Next-Generation Sequencing and Sepsis

Subjects: Critical Care Medicine

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1. Introduction

In the 1970s, Sanger, Maxam, and Gilbert developed the technique of DNA sequencing. Since then, the process has been further optimised. Today, next-generation sequencing (NGS) enables a higher capacity in sequencing, with faster results and lower costs than the first molecular methods.

In 2010, a study established a catalogue of 3.3 million non-redundant human intestinal microbial genes [1]. NGS is a novel procedure among molecular methods for pathogen diagnostics, and it is able to analyse the overall DNA fragments in the sample. The procedure can also differentiate between human DNA, bacteria, eukaryotes, archaea, and chloroplasts. In 2013, Skvarc et al. investigated molecular methods for pathogen diagnostics using PCR. At that time, the conclusion was that, despite certain limitations, molecular methods should be further investigated together with culture-based methods in everyday clinical practice [2]. Since the NGS method has broader applications than previous molecular methods, this statement is more valid than ever. NGS was also able to determine which bacteria were present in the blood of critically ill patients within 30 h. The results coincided with the results of blood cultures, and NGS also showed other microbiota that were not detectable in blood cultures [3]. In comparison, conventional blood cultures take between 24 and 120 h from collection to results [4]. Cell-free DNA found in NGS may also result from the translocation of DNA by gut bacteria into the blood. Therefore, NGS results from blood samples should be interpreted critically. Initial work on cell-free DNA sequencing with optimised workflows and nanopore-based real-time sequencing, which further develops NGS, detected bacteraemia within 6 h. It was also shown that this method achieved a detection rate of 90.6% in 239 retrospectively evaluated sepsis samples by extrapolation. Furthermore, by using NGS, the study investigated a six-fold increased positive rate for pathogen hits in the course of sepsis compared to blood cultures [5]. In another study with septic patients, NGS results would have resulted in a change in antibiotic therapy in 53% of cases [6]. Moreover, in pneumonia patients in an ICU, NGS could detect microbiota in 84% of the patients, whereas culture-based methods could only detect microbiota in 65% of the patients [7]. Otto et al. were able to show that there are three phases during sepsis and that these are probably related to changing pathophysiological mechanisms. In the late phase of sepsis, there is an increase in positive blood cultures [8]. In combination with the previously presented results, showing that blood cultures only select some of the microbiota, NGS methods could provide earlier and further insights into the pathophysiological mechanisms of sepsis and a possible organ failure. However, it must be emphasised that NGS results must then be examined to determine whether the pathogens found were viable or dead pathogen residues. For this purpose, the wet lab or bioinformatics must reconcile the results critically.

2. Sepsis-Induced Acute Respiratory Distress Syndrome

A study published in 2016 found that 10.4% among all patients admitted to the ICU and 23.4% of all ICU-ventilated patients suffered from an ARDS [9]. The current mortality rate of ARDS remains high and is up to 43% [10], even though there is still no consensus on what is meant by a healthy lung microbiome. Specific microorganisms are often found in patients with healthy lungs [11]. However, the detection of these microorganisms seems to be challenging in clinical practice. Even in critically ill ARDS patients who required mechanical ventilation, almost 43% of bronchoalveolar lavage cultures were negative, although NGS showed a positive result [12]. A lower alpha diversity indicates poor lung health, and the alpha diversity also decreases by the time of invasive ventilation [12][13][14][15]. Ventilated ARDS patients showed a significantly lower alpha diversity and a higher dominance with only one bacterial species (>50%) when their BAL cultures were also positive compared to BAL-negative patients [16]. Kyo et al. demonstrated that ARDS patients had a decreased

alpha diversity of the lung microbiome with increased in-hospital mortality. Additionally, they demonstrated that ARDS had abundant Betaproteobacteria, *Staphylococcus*, *Streptococcus*, and Enterobacteriaceae, which may play a pivotal role in the pathophysiology of ARDS patients [15]. Patients who had nursing-home and hospital-associated infection (NHAI) pneumonia had significantly more reduced alpha diversity than non-NHAI pneumonia patients [17]. Cigarette smoking trauma patients are more likely to suffer from an ARDS. They have a different microbiome with a higher abundance of *Streptococcus*, *Fusobacterium*, *Prevotella*, *Haemophilus*, and *Treponema* compared to non-smokers [18]. Smoking significantly alters the lung microbiome. In a mouse model, it was shown that mice exposed to smoke for 2 h each day for 90 days had increased levels of *Staphylococcus*, *Acinetobacter*, and *Bacillus*, all are considered pathogenic microorganisms. [19]. Finally, it is essential to distinguish whether a microorganism is responsible for a disease or just a part of the colonisation. For example, in pneumonia patients who had a positive BAL culture for methicillin-resistant *Staphylococcus aureus* (MRSA), it could be shown that this is often only colonisation without disease value [20], which makes the interpretation of microbiome data in a clinical setting a lot more demanding.

In the pathogenesis of sepsis and ARDS, a gut-lung axis has often been described. In a murine model, typical gastrointestinal bacteria were detected in a septic ARDS in the pulmonary tract, such as Bacteroidales order, *Enterococcus* species, and Lachnospiraceae species [21]. Whether this translocation is part of an unrecognised bloodstream infection or is the result of ongoing gastric reflux followed by microaspirations remains unclear.

3. Perioperative Sepsis and Complications

Anastomotic insufficiencies with resulting peritonitis are a common reason for a severe sepsis and can lead to organ failure. Changes in the microbiome composition seem to be associated with the incidence of these insufficiencies. Patients who underwent bowel surgery and showed a reduced alpha diversity were more prone to higher infection rates and anastomotic complications [22]. Patients with colorectal anastomoses showed reduced alpha diversity and the abundance of Lachnospiraceae and Bacteroidacae [23][24]. Furthermore, a microbial imbalance with a higher dominance of several bacteria also increased the risk of anastomotic leakage. [25].

In 32 patients who underwent pancreatic surgery, three distinct microbiome enterotypes were found. One of the enterotypes showed an increase in Enterobacteriaceae, *Akkermansia*, and *Bacteroidales* and a decrease in *Bacteroides*, *Prevotella*, and Lachnospiraceae. Patients that have shown colonisation with this specific enterotype at least once during their hospital stay suffered significantly more often from complications and had a significantly longer hospitalisation. [26].

A study conducted on liver transplant patients also demonstrated the advantage of NGS in diagnosing fungal infections. The invasive fungal disease could be distinguished from colonisation by comparing NGS and culture-based results [27]. Furthermore, it was possible to distinguish a fungal infection from colonisation in critically ill sepsis patients using NGS, thus demonstrating the usefulness of NGS to detect invasive fungal disease [28].

4. Discussion

The idea that the lungs are sterile has existed for a long time, but today, we know that the lung microbiota exists for various microorganisms. Many of them seem to be translocated from the oropharyngeal tract [29]. The microbiome of the lungs is shaped from birth. In the first years of life, the microbiome's composition can already cause a predisposition to later diseases such as asthma, lung infections, and allergies. In general, the lung microbiome of healthy people differs from that of people with diseases. In addition, the lungs are exposed to the environment and can be influenced by allergens, microorganisms, and pollutants. Furthermore, the gut microbiome can affect the balance of the lung microbiome through the gut−lung axis by the immune system [30]. Therefore, preserved alpha diversity appears to play a key role in critically ill patients' outcomes because an increased alpha diversity reflects a healthy microbiome [15]. In addition, with or without sepsis, critically ill patients seem to have a markedly altered microbiome compared to healthy patients. It is also not uncommon that the microbiome in these patients consists of 50% and sometimes more than 75% of only one bacterial genus [31], which paves the way for problematic bacteria such as *Pseudomonas aeruginosa*. Given the previous results, it seems that the lung microbiome is, to a certain extent, related to the oral microbiome. However, at the same time, this relationship must be balanced to prevent serious infections. An increased presence in the lungs of *Haemophilus influenza, Moraxella catarrhalis*, and *Streptococcus pneumoniae*, especially at a young age, leads more frequently to asthma. These results are further evidence of an existing gut−lung axis and its interaction [32].

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