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Functional genomics approach to understanding sepsis heterogeneity

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FUNCTIONAL GENOMICS APPROACH TO UNDERSTANDING SEPSIS HETEROGENEITY

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Functional Genomics Approach To Understanding Sepsis Heterogeneity

PhD thesis

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University of Groningen
on the authority of the
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and in accordance with
the decision by the College of Deans.

This thesis will be defended in public on
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INTRODUCTION

Sepsis, also known as blood infection, is a life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al., 2016). Sepsis is not a disease, but rather a heterogeneous syndrome in which the severity is classified into two clinical stages – sepsis and septic shock – depending on the degree of organ dysfunction. In 2017 the World Health Organization prioritized sepsis as a global health focus due to its high incidence, mortality and morbidity, and sepsis is estimated to affect more than 31 million people worldwide per year (Fleischmann et al., 2016). In the high-income countries, sepsis incidence was 437 cases per 100,000 people over the past ten years (2005-2015), and the mortality rate varied from 17–26% (Fleischmann et al., 2016). In middle- and low-income countries, sepsis is expected to have a similar, or even higher, incidence and mortality rate due to poverty and the lack of adequate hygiene, public health programs and supportive equipment for patient care (Rosenthal et al., 2016). Sepsis is also a threat for neonates and children. It is estimated that sepsis affects 3 million neonates and 1.2 million children every year, with a mortality of 11–19% (Fleischmann-Struzek et al., 2018). The outcome in sepsis varies between individuals, including in the degree of inflammation (hyper-inflammation vs. hypo-inflammation) and the degree of organ damage. Kidney, heart, lung, liver, gut and brain failure are all possible outcomes of sepsis patients, yet the factors that drive this process are not yet known (Iskander et al., 2013).

The inter-individual differences in sepsis development and outcome can be attributed to three main sources: pathogen-related factors, host non-genetic factors (such as age, immunosuppressive diseases, immunosuppressive medication, diabetes and indwelling catheters) and host genetic variations (Fleischmann-Struzek et al., 2018) (figure 1).

Pathogen-related factors in sepsis

Sepsis can be caused by any type of infection at different sites in the body. The infectious pathogens can differ according to which species are regionally endemic and to environmental factors, ranging from bacterial and fungal infections in high-income countries to dengue virus and hantavirus infections in middle- and low-income countries (Southeast

Asia Infectious Disease Clinical Research Network, 2017). However, bacterial and fungal infection are the most frequent causes in sepsis. Among positive blood-culture cases, Gram-negative bacteria, Gram-positive bacteria and fungi have been detected in 62%, 47% and 17% of patients, respectively (Vincent et al, 2009).

Figure 1.

Overview of the factors that can affect sepsis onset and the variability in host response that leads to organ dysfunction. These factors can be pathogen-related or host- specific, i.e. non-genetic or genetic. However, how these factors lead to injuries in different organs remain elusive. Since endothelial cells and immune cells are considered central in sepsis pathogenesis, in this thesis we explore how specific pathogens and host genetics affect the function of immune and endothelial cells.

Moreover, development of sepsis is also associated with particular sites of infection. Vincent et al. (2009) found that sepsis was initiated from infections in the lung (64% of cases), abdomen (20%), blood stream (15%) and renal and genitourinary tract (14%). Gram-positive bacterial sepsis is also more likely develop into organ dysfunction (31%) than Gram-negative bacterial or fungal sepsis (25-28%) (Esper et al, 2006). However, although sepsis can be induced by various types of pathogens, the clinical presentation of sepsis patients converges on some common phenotypes, and sepsis treatment was therefore generalized based on signs and symptoms. Broad-spectrum antibiotics, anti-inflammatory drugs and vasopressors (if necessary) are commonly used to treat sepsis. Recent research has now shown that sepsis pathogenesis can vary drastically depending on the type of infectious pathogens (Grewal et al., 2013; Yang et al., 2018). Therefore, more research needs to be done to explain the relationship between the types and sites of infection and the frequency of acute organ dysfunction.

Host non-genetic and genetic factors

In addition to the characteristics of the infectious pathogens, host comorbidities such as age, frailty, use of immune-suppressive medicines and chronic health issues (cancer, cirrhosis, AIDS, diabetes mellitus) are important factors in sepsis development and outcome (Cohen et al., 2015). In the US, 65% of sepsis cases are recorded in patients aged 65 and older, with an odds-ratio for mortality of 2.26 (Martin, Mannino & Moss, 2006). Type 2 diabetes mellitus, chronic renal disease, dementia and liver cirrhosis patients are at high risk for mortality in sepsis (Tiwari et al., 2011; Mansur et al., 2015; Bouza, Martinez-Ales & Lopez-Cuadrado, 2019). However, the impact of comorbidities on organ dysfunction has been suggested to vary according to the

site of infection, with intra-abdominal infections having a worse prognosis than community-acquired pneumonia (Sinapidis et al., 2018).

While more insights into the association between comorbidities and organ dysfunction and mortality are needed, on the other side of the puzzle greater light is being shed on the association between genetic factors and sepsis. Sepsis pathogenesis can be divided into two main stages: the development from local infection to systemic infection and the progression to organ dysfunction. Studies comparing immune defense between populations of different ethnicities have demonstrated that host genetics can predispose the host to different responses to infections. For instance, in terms of *Salmonella typhimurium* and *Listeria monocytogenes* infections in the American population, individuals of African descent have stronger inflammatory responses and the ability to kill intracellular bacteria compared to individuals of European descent (Nedelec et al., 2016). Moreover, a genome-wide association study (GWAS) on sepsis onset in neonates and premature babies has also suggested a strong association between genetic variations and the onset of sepsis (Srinivasan et al., 2017). On the other hand, although no GWAS has been conducted to identify genetic risks for organ dysfunction in sepsis, one GWAS have identified a genetic locus that is associated with patient survival: rs4957796 (Rautanen et al., 2015). The genetic contributions to sepsis progression and end-organ damage remain un-elucidated, and studies are needed to functionally validate the suggestive loci in sepsis pathogenesis identified thus far.

Host predisposition, including host non-genetic and genetic factors, affects the host's responses to pathogens. Although there is a gap in sepsis pathogenesis between systemic infection and end-organ damage, immune cell dysregulation and vascular leakage are ubiquitous symptoms among sepsis patients

and are suggested to be central to sepsis pathophysiology (Van Der Poll et al., 2017).

Immune response in sepsis

Immune cells are the frontier cells protecting the body against infection and are comprised of innate immune cells and adaptive immune cells. Innate immune cells include natural killer cells, mast cells, eosinophils, basophils, neutrophils, dendritic cells, monocytes and macrophages. These cells can recognize and kill infectious pathogens while presenting the pathogenic signatures to other cell types in the body, particularly the adaptive immune cells. The adaptive immune cells are made up of B cells and T cells, which can mediate pathogen clearance in a pathogen-specific manner. B cells secrete antibodies, while T cells secrete cytokines and attach to the infected cells. Both processes can induce programmed cell death, apoptosis or clearance of pathogens by phagocytes. These immune cells circulate in the blood stream or take up residence in different host tissues. Upon infection, immune cells play a central role in orchestrating host inflammation, in balancing pro-inflammatory signals and anti-inflammatory signals to kill the pathogens and in the return to normal homeostasis when the infection is cleared. In this thesis we use peripheral blood drawn from the vein and peripheral blood mononuclear cells (PBMCs). PBMCs are composed of most of the cell types of the innate and adaptive immune response, except for mast cells, eosinophils, basophils and neutrophils.

The dysregulated responses of the immune system observed in sepsis are known as the hyper-inflammation and hypo-inflammation stages. In hyper-inflammation, excessive inflammatory mediators, such as TNF- α , IL-1 β , IL-12 and IL-18, are produced and circulated. In hypo-inflammation, there is a reduction in T-cell activation, leukocyte movement and inflammatory responses.

In the past, cytokines serving as markers for each stage were used to differentiate whether patients were in the hyper- or hypo-inflammation group. However, due to the dynamic and complex interaction and feedback between cytokines, these two processes are now thought to happen simultaneously and are associated with mortality in sepsis patients (Wiersinga et al., 2014; Davenport et al., 2016). Moreover, once activated, the immune cells can interact with different components of the host defense system to regulate the host response, including complement, coagulation and different cell types such as the vascular endothelium (Van Der Poll et al., 2017). In sepsis this interaction is crucial as failure to regulate these interactions results in systemic inflammation, increased abnormal blood coagulation (coagulopathy) and vascular leakage. However, the extent to which host predisposition and pathogenic characteristics affect these interactions remains unknown.

Endothelial response in sepsis

The endothelium consists of a monolayer of cells lining the blood vessels. It maintains vascular homeostasis, regulates blood coagulation and coordinates with the immune cells to respond to infection. Being non-classical innate cell, endothelial cells express molecules that are inherent to innate immune cells such as Toll-like receptors, NOD-like receptors and RIG-I receptors to respond to pathogenic substances such as lipopolysaccharide (LPS) or to foreign genetic materials (RNA and DNA) (Dauphinee, Karsan, 2006; Davey et al., 2006). In sepsis, endothelial leakage leads to loss of barrier function that allows excessive inflammatory mediators, pathogens and fluid to access the tissue. This then causes tissue edema, potentially leading to organ dysfunction. Although endothelial and immune cell interaction are known to play a role in initiating inflammation and maintaining vascular homeostasis, how endothelial cells

respond to different types of pathogens and their interaction with immune cells in sepsis remain to be studied. Chapter 1 presents an intensive review of what is known about endothelial heterogeneous response in different organs in sepsis.

Current approaches in sepsis research

In the past 40 years, more than 20,000 studies have been published that aimed to elucidate sepsis pathogenesis and identify targeted molecular therapies. Despite promising effects in *in vitro* and *in vivo* models, clinical trials of the drugs identified in these studies have had disappointing outcomes. To date, no drug has been successfully approved for sepsis treatment. The translation of sepsis research into clinical treatment thus still faces many challenges, mainly driven by the heterogeneity of sepsis in patients. In the past, sepsis was described as a consequence of infection in the host, with an emphasis on the hyper-inflammation of the host immune responses (sepsis-2) that led to a focus on inflammation for both classification of patients and research. Basic knowledge on the cellular responses to different types of infectious pathogens has been growing rapidly. However, most research has been conducted as hypothesis-driven studies looking at specific pathways, and knowledge of the cellular interaction with pathogens on a global scale is still lacking. *In vivo* studies, on the other hand, move us a step closer to the host reaction by providing physiological factors such as blood flows, interaction between all tissues and metabolism. Mouse, rabbit and baboon models have often been challenged with an endotoxin, LPS, delivered either intravenously or intraperitoneally to mimic endotoxemia models. Cecal ligation and puncture animal models are also used to mimic peritonitis-induced sepsis (Seok et al., 2013). However, these animal

models are over simplified in comparison to humans and exhibit different hemodynamics and sensitivity to toxicity (Fink, 2014). Observations of how new therapeutic agents effect on sepsis in preclinical studies should thus be viewed skeptically (Fink, 2014).

Sepsis has now been redefined with a focus not only on systemic infections, but also on organ damage. Given the contributions of pathogen characteristics and non-genetic and genetic factors of the host to sepsis heterogeneity, a functional genomics approach can be used to acquire a global view of the changes in the host with a deep understanding at molecular mechanistic levels. It can not only distinguish patients based on their genotype, comorbidities and pathogen characteristics, it can also provide insights into the disease pathogenesis in those patients. By integrating multi-omics information (transcriptomics, proteomics, microbiomics and metabolomics) in patients versus controls, we can explain the mechanisms underlying the differences between groups. In complement to studies conducted in patients, which are often restricted in sample size and intervention approach, *in vitro* and *in vivo* experiments can be done to further investigate the mechanisms suggested by patient studies. Moreover, sepsis studies should be designed to observe the global view of host responses, for example accounting for immune responses and their interaction with different cell types. Collectively, in the future, we hope to develop markers and specific treatments for certain groups of patients based on the infectious pathogens and predisposing host factors (Wong et al., 2015).

OUTLINE OF THE THESIS

Chapter 1 is a review of the role of endothelial cells in organ-specific failure in sepsis. In this chapter we describe the heterogeneity of endothelial cells derived from different organs and the impact of this heterogeneity on the failure of specific organs, including lung and kidney.

In chapters 2 and 3, we explore the contribution of genetics to disease-onset and survival of sepsis patients. In chapter 2, we give an overview of the genetic variants that are associated with sepsis onset or sepsis survival based on the three current sepsis GWAS. We interpret the fact that there are many suggestive loci that do not reach genome-wide significance as a reflection of the limited of sample size against the broad heterogeneity of sepsis. However, by annotating and integrating the effects of suggestive GWAS loci with expression quantitative trait loci, gene expression and cytokine production, we can delineate the potential causal effect of these loci in sepsis. We also examine the causal effect of the GWAS SNP rs4957796 by looking at the alteration in transcription binding sites and the function of genes located nearby in endothelial cells.

In chapter 3, we investigate the genetic contribution to the amount of 92 circulating cytokines and inflammatory mediators in a more homogeneous group of sepsis patients: those with candidemia. We first identify the differences in protein profile between patients and healthy individuals. Then, for the set of proteins that differs between patients and controls, we explain how genetics can contribute to protein levels. To do so, we associate genetic polymorphisms with the abundance of proteins. Here we use cytokine levels measured in an *in vitro* setting where

PBMCs are challenged with *Candida albicans* yeast. We also aim to explain the mortality and susceptibility using the cytokine profiles.

In chapter 4 we delineate the interaction of leukocytes and endothelial cells in the context of infection. For the first time, we systematically profile leukocyte responses to a wide range of infections at RNA-level and investigate the effect of pathogens on leukocyte interaction with the vascular endothelium. We also reveal the transcriptomic changes in endothelial cells upon direct exposure to different types of infection: Gram-positive bacteria, Gram-negative bacteria and fungi. We then investigate the effect of IL1, TNF- α and IFN pathways on the interaction between leukocytes and endothelial cells and their dependence on different types of pathogens.

Chapter 5 discusses what the findings in this thesis have added to the field. We also discuss the current stages of sepsis research and perspectives.

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