Early-Onset Group B Streptococcus Sepsis in High Risk Neonates Born After Prolonged Rupture of Membranes

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ABSTRACT:

Background: According to the U.S. Centers for Disease Control guidelines, prolonged rupture of membranes mandates intrapartum antimicrobial prophylaxis for group B Streptococcus whenever maternal GBS status is unknown.

Objectives: To evaluate the local incidence, early detection and outcome of early-onset GBS sepsis in neonates born at 35–42 weeks gestation after PROM to women with unknown GBS status who were not given intrapartum antimicrobial prophylaxis.

Methods: During a 1 year period we studied all neonates born beyond 35 weeks gestation with maternal PROM ≥ 18 hours, unknown maternal GBS status and without prior administration of IAP. Complete blood count, C-reactive protein, blood culture and polymerase chain reaction amplification of bacterial 16S rRNA gene were performed in blood samples collected immediately after birth. Unfavorable outcome was defined by one or more of the following: GBS bacteremia, clinical signs of sepsis, or positive PCR.

Results: Of the 3616 liveborns 212 (5.9%) met the inclusion criteria. Only 12 (5.7%) of these neonates presented signs suggestive of sepsis. PCR was negative in all cases. Fifty-eight neonates (27.4%) had CRP > 1.0 mg/dl and/or complete blood count abnormalities, but these were not significantly associated with unfavorable outcome. Early-onset GBS sepsis occurred in one neonate in this high risk group (1/212 = 0.47%, 95% CI 0.012–2.6).

Conclusions: In this single-institution study, the incidence of early-onset GBS sepsis in neonates born after PROM of ≥ 18 hours, unknown maternal GBS status and no intrapartum antimicrobial prophylaxis was 0.47%.

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KEY WORDS: sepsis, newborn infant, intrapartum antimicrobial prophylaxis, prolonged rupture of membranes, polymerase chain reaction, group B Streptococcus

GBS = Group B Streptococcus
PROM = prolonged rupture of membranes
IAP = intrapartum antimicrobial prophylaxis
PCR = polymerase chain reaction
CRP = C-reactive protein

Neonatal early-onset group B Streptococcus sepsis is a life-threatening condition mandating early diagnosis and treatment. The major risk factors for early-onset GBS sepsis are maternal GBS carriage, prematurity, maternal intrapartum fever, amnionitis, and prolonged rupture of membranes (≥ 18 hours) [1,2]. In high risk neonates born after PROM of ≥ 18 hours, the incidence of proven early-onset GBS sepsis was 4.2% and 10% in term and preterm neonates respectively [3]. However, in 41% of neonates with early-onset GBS sepsis, no maternal risk factors for infection were present [4]. The risk of early-onset GBS sepsis was reported to be 10.4, 10, and 25.8-fold higher because of prematurity, maternal fever and PROM, respectively [2].

In an effort to reduce the perinatal GBS disease in the United States, the Centers for Disease Control issued guidelines recommending screening for GBS at 35–37 weeks gestation [5]. According to earlier reports this approach was seemingly successful in the U.S., with a 65% decline from 1.7 to 0.6 cases/1000 live births [4,6-9]. According to a recent CDC report [10], the decline of perinatal GBS disease from the period 2000–2001 (0.49 cases per 1000 live births) to 2003–2005 (0.33 cases per 1000 live births) was 33%. However, the relevance of these CDC guidelines to non-U.S. populations remains unknown, as acknowledged by the CDC itself [5].

In Israel the incidence of GBS sepsis is very low (0.1–0.5/1000 live births) [11-18]. Therefore, in 2006 the Ministry of Health decided not to recommend GBS screening. Hence, the GBS carriage status is not known for some parturient women presenting at delivery rooms beyond 35 weeks gestation. Accordingly, the main indication for intrapartum antimicrobial prophylaxis administration in Israel is GBS carriage status as defined by the CDC [5]. This included women with GBS bacteriurea, previous birth of a neonate with GBS disease, and evidence for GBS carriage during the current pregnancy.

As a result, not all women with PROM ≥ 18 hours and unknown GBS status, considered by the CDC [5] to be a *high risk group* for which intrapartum antimicrobial prophylaxis is recommended, receive prophylaxis against GBS in Israel. Under such circumstances, the CDC guidelines regarding

CDC = Centers for Disease Control

neonatal management state that "if no maternal IAP was administered despite an indication being present, data are insufficient on which to recommend a single management strategy" [5]. Accordingly, it is not always clear to neonatologists outside the USA which maternal and neonatal risk factors justify evaluation for potential early-onset GBS sepsis.

The aims of the present study were to assess the local incidence, rate of early detection and outcome of early-onset GBS sepsis in a high risk subgroup of neonates born after PROM, unknown maternal GBS status and no prior intrapartum antimicrobial prophylaxis.

PATIENTS AND METHODS

This prospective study was conducted in a well-baby nursery at Rambam Health Care Campus, Haifa, Israel. The study was approved by the local Helsinki Committee, and informed consent was obtained from all mothers of neonates enrolled in the study.

STUDY POPULATION

During a 1 year period (7 February 2006 to 6 February 2007), 3616 neonates were born in our institution. Of these neonates, 212 (5.9%) met the following criteria: a) gestational age ≥ 35 weeks, b) PROM ≥ 18 hours, c) unknown maternal GBS status, and d) lack of IAP.

We chose the inclusion cutoff of 35 weeks gestation because such neonates stay in our well-baby nursery, and also because the CDC has the same cutoff in its algorithm for neonatal management [5]. Five neonates who met only the first three inclusion criteria but not the fourth criterion were not included in the study analysis. Three additional neonates with PROM of ≥ 18 hours were not enrolled in the study due to refusal to consent (n=2) or logistic problems (n=1). None of these eight excluded neonates was symptomatic or developed sepsis.

DATA COLLECTION

Relevant maternal, perinatal and neonatal data were collected [Table 1]. Blood samples were drawn after birth for culture, complete blood count, C-reactive protein and 16S rRNA gene polymerase chain reaction amplification. External ear and gastric aspirate cultures were obtained as well. Neonates were followed for 48–72 hours for clinical signs suggestive of sepsis. Unfavorable outcome was defined by one or more of the following: clinical signs raising suspicion of sepsis, bacterial growth in blood, or positive 16S rRNA gene PCR product.

LOCAL PROTOCOL FOR ADMINISTRATION OF IAP

At our institution, the indications for maternal IAP include: a) PROM in premature neonates < 35 weeks (if vaginal delivery), b) maternal intrapartum fever > 38.5°C (at all

gestational ages), and c) maternal chorioamnionitis (at all gestational ages).

PROTOCOL FOR NEONATES BORN AFTER PROM OF ≥ 18 HOURS

After physical examination, we obtained blood for a complete blood count with differential counts and blood culture, and swabs from external ear and gastric aspirate. If PROM duration was 18–24 hours, antibacterial therapy was administered

Table 1. Schedule of DVIP regimen given every 3 weeks

	Mean ± SD	n (%)
Demographic data		
Gestational age (wks)		
Mean ± SD	38.6 ± 1.8	
Range	(35–42)	
Birth weight (g)	3118 ± 509	113 (53.3%)
Male gender		158/54
Jewish/Arab origin		(74.5%/25.5%)
Intrapartum course		
PROM (hrs)		
Mean ± SD	32.4 ± 37.7	
Range	(18–504)	
Suspected amnionitis		4 (1.9%)
Maternal intrapartum fever		9 (4.2%)
Foul smell		2 (0.9%)
Maternal WBC › 15,000/mm³		34 (16%)
Spontaneous delivery		164 (77.4%)
Singletons		204 (96.2%)
Neonatal resuscitation		8 (3.8%)
5 minute Apgar score		
7		3 (1.4%)
8		4 (1.9%)
9		29 (13.7%)
10		176 (83%)
Neonatal course		
Respiratory distress		11 (5.2%)
Fever (≥ 37.8°C)		0
Cyanosis		5 (2.3%)
Pneumonia		1 (0.5%)
Oxygen needed		8 (3.8%)
IMV/NCPAP		3 (1.4%)
Transfer to NICU		7 (3.3%)
Laboratory tests		
WBC › 25,000/mm³		29 (13.7%)
WBC › 30,000/mm³		9 (4.2%)
WBC ‹ 5000/mm³		1 (0.5%)
I/T ratio › 0.2*		29 (13.7%)
Platelets ‹ 15,0000/mm³		6 (2.8%)
CRP › 1.0 (mg/dl)**		7 (3.3%)
Neonatal antimicrobial prophylaxis		160/212 (75.4%)

*I/T ratio missing in 5 subjects
**CRP values missing in 7 subjects
IMV = intermittent mandatory ventilation, NCPAP = nasal continuous positive airway

only if the blood count was abnormal. If PROM was > 24 hours, antibacterial therapy was administered (regardless of blood count results) for 48–72 hours until culture results became available. Antibacterial therapy included oral amoxicillin + intramuscular gentamycin if the baby was asymptomatic, or intravenous ampicillin + intravenous gentamicin if signs of sepsis were present.

16S RRNA GENE PCR AMPLIFICATION

PCR was performed in order to obtain early evidence (within 4–6 hours) of a bacterial presence in blood. DNA was extracted from 200 μl of blood, using a commercial kit (QIAamp DNA Mini Kit QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Amplification was then performed for a variable segment of the gene encoding 16S rRNA, using primers that identify universally conserved flanking sequences. Identification of DNA amplification products was performed by electrophoresis on 1.5% agarose gel. PCR results were available within 6 hours. Each PCR reaction included four controls: a) one negative control sample controlling for PCR contamination where water was substituted for the DNA sample, b) a second negative control sample controlling for contamination during DNA extraction where a blank sample was processed in parallel with the patient sample, c) an inhibition control for each sample, and d) a positive control sample consisting of bacterial DNA. Altogether, these four control samples ensured the reliability of the PCR reaction.

STATISTICAL ANALYSIS

Data were analyzed using SPSS 14 (Chicago, IL, USA). We tested for possible statistical relationships between each clinical or laboratory variable and development of unfavorable outcome. For continuous/ordinal variables we used the Mann-Whitney test. For categorical variables we used the chi-square test or Fisher's exact test. Sensitivity, specificity, positive predictive value and negative predictive value of CRP < 1 and blood count pathology were calculated as well as their 95% confidence interval (the exact method was performed when appropriate). Logistic regression was used to identify independent predictors of developmental unfavorable outcome. A P value of less than 0.05 was considered statistically significant.

RESULTS

Table 1 shows the demographic, intrapartum, neonatal and laboratory variables of the 212 neonates (35–42 weeks gestational age) enrolled in this study. Most neonates were full-term vaginally born singletons with normal 5 minute Apgar score. Few neonates were born after maternal risk factors for infection, such as suspected maternal amnionitis, fever (> 38.5°C) or foul smell at birth. Seven of the 212 mothers had PROM beyond 72 hours (76, 115, 129, 130,144, 144, 504

hours). Vaginal cultures were obtained from five of these seven women and were negative for GBS. All of these women delivered within 24 hours of vaginal culturing and, therefore, their GBS status was available only 48–72 hours postpartum.

Only 12 (5.7%) of the neonates presented with one or more clinical signs suggestive of sepsis. Univariate analysis showed that abnormal clinical signs occurred significantly more in twins ($P < 0.0001$), and in neonates with low 5 minute Apgar score ($P < 0.0001$), low birth weight ($P < 0.007$) and low gestational age ($P < 0.0001$), but were not associated with gender, ethnic origin, maternal leukocytosis or duration of PROM. Using multiple logistic regression analysis, only lower 5 minute Apgar score and decreasing gestational age remained significant ($P < 0.0002$ and $P = 0.01$, respectively).

CRP > 1.0 mg/dl alone, blood count abnormalities (one or more), or combined abnormalities of both CRP and blood count were not significantly associated with an unfavorable outcome. The sensitivities of elevated serum CRP alone, abnormal blood count or elevated CRP and/or abnormal blood count for the prediction of unfavorable outcome were 8.3–6.2%; specificities were 73.5–97% and positive predictive values 10–14.3%, however negative predictive values were > 94%. Considering neonatal leukocytosis as white blood cell count > 30,000/mm^3 gave similar results as with WBC > 25,000/mm^3.

Bacteremia was rare, occurring only in three cases with only one case of genuine GBS infection and two other isolates considered as contaminations. External ear cultures were positive in 54 (26.1%) of the cultures for various pathogens, but only 6 (2.9%) of them were identified as GBS. Only 12 gastric aspirate cultures (5.9%) were positive, 4 (2%) of which grew GBS. Simultaneous GBS growth in ear and gastric aspirate occurred in two cases, in the ear only in four cases and in the gastric aspirates only in two cases. Hence, the overall GBS colonization rate in this high risk group was as low as 3.8% (8/212). Colonization with microbes at birth was not significantly associated with gender, ethnic origin, multiple births, maternal leukocytosis, mode of delivery, Apgar score, birth weight, gestational age or duration of PROM.

Three-quarters of the neonates received antimicrobial prophylaxis according to our institutional protocol until the culture results became available. There was no mortality. The only infant with proven early-onset GBS sepsis was a term female neonate, born vaginally following 27 hours of PROM and fever of 38°C as well, but without IAP. She was asymptomatic and had a WBC of 27,450/mm^3, immature/total neutrophil ratio of 0, and a CRP of 0.015 mg/dl. Although PCR from blood samples was negative, GBS was isolated in this patient from blood, ear swab and gastric aspirate. She was treated with intravenous ampicillin. In two newborns, coagulase-negative Staphylococcus and alpha-hemolytic Streptococcus grew in

WBC = white blood cell count

the blood cultures, but they were considered as contaminations. These infants were born at term after 33 and 20 hours of PROM, respectively, had normal 5 minute Apgar scores, were asymptomatic, had normal blood count, CRP < 1.0 mg/dl, negative PCR and negative ear and gastric cultures.

16S rRNA gene PCR amplification was negative in all cases, corresponding to 0% sensitivity and a specificity of 100% (95% CI 98.3–100%). Contrary to our expectations, PCR did not detect bacteremia in the infant with a true positive blood culture. The negative predictive value of PCR for predicting bacteremia was 99.5% (95% CI 97.4–100%) or 98.6% (95% CI 95.9–99.7%), depending on whether we consider bacteremia in one or three cases, respectively.

DISCUSSION

Our results show that the incidence of early-onset GBS sepsis among high risk neonates (gestational age 35–42 weeks) born after prolonged PROM, unknown GBS status and no IAP was relatively high (1/212, 0.47%; 95% CI 0.012–2.6) and the neonatal GBS colonization rate was 3.8%. Most of these neonates were asymptomatic. 16S rRNA PCR amplification did not detect genuine bacteremia in the infant. Early blood count and CRP measurements had value only in ruling out sepsis.

According to the CDC guidelines, all the mothers in our study group should have received IAP [5]; however, according to our institutional protocol, they did not. Maternal GBS carriage rates in Israel range between 5.3% and 19%, with significantly higher rates in mothers of North American or former USSR origin [13-17]. At our institution the maternal GBS colonization rate is not known; the reported rates of GBS colonization among Israeli neonates are 1.1–4.1% [13,14]. In our group, despite being a high risk group for early-onset GBS sepsis, the GBS colonization rate was only 3.8%. This low rate could be due to the exclusion of neonates of known GBS-carrier mothers.

The rate of early-onset GBS sepsis in Israel is low (0.1–0.5/1000 live births) [12–18]. The Israeli Ministry of Health in 2006 decided not to implement routine GBS screening. Hence, the GBS carrier status of some women in active labor or with PROM beyond 35 weeks gestation is unknown. The rate of early-onset GBS sepsis in our high risk group was 4.7/1000 high risk neonates. While this is not statistically significantly higher than that of the historical controls, it was approximately tenfold higher than that reported among the general neonatal population in Israel (0.1–0.5/1000 live births) [13-17], and 7.5-fold higher than that in the whole neonatal population in our institution (0.6/1000 live births). Thus, what is needed is a larger multi-institutional study to determine the actual GBS attack rate in this population and

to ultimately formulate appropriate treatment recommendations for Israel

One-quarter and 5.9% of the cultures from external ear and gastric aspirates, respectively, grew various pathogens. Colonization with GBS would have been missed in seven neonates had cultures of ear and gastric aspirates not been obtained, but none of these neonates developed late-onset sepsis or meningitis. Thus, the value of detection of neonatal GBS colonization is questionable, as also suggested previously [19]. In addition, ignoring the results of surface cultures in neonates was not risky and led to considerable saving of time, effort and cost [20]. Our results confirm the above-mentioned reports even in a high risk group for sepsis.

In our study, only 5.9% of neonates were clinically symptomatic but none of them had bacteremia. Blood count abnormalities (WBC > 30,000/mm^3, WBC < 5000/mm^3, absolute neutrophil count < 1500/mm^3, and I/T ratio > 0.2) were not reportedly reliable predictors of early-onset sepsis [21], and WBC abnormalities occurred in 41% and 27% of septic and non-septic neonates, respectively [21]. In our study, the blood count abnormalities appear to be of limited value in predicting unfavorable clinical outcome in spite of their high negative predictive value.

CRP levels were reportedly low in cord blood or immediately after birth and thus were not reliable predictors of early-onset sepsis. However, CRP levels obtained at 12–48 hours after birth might still be useful in excluding early-onset sepsis by virtue of the high negative predictive value of normal/low CRP levels in ruling out sepsis [19,20]. In the present study, the low CRP levels in most cases could be due to the early blood sampling or to the rarity of proven sepsis. Overall, the sensitivities of elevated serum CRP alone, abnormal blood count or elevated CRP and/or abnormal blood count for the prediction of unfavorable outcome were low, specificities were reasonable and positive predictive values were very low; however, negative predictive values were all high (> 94%).

Molecular techniques are a useful adjunct to standard blood culture for diagnosing early-onset sepsis. Using PCR, evidence for bacteremia can be available earlier (within 4–6 hours) than blood culture. Jordan and Durso [22] reported an overall agreement of 94.1% between results of blood culture and real-time PCR. Contrary to this, 16S rRNA PCR amplification showed high specificity and negative predictive value but low-to-moderate sensitivity in detecting bacteremia [23,24]. In our study, genuine bacteremia occurred only in one of the 212 studied neonates, but all of them had negative PCR, including the infant with GBS bacteremia. This could have been caused in part by a low bacterial concentration in blood samples obtained immediately after birth. Given the fact that our PCR assay detection threshold is about 10 cfu/

CI = confidence interval

I/T = immature/total neutrophil ratio
cfu = colony-forming units

ml [24], samples containing fewer bacteria would be diagnosed as PCR negative. The small sample size and the very low prevalence of bacteremia in our group preclude definitive conclusions regarding the value of molecular techniques in the management of neonates born after PROM. The failure of PCR to detect any of the cases of bacteremia makes it of limited value in our setting.

The CDC guidelines regarding the management of PROM are clear only for newborns whose mothers received intrapartum antimicrobial prophylaxis [5]. Nonetheless, these guidelines clearly state that "variations that incorporate individual circumstances or institutional preferences may be appropriate." A survey of the 17 major neonatal nurseries in Israel (questionnaire via e-mail) regarding their protocols for management of asymptomatic term neonates born after PROM of ≥ 18 hours revealed marked inter-center differences. In the absence of additional maternal risk factors (amnionitis, fever), in only 53% of the centers are blood count and blood culture obtained and empiric antibiotics administered. When maternal risk factors are present, however, this rate rises to 88%.

The moderately high rate of early-onset GBS sepsis in our sepsis-prone neonates in a single institution supports the development of local guidelines for management and reevaluation of empiric antibacterial therapy of such neonates. This would include obtaining blood culture, complete blood count and blood-PCR after birth and a subsequent decision regarding initiation of empiric antibacterial therapy, based on clinical signs and results of blood count and PCR.

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