Patient-related determinants of host response and sepsis outcome

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Abstract
Sepsis is an important cause of morbidity and mortality in intensive care unit patients. In this review, the results of studies with specific research questions performed within a large prospective observational study on sepsis in the Netherlands are discussed. The studies investigated various factors that may affect the host response during sepsis, and may have an impact on the outcome, including prior medication (antiplatelet agents, calcium channel blockers, and statins), HIV infection, and hypothermia. Neither prior medication nor HIV infection were associated with large differences in the host response during sepsis, whereas only prior use of calcium channel blockers was associated with an improved outcome. In addition, while hypothermia was not associated with an altered immune response, there was an independent association between hypothermia and mortality.

Introduction
Worldwide, more than 19 million cases of severe sepsis are estimated to occur annually, resulting in more than 5 million deaths.[1] The incidence rate of sepsis is estimated at 270 per 100,000 person years in high-income countries.[1] The incidence of sepsis has increased over the past decades[2,3] and this trend is expected to continue due to age of the population, increased burden of comorbidities, cumulative use of immunosuppressive drugs, chemotherapy, transplantation and invasive procedures.[4] Hence, sepsis is a large burden on human health.

One essence of sepsis pathogenesis lies in the failure of the body to effectively eliminate the invading pathogen, repair tissue and reconstitute homeostasis upon infection. Sepsis is in part the consequence of a dysregulated host response to infection, characterised by disproportionate pro- and anti-inflammatory components, coagulation and complement factors, and disturbed vascular responses, including increased leukocyte adhesion, vasodilation, and loss of endothelial barrier function, leading to tissue and organ damage.[5,6]

This review discusses various studies with specific research questions performed within the ‘Molecular Diagnosis and Risk Stratification of Sepsis’ (MARS) study, a large prospective observational study of sepsis in two academic intensive care units (ICUs) in the Netherlands. Various factors that may have an effect on the host response during sepsis, and may impact outcome were studied, including cardiovascular medication (antiplatelet agents, calcium channel blockers, and statins), HIV infection, and hypothermia (figure 1). These studies were bundled in an academic thesis.

Figure 1. Association between host factors and survival in critically ill patients with sepsis. Hypothermia was associated with increased mortality, calcium channel blocker use with reduced mortality. HIV, antiplatelet and statin therapy were not associated with altered mortality. Host response biomarkers were largely similar in patients with and without the particular host factor.

The MARS study
The MARS study was a collaboration of multiple partners and registered at clinicaltrials.gov (identifier NCT01905033). The primary aim of the MARS study was to develop tools that can provide rapid and accurate information on pathogen and host immune response or status. These tools should be easy to use,
available at or close to the bedside, aiding the clinician in the determination of the optimal treatment in an individual patient. From 2011 until 2013, consecutive patients aged 18 years or older and admitted to the mixed medical-surgical ICUs of the Academic Medical Center in Amsterdam and the University Medical Center Utrecht were included if they were expected to have a length of stay of at least 24 hours; the project had no other inclusion and exclusion criteria.\[7-11\]

Clinical data were prospectively collected from all patients, including demographics, premorbid comorbidities, use of prior (cardiovascular) medication, ICU admission characteristics, daily physiological measurements, disease severity scores, complications such as acute kidney injury and acute respiratory distress syndrome, and microbiology test results and antibiotic prescriptions. For all patients, leftover plasma was stored for protein biomarker analysis, including measurement of cytokines, endothelial activation and coagulation markers; also, whole blood was collected for genome wide RNA expression profiling of leukocytes. During the three-year study period, 6984 unique patients were enrolled in the MARS study, amounting to 8305 ICU admissions.

**Prior cardiovascular medication**

Cardiovascular disease is among the most common premorbid diseases in sepsis patients.\[12,13\] Preclinical studies using animals and observational studies in humans suggest that several cardiovascular drugs possess anti-inflammatory, antioxidant and other immune-modulatory effects, which, when used during infection, may modify the host response to sepsis.\[14-17\]

**Antiplatelet therapy**

Besides their role in primary haemostasis, platelets also exert important immune functions.\[14,18\] While platelets have been implicated in multiple inflammatory and procoagulant reactions, knowledge on effects of antiplatelet therapy on host response in sepsis patients is very limited. In the MARS study it was hypothesised that antiplatelet therapy modifies the host response during sepsis. In the MARS cohort, severity of illness upon ICU admission was similar in antiplatelet users compared with non-users and there was no association with an altered risk of mortality.\[19\] There was also no association between prior antiplatelet therapy and 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.

Previous observational studies investigating associations between antiplatelet therapy and outcomes in patients admitted to an ICU with sepsis have reported variable results.\[20-23\] Two of these studies used propensity matching to correct for baseline differences between antiplatelet users and non-users.\[21,23\] One of them entailed patients admitted to a medical ICU with severe sepsis or septic shock, and observed no influence of antiplatelet therapy after adjusting for the propensity to receive antiplatelet therapy and severity of illness, calculated using the APACHE III score.\[21\] The other one encompassed patients admitted to the ICU with systemic inflammatory response syndrome (SIRS), a subgroup of which was classified as sepsis; propensity analysis revealed a mortality reduction in acetylsalicylic acid users in both the overall SIRS population and the sepsis subgroup.\[23\]

While both studies adjusted for concurrent statin use, other prior medication use, such as beta-blockers, calcium channel blockers, and ACE inhibitors, was not taken into account.\[21,23\]

The third study consisted of a regression analysis to establish the impact of prior antiplatelet therapy on sepsis outcome showing an association between low-dose acetylsalicylic acid therapy with decreased hospital or ICU mortality.\[22\] Another study concerning the continuous use of acetylsalicylic acid during ICU stay reported similar findings.\[24\] Lastly, an investigation reported an association between prior antiplatelet treatment and reduced sepsis mortality using a medical claims database.\[25\]

The analysis of the MARS data was different from these previous reports in several aspects. First, in the MARS study patients were prospectively enrolled and classified based on strict diagnostic criteria and post-hoc assessment of trained research physicians, taking into account all available clinical and microbiological information. Second, propensity matching allowed us to compose comparable patients cohorts with respect to multiple relevant patient characteristics. Sepsis patients are heterogeneous in terms of age, comorbidity, genetic background, severity, site and microbiology, and plasma biomarker levels demonstrated variability between patients, which may explain the lack of effect by prior antiplatelet therapy. Nonetheless, these data argue against a beneficial effect of prior antiplatelet therapy on sepsis severity and outcome. To our knowledge no randomised controlled trials of antiplatelet therapy have been performed in critically ill sepsis patients.

**Statins**

Several investigators have looked at the association between prior statin use and outcome in hospitalised patients with infections. Considering the abundant literature on pleiotropic non-lipid-lowering properties of statins, the MARS study was used to explore associations between prior statin use and host response characteristics in critically ill patients with sepsis. For this, plasma biomarkers were measured to provide insight into systemic inflammatory reactions, activation of the endothelium and the coagulation system, and studied whole genome expression profiles in blood leukocytes. Sepsis patients who were on prior statin therapy prior to admission were compared with those who were not on prior statin therapy, in both the complete and in a propensity score matched cohort. The results of this analysis suggest that prior statin therapy does not affect any of the host response pathways in ICU patients with sepsis.
Host factors influencing sepsis

Calculating channel blockers

Studies in animals have suggested that calcium channel blockers (CCBs) can lower mortality induced by endotoxaemia or sepsis by restoring intracellular calcium homeostasis; however, knowledge on the association between prior CCB use and outcome in humans with sepsis is limited. Therefore, this association was studied in the MARS cohort of critically ill patients with sepsis. Prior use of CCBs was associated with improved survival 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 prior medication. The influence of CCBs on three key host response systems implicated in sepsis pathogenesis was studied: activation of the cytokine network, the vascular endothelium and the coagulation system. Biomarkers indicative of these responses during the first four days after ICU admission were similar in 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 reduced cytokine release and blunted reductions in the anticoagulant proteins antithrombin and protein C, which suggests some effect of CCBs in patients who also receive other cardioprotective and/or vasoactive drugs. Since the finding on the potential beneficial effect of prior CCB use in patients with sepsis was the first report on this association, these data should be confirmed in another sepsis cohort. One earlier study reported on the link between CCB use and outcome of severe infection. This retrospective analysis of the clinical records of 388 bacteraemic patients caused by aerobic Gram-negative bacilli and Staphylococcus aureus, prior CCB use was not associated with altered mortality in multivariate analysis. This study differed from the MARS study in various ways, i.e., its retrospective nature, and patient inclusion criteria (only bacteraemic patients and the absence of information about the type of care).

In multivariate analyses the relation between prior use of other cardioprotective medication and sepsis mortality was investigated. In particular the role of beta-blocker therapy in sepsis is currently under debate; a randomised controlled trial demonstrated a significant difference in 28-day mortality between septic shock patients treated with esmolol (49.4%), compared with the placebo group (80.5%). In the MARS cohort, prior use of beta-blockers was not associated with altered 30-day mortality. One earlier observational study reported on prior beta-blocker use and sepsis outcome. Patients previously prescribed beta-blockers had a lower frequency of death at 28 days than those previously untreated. Of importance, patients in this study were included based on hospital discharge records versus prospective inclusion by dedicated research physicians in the MARS study, and the fact that all hospitalised patients were enrolled versus ICU patients. In a cohort of patients with pneumonia, prior beta-blocker use was not associated with mortality. This study is different to the MARS study in that only pneumonia patients requiring hospital admission were included, whereas the MARS study was conducted in sepsis patients requiring intensive care and with different sources of infection.

HIV infection

The epidemiology of sepsis in patients infected with the human immunodeficiency virus (HIV) has changed dramatically upon the introduction of combination antiretroviral therapy. The incidence of opportunistic infections has decreased and long-term survival improved; however, invasive bacterial infections and sepsis remain an important cause of morbidity and mortality in HIV patients. The MARS investigators studied the impact of chronic HIV infection on the presentation, outcome and host response of sepsis patients. ICU admissions of HIV-positive patients for sepsis more often involved pneumonia compared with admissions of 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. In ICU patients with sepsis, HIV/AIDS was independently associated with inhospital mortality in other studies. However, these differed from the MARS investigation in patient outcomes.
selection\(^{[43,44]}\) and setting\(^{[45]}\) resulting in a cohort of patients with more severe disease\(^{[43-45]}\). Over time, standards of care for HIV patients have improved considerably and studies suggest that survival of critically ill HIV-infected patients has improved in the era of widespread cART availability\(^{[46,47]}\). These findings indicate that in a setting with excellent access to care and HIV treatment, the prognosis of sepsis patients with HIV infection admitted to the ICU has become similar to that of patients without HIV infection.

As there were large demographic differences between HIV-positive and -negative patients, a control cohort of 90 admissions of HIV-negative pneumonia patients matched for age, sex and race was composed to study the host response. The levels of most host response biomarkers were similar in admissions of HIV-positive and HIV-negative patients, with the exception of interferon-γ and soluble ICAM-1, which were higher in HIV-positive patients at day 0 and 2.

Two earlier studies on the host response in adult HIV patients with sepsis have been published, reporting plasma cytokine levels\(^{[48,49]}\). HIV status had little impact; one of these studies reported elevated plasma IL-10 levels in the presence of unaltered plasma IL-6 levels in HIV-positive patients with sepsis\(^{[49]}\). However, these only included patients with advanced AIDS defining disease and high mortality rates (around 50%).\(^{[44,49]}\) Chronic HIV infection seems to induce endothelial cell activation and disturbances\(^{[50]}\), responses that are also observed in patients with sepsis\(^{[51]}\). In Malawian children with severe bacterial infection, plasma angiopeptin-2, an angiogenic peptide that increases endothelial activation and vascular permeability, was more elevated in patients with HIV co-infection compared with controls.\(^{[52]}\) In the adult ICU patients with pneumosepsis from the MARS cohort, HIV status did not influence plasma levels of specific endothelial cell activation markers (angiopeptin-1 and -2, and soluble E-selectin). Interestingly, HIV-positive patients displayed higher circulating levels of soluble ICAM-1, which can be shed by both endothelial cells and leukocytes. HIV infection can enhance the release of exosomes containing ADAM17 (ADAM metalloproteinase domain 17), the cleaving protease for ICAM-1, which promotes ICAM-1 shedding.\(^{[53]}\) Increased levels of IFN-γ, as observed in the MARS study, may also contribute to the release of sICAM-1.\(^{[54]}\)

**Hypothermia**

Patients with sepsis can display profound hypothermia, being observed in 9–35% of septic patients\(^{[55,56]}\) the underlying mechanism of which is poorly understood. Immune suppression, due to an excessive anti-inflammatory response, has been proposed as a mechanism for hypothermia.\(^{[59]}\) Also, a recent study showed that hypothermia was associated with lymphopenia following diagnosis of sepsis,\(^{[60]}\) thereby potentially accounting for the association with adverse outcome.\(^{[55,56,46,61]}\) The MARS study aimed to determine risk factors of the occurrence of hypothermia during the first 24 hours of ICU admission and to characterise the host response in patients with hypothermic sepsis.\(^{[57]}\) 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.\(^{[55,56]}\)

In order to obtain insight into the pathophysiological mechanisms, pro- and anti-inflammatory cytokines and endothelial activation markers were measured. Pro- or anti-inflammatory cytokine levels were not different between hypothermic and non-hypothermic patients in the MARS study, even after correction for disease severity, which is in line with a study in hypothermic patients reporting no difference in circulating levels of proinflammatory cytokines.\(^{[62]}\) The host response biomarkers were similar in groups of patients with and without hypothermia, except for plasma fractalkine, which was higher in patients with hypothermia, and this difference remained after correction for disease severity. Fractalkine is a chemokine that has been implicated as a mediator in a diverse spectrum of inflammatory conditions and is associated with adverse outcome in critically ill patients with sepsis.\(^{[63]}\)

Endothelial cells have been described as an important source of fractalkine.\(^{[64]}\) Considering the presence of mainly cardiovascular risk factors in patients with hypothermia, the MARS data may suggest that the endothelium is implicated in hypothermia. The association between hypothermia and enhanced circulating levels of fractalkine in a population with significantly increased disease severity requires further evaluation.

**Discussion**

Within the MARS consortium a large prospective cohort of critically ill patients was studied, in which patients were methodically categorised and followed by committed research physicians. Besides information on demographics, comorbidities, and other patient-related factors, the MARS database also included extensive data on the septic episodes, i.e., timing of the infection, sepsis-induced organ failure and causative pathogens. In addition, a large set of host response biomarkers were measured, which provided further insight into the immune response of sepsis patients admitted to the intensive care unit. Although the studies on the association between prior medication, disease severity, host response and outcome were performed in an extensive cohort of patients, the observational nature of MARS studies does not allow for assessment of causal relationships. Propensity score matching was implemented to enable estimation of the independent effect of individual factors. The size of the MARS study population allowed it to perform matching by many important covariates; however, bias can remain as a result of unmeasured confounders. Another limitation involves the fact that this study was performed in two centres in the Netherlands, limiting generalisability of the results.
Conclusion
In the future, the number of patients who will present with 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 the MARS study several ‘external’ and host factors that might influence sepsis outcome and the accompanying host response were studied, particularly prior 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. In addition, the influence of an acute manifestation of severe infection (i.e., hypothermia) on sepsis outcome and the host response was examined, revealing an independent association between hypothermia and mortality without evidence for an altered immune response. Sepsis patients are heterogeneous in terms of comorbidity, age and host response, therefore 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 individualised treatment of patients with sepsis.

Disclosures
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