Host response and outcome of sepsis in the critically ill
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Chapter 10

Summary and general discussion
SUMMARY AND GENERAL DISCUSSION

Sepsis remains a continuing challenge for critical care specialists around the globe. In this thesis, we studied the outcome and host response of critically ill sepsis patients admitted to the intensive care units (ICUs) of two large tertiary teaching hospitals in the Netherlands, the Academic Medical Center in Amsterdam and the University Medical Center Utrecht. We prospectively enrolled a large cohort and performed several studies, providing further insight into sepsis pathogenesis and the impact of several host characteristics on disease severity and outcome. Circulating biomarkers reflective of key pathways implicated in sepsis pathogenesis were determined in a large group of patients. In addition, several other approaches were taken to study the host response. A human endotoxemia model, in which healthy volunteers were intravenously challenged with Escherichia coli lipopolysaccharide, was used to dissect aspects of the host response observed in critically ill sepsis patients. Also, whole blood of critically ill patients and healthy volunteers was stimulated ex vivo. Additionally, we used an unbiased approach by analyzing the whole genome expression profiles of whole blood leukocytes circulating in sepsis patients.

Host response biomarkers in sepsis

Sepsis is characterized by a dysregulated host response to infection. Important features of the septic host response include activation of the cytokine network, the vascular endothelium and the coagulation system. In Chapters 3-7 and 9 we measured plasma biomarkers reflecting activation of these pathways. As expected, patients with sepsis displayed signs of systemic inflammation, reflected by a profound activation of the cytokine network, elevated levels of matrix metalloproteinase (MMP)-8 and TIMP metalloproteinase inhibitor (TIMP)-1 and an increased acute phase protein response (elevated plasma C-reactive protein concentrations). In addition, sepsis was associated with activation of the vascular endothelium, shown by increased plasma concentrations of soluble E-selectin, soluble intercellular adhesion molecule (ICAM)-1, fractalkine and angiopoietin-2, and reduced levels of angiopoietin-1. Moreover, sepsis patients displayed signs of a stimulated coagulation system, with elevated D-dimer levels, reduced levels of the anticoagulant proteins protein C and antithrombin, and prolonged prothrombin time and activated partial thromboplastin time.

In Chapter 2 we studied the potential of fractalkine as biomarker in critically ill patients with sepsis. Plasma fractalkine levels were elevated in sepsis patients compared to healthy volunteers, and did not decrease during the first 4 days of ICU admission. Non-surviving sepsis patients displayed higher fractalkine levels than sepsis patients who survived, and the association between fractalkine concentrations and mortality remained after adjustment for factors known to influence sepsis outcome. These data suggest that fractalkine can be of value in models for prognostication of critically ill sepsis patients. Although fractalkine can be produced by a variety of cell types, arterial and capillary endothelial cells have been identified as a major source during endotoxemia. As endothelial cells are not easily accessible for studying production of inflammatory mediators in patients with sepsis, we used the model of human endotoxemia to obtain indirect evidence for the endothelium as an
important source of fractalkine, comparing the release of fractalkine with that of the specific endothelial cell marker soluble E-selectin. Fractalkine release induced by intravenous endotoxin followed highly similar kinetics as soluble E-selectin, suggesting that fractalkine is primarily derived from the endothelium during systemic inflammation. We also assessed whether elevated fractalkine concentrations are specific for infection in critically ill patients. A substantial number of biomarkers have been proposed for the diagnostic stratification of infectious and non-infectious ICU patients, most notably procalcitonin. However, fractalkine was equally elevated in community-acquired pneumonia patients and patients treated for community-acquired pneumonia but in whom the diagnosis was retrospectively refuted, indicating that enhanced fractalkine release is a common feature in critically ill patients irrespective of the presence of infection.

**Comorbid conditions and chronic medication in sepsis**

The host response and outcome of sepsis can be influenced by pre-existing conditions such as chronic medication and comorbidity. Animal studies and observational human studies suggest that several cardiovascular drugs possess anti-inflammatory, antioxidant and other immune-modulatory effects that, when used during infection, may modify the host response to sepsis. Several observational studies investigated the association between antiplatelet therapy and outcome in patients admitted to an ICU with sepsis, reporting variable results. In our cohort, severity of illness upon ICU admission was similar in antiplatelet users compared to non-users and there was no association with an altered risk of mortality (Chapter 3). Besides their role in primary hemostasis, platelets exert important immune functions. While platelets have been implicated in multiple inflammatory and procoagulant reactions, knowledge on the effect of antiplatelet therapy on the host response in sepsis patients is highly limited. We hypothesized that antiplatelet therapy modifies the host response in sepsis, but did not find an association between antiplatelet use and the plasma concentrations of biomarkers indicative of key host responses to severe infection. Specifically, antiplatelet therapy was not associated with alterations in systemic inflammation, coagulation, endothelial activation, or renal injury during sepsis. Sepsis patients are heterogeneous in terms of site and microbiology, severity, genetic background, age and comorbidity, and plasma biomarker levels demonstrated large inter-individual variability, which may explain the lack of effect by antiplatelet therapy. Nonetheless, our data argue against a beneficial effect of pre-existing antiplatelet therapy on sepsis severity or outcome.

In Chapter 4, we show that prior use of calcium channel blockers (CCBs) is associated with improved survival in critically ill patients with sepsis in multivariable analysis of the complete cohort as well as in analysis of a cohort in which CCB users were matched to controls by demographics, comorbidities and chronic medication. We studied the potential influence of CCBs on three key host response systems implicated in sepsis pathogenesis (i.e., activation of the cytokine network, the vascular endothelium and the coagulation system) by measuring biomarkers indicative of these responses during the first 4 days after ICU admission, but did not find differences between propensity-matched CCB users and non-users except for less reduction in antithrombin levels relative to normal
values in CCB users. In the unmatched cohort CCB use was associated with attenuated cytokine release and blunted reductions in the anticoagulant proteins antithrombin and protein C, suggesting some effect of CCBs in patients who also receive other cardioprotective and/or vasoactive drugs. The mechanism by which chronic CCB use may influence sepsis outcome was not revealed by our analysis of the host response and we speculate that this may involve partial prevention of cellular toxicity related to sustained elevations in intracellular Ca²⁺ levels.

In the past years, multiple groups have looked at the association between statin use and outcome in patients hospitalized with infection. Several, but not all, observational studies have shown a survival benefit for patients with sepsis on statin therapy. Considering the abundant literature on pleiotropic non-lipid lowering properties of statins, we aimed to determine a possible association between prior statin use and host response characteristics in this population of critically ill patients with sepsis (Chapter 5). We measured plasma biomarkers providing insight into systemic inflammatory reactions, activation of the endothelium and the coagulation system, and studied whole genome expression profiles in blood leukocytes, and compared these between sepsis patients who were on statin therapy prior to admission and those who were not, in both an unmatched and a propensity score matched cohort. Our results suggest that prior statin therapy does not affect any of the host response pathways studied in patients with sepsis requiring intensive care. To our knowledge only one earlier study focused on sepsis patients admitted to the ICU: in a randomized trial of 250 critically ill patients with severe sepsis, prior statin users had lower baseline levels of interleukin (IL)-6 compared to statin-naive patients; treatment with atorvastatin during admission did not alter IL-6 levels compared to placebo in either prior statin users or statin-naive patients.

Although our studies on the association between chronic medication, disease severity, host response and outcome were performed in a large cohort of patients, the observational nature of our studies does not allow for assessment of causal relationships. We implemented propensity score matching to enable estimation of the independent effect of individual drugs. The size of our study population allowed us to perform matching by many important covariates, however bias can remain as a result of unmeasured confounders.

Patients with sepsis can display profound hypothermia, the mechanism of which is not yet fully elucidated. In Chapter 6 we aimed to determine risk factors of the occurrence of hypothermia during the first 24 hours of ICU admission. Lower body mass index, hypertension and chronic cardiovascular insufficiency were associated with hypothermic sepsis. Hypothermia was independently associated with mortality in multivariate analysis, confirming previous studies. In order to obtain insight into the pathophysiological mechanisms, we measured pro- and anti-inflammatory cytokines and endothelial activation markers. Plasma fractalkine levels were higher in patients with hypothermia, and this difference remained after correction for disease severity. Further studies are needed to establish a possible direct link between hypothermia and function of the vascular endothelium and fractalkine release.
In Chapter 7 we studied the impact of chronic HIV infection on the presentation and outcome of sepsis patients. ICU admissions of HIV positive patients for sepsis more often involved pneumonia compared to admissions of HIV negative patients. Previously, pneumonia was shown to be a major source of morbidity in HIV patients, even in those with high CD4 cell counts. Prior to the wide availability of combination antiretroviral therapy, Pneumocystis (P.) jirovecii pneumonia was a common reason for ICU admission. In our cohort P. jirovecii was a more common pathogen in HIV positive patients, as well as cytomegalovirus, but the number of infections caused by these opportunistic pathogens was relatively small, with the majority of pneumonia cases being caused by bacterial pathogens also found in HIV negative patients. There were no significant differences in mortality up to one year after admission between HIV positive and HIV negative patients in the total sepsis cohort, as well as in the pneumosepsis subgroup. These findings indicate that in a setting with excellent access to care and HIV treatment, the prognosis of ICU-admitted sepsis patients with HIV infection has become similar to that of patients without HIV infection. Considering the large demographic differences according to HIV status, we composed a control cohort of 90 admissions of HIV negative pneumonia patients matched for age, sex and race to study the host response. The concentrations of most host response biomarkers were similar in admissions of HIV positive and HIV negative patients, with the sole exception of interferon-\gamma and soluble ICAM-1, which were higher in HIV positive patients at day 0 and 2. HIV infection can stimulate the release of exosomes containing ADAM17 (ADAM metallopeptidase domain 17), the cleaving protease for ICAM-1, which promotes ICAM-1 shedding. However, other coagulation and endothelial markers were unaffected, which indicates that HIV infection has little additive effect on activation of these systems in critically ill patients with pneumonia.

**Immune suppression in sepsis**

In the past decades, many clinical trials have been performed to investigate inhibition of the exaggerated inflammatory response generally held responsible for sepsis mortality. In more recent years, the subject of immune suppression has become of increasing interest, which has been implicated as an important cause of secondary infections and late mortality. Exhaustion, apoptosis and hyporesponsiveness of immune cells are common features of immune suppression in sepsis patients. During HIV infection, both T-cell numbers and function become compromised. Our finding of higher plasma levels of interferon-\gamma in sepsis patients with HIV co-infection, which was sustained up to two days after ICU admission (Chapter 7), is remarkable, since HIV patients generally have reduced numbers of circulating NK cells and T-helper-1 cells. We have not extensively executed experiments to investigate immunosuppression in our pneumosepsis cohort, but did not find any suggestion that HIV patients were more immunosuppressed compared to HIV negatives.

Immunosuppression, due to an excessive anti-inflammatory response, has been proposed as a mechanism for hypothermia. Also, a recent study showed that hypothermia was associated with lymphopenia following diagnosis of sepsis, thereby potentially accounting for the association with adverse outcome. We found no difference in levels of either pro- or anti-inflammatory cytokines.
between hypothermic and non-hypothermic patients, even after correction for disease severity (Chapter 6). In accordance with previous investigations, whole blood leukocytes of critically ill patients obtained the first morning after ICU admission showed a decreased capacity to mount a cytokine response compared to healthy subjects, when stimulated ex vivo with the bacterial component lipopolysaccharide. However, whole blood stimulations resulted in similar cytokine release in hypothermic versus non-hypothermic patients. In addition, the incidence of ICU-acquired infections was similar between groups. Together these results suggest that hypothermia is not associated with clear immune suppression in patients admitted to the ICU with sepsis.

To obtain insight into pathophysiological mechanisms initiated after ICU admission and contributing to the development of a new infection while on the ICU, we compared the host response in patients with and without subsequent ICU-acquired infections in Chapter 8 and 9. No relationship was observed between cytokine release in whole blood ex vivo stimulated with lipopolysaccharide and the subsequent development of ICU-acquired infections (Chapter 8). These results argue against the use of whole-blood stimulation as a functional test of innate immunity applied early after ICU admission to predict nosocomial infection. Previous studies investigated the value of surrogate markers of immune suppression of the adaptive immune system to predict nosocomial infections in ICU admitted sepsis patients. Notably, reduced expression of human leukocyte antigen-DR and increased expression of programmed cell death (PD)-1, PD-ligand 1, and PD-ligand 2 on blood monocytes, determined by flow cytometry 3 to 5 days after ICU admission, correlated with an enhanced incidence of secondary infections in patients with septic shock.

In Chapter 9 we hypothesized that sepsis patients who acquire a secondary infection during their ICU stay, besides immune suppressive features, also display more profound “hyperinflammatory” responses when compared with those who do not develop a secondary infection. Sepsis patients who developed an ICU-acquired infection indeed demonstrated elevated plasma cytokine and MMP8 concentrations and stronger endothelial cell and coagulation activation than those who did not acquire a secondary infection. The more pronounced dysregulated host response on admission remained when the groups were adjusted for disease severity and source of infection. The hyperinflammatory host response detected at the time of an ICU-acquired infection was not different from that measured at the time of a non-infectious ICU-acquired complication (i.e., acute lung injury or acute kidney injury). Our host response analyses in patients with sepsis demonstrate concurrent immune suppression (Chapter 8) and hyperinflammation (Chapter 9), wherein hyperinflammatory reactions, but not the reduced whole blood leukocyte responsiveness to lipopolysaccharide, were more pronounced in patients who went on to develop an ICU-acquired infection.
CONCLUSION AND FUTURE PERSPECTIVES

In the future, the number of people who will develop sepsis is likely to increase due to ageing of the population, aggressive therapies for chronic diseases (most notably cancer) and the emergence of multidrug resistant pathogens. As a consequence, the management of sepsis will remain an important issue in the years to come. In this thesis we studied several “external” and host factors that might influence sepsis outcome and the accompanying host response, particularly chronic medication (antiplatelet agents, CCBs, statins) and comorbidity (HIV infection). None of these factors had a major impact on the host response to sepsis, while only the use of CCBs was associated with an altered (improved) outcome. Since our finding on the potential beneficial effect of chronic CCB use in patients with sepsis was the first report on this association, these data should be confirmed in another cohort.

In addition, we examined the influence of an acute manifestation of severe infection (i.e., hypothermia) on sepsis outcome and the host response, revealing an independent association between hypothermia and mortality without evidence for an altered immune response. Finally, the research presented in this thesis provides evidence that a disturbed host response towards a more hyperinflammatory phenotype renders sepsis patients more vulnerable to develop a secondary infection while on the ICU.

Current sepsis treatment predominantly drives on a “one size fits all” approach irrespective of specific features of individual patients. Given the fact that sepsis is characterized by non-specific signs and symptoms, and that the host response and severity can be influenced by many factors, including virulence of the pathogen, site of the infection, host genetics and therapeutic interventions, adjuvant therapies for sepsis may differ from patient to patient. Great advances have been made in terms of understanding the pathogenesis of sepsis in recent years, and implementation of this increased knowledge in clinical practice will likely facilitate individualized treatment of patients with sepsis. The challenge is to characterize the host response of the individual patient, which can be done by measuring biomarkers at the bedside that together with clinical readouts provide useful information for detection of infection, the predominant type of the immune response and risk stratification. This may select patients for targeted therapies, for example seeking to inhibit proinflammatory reactions in some patients, while boosting certain immune functions in other.
REFERENCES

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