Lung Microbiome in Critically Ill Patients

Subjects: Critical Care Medicine | Infectious Diseases

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The microbiome is a diverse ecosystem that includes all host-associated microorganisms and their genomes. These microorganisms belong to various kingdoms including some potential pathogens such as bacteria, viruses and fungi. To obtain a comprehensive view of the lung microbiome, including not only bacterial but also viral and fungal data, is of great value to improve our understanding of critical lung illnesses such as VAP or ARDS. The evolution of the lung microbiome over time and the description of its dysbiosis will be key elements to improve diagnosis and preventive measures in ventilated patients.

lung microbiome | lung mycobiota | acute respiratory distress syndrome | ventilator associated pneumonia | mechanical ventilation

1. Lung Microbiome in Critically Ill Patients

1.1. Lung Bacterial Microbiota

1.1.1. Lung Bacterial Microbiota and Invasive Mechanical Ventilation

Studies to date have been mostly descriptive. A first work demonstrated in 2007 the considerable diversity of microbial populations in bronchial aspirates collected from ventilated patients colonized with P. aeruginosa [1]. Since high-throughput sequencing was not gold standard, this very first study used 16S-rRNA clone libraries (PCR amplification, cloning into a vector and sequencing). In 2012, based on a similar methodology for bacterial identification, Bousbia et al. also observed a high bacterial diversity in bronchoalveolar lavage (BAL) from ICU patients mostly ventilated for community-acquired pneumonia [2]. A large repertoire of 146 bacterial species belonging to seven phyla was identified, of which 73 bacterial species had never been described in infected lungs. Subsequently, most studies used high-throughput sequencing of 16S-rDNA hypervariable sequences to explore the lung microbiota. Smith et al. studied the microbiota of 15 uninfected ventilated patients admitted to a surgical unit whose BAL was negative in conventional culture [3]. The same phyla were identified in BAL using sequencing of the V4 hypervariable region of 16S-rRNA genes with an Ion Torrent® sequencer. Most patients had profiles with a high degree of alpha diversity, and inter-individual variation was mostly apparent at the genus level (species diversity within a sample from a given individual). These data were snapshots at a given time point, and the question of how the respiratory microbiota changes under mechanical ventilation overtime, likely the most relevant element, has been addressed in more recent works.

1.1.2. Lung Bacterial Microbiota and Acute Respiratory Distress Syndrome

Beyond the specific effect of mechanical ventilation on the lung microbiota, acute respiratory distress syndrome (ARDS) or severe systemic inflammatory response syndrome (SIRS) may have an impact on its composition, directly or by enrichment from the gut microbiome [4]. Only a few studies have explored these aspects in critically ill patients. However, the relationship between the gut and the lung microbiome has been well described in asthma or cystic fibrosis and is referred to as the “gut−lung” axis [5][6].

Table 1 summarizes the results of the different comparative studies. Further studies, with comparable methodologies, are needed to better characterize the role of the different actors in the vicious circle between dysbiosis, inflammation and lung injury, and to determine the role of enrichment of the lung microbiota with bacteria from the gut microbiota.

Table 1. Main comparative studies exploring the lung microbiota in ventilated patients with acute respiratory distress syndrome.

Study	Enrolled Patients	Methods (Sampling and Sequencing)	Main Results
Panzer et al., 2018 [7]	30 ventilated patients (severe blunt traumatism) - 13 ARDS [1] patients - 17 non-ARDS patients	ETA [2] on admission and 24 h after V4 16s-rRNA MiSeq Illumina sequencer	- Association between ARDS development and lung community composition at 48 h (r2 = 0.08, p = 0.04) - ARDS patients: microbiota enriched with Enterobacteriaceae, Prevotella and Fusobacterium
Kyo et al., 2019 [8]	47 ventilated patients: - 40 ARDS - 7 non-ARDS	BAL [3] within 24 h after intubation V5-6 16s-rRNA Ion One Touch sequencer	- Decreased alpha diversity in ARDS patient compared to controls (p = 0.031) - Copy number of 16S rRNA gene of Betaproteobacteria decreased in non-surviving (n = 16) vs. surviving patient (n = 24). (10^6 vs. 10^4; p < 0.05)
Dickson et al., 2020 [9]	91 ventilated patients - 17 ARDS - 84 non-ARDS	BAL within 24 h of ICU admission V4 16s-rRNA MiSeq Illumina sequencer	- Increased relative abundance of Enterobacteriaceae in ARDS patient (12.5% vs. 0.8%) (p = 0.002). - Association between presence of gut associated bacteria in the lung microbiota and the ventilator-free days at day 28 (p = 0.003)
Schmitt et al., 2020 [10]	30 ventilated patients (surgical) - 15 patients with sepsis-induced ARDS - 15 controls	BAL at ARDS onset (D0 [4], D5 [5], D10) V4 16s-rRNA MiSeq Illumina sequencer	- Lower alpha diversity in BAL of ARDS patients vs. controls (Shannon index 3 (2;3.6) vs. 1 (0.5;1.5); p = 0.007) - Decrease in anaerobic bacteria Prevotella spp (p = 0.0033) and Veillonella spp (p = 0.0002) in ARDS patient - Decreased alpha diversity associated with

References

Study	Enrolled Patients	Methods (Sampling and Sequencing)	Main Results
			increased length of mechanical ventilation (ρ = −0.48, p = 0.009)

[1] acute respiratory distress syndrome; [2] endotracheal aspirate; [3] bronchoalveolar lavage; [4] day following intubation, [5] five days post-intubation.

1.1.3. Bacterial Microbiota and Lung Infections

The bacterial lung microbiota has not been extensively studied in the context of acute lung infections, in particular under mechanical ventilation. Flanagan et al. were the first in 2007 to clone and sequence r16S DNA from bronchial aspirates and BAL of mechanically ventilated ICU patients who were colonized with P. aeruginosa [1]. Identified bacteria belonged mainly to the three major phyla previously described: Bacteroidetes, Firmicutes and Proteobacteria, and among them the less abundant species belonged to the flora of the oropharyngeal, nasal and gastrointestinal tracts such as Lactobacillus, Enterococcus and Veillonella. During the antibiotic course, a decrease in the diversity of the microbiota was observed along with the significant predominance of P. aeruginosa despite its in vitro susceptibility to the administered treatment. From these results, it appears, on the one hand, that the oropharyngeal and digestive microbiota could be an important source of the pulmonary microbiota change during mechanical ventilation, and, on the other hand, that certain non-pathogenic species could have a protective effect against the development of a ventilator-associated pneumonia (VAP). This could act as a commensal barrier flora of which the reduction could be deleterious [4][9].

Identification of risk markers within the lung microbiota is probably the most relevant question. Emonet et al. recently attempted to identify metataxonomic risk markers for the occurrence of VAP from the time of intubation to the day of VAP diagnosis using V3-V4 regions MiSeq Illumina sequencing of BAL samples [11]. They did not observe a significant difference in the lung microbiota evolution between patients with VAP and control ventilated patients at any time point. However, tracheal aspirates from patients with VAP contained more Gammaproteobacteria (including notably Pseudomonas spp, Enterobacteriaceae) three days before VAP diagnosis [11]. In parallel, oropharyngeal swabs from these same patients with VAP contained fewer Bacilli (Enterococcus spp, Streptococcus spp, Lactobacillus spp, and Staphylococcus spp) on ICU admission. The authors used this difference to classify patients between a VAP group and a control group, with good diagnostic performance. However, their results need to be confirmed in other settings and with a greater number of patients. The results of the main studies concerning mechanically ventilated patients and VAP are summarized in **Table 2**.

Table 2. Main comparative studies exploring the lung microbiota in ventilated patients with ventilator-associated pneumonia.

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Study	Enrolled Patients	Methods (Sampling and Sequencing)	Main Results
Kelly et al., 2016 [12]	- 15 MV [1] patients from medical intensive care unit - 12 healthy unventilated patients	ETA [2] and OS [3] within 24 h of orotracheal intubation and every 72 h after V1–V2 16s-rRNA MiSeq Illumina sequencer	- Lower alpha diversity in intubated patients than healthy controls ($p = 2.3 \times 10^{-13}$) - Decreasing alpha diversity overtime in URT [4] of VAP [5] patient ($p = 0.0015$) - Higher beta diversity in MV patients than in healthy controls
Zakharkina et al., 2017 [13]	- 11 ventilated patients with VAP [5] - 18 ventilated patients without VAP - 6 HAP [6]/CAP [7] - non ventilated control patients	- BAL [8] for VAP suspicion - ETA at ICU [9] admission and twice a week thereafter 16s-rRNA 454 platform	- Decreased alpha diversity associated with increased length of mechanical ventilation (fixed effect regression coefficient (β): −0.03 CI95% [−0.05; −0.005]) - Increase in β diversity for VAP patients ($p = 0.03$)
Emonet et al. 2019 [11]	- 16 late onset confirmed VAP patient - 38 matched ventilated controls	- ETA and OS at five time points during MV including the diagnosis of VAP (DVAP) and three days later (DVAP +3) V3-V4 16s-rRNA MiSeq Illumina sequencer	- Progressive increase in Proteobacteria and decrease in Firmicutes (40% vs. 30%) in OS and ETA of VAP patients - Greater initial abundance of the Bacilli class in ETA from control patients - Association between presence of gut associated bacteria in the lung microbiota and the ventilator-free days at day 28 ($p = 0.003$)

[1] mechanically ventilated; [2] endotracheal aspirate; [3] oropharyngeal swab; [4] upper respiratory tract; [5] ventilator-associated pneumonia; [6] hospital-acquired pneumonia; [7] community-acquired pneumonia; [8] bronchoalveolar lavage; [9] intensive care unit.

1.2. Lung Virome

1.2.1. Virome and Invasive Mechanical Ventilation

The impact of mechanical ventilation on respiratory virome is still unclear and has not been studied much. However, the human virome seems to be strongly altered during hospitalization in ICUs, in connection with viral reactivations, in particular from the herpes group. The latter could be associated with a prolongation of the length of hospitalization and excess mortality [14][15]. In a population of patients with ARDS or VAP, lung biopsies revealed cytomegalovirus (CMV)-related lung damage in 29 to 50% of subjects [16]. In septic patients, CMV reactivation (17%) as well as viral reactivations of Epstein−Barr Virus (EBV) (48–53%), HSV1 (14–26%), HSV2 and HHV6 (10–24%) have also been described [17][18].

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Although the impact of mechanical ventilation and ICU inflammation on the lung virome is difficult to determine with precision, several elements underline the importance of studying it in order to obtain a more comprehensive view of the lung dysbiosis of ICU patients.

1.2.2. Virome and Pulmonary Infections

Virome and Community-Acquired Pneumonia

- Eukaryotic Virome and Community-Acquired Pneumonia

Viral infections are a major etiology of acute community-acquired pneumonia [19][20]. The most frequently identified pathogenic viruses, including in ventilated ICU patients, include rhinoviruses and influenza viruses, followed by human metapneumoviruses, parainfluenza viruses, respiratory syncytial virus, coronaviruses and adenoviruses.

At the same time, the presence of viruses, such as influenza virus or rhinovirus, in the airways may favor the occurrence of bacterial infections, possibly through a bacterial lung dysbiosis, and could be associated with significant excess mortality [21][22][23]. The reciprocal mechanism could also occur [24].

- Prokaryotic Viruses and Community-Acquired Pneumonia

The interaction between phages and bacteria in the gut microbiota is an example of symbiosis, which may play an important role in controlling bacterial populations [25]. Bacteriophages exert selective pressure on their bacterial hosts and directly influence the human microbiota, notably by infecting dominant bacterial populations more frequently and thus favoring the persistence of less competitive bacterial populations but also by conferring antibiotic resistance genes [26][27].

Virome and Ventilator-Associated Pneumonia

The role of viruses in the occurrence of VAP and their impact on patient outcome depends on the viral species [28]. For instance, CMV reactivation was associated with bacterial superinfections [29]. CMV pulmonary reactivation was also associated with increased duration of mechanical ventilation, ICU length of stay and mortality in a population of 93 mechanically ventilated patients with suspected VAP, whereas the effect of HSV replication in the lung is less clear [18]. However, Luyt et al. observed that HSV bronchopneumonitis could develop in a fifth of all ventilated patients. Such viral reactivation was associated with a worse outcome [30].

Viruses of the Herpesviridae, Paramyxoviridae and Picornaviridae families have been identified in all ventilated ICU patients in the pioneer study of Bousbia et al. that have included lung viral analysis (targeted PCR) [2]. In this study, HSV and CMV were the most commonly identified viruses, and CMV was more frequently identified in patients with a pneumonia than in controls. Interestingly, parainfluenza virus-1 was detected in three VAP patients [2].

1.3. Lung Mycobiota

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The study of dysbiotic mycobiota and its correlation with pulmonary disease is in its infancy, and the lung mycobiota in ICU patients is almost unexplored [31].

Few studies have evaluated this lung mycobiota using high-throughput sequencing [32][33][34]. In healthy individuals, studies revealed many environmental fungi including Aspergillus sp., mold (Penicillium and Cladosporium) and yeasts belonging to the two main phyla Ascomycota (Candida) and Basidiomycota (Malassezia) [31][33][35]. In contrast, the respiratory mycobiota of patients with chronic respiratory diseases is characterized by a dysbiosis with a restricted diversity and a clear predominance of Candida species [33][36].

In most environments, an interaction between bacterial and fungal communities exists, and the evolution of one community induces a modification of the other. Airway colonization by certain yeasts, notably the genus Candida, has been observed in 25 to 50% of patients after a few days of invasive mechanical ventilation [37][38]. This colonization was statistically associated with the development of bacterial lung infections [37][39]. It is therefore plausible that bacterial–fungal interactions play an important role in the pathophysiology of VAP. In a multicenter study of critically ill immunocompetent patients over a 4-year period, 214 patients (26%) with airway colonization were matched and compared with 214 unexposed patients [37]. Bronchial Candida colonization was found to be an independent risk factor for Pseudomonas pneumonia (9 vs. 4.8%) with an adjusted odds ratio of 2.22 [1.00–4.92] ($p = 0.049$). Interestingly, airway colonization with C. albicans in a murine model induced a Th1-Th17 immune response that promoted the development of bacterial pneumonia through the inhibition of bacterial phagocytosis by alveolar macrophages [40]. The same team showed in vitro that C. albicans impaired ROS production by alveolar macrophages and that this correlated in vivo with an increased prevalence of P. aeruginosa pneumonia in rats. In addition, the same fungal colonization promoted A. baumannii, E. coli and S. aureus pneumonia in rats [41][42], and that such colonization was an independent risk factor for A. baumannii pneumonia in mechanically ventilated ICU patients [39].

2. Lung Microbiome in Intensive Care Medicine: Limits and Future Research

2.1. Limits in 2021

Most studies of the lung microbiome have until recently been limited to the sole bacterial microbiota, using 16S rDNA genes (genes encoding 16S ribosomal RNA) sequencing. No study has really focused on the evolution of the mycobiota or the virome in ventilated patients, whereas fungal lung colonization and viral reactivation are extensively described in this particular population [18][43]. Definitely, inter-kingdom interplay in the lung microbiota and its interaction with the host likely play a key role in the pathophysiology of VAP and have to be considered. Addressing the dynamic evolution of the whole lung microbiome composition (including bacteria, fungi and viruses) is thus one of the main challenges in acute respiratory medicine to redefine our understanding of VAP pathophysiology.

2.2. Future Research

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Further longitudinal metagenomic studies are now needed to fully characterize pulmonary dysbiosis in ventilated patients who have developed a VAP or an ARDS to understand whether pulmonary dysbiosis is a cause, a consequence or both. These studies will have to use standardized methods that will allow their comparability.

One of the daily issues intensivists face is the accurate diagnosis of VAP in ventilated patient. Regardless of the type of respiratory specimen, pathogen identification by conventional culture-based microbiology techniques is time-consuming and requires a minimum delay of 24–48 h. Promising results were performed with next-generation specific platform BIGISEQ→ platform [44], or Oxford Nanopore→ MinION device (Oxford Nanopore Technologies, UK) [45], techniques that are not currently available in every country or not available enough to respond to the clinical demands of ICUs. Moreover, these studies have been performed with different experimental protocols, sequencing platforms and bioinformatic tools. Further larger studies are therefore required with a similar protocol to confirm the usefulness of such techniques for a large panel of microorganisms, including virus.

In parallel to the challenges of VAP diagnosis, VAP prevention is of high importance for the management of ICU patients. Obviously, a better understanding of pathophysiological infectious steps can help to define targeted interventions on the bacterial microbiota, the mycobiota and the virome. Targeting very specific bacterial strains with bacteriophages may also be an interesting field to treat lung dysbiosis and restore normal flora. The same reasoning may be held with antiviral treatment of viral colonization or co-infection.