Metabolomics in community-acquired pneumonia: exploring metabolomics-based biomarkers for diagnosis and treatment response monitoring of community-acquired pneumonia

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CHAPTER 1

Introduction

1.1 Community-acquired pneumonia

Community-acquired pneumonia (CAP) is a common infection of the lower respiratory tract that results in the hospitalization of approximately 1 in every 500 adults every year [1, 2]. CAP has the highest burden of mortality and morbidity in the elderly [3, 4] and is among the most common causes of sepsis in all age groups [5]. The most common cause of CAP is the bacterial pathogen *Streptococcus pneumoniae*, followed by *Mycoplasma pneumoniae*, and viral infections such as influenza [2, 6].

The clinical diagnosis of CAP is based on symptoms such as shortness of breath, cough, fever, and new-found focal chest signs, and can be confirmed by the presence of a visible lung infiltrate on a chest radiograph [3]. Several microbiological tests are available to establish the microbial etiological diagnosis of CAP, including pathogen culturing, antigen testing, and polymerase chain reaction (PCR)-based diagnostics [7]. These tests are not applied to every CAP patient but merely those with more severe symptoms or specific risk factors for worse outcomes. Microbial etiological diagnosis using current laboratory techniques can take up to 48 hours and remains inconclusive often. In over 50% of CAP patients, no causative pathogen is identified because a sputum sample cannot be obtained, or because microbiological testing yields no causative pathogen [6, 8].

1.2 Antibiotic treatment and antimicrobial resistance

Hospitalized patients with moderate to severe CAP typically receive empirical broad-spectrum antibiotic therapy to provide broad microbial coverage prior to further determination of potential causal pathogens. Empirical antibiotic treatment of CAP usually consists of a beta-lactam antibiotic, which in more severe cases can be combined with a macrolide or fluoroquinolone monotherapy [9]. If the causative pathogen and its susceptibility profiles have been determined, more targeted narrow-spectrum antibiotics can be selected, avoiding undesired effects on nonpathogenic commensal bacteria. Importantly, in a subset of patients receiving empirical antibiotic therapy,

viral pathogens such as influenza are responsible for the symptoms. In those patients, antibiotic treatment is not useful and should be avoided as antibiotic use promotes the emergence of antimicrobial resistance (AMR). In conclusion, the availability of rapid and well-performing diagnostic tools to guide microbiological etiological diagnosis of CAP is essential to ensure effective treatment with antibiotics while limiting the risks of promoting AMR.

1.3 Treatment response biomarkers

Biomarkers predictive of treatment response in individual (hospitalized) CAP patients are important to optimize the effectiveness of therapeutic strategies. Such longitudinal monitoring of the treatment response can guide decisions about adapting the antibiotic treatment if the therapy is working insufficiently or terminating the antibiotic treatment when the infection is successfully treated. Early termination of antibiotic treatment because the bacterial infection has been effectively treated likely reduces the risk for development of AMR [10, 11]. Monitoring the treatment response is commonly performed through a combination of monitoring the clinical symptoms, such as fever, and through measurement of biochemical markers reflecting inflammation, such as C-reactive protein (CRP) [12, 13]. While CRP is commonly used for this purpose, it has limitations in terms of its correlation to clinical outcomes such as length of hospitalization or mortality, its specificity towards infectious causes alone and not towards other non-infectious inflammatory conditions, and its correlation with the underlying disease progression dynamics. As such, an unmet need exists for additional biomarkers that can further improve effective monitoring of the clinical response in CAP patients to guide treatment strategies [14].

1.4 Metabolomics as platform for discovery of biomarkers

The field of metabolomics concerns the large-scale measurement of biomolecules, or metabolites, with a molecular weight of <2000 Dalton in tissues, cells, or body fluids [15]. Metabolomics allows for the identification of metabolites that are associated with a state of health or disease. Because the metabolome is closely related to the biochemical state of organisms, it is a relevant source of potential biomarkers for various diseases [16]. For diagnosis and treatment response monitoring in several infectious diseases, metabolomics approaches have been explored [17].

The measurement of metabolites as part of metabolomics workflows is performed using mass spectrometry (MS) or nuclear magnetic resonance (NMR) based techniques [17], where MS-based approaches are more commonly used due to improved sensitivity and selectivity as well as increased throughput [18]. Metabolomics studies can be designed as targeted or untargeted studies. Untargeted approaches are useful for hypothesis-free discovery of new metabolites associated to a certain health condition, while targeted metabolomics can help to quantify the levels of metabolites that are known to be

involved in certain biological processes [19]. Targeted metabolomics is therefore more relevant for the discovery of biomarkers to be used for diagnosis or treatment response monitoring in patients.

Currently, the microbial etiological diagnosis of CAP is established through tests focused on the causative pathogen. To identify new biomarkers for the etiological diagnosis of CAP, the specific host-response of the patient could also be an interesting target. The immunometabolome, which concerns the interplay between metabolism and immunology, could be a source of such biomarkers for viral or bacterial infections. The relevance of immunometabolomics has been shown in previous studies, for example, to separate tuberculosis patients from patients with community-acquired pneumonia and healthy controls [20], and to separate sepsis patients from emergency room controls [21]. Also, some small studies have shown the potential of metabolomics for pathogen identification. For example, patients with H1N1 influenza pneumonia could be discriminated from patients with bacterial pneumonia based on serum metabolite profiles of CAP patients admitted to the ICU [22], and CAP patients with *Streptococcus pneumoniae* could be differentiated from CAP patients with other bacterial and viral infections based on their urinary metabolite profile [23].

For monitoring the treatment response, metabolic biomarkers could have potential because the metabolome closely represents the current state of the patient, which may be highly dynamic. This has been shown, for example, in patients with chronic hepatitis B infections, of who the progression of the disease could be associated with increased concentrations of long-chain triglycerides, together with citrulline and ornithine [24]. Also, a study in CAP patients has demonstrated that the change in lysophosphatidylcholines (LPC) mirrors the transition from acute illness to recovery after the start of antibiotic treatment [25].

For biochemical and functional interpretation of metabolomics study results, published research on individual metabolites and the biological processes they are involved in, can be evaluated. In addition, several computational tools are available for biochemical and functional analysis [26, 27, 28]. However, determining the relationships between metabolism and immunological processes remains challenging because of the many possible interactions and sparse literature on these interactions. As such, there is a need for new methodologies to determine associations between metabolites and immune processes to aid in the biological interpretation of metabolomics data.

1.5 Scope of this thesis

For this thesis, our central hypothesis is that changes in the host metabolome associated with the immune response in patients with CAP may be a potential source for novel biomarkers. To this end, the overall aim of this thesis is to assess the potential utility of metabolomics-based biomarkers for the diagnosis and the monitoring of the treatment response in patients with CAP.

In **Chapter 2** we aimed to investigate if predictive metabolic biomarkers for the microbial etiological diagnosis of CAP could be identified. Serum samples from CAP patients with confirmed microbial etiologies: *S. pneumoniae*, atypical bacteria, or viral infections were analyzed using targeted mass-spectrometry-based metabolomics techniques. In **Chapter 3** we further studied the specific differences in the metabolic host response to distinct CAP-associated pathogens. We performed a systematic characterization of differential metabolite profiles for different pathogens, which can support evaluation of diagnostic performance and may contribute to insights in disease pathogenesis. In **Chapter 4** we aimed to characterize longitudinal metabolite profiles in 25 hospitalized CAP patients with *S. pneumoniae* to determine their potential relationship to disease severity and treatment response, and thereby their utility as treatment response biomarkers. Finally, in **Chapter 5** we describe the development of the Immunometabolic Atlas, which is a computational tool for the interpretation of metabolomics data to aid in the design and interpretation of metabolomics studies.