Early onset sepsis in Suriname

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Immature-to-total-granulocyte Ratio as a Guide for Antibiotic Treatment in Suspected Early Onset Sepsis in Surinamese Newborns

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Submitted

ABSTRACT

Objective

Measurement of immature granulocytes may be helpful in management of early onset sepsis (EOS) in newborns in developing countries. We evaluate early negative prediction of automated measurement of a one-point measurement of immature-to-total-granulocyte (I/T) ratio in newborns with suspected EOS to help decisions on duration of antibiotic treatment.

Methods

A retrospective study was performed amongst newborns with a gestational age ≥34 weeks with suspected EOS in whom local protocol had been followed for start and duration of antibiotic treatment, and in whom granulocyte counts had been measured with a Sysmex XT 2000i automated hematology analyzer.

Results

I/T and I/T^2 were significantly lower (*P* 0.048 and *P* 0.015, respectively) in newborns with favorable clinical course and in whom EOS was unlikely. In these newborns antibiotics were stopped 48-72 hours after start after which they all remained healthy.

Conclusion

Low I/T and I/T^2 may help to increase the threshold to start empirical antibiotics or to guide safe stoppage of antibiotics after 48-72 hours. Ultimately, this may help to reduce the antibiotic burden in developing countries.

INTRODUCTION

An estimated tenfold of newborns is empirically, and often unnecessarily, treated with antibiotics in the first 72 hours of life for suspected early onset sepsis (EOS) [1]. Poor specificity of clinical symptoms and limited utility of infection biomarkers complicate decisions on start and duration of antibiotic treatment [1]. Over 60% of antibiotics given empirically before 72 hours of life are prolonged beyond 48-72 hours despite a negative blood culture and a stable clinical condition [2]. Furthermore, the threshold to start antibiotics in newborns in developing countries is low [3]. It remains a challenge to safely avoid unnecessary antibiotics in newborns with suspected EOS, especially in developing countries.

Recent studies indicate that the immature-to-total-granulocyte (I/T) ratio may be promising tool to identify infection at an early stage and to guide duration of antibiotic therapy (4-8). Newman et al. proposed combination of the absolute neutrophil count (ANC) and I/T into the I/T^2, to be used in a prediction model for EOS [4]. Higher I/T^2 predicted positive blood culture in suspected EOS. Moreover, low serial immature-to-total-granulocyte ratios (I/T), in combination with negative blood culture, can be used to discontinue antimicrobial therapy at 36-48 hours [5]. Whether a single point measurement of I/T or I/T^2 at start of antibiotics in EOS is already predictive for negative blood cultures is unknown.

Nowadays, the global availability of high-throughput flow-based automated hematology analyzers (AHA) facilitates simple and fast measurement of immature granulocytes in developing countries [6]. The aim of this study is to retrospectively evaluate early negative prediction of automated measurement of I/T and I/T^2 in Surinamese newborns with suspected EOS. We hypothesized that I/T and I/T^2 at start of antibiotic treatment were lower in newborns with unlikely EOS in whom antibiotics were discontinued after 48-72 hours.

SUBJECTS AND METHODS

Study Design

A retrospective study was performed amongst newborns admitted to the neonatal care facility at the Academic Hospital Paramaribo (AHP) from September 1st 2015 to May 30th 2016. Included were newborns with a gestational age (GA) ≥34 weeks, who received antibiotic therapy within 72 hours after birth and with available laboratory results on immature granulocytes. Antibiotics (intravenous ampicillin and gentamycin) were started for presence of maternal risk factors for EOS or clinical suspicion. According to local protocol, EOS was considered unlikely if clinical course had been uneventful (i.e., clinical improvement and no support other than normal volumes of IV fluids or tube feeding), serial measurement of C-reactive Protein (CRP) was normal (i.e., levels <0.5 mg/dL), and blood culture was negative. Consequently, antibiotic treatment was discontinued after 48-72 hours. Otherwise treatment was continued for 7 days. Newborns were divided in two groups, namely: 1) Unlikely EOS: antibiotics discontinued after 48-72 hours; and 2) Probable EOS: full 7-day treatment. The study protocol was part of a larger study on EOS, which was made available on clinicaltrials.gov (NCT02486783) and was approved by the Surinamese Medical-Ethical Board (VG-021-14A).

Data and Sample Collection

The following data were collected: maternal and prenatal conditions and risk factors, delivery mode, GA, birth weight, ethnicity, gender, leukocyte differentials including immature granulocytes and hour of life at blood draw, and hospital course. According to local protocol, determination of leukocyte count was done in whole blood which was collected as part of standard draws for infection parameters at t=0 (start of antibiotic treatment). In all newborns blood was collected for bacterial culturing after insertion of a cannula before start of treatment.

Automated Measurement of Leukocytes

White blood cell (WBC) and platelet counts and ANC and IG (i.e., metamyelocytes, myelocytes and promyelocytes) counts were collected from a Sysmex XT 2000i analyzer (Sysmex, Kobe, Japan). I/T ratios were calculated as IG count divided by the total (ANC+IG) granulocyte count. We calculated the I/T^2 according to Newman et al. [7] by dividing the I/T by the ANC again.

Statistical Analysis

Categorical variables were presented as numbers and percentages and continuous variables as mean with SD or, if not normally distributed, as median with IQR. Categorical variables were compared with chi-square. Normality for continuous variables was assessed by a Shapiro-Wilk test and further analyzed by a Student t-test or two-tailed Mann Whitney U-test. P <0.05 was considered statistically significant.

RESULTS

Twenty-seven newborns were included. Between both groups maternal, perinatal and neonatal demographics were comparable (Table 1). In 13 (48.1%) newborns EOS was considered unlikely and antibiotics were discontinued after 48-72 hours. Clinical course of these newborns had been uneventful and they all remained healthy after stop of antibiotics. In the probable EOS group delta CRP and need for additional support at t=48-72 was higher (Table 1). Of these newborns one had a positive blood culture (i.e., *Enterobacter cloacae*) and one died after cardiorespiratory failure.

I/T and IT^2 were significantly lower (P 0.048 and P 0.015, respectively) in newborns with unlikely EOS versus probable EOS (Figure). When newborns under 4 hours of age were analyzed separately, lower I/T^2, but not lower I/T, was found in newborns with unlikely EOS (P 0.05 and P 0.15 respectively).

DISCUSSION

In this study, newborns with unlikely EOS had significantly lower I/T and I/T^2 at start of antibiotic treatment compared to newborns with probable EOS. The retrospective nature of this study prevented bias in decision making for duration of antibiotic treatment based on AHA results. For these reasons I/T and I/T^2 may be highly useful in the clinic to guide antibiotic therapy in newborns with suspected EOS in two ways. First, a low I/T and I/T^2 may help to increase the threshold to start

Table 1. Descriptive statistics and laboratory results of Surinamese newborns with suspected early onset sepsis (EOS).

		Unlikely EOS (N=13)	Probable EOS (N=14)	*P* value[1]
Maternal morbidity,	Diabetes Mellitus	0	2 (14)	
n (%)	Hypertension	0	1 (7)	
	Preeclampsia	0	0	
	HIV	0	1 (7)	
Mode of delivery,	Vaginal	11 (85)	8 (57)	
n (%)	Caesarean	2 (15)	6 (43)	
Male (female)		10 (3)	9 (5)	
Ethnicity, *n (%)*	Maroon or Creole	10 (77)	10 (71)	
	Other[2]	3 (23)	4 (29)	
Gestational age, *median (weeks+days)*		37+1	37+4	
Birth weight, *mean±SD (grams)*		2,628±245	2,731±152	
Age at presentation[3],	<4 hours	6 (46)	10 (71)	
n (%)	≥4 hours	7 (54)	4 (29)	
Clinical symptoms[3],	Respiratory distress	6 (46)	10 (71)	
n (%)	Circulatory instability	0	2 (14)	
	Feeding intolerance	1 (8)	2 (14)	
	Hypoglycemia	2 (15)	2 (14)	
	Lethargy	1 (8)	0	
Reason for antibiotics,	Prenatal risk factors	6 (46)	4 (29)	
n (%)	Clinical suspicion	7 (54)	10 (71)	
Clinical Course[4],	CPAP	0	4 (29)	
n (%)	Mechanical Ventilation	0	4 (29)	
	Cardiotonics	0	2 (14)	<0.001
	Positive blood culture	0	1 (7)	
	Mortality	0	1 (7)	
CRP,	Initial[3]	<0.5 (0)	<0.5 (2.8)	0.006
median (IQR) (mg/dL)	Delta[4]	0 (0.3)	2.1 (3.7)	
AHA measurements[3],	WBC (x10^9/L)	16.4 (12.5)	17.0 (16.1)	
median (IQR)	Platelets (x10^9/L)	219 (119)	193 (119)	
	ANC (x10^9/L)	11.7 (8.0)	7.9 (8.4)	
	IG%	0.7 (1.0)	1.2 (3.9)	

AHA = automated hematology analyzer; HIV = human immunodeficiency virus; CPAP = continuous positive airway pressure; CRP = C-reactive protein; WBC = white blood cell count; ANC = absolute neutrophil count; IG% = immature granulocyte fraction.

[1] *P* >0.1 unless stated otherwise.

[2] Includes: Hindo-Surinamese, Javanese and Amerindian.

[3] At start of antibiotics.

[4] At t=48-72 hours after start of antibiotic treatment.

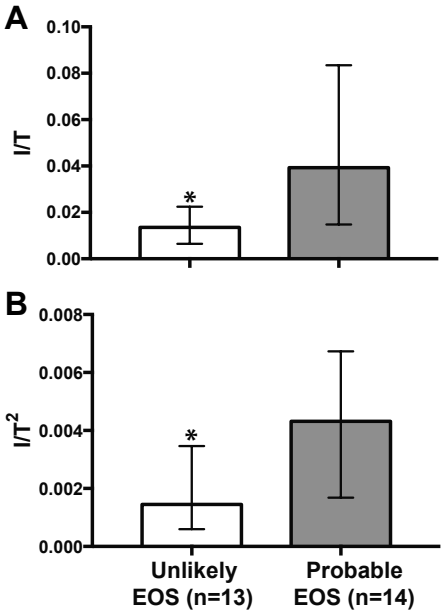

Figure 1. Immature-to-total-granulocyte ratios (I/T and I/T2) in Surinamese newborns treated with antibiotics for suspected early onset sepsis (EOS). A: I/T; B: I/T2. Data represent ratios calculated from results of automated measurement of granulocytes with a Sysmex XT 2000i analyzer in whole blood obtained from newborns with suspected EOS at start of antibiotic treatment. I/T2 is calculated according to Newman et al. (7). * P <0.05 compared to probable EOS. Bars represent median values and error bars interquartile range.

empirical antibiotic therapy. Second, it may guide clinicians to shorten antibiotic treatment to 48-72 hours in case of negative blood cultures.

Of the measured parameters, I/T^2 showed the most significant difference between short and full treatment duration. This is in line with the conclusions made by Newman et al. [4,8]. They postulated that measurement of both the ANC and I/T have separate predictive values but are two dependent tests since the ANC is a variable in the denominator of I/T. The I/T^2 was designed to capture both tests into one ratio, which showed increasing performance in newborns older than 4 hours of life and when there was high suspicion of EOS. In contrast to Newman et al., we were particularly interested in the negative predictive ability of I/T^2 during the transition period of newborns. In our cohort most newborns started within 4 hours of life with antibiotics. In these newborns the threshold to start empirical antibiotic therapy is lower due to ambivalent clinical symptoms during transition to extrauterine life. When measured within 4 hours after birth, we observed a trend in lower I/T^2 in cases of unlikely EOS. This is a clinically relevant finding, which may favor the I/T^2 in increasing the threshold to start antibiotics during the transition period in newborns with a low suspicion of EOS.

Nowadays, CRP is frequently used in clinical settings to determine safe stoppage of antibiotics for EOS, also in Suriname. Serially measured low levels of CRP predict absence of EOS with a negative predictive value (NPV) of 99%, yet only in combination with a negative blood culture and clinically

improved newborn [2,9]. However, in practice, despite this high NPV, serial measurement of CRP can also lead to additional testing and longer duration of antibiotic treatment [10]. Based on our results, the NPV of a one-point measurement of I/T^2 may be comparable with serial measurement of CRP and should now be further evaluated in prospective studies. The I/T^2 could then also be added to an algorithm with serial measurement CRP in order to enhance NPV and clinical utility of both markers [11]. Last, automated measurement of immature granulocytes performed at least equal to manual differentiation [12]. The obvious advantages of automated measurement are the large sample size, the immediate availability of test results for the clinician, and its cost-effectiveness for the low-resource setting.

Because measurement of immature granulocytes was not part of standard care in Suriname, the sample size for this study was relatively small. Larger and prospectively included cohorts are necessary to increase prevalence of positive blood cultures and further controlling for maternal (e.g., preeclampsia, mode of delivery) and neonatal (e.g., sex, ethnicity, hours of life) factors that may influence leukocyte differentials [8,13]. However, we studied a homogeneous group of near term and term newborns so we do not expect our results to differ in larger cohorts. Additionally, establishing larger cohorts with blood culture confirmed EOS is complicated since prevalence of positive bacterial blood cultures is low [1].

CONCLUSION

Our results indicate clinical utility of automated measurement of I/T and I/T^2 in clinical decision-making on start and duration of antibiotic treatment in EOS. Ultimately this may contribute to reduction of the antibiotic burden in Suriname and other developing countries.

Abbreviations and Definitions

ANC = absolute neutrophil count
IG = immature granulocyte
IG% = immature granulocyte fraction of total leukocytes
I/T = immature-to-total-granulocyte ratio
I/T^2 = immature-to-total-granulocyte ratio divided by the absolute neutrophil count
Maroon = descendant from Africans that escaped slavery and established independent societies (e.g., term predominantly used in South America and on Caribbean Islands)

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Author Contributions

R Zonneveld and FB Plötz designed the study. R Zonneveld, S Simson, A Juliana and J Codrington collected and analyzed the data. R Zonneveld and FB Plötz drafted the manuscript. S Simson, A Juliana and J Codrington revised and helped draft the final manuscript. All authors have read and approved the final manuscript. The authors declare they have no conflict of interest.

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The Vascular Pathophysiology of Early Onset Sepsis