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The Pulmonary Microbiome, Mechanical Ventilation, and Trauma

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Abstract

Recent advances in molecular technology have facilitated a more thorough investigation of the human microbiome. These developments have allowed examination of associations between disease states and a person's microbiome. While the majority of scientific literature has been focused on the microbial flora in the intestines, attention has recently been directed at the pulmonary microbiome and chronic disease states. Of particular interest is the microbiome's effect on mechanical ventilation in trauma patients, where ventilator-associated pneumonia leads to significantly increased mortality rates. However, within the trauma population, many patients that exhibit the clinical symptoms of a pulmonary infection fail to culture or exhibit only "normal respiratory tract flora." Herein, we discuss the current state of pulmonary microbiome research, risk factors, and future avenues of research involving the pulmonary microbiome as it relates to trauma patients.

Keywords: Microbiome; Pulmonary; Trauma; Bronchoalveolar

Introduction

Trauma patients in the ICU exhibit a diverse array of clinical problems that relate to their injury [1]. As a response to injury, trauma patients have clinical biomarkers that are commonly out of the normal range of a healthy patient, even in the absence of infection. These biomarkers are typically used to detect when patients have an infection [2]. However, in the case of trauma patients it is increasingly difficult to identify when patients have infections based upon these biomarkers alone. This is especially true when identifying pulmonary infections and pneumonia in mechanically ventilated patients [3]. One of the risk scores utilized to determine when a trauma patient has a pulmonary infection is the Clinical Pulmonary Infection Score (CPIS) [4,5]. However, many of the parts that make up this score are normally out of range in trauma patients. This can make it difficult to identify and treat pulmonary infections and pneumonia in mechanically ventilated trauma patients. Criteria such as the CPIS are utilized to determine when a patient needs to have a bronchoalveolar lavage (BAL). The BAL is utilized to make a clinical assessment of whether or not a trauma patient has a pulmonary infection. Collected BAL samples are sent to a pathology lab for clinical diagnosis of what specific organisms are cultured from the BAL. The cultures are then used to detect and identify the presence of disease–causing microorganisms and, if growth is present, which antibiotics should be used to treat the infection based upon that organism's antibiotic susceptibility. However, often there are clinical signs of infection in the pulmonary tract, but traditional culture-based techniques fail to identify an organism, or alternatively cultures are identified as "normal respiratory tract flora [6]". This lack of detection and identification presents a persistent barrier to the proper diagnosis of respiratory infections and pneumonia in trauma patients. Without proper diagnoses, clinicians are unable to properly treat infections, which then can delay recovery or worsen the clinical outcome, potentially including mortality.

In order to identify potential pathogens or define the infected state, it is first necessary to understand what constitutes "normal lung flora." Recent advances in molecular technologies including phylogenetic microarrays and highly parallel sequence-by-synthesis methodologies (Next Generation Sequencing or NGS) have facilitated the robust examination of the microbiome in both the patient environment as well as different organ systems. The majority of the early microbiome research in humans was primarily descriptive. These studies focused on samples or disease states that were relatively easy to sample. Among all these the region of the body that has received the most extensive characterization in research was the gut microbiome. These and subsequent studies have disproven many long held dogmas of the gut microbiome, including its diversity, abundance, and its contribution to clinical phenotypes. This extensive research on the gut provided the seed for additional hypotheses in other disease states and other areas of the body, including the contribution of the pulmonary microbiome to health.

The early studies of the lung and pulmonary microbiome have already over turned the long-held dogma of the lung and pulmonary tract as a sterile environment. In retrospect, this should not have been surprising since the lung is constantly exposed to bacteria through the air. Efforts have continued to identify numerous bacterial members comprising a healthy pulmonary microbiome, as well as have investigated the alteration or dysbiosis of the lung microbiome under various conditions and disease states.

The Healthy Lung Microbiome

Several culture-independent studies on BAL samples illustrate that lungs are not sterile and reveal the existence of a core pulmonary bacterial microbiome in healthy lungs [7-9]. Microbes present in the lung were first considered to come from the upper respiratory tract. Microbial community overlaps were observed between BAL samples and samples from the upper airway. The ebb and flow of air in and out of the lungs likely results in a compositional continuum rather than a clearly demarcated line of separation between upper and lower respiratory tract. Nevertheless, the core microbiota in healthy human

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lungs has been found to be comprised of a diverse assemblage of bacterial genera including species of *Streptococcus*, *Pseudomonas*, *Prevotella*, *Fusobacterium*, *Haemophilus*, *Veillonella*, and *Porphyromonas* [7]. Debate continues on whether the organisms present in the lower respiratory tract truly represent a resident microbiome or are transient contaminations from air and the upper respiratory tract, however, the presence of microbes in the lower lung in the disease state is far less controversial (reviewed in Nagalingam et al.) [10].

Lung Microbiome in Chronic Disease states

Most studies on the lung microbiome have investigated conditions within the chronic disease state, such as in patients with AIDS, asthma, cystic fibrosis, and chronic pulmonary obstructive disorder (COPD). It is possible that these disease conditions may serve as models for trauma patients, whose physiological systems are already under stress from other conditions. For example, a variety of pathogenic and opportunistic infections are frequently observed in HIV-infected patients due to their impaired immune system. 16S rRNA-based phylogenetic analysis from deep sequencing projects have shown that HIV infected and uninfected individuals had substantial differences between their lung microbiota. Compared with uninfected individuals, 14 bacterial genera from *Proteobacteria* increased in HIV infected patients, while 12 different bacterial genera increased in the uninfected individuals [11]. Another study showed that the bacterium *Tropheryma* was more frequently distributed in HIV infected individuals than in uninfected individuals [12].

The diagnosis of asthma actually spans a continuum of diseases subdivided into at least 11 different phenotypes ranging from non-allergic asthma, to allergic bronchopulmonary aspergillosis, to the virus-induced wheeze of bronchitis, with several in between [13]. The microbiota associated with asthma includes bacteria, viruses, and fungi [14,15]. A study by Hilty and colleagues analyzed bronchial scrapings and BAL from adult and child asthmatics and found that both adult and child asthmatics were colonized by *Proteobacteria* such as *Haemophilus* spp., *Moraxella* spp., and *Neisseria* spp. These organisms were in contrast to *Provetella* spp. and Bacteroidetes, which were dominant in control non-asthmatics adults and children, respectively [16]. Several studies have shown that infection with atypical bacteria, specifically *Chlamydophilia pneumoniae* and *Mycoplasma pneumoniae*, are related to asthma pathogenesis [17]. Just as adult and child asthmatics have similar microbial composition, mild and severe asthmatics also have similar bacterial colonization. Mild asthmatics not only are inhabited by Proteobacteria more frequently than non-asthmatics, but sputum samples also prove to exhibit more bacterial diversity [18].

Viruses such as the human rhinovirus (HRV), respiratory syncytial virus (RSV), and Influenza A virus lead to approximately 80% of asthma exacerbations. In addition to exacerbation of asthma in previously diagnosed patients, these viral infections in infants may lead to predisposition for the development of asthma phenotypes [19,20]. Fungi are also a factor of the lung microbiota that is affected by respiratory conditions such as asthma. An investigation of sputum samples from asthmatics and healthy individuals found that out of 136 fungal species identified by pyro sequencing, 90 were more common in samples from asthmatic patients than in controls. *Malassezia pachydermatis*, *Psathyrells candolleana*, and *Termitomyces clypeatus* are a few examples of fungi found at a higher percentage in asthmatic sputum, while *Eremothecium sinecaudum*, *Cladosporium cladosporoides*, and *Vanderwaltozyma polyspora* were found more in sputum from healthy patients [15].

Chronic obstructive pulmonary disease (COPD) is defined as "a common preventable and treatable disease, characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases" [21]. COPD is currently the third-most common cause of death in the United States of America with smokers having the highest-risk for developing the disease [22]. The lung microbiome associated with COPD has been studied in individuals with varying degrees of severity including those who are considered stable and those with an acute exacerbation [23,24]. Microorganisms found in the bronchial trees in COPD patients may be classified into two categories: potentially pathogenic microorganisms (PPM), which are known to cause respiratory infection and Non-PPMs which are oropharyngeal or gastrointestinal flora that are not usually associated with respiratory infections in non-immunocompromised individuals. PPMs include *Pseudomonas aeruginosa*, members of Enterobacteriacae, *Haemophilus* spp., *Staphylococcus aureus*, *Streptococcus pneumonia*, and *Moraxella catarrhalis*. Examples of non-PPMs include: *Streptococcus viridans* group, *Candida* spp., *Corynebacterium* spp., and *Neisseria* spp., along with others [25]. COPD patients with no current clinical signs or symptoms are referred to as stable. A study by Cabrera-Rubio and colleagues investigated six stable individuals with moderate COPD. Sputum, tissue, bronchoalveolar lavage (BAL) and bronchial aspirate samples were collected and analyzed by PCR amplification of the 16S rRNA genes and pyrosequencing [26]. Each sample exhibited diversity of over 500 species and between 80 and 140 genera per patient. The most common phyla identified were Proteobacteria, Bacteroides, Actinobacteria, Firmicutes with *Streptococcus*, *Prevotella*, *Moraxella*, *Haemophilus*, *Acinetobacter*, *Fusobacterium*, and *Neisseria* making up approximately 60 percent of total sequences [26]. A larger scale study found similar genera in COPD stable patients including *Haemophilus influenza*, *Streptococcus pneumonia*, *Moraxella catarrhallis*, *Enterobacteria* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. In contrast to the Cabrera study, only a minority of individuals studied were found to have bronchial colonization with the percentages increasing from 0% in those with mild COPD to a peak of 45% in patients with severe disease [27]. This difference in amount of species identified between studies may be attributed to the mode of analysis as the study was done with traditional selective media culturing rather than advanced molecular culture-independent techniques. Specific types and strains of PPMs differ between patients and over time within individuals with stable moderate COPD. Comparisons of baseline sputum and 9 month follow-up samples showed that a persistent PPM strain was only found in approximately 15% of cases [28]. In addition to bacteria, filamentous fungi are also found to inhabit the stable COPD patient, predominantly *Aspergillus fumigatus* and *Penicillium* spp. The prevalence of these filamentous fungi is not altered during acute exacerbation [29]. However, during an acute exacerbation, the lung microbiome of COPD patients changes [24]. Bacterial families such as Pseudomonaceae, Pasturellaceae, Helicobacteraceae, Enterobacteriaceae, Comamonadaceae, Burholderiaceae, and Alteromondaceae were found in endotracheal aspirates from patients with severe exacerbation leading to a stay in an ICU. Bacterial diversity in each person is associated with the types of bacteria found [30]. *Pseudomonas aeruginosa* and *Haemophilus influenza* are most commonly found during exacerbations and their presence correlates to greater respiratory impairment [31].

Comorbidities are known confounders for the recovery from trauma. In addition to comorbidities like COPD and asthma that affect the pulmonary microbiome, the effect of smoking on the pulmonary

microbiome has been investigated in healthy individuals. Upper airway microbial communities from nonsmokers was significantly less diverse than smokers [9]. However, some other studies reported that lung microbial community composition of healthy smokers was similar to that of healthy nonsmokers [7,32]. How this variation in diversity and microbial community composition will affect the recovery from trauma, especially in mechanically ventilated patients, remains to be determined. It is foreseeable that the management of pulmonary microbiome in mechanically ventilated trauma patients may differ based upon risk factors that alter a person's core microbiota (i.e. COPD, asthma, smoking, etc.)

NGS vs. Culturing in Identification of Lung Infections

One pyrosequencing study on patients with lower respiratory tract infections revealed complicated microbial communities in the sputum samples including *Streptococcus*, *Staphylococcus*, *Mycoplasma*, *Haemophilus*, *Moraxella* and etc. [33]. However the parallel cultural based tests were unable to detect some of the causative pathogens. Similarly, Huebinger et al. investigated BAL samples from ventilated patients after traumatic injury using culture-independent NGS techniques and detected more bacteria species than the standard culture methods [34]. In fact, in only one case (n=12) was the same organism identified as the predominant bacterium in both NGS and standard culture assays. Interestingly, the bacterial diversity measured by NGS was positively correlated with the number of days patients spent on a ventilator. However, such a correlation was not observed with culture-dependent tests. They also reported that antibiotic treatment has no significant effect on the number of bacterial species detected in the lung.

Risk Factors Contributing to Infection

There are other risk factors that may contribute to pulmonary infections in mechanically ventilated patients. The timing and location of intubation (pre-hospital vs. hospital) have been examined as risk factors for the development of VAP. Early intubation has been identified as a potential source of microbial contamination and increasing the risk of developing pneumonia [35]. Several retrospective analyses of pre-hospital intubations have attempted to determine if the location of intubation was a risk factor for the development of pneumonia [36-38]. A study examining pre-hospital versus emergency department intubations found no significant difference in the development of VAP [38]. However, other analyses that compared pre-hospital, emergency department and inpatient intubations found a reduced incidence of pneumonia with inpatient intubations. In this study, patients that received urgent intubation (pre-hospital or emergency department) were less severely injured and younger compared to the in-patient intubation group [36]. The site of intubation has also been associated with the type of bacterial colonization found within the lower airways [35,37]. Endogenous colonization is when PPMs carried in the throat migrate to the lower airways. In contrast exogenous colonization is colonization of the lower respiratory tract with PPMs not in the throat; these could be either community or hospital acquired. Endotracheal ventilation is associated with endogenous colonization, while ventilation via a tracheotomy more commonly results in exogenous colonization [39]. Method of ventilation is also a risk factor for respiratory infection. Invasive mechanical ventilation (IMV) is associated with higher risk of nosocomial respiratory infection than Noninvasive ventilation (NIV). However, NIV failure leading to the need for more invasive intervention is associated with airway colonization of non-fermenting Gram-negative bacilli prior to ventilation and should be taken into account when predicting NIV outcome [40]. Ventilator bundles are another way to reduce the occurrence of ventilator-associated pneumonia (VAP). The Institute for Healthcare Improvement developed a bundle including four evidence based practices that were shown to decrease the risk of VAP. The bundle components are: 30-45 degree elevation of the head of the bed, daily "sedation vacation" and assessment of readiness to extubate, peptic ulcer prophylaxis, and deep venous thrombosis prophylaxis. The use of bundles has been found in several studies to decrease the incidence of VAP, however some studies were found to have flaws [41]. Bundle components are not finite and the traditional four component system can be altered to include/exclude components. Other components that may be added include oral care with antiseptic solution, use of NIV whenever possible, and sub-glottic suctioning among others [42,43]. Recently, Croce and colleagues conducted a study using trauma patients admitted to the ICU of six Level I Trauma Centers. This study found that in a study of 630 patients, development of VAP was independently associated with the male sex and severity of chest injury and the use of bundles had no involvement [44].

Oral Care

A connection has been found between the oral cavity and lung infections; poor oral health can contribute to an increased risk of developing pneumonia especially in high risk populations such as mechanically ventilated individuals [45]. One crucial factor in reducing the risk of VAP development is reducing PPM colonization of the oropharyngeal cavity. Antiseptics and antimicrobial peptides such as chlorhexidine (CHX) and colistin (COL) have been used for oral decontamination and have been shown to significantly decrease the risk of VAP by treating with either CHX alone or in conjunction with COL when applied to the oral cavity every six hours [46]. Success of an oral care regimen may be affected by who performs the task. A study by Arroliga and colleagues found that when chlorhexidine gluconate was administered by respiratory therapists who were comfortable dealing with the oral endotracheal tube, adherence increased approximately three times from when oral care was administered by a nurse, this phenomena possibly contributed to the decrease in VAP cases [47]. Several studies have been conducted to assess the effect of tooth brushing in mechanically ventilated individuals on VAP incidence. A compilation of six studies with proximately 1,400 patients found that tooth brushing showed a trend for reduced risk of VAP; however this trend was only a significant decrease in one study [48]. The use of oral and parenteral antibiotics to reduce VAP is controversial, while studies have shown that selective decontamination of the digestive tract (SDD) may reduce VAP and mortality in ICU patients, there is a fear that administration of broad-spectrum antibiotics may lead to resistance [49]. A large amount of equipoise relative to SDD and its benefits to mechanically ventilated patients exists [50,51]. With the conflicting results from SDD and the worries about increasing the prevalence of antibiotic resistant bacteria, recent attention has been focused on using probiotics as a prophylaxis for the development of VAP [52-55]. In a clinical trial, the administration of a probiotic was able to significantly reduce the incidence of microbiologically confirmed VAP in mechanically ventilated patients [54]. Although different in their approach, the targeting of the gut microbiome by SDD and probiotics allude to the importance that gut microbiome may play in the development and/or prevention of VAP.

Conclusions

While many questions remain, the organisms that make up the lung microbiome have begun to be elucidated. Investigations of disease states and their correlation to various forms of dysbiosis shed light on

the important players not only of pulmonary disease, but also those possibly involved in the promotion of health. Improper application of antibiotics may actually worsen lung infection, presumably by removal of beneficial organisms [56]. This phenomenon has precedent in the gastrointestinal tract, where long-term antibiotic usage can lead to colonization of pathogenic *Clostridium difficile* resulting in pseudo membranous colitis. Interestingly, new treatments for the latter by fecal transplantation have shown dramatic effects [57,58]. This technique is already likely to give way to more targeted bacteriotherapy approaches using a defined bacterial consortium [59]. Beyond this simple analogy, it has also been well established that the health of the gut influences the function of other organ systems, including the lungs. Communication occurs by virtue of immunomodulatory interactions between gut microbes and the gut mucosa, and the subsequent distribution of immune cells throughout the body [60]. These effects have implications for COPD and asthma [61], as well as respiratory infection [62].

It would seem that the lung is a more hospitable environment for fungi than the mostly anaerobic gut; however, few deep sequencing studies have examined the fungal component of the lung. This is surprising given the variety of pulmonary fungal diseases known to exist [29,63]. One example, Valley Fever (coccidioidomycosis), is of increasing concern in the US [64]. A similar dearth of data exists for the pulmonary "viriome." Interestingly, recent work with bacterial viruses (bacteriophage) inhabiting the mucosal surfaces of the upper respiratory tract led the authors to suggest that surface-associated bacteriophage may serve a non-host-derived immune function [65].

Critical to future research, not to mention medical diagnostics and treatment, is the apparent lack of correlation between molecular-based identification (e.g. 16S rRNA gene sequence) and traditional, culture-based techniques [33,34]. The latter still serves as the gold standard in medicine, and a necessary component in the fulfillment of Koch's postulates. By contrast, microbial ecologists have dealt with "the Great Plate Count Anomaly" for years [66]. Simply put, it states that in any particular environment roughly 100 times more bacteria can be seen under a microscope than can be grown on a petri dish. Moreover, it is also well known that several entire phylogenetic divisions of bacteria lack a single, cultivated member. The reasons for this are many and complex, but often involve unknown nutritional requirements. Given the ratio of microbial species to microbiologists, the anomaly is not yet in danger of being resolved. It would therefore be naïve to assume that all pathogens of clinical importance have been cultivated and studied in pure culture. Application of molecular techniques, albeit with their own inherent limitations, will provide valuable insight into what organisms are still missing best practices for their exclusion in clinical settings, elucidation of what constitutes a "healthy" lung microbiome, and a deeper understanding of how these organisms promote or resist disease.

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