Early onset sepsis in Suriname

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9

Summary & Future Perspectives

This thesis focuses on early onset sepsis (EOS) amongst newborns in Suriname. In **Part 1** demographics of newborns treated at the neonatal care facility in Suriname and the impact of EOS are described, followed by the clinical dilemmas arising in the prediction of EOS in **Part 2.** In **Part 3** the vascular pathophysiology of EOS and potential for novel diagnostic methodologies are described. This chapter summarizes and discusses the main findings of the various studies in this thesis with implications for future research towards a novel diagnostic approach for EOS.

EPIDEMIOLOGY OF EARLY ONSET SEPSIS IN SURINAME

To date, detailed demographics of newborns in Suriname are absent. In 2008 the first referral neonatal care facility in Suriname, with the ability for neonatal intensive care for newborns, opened its doors at the Academic Hospital Paramaribo. In 2015, this facility transitioned to a modern environment, along with implementation of interventions to improve neonatal care. In **Chapter 2** of this thesis we evaluated the impact of this transition on mortality and morbidity of newborns. We performed a retrospective study amongst 601 newborns admitted to the facility. We compared outcomes of newborns between two 9-month periods before and after the transition in March 2015. After the transition more intensive care was delivered and more outborn newborns were treated. Overall neonatal mortality rate of all inborn and outborn newborns was reduced from 23.4 to 13.4 deaths per 1,000 live births, along with a reduction in mortality of sepsis and asphyxia. At the same time, mortality of newborns with a birth weight below 1,000 grams and incidence of sepsis increased after the transition.

This study makes clear that two major challenges for future neonatal care in Suriname remain. First, the reduction in neonatal mortality indicates a substantial improvement in the quality of tertiary function of the facility and neonatal care in Suriname. However, despite these promising results, it is important to realize that Suriname remains a developing country where political, economic, and logistic challenges may negatively impact sustainability of this reduction in mortality. We are currently designing a nationwide perinatal registry system and follow-up epidemiological studies to monitor tertiary function, referral patterns, and morbidity and mortality amongst Surinamese newborns. Second, incidence of sepsis increased after the transition of the neonatal care facility. Although part of the increased sepsis incidence results from late onset sepsis due to invasive lines and procedures, still half of all blood culture confirmed cases of sepsis were cases of EOS. Additionally, over 30% of all admitted newborns were suspected of EOS and empirically received antibiotics. Therefore, the main focus of this thesis was to summarize evaluate clinical and pathophysiological aspects of EOS that may aid in improvement of early identification and exclusion of EOS, with the final aim to initiate prompt treatment of infected newborns and reduce unnecessary antibiotic usage amongst uninfected ones.

PREDICTION OF EARLY ONSET SEPSIS

Prediction of EOS is complicated due to the fact that clinical symptoms and inflammatory biomarkers are often not specific for presence of EOS [1]. Like in many Western countries, clinical protocol at the neonatal care facility in Suriname prescribes a combination of perinatal risk factors,

clinical symptoms of the newborn, and serial assessment of inflammatory biomarkers C-reactive protein (CRP) and white blood cell count, to predict presence or absence of EOS within 72 hours after birth. In **Part 2** of this thesis we describe tools that may be of additive value in the prediction of EOS in the clinic.

The online available EOS calculator is a novel tool to predict EOS and help decision making on start and duration of antibiotic treatment [2]. The EOS calculator is based on five objective maternal parameters and clinical evaluation of the newborn straight after birth, and provides a risk estimate on EOS. It is unclear how the EOS calculator relates to levels of CRP and leukocyte and thrombocyte counts in the first 72 hours of life. Increase of CRP and leukopenia have been shown to be associated with blood culture positive EOS. In **Chapter 3** we investigated the hypothesis that higher EOS calculator results are associated with increase in CRP within 24-48 hours and low leukocyte counts. EOS risk estimates were calculated for 108 newborns of 34 weeks of gestational age, in whom antibiotics were started for suspected EOS. EOS risk estimates were retrospectively compared to infection parameters CRP, and leukocyte and trombocyte counts. In contrast to our hypothesis, high EOS risk at birth was consistently correlated with lower CRP and leukocyte counts within 24 hours after the start of antibiotics, but not with infection parameters after 24 hours.

The study in Chapter 3 does not show correlation with infection parameters CRP and leukocyte counts that are currently commonly used in the clinic to help decision making on start and duration of antibiotic treatment in the clinic. However, in a large recent study amongst 204,485 newborns in the United States determination of EOS risk with the EOS calculator reduced the number of newborns that received laboratory testing and empirical antibiotic treatment [3]. Retrospective analysis of the Dutch cohort used in Chapter 3 showed that application of the EOS calculator could reduce antibiotic treatment with 50% [4]. To further enhance its clinical utility, it may be useful to investigate association of the EOS calculator with biomarkers, such as immature granulocytes and markers of endothelial cell activation, discussed in this thesis. Integration of the EOS calculator with these biomarkers into a novel diagnostic approach is proposed in the last paragraph of this chapter.

In **Chapter 4** we investigated another option to predict EOS and to help decisions on start and duration of antibiotic treatment. Automated measurement of immature-to-total-granulocyte (I/T) has been shown to have negative predictive value of EOS [5]. We retrospectively evaluated a one-point measurement of immature-to-total-granulocyte (I/T) ratio in predicting duration of antibiotic treatment in EOS in a cohort of Surinamese newborns. I/T ratio was lower in newborns in whom antibiotics were discontinued at 48-72 hours after start after which they all remained healthy. We conclude that low I/T ratio may help to increase the threshold to start empirical antibiotic treatment or to guide safe stoppage of antibiotics after 48-72 hours. Further prospective investigations in larger cohorts of newborns are necessary to evaluate clinical utility of a one-point measurement of I/T ratios, and to establish appropriate cut-off values. Nonetheless, it is a quickly available measurement (i.e., within 10 minutes after blood draw) to help decision-making on start of antibiotics in suspected EOS. Additionally, the fact that automated hematology analyzers are becoming universally available in the non-Western world favors their implementation there.

THE VASCULAR PATHOPHYSIOLOGY OF EARLY ONSET SEPSIS

The dilemmas in prediction of EOS in the clinic occur because the pathophysiology of EOS is poorly understood. In **Part 3** of this thesis we focus on the vascular pathophysiology of EOS and the potential of its molecular aspects for translation into novel diagnostic methodologies.

The vascular pathophysiology of sepsis is associated with interactions between leukocytes and the vascular endothelium [6]. During sepsis, adhesion molecules are expressed on the cell membranes of both cell types that orchestrate leukocyte rolling on, adhesion to, and transmigration across the endothelium [7]. As inflammation progresses, adhesion molecules accumulate in the blood as soluble forms after shedding by shedding enzymes, or 'sheddases', such as matrix metalloproteinase-9 (MMP-9) and neutrophil elastase [8]. In **Chapter 6** we review the studies that have tested the predictive value of soluble adhesion molecules (sCAMs) in sepsis pathophysiology in newborns, children and adults. Four endothelial sCAMs, specifically P-selectin, E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1), along with one leukocyte adhesion molecule, namely L-selectin, were associated with sepsis. While increased levels of these sCAMs generally correlated well with the presence of sepsis, their degree of elevation was poorly predictive of sepsis severity scores, outcome and mortality. Separate analyses of newborns, children, and adults demonstrated significant age-dependent differences in both basal and septic levels of sCAMs. Based on the results reported in this review, we proposed two novel directions for improving clinical utility of sCAMs: 1) the combined simultaneous analysis of levels of soluble adhesion molecules and their sheddases, and 2) taking age into account in the interpretation of their levels.

In **Chapter 7** we applied this approach in a Surinamese cohort of 20 healthy newborns and 71 newborns with suspected EOS, included within 72 hours after birth. We hypothesized that sCAMs and sheddases circulate at higher levels in blood culture positive EOS in newborns and that they are useful as biomarkers for EOS. Soluble CAMs sP-selectin, sE-selectin, sVCAM-1, sICAM-1, and platelet and endothelial cell adhesion molecule-1 (sPECAM-1), sheddases MMP-9 and neutrophil elastase, and sheddase antagonist tissue-inhibitor of metalloproteinases-1 (TIMP-1) were measured simultaneously in serum of 91 newborns. Of the newborns, six (8.5%) had a positive blood culture. At start of antibiotic treatment and after 48-72 hours no differences were found in levels of sCAMs and sheddases between blood culture positive EOS, blood culture negative EOS and controls. In contrast to our hypothesis, these data show that endothelial CAM shedding was not increased in EOS. Additionally, levels of sCAMs and sheddases were similar in all newborns between straight and six days after birth, indicating that sCAM shedding remains unchanged in early newborn life. Levels of sCAMs found in our study corresponded well with levels in other studies in newborns. However, when compared with earlier reports in adults sCAM levels in newborns are higher. We concluded that other mechanisms, such as perinatal stress during birth, may drive overall high levels in all newborns which precludes discrimination between septic and healthy newborns based on these levels. For these reasons, sCAMs and sheddases may not prove to be useful as biomarkers for EOS. Thus, our study indicates that simultaneous measurement of sCAMs and sheddases, as proposed in our review in Chapter 6, does not provide a satisfactory improvement of clinical utility of sCAM levels in prediction of EOS.

Vascular inflammation and leakage in sepsis is mediated by Angiopoietin (Ang)-1 and Ang-2 and their binding associated phosphorylation of the endothelial Tie-2 receptor. Levels of Ang-2 change in adults and children during sepsis, which is associated with severity of sepsis. In **Chapter 8** we investigated serum levels of Ang-1 and Ang-2 in newborns within 72 hours after birth. A prospective study was performed in the same cohort of newborns as described in Chapter 7. At start of antibiotic treatment Ang-1 serum levels were significantly lower and Ang-2 and Ang-2/Ang-1 serum protein ratios higher in newborns with blood culture positive EOS than in blood culture negative EOS and controls. These levels were not dependent on timing of first blood draw after birth. After 48-72 hours, levels of Ang-1 further decreased in blood culture positive EOS, while in the other groups no change was observed. These findings support the hypothesis that a dysbalance in the Angiopoietins is associated with the (vascular) pathophysiology of EOS. Additionally, these findings suggest potential for the Angiopoietins as biomarkers for EOS.

We propose further investigations into the Angiopoietin in EOS at two complementary levels. First, we propose to measure Ang-1 and Ang-2 levels in peripheral and cord blood in a mouse model of pregnancy to study both maternal and perinatal factors that may influence Ang-1 and Ang-2 levels in neonatal mice [9]. In addition, inoculation of neonatal offspring born in this study with *Group B Streptococcus* (GBS), to model EOS, within 72 hours could further reveal the vascular pathophysiology of EOS [10]. Second, from the results of our first study we hypothesize that serial measurement of high Ang-1, low Ang-2, and low Ang-2/Ang-1 ratio may negatively predict presence of EOS, and thus be extra arguments for safe stoppage of antibiotics. We propose further evaluation of Ang-1 and Ang-2 as biomarkers for EOS by repeating the study in Chapter 8 in a larger Surinamese cohort to establish sensitivity, specificity and appropriate cut-off and predictive values of the Angiopoietins for EOS. Maternal and perinatal factors may influence levels of Ang-1, Ang-2 and Ang-2/Ang-1 ratios in newborns, for which logistic regression analysis should be performed. Additionally, the methodological possibilities to enable quick measurement of Ang-1 and Ang-2 levels for clinical purpose should be explored.

While the results from earlier chapters in this thesis provide new avenues for identification and exclusion of EOS, a continuing need for (development of) novel and clinically useful diagnostic tools remains. Alterations in neutrophil morphology (size, shape and composition), mechanics (deformability), and motility (chemotaxis and migration) have been observed during sepsis. In **Chapter 9** we summarized features of neutrophil morphology, mechanics, and motility that change during sepsis and combined that with an investigation into their clinical utility as markers for sepsis through measurement with existing and novel technologies. When compared to resting conditions, sepsis causes an increase in circulating numbers of larger, more rigid neutrophils that show diminished granularity, migration and chemotaxis. Combined measurement of these parameters may provide a more complete view on neutrophil phenotype manifestation. For that purpose, sophisticated automated hematology analysers, microscopy and bedside microfluidic devices provide clinically feasible, high throughput, and cost limiting means. We propose that integration of features of neutrophil morphology, mechanics and motility with these new analytical methods can be useful as markers for diagnosis, prognosis and monitoring of sepsis, and may even contribute to our basic understanding of its pathophysiology (See also **Appendix I**).

OPTIONS AND CHALLENGES FOR A NOVEL DIAGNOSTIC APPROACH FOR EARLY ONSET SEPSIS

As discussed above, the major challenge in EOS is its timely identification for prompt initiation of antibiotic treatment, while preventing unnecessary antibiotic treatment in uninfected newborns. This is especially true for low resource settings, such as Suriname, where the risk for EOS is relatively high, and the threshold to start empirical antibiotic treatment is relatively low, when compared to Western countries. To date, only serial measurement of low levels of CRP, combined with a negative blood culture and clinically improved newborn, showed negative predictive value for EOS [11]. In a recent randomized controlled trial amongst 1,710 newborns in eighteen clinics in four European countries and Canada, a clinical decision-making regimen, in which a four point measurement of procalcitonin was added to the standard regimen of measurement of CRP and white blood cell counts alone, was superior in reducing duration of antibiotic treatment [12]. Data in this thesis show that incidence of blood culture positive EOS in Suriname is higher than reported in this trial (i.e., estimated between 8-10% versus 1-2%, respectively) indicating that newborns in Suriname are at higher risk for EOS. Additionally, the safety nets and facilities to properly follow-up clinical evolution of sent home newborns in whom antibiotics are discontinued, such as home maternity care, are absent in Suriname. For these reasons, the reported superior new regimen including procalcitonin cannot be safely applied directly to the Surinamese situation. However, the standard regimen described in this study was similar to the regimen that is currently used in Suriname. Thus, it would be interesting to perform a similar randomized controlled 'non-inferiority' trial to compare a novel diagnostic approach with the standard regimen in Suriname.

Starting point of this proposed novel diagnostic approach is the EOS calculator. Since the EOS calculator is freely available online (also as an app for a smartphone), it is the most affordable tool to guide clinicians in decision-making on start of antibiotics in cases of suspected EOS in low resource settings such as Suriname. Especially at medical posts in rural areas of Suriname, the EOS calculator is an easy-to-use tool to help decision-making on transport of newborns to the neonatal care facility in the capital Paramaribo for evaluation and treatment. Before implementation in Suriname, it is important to realize that for optimal performance the EOS calculator algorithm uses local incidence of EOS is needed. Another variable in the algorithm is maternal GBS colonization status, which is usually unknown in pregnant women in the Surinamese setting. Moreover, our prospective studies showed that only gram-negative EOS occurred in our cohort, indicating that GBS colonization may play an inferior role as causative pathogen of EOS in Suriname. We are currently performing a prospective observational cohort study in pregnant women and their newborns in Suriname to evaluate the exact incidence and local risk factors for EOS and performance of the current EOS calculator. This is intended to ultimately create a customized EOS calculator with variables that are specific for Suriname. Further improvement of EOS calculator performance can be achieved when used in combination with novel biomarkers discussed in this thesis. As discussed above, appropriate cut-off values for both I/T ratio and levels of Ang-1 and Ang-2 have to be established in larger prospective studies. Once these have been established, in Suriname the novel diagnostic approach could consists of the following if a newborn

presents with suspected EOS: 1) immediate determination of EOS risk with a Suriname-specific EOS calculator, combined with 2) immediate measurement of I/T ratio to help decision-making on whether to start antibiotic treatment, and then, if antibiotics are started, 3) repeated Ang-2/Ang-1 ratio determination to help decide whether to continue antibiotic treatment after 48-72 hours after start. This novel diagnostic approach could reduce antibiotic treatment in suspected EOS in two complementary ways, namely the prevention of start of unnecessary antibiotics, and safe stoppage of antibiotic treatment after 48-72 hours in the high-risk low resource setting. If successful, both scenarios would be a major step forward in reducing the burden of antibiotic treatment in newborns in Suriname and similar low resource settings.

A FUTURE CASE OF SUSPECTED EARLY ONSET SEPSIS IN SURINAME IN 2030

After a boat ride from her village down the *Suriname* River, the mother arrives at the nearest mission post in *Debike*[1]. She is pregnant for eight months and her water has broken a few days earlier. She has *korsu*[2]. The *datra*[3] at the mission post uses an application on his smartphone and puts in her temperature. He explains that the result of the app tells him to send her to the new hospital in the city of *Paramaribo* to give birth because her unborn child may have an infection. She gives birth to a daughter there the next day. The doctors take her to the baby ward and take her blood. The results of the blood test come back within 10 minutes, after which they start antibiotics. After two days the baby is healthy and feeding well. The doctors take her blood again. The nurse tells her the results are fine and that they will stop the antibiotic treatment. The next day the mother rides the boat home to her village with a healthy daughter.

[1] Village located along the Suriname River in the district Sipaliwini in the interior of Suriname

Translated from the Surinamese language (Sranan Tongo):

[2] physician;

[3] fever.

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