

Keywords

Lung, gut, microbiota, respiratory disease, pneumonia, coronavirus, probiotics, metabolomics.

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Introduction

Healthy lungs are not sterile [1]. Until 2010 it was thought that the lung was a sterile organ and that was inhabited by microorganisms (bacteria, fungi, viruses) on the inside only in pathological conditions such as cystic fibrosis, emphysema, bronchiectasis, or pneumonia.

In the last decade, it was demonstrated that the healthy lung has its specific microbiota. It is much smaller in numerical terms, compared to the gut microbiota, but it is a unique microbiota that can affect the health and the diseases. With an estimated number of 10-100 bacteria for 1.000 human cells, the lower respiratory tract is one of the less populated surfaces by the bacteria of the whole human body [2].

If you think about it, at every single breath, the lung is exposed to any kind of particulates, contaminants, pathogenic, and non-pathogenic bacteria [3].

Similarly to the gut, the two predominant phyla detected in the airways are *Firmicutes* and *Bacteroidetes*, while *Actinobacteria*, *Proteobacteria*, and *Fusobacteria* are minor constituents of the local microbiota [2, 4, 5]. *Veillonella spp.* and *Prevotella spp.* are minor constituents of the local flora, they are anaerobic bacteria originating from the oropharyngeal microbial flora, that is generally non-pathogenic. It must be said that *Veillonella spp.* and *Prevotella spp.* are associated with the presence of inflammatory cells in the lung. This behavior could have a compensatory significance. Even *Neisseria spp.* and *Acinetobacter spp.*, and some species of *Streptococcus spp.* are generally present in the lung. On the contrary, *Pseudomonas spp.* is rarely found in a healthy lung.

Bacteria from different body areas dialogue with each other with a system of double communication. There is a vital crossed dialog among the mucosae of our body, both in healthy condition and in a pathological condition, as demonstrated by, for instance, the presence of intestinal complications during the respiratory diseases and vice-versa [6]. Furthermore, the inflammation in the gut can affect the inflammation of the airways in case of asthma and cystic fibrosis.

Moreover, lungs do not show the same microbial population in every district (bronchi, bronchioles, alveoli) [7]. The alveolar space is never sterile. Even if in small quantities, the bacteria present in the alveoli contribute to the maintenance of the equilibrium of the immune system of the lung. The lung microbiota varies with age. Already at birth, microbial colonies are present in the oral cavity (*Staphylococcus spp.*, *Streptococcus spp.*, and *Moraxella spp.*). In the respiratory tract of intubated preterm newborns, there can be found *Proteobacteria*.

What does the lung microbiota depend on?

The composition of the lung microbiota depends on several factors, among which the most important are: a) the so-called "microbial immigration" from micro-aspiration and inhalation of microorganisms (the observation that the lung microbiota is very similar to that of the oropharynx confirms the mechanism of micro-aspiration); b) microbial elimination (cough, mucociliary clearance, innate and adaptive immunity); c) local conditions (nutrition, temperature, local oxygenation, quality and quantity of the anti-inflammatory cells). The

lung microbiota is essential both in the acute and chronic diseases [8].

For instance, in adults, we know that in case of chronic obstructive pulmonary disease (COPD), pulmonary emphysema, and chronic bronchitis, the bacteria have a substantial role in perpetuating the inflammation that leads to frequent pulmonary exacerbations. We have 2 ways to use the microbiota efficiently [9]. The first and simplest is to stratify the patients in order to predict better how they would respond to a particular therapy. To do so, we can build data banks that link the profile of their microbiota, the profile of their immune system and their medical history, and use Big Data and the artificial intelligence to make predictive evaluations. The second way is the therapeutic intervention, trying to identify which microbiota is exacerbating for the diseases, and thus exploring the way to manipulate it and improve the health status.

The intestinal microbiota and the lung microbiota go hand in hand: the gut-lung axis

The connection between the lung and the gastrointestinal tract is not entirely understood. Patients with respiratory infections generally have gut dysfunctions complications, which are related to a more severe clinical course, thus indicating gut-lung crosstalk. A study concerning cystic fibrosis provides evidence that the gut microbiota and the lung microbiota develop at the same time after birth, and it is evident that there is a constant crossed dialog between these two areas [10]. A certain number of bacteria appear in the gut before being detected in the respiratory tract; this indicates a contribution of micro-aspiration of gut microbes in the development of the microbiota of the airways.

The bacteria producing lactic acid and *Lactobacilli*, in particular, increase the activity of the natural killer cells, increase the antiviral immune response, reduce the production of pro-inflammatory cytokines and determine an up-regulation of the cell-mediated cytotoxicity after a respiratory infection.

It has been demonstrated that factors such as diet can affect both the gut microbiota composition and that of the lung microbiota [11, 12]. Recent studies on mice highlighted that dietary fibers and the short-chain fatty acids (SCFA) can protect against allergies and airways inflammations, modulating the immune system. The intake of fibers leads to an increase of SCFA, together with the modification of gut microbiota and, to a lesser extent, modification of the microbiota of the airways. Similar correlations have been made in humans between the modification of gut microbiota after the intake of fiber and a low incidence of asthma.

A part of the microorganisms of the lung derives from the intestine. *Firmicutes* and *Bacteroidetes* prevail and characterize the gut microbiota [5]. The gut-lung microbiota relies fundamentally on the ability of the gut microbiota to modulate that of the lung and its immunologic activity. The latter manifests itself through different elements: the production of substances such as lipopolysaccharides (LPS), the production of bacterial metabolites such as SCFA, the migration of immune cells (T-cells, in particular) from the gut to the lung through the blood. What is now evident from the literature is that a microbial dysbiosis of the lung can contribute to trigger the pulmonary diseases and to their progress, and this could be preceded by an intestinal dysbiosis [10]. The gut microbiome does not affect the intestine only, but it can affect very distant areas and alters the immune response in other organs, such as the heart, the liver, and the kidney [10]. The same can likely happen in the lung. In the immediate future, the microbiota could be individually managed at birth and in the different stages of life, and it can be thought of as a microbial GPS (global positioning system) to detect the phenotypes and to predict diseases and responses to different treatments.

The lung microbiota in perinatal and pediatric period

The fetal and placental lung microbiota

Human fetal lungs host “a signature” of the microbiota [13]: after only 11 weeks after the conception, the lungs already show a bacterial signature of the microbiota; this suggests that the bacteria can colonize the lungs long before birth. This discovery, for the first time, sheds light on the mystery of how the microbes or the microbial products reach these organs before birth and which role they have in the healthy development of the lung and the immune system. The maturation of the microbiota seems to advance with gestational age. Finally, in this study, they found a placental microbiota in the human fetal tissue, and this “signature” of the microbiota showed some taxonomic overlaps with the correspondent human fetal lung microbiota. The latest could be useful

to "trigger" the development of the innate immune system and to help in establishing a healthy relationship between host and bacteria.

The lung microbiota in the newborn

The lung microbiota changes during the first postnatal weeks. In particular, there are several factors related to preterm birth that can alter its development. Chorioamnionitis or trans-placental infections can activate an inflammatory state that acts as a triggering factor for the development of broncho-pulmonary dysplasia (BPD), a severe chronic pulmonary pathology of the very preterm and very low birth weight newborn. The subject has been discussed very well [14-18]. Furthermore, the use of antibiotics, both in the uterus and after birth, has been associated with an increase in the incidence of BPD. Other factors are the ventilatory assistance, enteral nutrition, and the onset of sepsis. Intestinal dysbiosis has been considered among the causes of necrotizing enterocolitis (NEC) and, if this process of dysbiosis happens at the level of the lung as well, it could trigger the inflammatory process at the base of BPD. This could be explained by the presence of commensal bacteria in the lungs that are involved in the formation of the immune system. If these commensal bacteria are eliminated or damaged by the abovementioned factors, an abnormal immune response responsible for the pathogenesis of BPD could take place. Some authors demonstrated that there is a faster turnover of the microbiota with postnatal age in newborns with more severe BPD. In particular, the concentration of *Staphylococcus spp.* is lower in the first days of life, while there is a higher concentration of *Ureaplasma spp.* This suggests a possible role of the microbiota in the delineation of the severity of BPD. It has been found a significant presence of *Corynebacterium spp.* in newborns with severe BPD, especially those treated with more extended invasive ventilation. Furthermore, the lungs of preterm newborns are already colonized by *Acinetobacter spp.*, and the number of *Lactobacilli* in the lung microbiota of the preterm newborns that developed BPD is significantly lower than those that did not develop BPD. This low concentration is present in preterm newborns whose mothers suffered from chorioamnionitis, as well [19].

The lung microbiota in infants

Bronchiolitis, an acute viral infection of the lower respiratory tract, is a significant public health problem in the world. Although almost 40% of infants develop clinical bronchiolitis in the first 2 years of life, its severity varies considerably from mild forms to fatal forms. Several studies showed several clinical risk factors for higher severity (i.e., prematurity, comorbidity). Nevertheless, it is not clear which infants will develop a severe pathology with admission in intensive care [20]. Emerging evidence indicates that the pathobiology of the bronchiolitis involves a complex interaction between the virus, the microbiota of the airways, and the immunity of the host [21]. For instance, the predominance of *Streptococcus spp.* or of the *Haemophilus spp.* in the upper respiratory tract has been associated with higher severity of the disease, while *Moraxella spp.* has been related to a reduction in the disease severity in hospitalized infants. It should be remembered that even recurrent infections of the respiratory tract are related to an intestinal dysbiosis: in particular, there is a reduction of the bacteria that produce butyrate, the species of *Fecalibacterium*, and *Eubacterium* [22].

The lung microbiota in adults

The microbiota and acute pulmonary diseases in animal experiments

If we consider the relationship with the flu syndrome and the impact of gut and lung microbiota, our knowledge is still fragmentary, data in humans are not yet available. Nevertheless, the antibiotic treatment determines a significant reduction of the immune responses against the influenza virus in mice [10]. On the contrary, mice infected by the influenza virus fed by high-fat diets show higher survival rates compared to infected controls thanks to an increased generation of Ly6c-patrolling monocytes. These monocytes increase the number of macrophages that had a limited ability to produce CXCL1 locally, reducing the recruitment of neutrophils in the respiratory tract and thus the tissue damage. In addition to these data, the most crucial element that emerges from some experimental studies is the following: the reduction in bacterial diversity in the gut microbiota is related to a significant increase of mortality from viral infections [23].

In a study, Groves et al. have systemically evaluated the events of the pulmonary infection by the respiratory syncytial virus (RSV) and the modifications in the gut microbiota. It has been shown that the cellular immune response to

the viral pulmonary infection induces a lack of appetite, which in turn alters the gut microbiota and the metabolome. The viral infection also leads to a modification in the fecal intestinal metabolome, with a significant change in the lipid metabolism. The sphingolipids, the polyunsaturated fatty acids (PUFA) and valerate (a SCFA) were all abundantly increased in the fecal metabolome after the infection of RSV. Surprisingly, there was an increase in lipids associated with the resolution of the disease. It remains to be seen if the impact of the lack of appetite induced by the infection and the gut microbiota modifications are parts of a protective anti-inflammatory response during the respiratory viral infections [24].

Both the gut and lung microbiota are essential against bacterial pneumonia.

The lung microbiota can protect against respiratory infections with *S. pneumoniae* and *K. pneumoniae*, triggering the pulmonary production of Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) through the stimulation on interleukin 17 (IL-17) and Nod2 [5]. Also, the gut microbiota plays a crucial role in the response of the lung to bacterial infections. Studies on germ-free mice showed an increase in the morbidity and mortality during the acute pulmonary infection by *P. aeruginosa*, *K. pneumoniae* and *S. pneumoniae* [5].

Segmented filamentous bacteria [4], commensal bacteria of the ileum of several animals, humans included, seem to be an essential component of the gut microbiota for the pulmonary defense against bacterial infections thanks to their ability to induce the production of the cytokine Th17, and IL-22, and to increase the count of the neutrophils in the lung during pneumonia by *S. aureus*.

A recent report in animals found that the coronavirus receptors, including angiotensin-converting enzyme 2 (ACE2) for Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and SARS-Cov-2, are digestion-related enzymes in human enterocytes. Coronaviruses are continually altering the binding receptor and binding modes during their evolution, but the potential target cell in the small intestine is constant when in the lung is inconstant. Enterocytes may act as a conserved cell reservoir for coronaviruses, which may be partially explained by the Red Queen hypothesis. The authors also found that coronaviruses receptors could be elevated in the presence of both invasive bacteria and their counterpart, probiotics. All these things should not be ignored in the investigation of coronavirus diagnosis and treatment strategies [25].

The microbiota and acute pulmonary diseases

The composition of the lung microbiota has been mostly investigated in chronic pulmonary diseases, both infective and noninfective [26].

In acute pulmonary diseases such as sepsis, trauma, and acute respiratory distress syndrome (ARDS), the lung microbiota becomes rich in gut bacteria, such as *Bacteroidetes* and *Enterobacteriaceae*. This phenomenon is also called "more gut in the lung" [5]. In acute situations, the gut becomes hyper-permeable (leaky gut), and the bacteria can translocate through the colon wall and reach the lung affecting the inflammation, the infection, and the acute pulmonary damage. The increased gut permeability is associated with an increased alveolus-capillary hyper-permeability, as well [27]. This increased vascular permeability, associated with the presence of mucus, is such that the Gram-negative "pathogens", commonly found in the lung, benefit from it, triggering a pro-inflammatory vicious circle.

According to the hypothesis of the intestinal lymph [28], ARDS requires that harmful mediators originated from the intestinal lumen travel through the mesenteric lymph to the lung where they cause tissue damage. Numerous researches support this hypothesis. The ligation of the mesenteric lymphatic duct reduces the lung lesions and attenuates the activation of the neutrophils in the murine models of chronic disease, improving survival. Furthermore, the injection of mesenteric lymph from trauma hemorrhage induces pulmonary hyper-permeability and pulmonary lesions [28]. Complementary to these data, there is the behavior of the gut-lung axis related to significant challenges. In a mice model of sepsis, the lung communities are dominated by bacteria associated with the gut, and the ecological analysis revealed that the gut is quite likely the source of pulmonary bacteria. This is coherent with the abundance of specific bacteria of the intestine in patients with ARDS in intensive care.

The pulmonary dysbiosis is associated with an increase of the markers of inflammation, such as IL-6 and IL-8 [29]. In adults there is a growth of an only one prevalent species (*E. coli*, *Enterococcus spp.*, *C. difficile*, *Pseudomonas spp.*, *Salmonella spp.*) due to multiple factors, such as intestinal ischemia, interruption of the nutrition, vasoactive drugs, prophylaxis of the stress ulcers, antibiotic therapy during the hospitalization in intensive care, that considerably lower the lung defenses. In animal experiments, they found that bacteria

such as *S. pneumoniae* and *K. pneumoniae* are associated with a reduction of IL-17 and GM-CSF, that are essential for the lung defenses. When patients recover, if they survive, a gut or lung dysbiosis can remain, that facilitates recidivism, until reaching the "collapse of the microbiota". The latter is related to immune exhaustion that facilitates multiple organ failure associated with high mortality, up to 40% [5].

The lung microbiota and the admission in intensive care

Among the hospitalized patients in intensive care, the most common nosocomial infection is ventilation-associated pneumonia (VAP), related to an alteration of the lung microbiota of the low respiratory tract, for the absence of anaerobic bacteria or the abnormal growth of particular bacterial species, deriving from the gut. Also, recent and interesting data suggests tight correlations between the lung microbiota and the admission in intensive care [27]. In particular, the modifications of the lung microbiota can help in predicting in which way the patients in critical condition will respond to the treatments. In a recent study on 91 adult subjects, only the patients that had *Enterobacteriaceae* in the gut were admitted in intensive care. Furthermore, the patients that had a high number of bacteria in the lungs and prevalence of typical ("good") bacteria of the lung flora, the next day after the admission in intensive care for ARDS, clinically improved (fewer days of ventilation). On the contrary, the presence in the lung of two groups of bacteria usually presents in the gut – *Lachnospiraceae* and *Enterobacteriaceae* – was frequent in the lung microbiota of patients with worse outcomes [27]. In conclusion, it can be affirmed that the type of bacteria detected is predictive of the outcomes as well. We must understand whether it is possible to modify the lung microbiota, for both preventing and treating the pulmonary lesions.

In acute cases, generally, an antibiotic cover is not directed against gut bacteria (usually, clinicians do not take into account these infections at the level of the lung). The clinicians can have the false feeling of giving an extensive antibiotic cover to the patient, while the responsible bacteria do not respond to the therapy. The individuation of the bacteria could be done with the bronchoalveolar lavage (BAL), but the execution of BAL, as well as being uncomfortable, has the bacterial super-infection of the lung as a side effect.

Can the severity of COVID-19 be affected by the bacteria?

In broads terms, pregnant women are considered to be an at-risk population for viral respiratory infections. We know how, for instance, the flu syndromes and recently the infections by Novel COronaVIrus Disease 2019 (COVID-19) are dangerous in pregnant women during the third trimester for the possible consequences both on the mother and on the fetus [30]. For the seasonal flu, the vaccination is recommended during pregnancy at the beginning of the flu season.

It has been investigated if the different incidence depending on age and the different courses between adults and children for COVID-19 could be due to the different concentrations and/or activation of ACE2 at the intestinal and pulmonary level [31].

In contrast to what had been supposed in preclinical studies, in one study, they analyzed BAL in newborns, children, and adults with ARDS. The authors found differences in the biomarkers of neutrophil response. Instead, for what concerns ACE and ACE2, their concentration and their activity at the pulmonary level do not seem to change with age. On the other hand, it is possible that ACE2 is more or less activated at the gastroenteric level and that its presence and its activity is affected by the microbiota.

The expression of ACE2 is mainly localized on the luminal surface of the intestinal epithelial cells and that it is less expressed at the level of the crypts. Moreover, the gut microbiota influences the action of ACE2. Thus, a close relationship between COVID-19 and the microbiota can be hypothesized. Nevertheless, the connections between the lung and the gastroenteric tract are not entirely understood, but it is well known that the infections of the respiratory tract can get complicated with gastrointestinal dysfunctions, and this phenomenon is observed in patients with infection by COVID-19 as well. Recent studies suggest that the involvement of the gut in COVID-19 is even greater and more prolonged compared to the lung. Thus, the rectal swab could be useful in monitoring and evaluating the possibility of transmission [32].

Several studies demonstrated that by acting on the gut microbiota, the episodes of enteritis and the associated respiratory pathologies could be reduced, and the side effects of the antibiotics avoiding the replication of the virus at a respiratory level can be reduced as well [33]. To date, there is

no evidence that probiotics can exert a therapeutic effect, but it can be supposed that they can facilitate the resolution of gastroenteric and respiratory symptoms.

In this regard, so far, only experimental animal studies have been performed. In one of these [34], performed on cats, Meazzi et al. studied the fecal microbiota composition in healthy cats and cats infected by feline coronavirus (FCoV), with and without peritonitis. Although there were no statistically significant differences, some conclusions can be made: the most represented bacteria were *Firmicutes*, followed by *Bacteroidetes* and *Actinobacteria*. In the group of FCoV-positive cats, *Firmicutes* and *Bacteroidetes* were over- and underrepresented, respectively, compared to the other groups. Among the 5 cats with peritonitis, 3 presented a similar microbiome. Thus, some differences can be already detectable. Nevertheless, the sample was limited to 15 cats. Further studies could elucidate the interaction between coronavirus and intestinal microbiota.

The future

Potential interventions for COVID-19

The discussion of potential interventions for COVID-19 does not fall within the aims of this review. Recently, it has been published a systematic review [35] that summarizes suggested potential interventions: nutritional, antiviral, anti-coronavirus, and miscellanea.

Also, we would like to highlight the lack of some potential interventions in the abovementioned review, such as, for example, the inhibitors of hyaluronic acid (HA) [36], lactoferrin (LF) and probiotics.

The metabolism of HA is a delicate equilibrium between synthesis (HA synthase) and degradation (hyaluronidase). It can be fragmented by reactive oxygen and nitrose species. [37]. Some bacteria (*Streptococcus spp.*, *E. fecalis*, *E. coli*, *L. lactis*, *B. subtilis*, *Agrobacterium spp.*) can produce HA [38]. Other bacteria (*Streptococcus A*, *B*, *C*, and *G*, *S. pneumoniae*, *C. perfrigens*) can produce hyaluronidase and can reduce HA [39]. HA plays essential roles in different situations, including inflammation, viral infection, and tumors. For example, in RSV infection, an accumulation of HA is present in smooth muscle cells [40].

Also, LF is an 80-kDa globular glycoprotein with a high affinity for metal ions, particularly for iron, that has to be taken into account since it acts as a potent inhibitor of several pathogens. LF has efficacious antibacterial and antiviral activities against a wide range of Gram-positive and Gram-negative bacteria and both naked and enveloped DNA and RNA viruses. In its antiviral pursuit, LF acts predominantly at the acute phase of the viral infection or even at the intracellular stage. In particular, LF inhibits the entry of viral particles into host cells, either by direct attachment to the viral particles or by blocking their cellular receptors [41]. LF participates in the host immune response against SARS-CoV invasion by enhancing NK cell activity and stimulating neutrophil aggregation and adhesion. Some results reveal that LF inhibits SARS pseudovirus infection in a dose-dependent manner, blocking the binding of spike protein to host cells, indicating that LF exerts its inhibitory function at the viral attachment stage. However, LF did not disrupt the interaction of spike protein with ACE2, the functional receptor of SARS-CoV [42].

Probiotics and acute pulmonary diseases

Studies that have been published on probiotics and acute pulmonary diseases were performed on mice. In addition to apparent general differences between humans and mice that we all know very well, it must be taken into account that we share only 15% of lung microbiota with mice. Furthermore, the studies did not consider fungi and viruses. Surely, it is crucial to investigate further the mechanism and the consequences of gut dysbiosis [43]. The use of probiotics implied a recovery of the optimal microbiota both at intestinal and at pulmonary level, through composite actions on the immune system and specific actions on the lymphocytes. Numerous studies have shown that the modulation of gut microbiota can reduce enteritis and ventilator-associated pneumonia; moreover, it can reverse the side effects of antibiotics used for influenza coverage [44].

A key area for the future is the use of probiotics in pulmonary diseases, as already reported by Chen et al. for the use of probiotics in intestinal diseases [33]. In the same way, more detailed studies are needed to face how probiotics (a) modulate the community of the residential lung microbiota, (b) persist and are localized in the airways, (c) interact with cells and residential leucocytes and (d) affect the homeostasis of lung immunity during and after the infection by several pathogenic agents.

The dark side of the coin is that the complexity of microbial communities, in terms of the number and percentage of different bacterial species in an environment, could be a critical factor in identifying a feasible intervention to manipulate microbiomes [45].

The use of probiotics is one possible way to prevent influenza virus infection. In this context, some authors conducted a clinical trial in humans to confirm the effectiveness of a probiotic strain in improving immune function [46].

Recently, it has been reported that probiotic bacteria extracted from fermented sea buckthorn berry had lots of *L. gasseri*, which represses the activation of purine, an energy source required for the mutation of new coronavirus [47].

The role of metabolomics

Several authors believe that "omics" sciences, metabolomics, in particular, allow to solve some mysteries of the lung microbiota, with essential health improvements in the daily practice.

It must be stressed that the microbiota interacts with the host through the secretion of metabolites that are identified by the immune system and provide information concerning the metabolic status and the quality and quantity of the colonization. This continuous crosstalk, essential for the reaching and the maintenance of the equilibrium of the organism [29], takes place at the pulmonary level as well [48]. How does this crosstalk happen? Through metabolites. The metabolites, meaning the low molecular weight substances present in all our biofluids, the object of the study of metabolomics, are the secret language of bacteria, cells, and organism, they are the biological SMS with which these worlds so far and so close, so necessary to each other, communicate. Thanks to metabolomics, the secret language has been, to no small extent, deciphered, and this gives us significant advantages from the preventive and therapeutic point of view. The metabolites are the language readable at the same time by bacteria and by our body [49].

In the light of the above, a specific metabolomics study, performed through the analysis of urines and/or blood, could predict the behavior of the patients and orient from the therapeutic point of view, discriminating earlier the "responders" or "not responders" patients to a given type of treatment, e.g. the antibiotic therapy [50]. At the moment, to our knowledge, no studies have been performed on coronavirus with metabolomics.

Conclusions

In light of the above, it must also be suggested more considerable attention to the microbiomics aspects of the lung in acute respiratory pathology conditions. The deepening of these aspects could potentially predict the clinical outcome of great interest and utility in the present clinical management of the patients with COVID-19. A practical suggestion could be to pay more attention to patients with an initial presentation with gastrointestinal symptoms, especially those from epidemic areas. The patients could have a leaky gut with bacterial traslocation from the gut to the lung. In patients with pneumonia, the pulmonary microbiota could rapidly and drammatically change with the presence of gut bacteria in the lung. The gut bacteria can significantly influence the need of ventilation and the survival. These concepts must be taken in mind when managing a patients with ARDS for an adequate antibiotic coverage. Several suggestions have been proposed for COVID-19 treatment. These options could include also probiotics, especially *Bifidobacteria* and *Lactobacilli*, namely *L. gasseri*.

In the next future, metabolomics could be applied in the study of COVID-19, deciphering the secret languages between viruses, bacteria and the organism.

Declaration of interest

The Authors declare that there is no conflict of interest.

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