Assessment of Neonatal Risk Stratification Methods for the Detection of Early Onset Neonatal Sepsis

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Early onset sepsis (EOS) occurs infrequently in newborns but can result in life-long deficits or even death. There is tremendous uncertainty about how to best identify infected infants. We aimed to validate the new obstetric diagnoses for intraamniotic infection, collectively known as Triple I, for their ability to identify EOS among infants ≥ 35 weeks gestational age and compare it to other approaches. We first determined that the obstetric diagnosis, suspected intraamniotic infection, modestly improves identification of infants with EOS compared to clinical chorioamnionitis with a numerically higher sensitivity and significantly higher area under the receiver operating curve (AUC). This solidifies use of this diagnosis in obstetric and pediatric practice over previous criteria. However, its test characteristics were suboptimal with a sensitivity of only 53% (95%CI: 40-66) and an AUC of 0.752 (95%CI: 0.682-0.821). Next, we combined diagnosis of suspected intraamniotic infection with the infant's clinical appearance after birth and assessed test characteristics of this categorical approach for EOS and compared it to the multivariate EOS risk calculator, an alternative, evidence-based approach to EOS screening. We identified that the categorical approach had sensitivity of 90% (95%CI: 79-96%) and AUC of 0.875 (0.825-0.924). While this approach identified EOS better than the calculator, the calculator maintained higher specificity. We then evaluated if placenta data can enhance specificity of the categorical approach. Among infants ≥ 35 weeks gestational age exposed to suspected

intraamniotic infection *in utero*, we identified that combining absence of umbilical cord inflammation and placenta culture growth could successfully rule-out 90% of non-infected but exposed infants. However, the maximum benefit of incorporating placenta data occurs if it is obtained shortly after delivery, a practice that is not commonly done. In conclusion, we successfully validated that a categorical approach combining diagnosis of maternal suspected intraamniotic infection and infant clinical appearance will identify the majority of EOS cases. However, it lacks specificity. While this can be improved using placenta histopathology and culture, it would require significant practice change. As institutions re-consider their approach to EOS screening given recent guideline changes, it is necessary to evaluate the strengths and limitations of each approach.

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1.0 Introduction

Early onset sepsis (EOS) causes severe morbidity and mortality in neonates but occurs infrequently. EOS is typically the result of an ascending intrauterine infection that presents in the first 48 hours of life.(Kuzniewicz et al., 2017; Ottolini et al., 2003) Even with timely and appropriate antibiotic therapy, EOS can lead to death or severe disability in up to 39% of affected neonates.(Brocklehurst et al., 2011) Fortunately, Group B streptococcus (GBS) screening and intrapartum antibiotic prophylaxis reduced incidence of GBS-associated EOS by over 85% in the past 20 years.(Verani et al., 2010) Current all-cause EOS incidence is between 0.5-0.8 per 1000 live-births among term infants, making it relatively uncommon.(Verani et al., 2010)

1.1 Clinical Chorioamnionitis as a Screening Tool for Neonatal Early Onset Sepsis

Until recently, national guidelines recommended that the maternal diagnosis of clinical chorioamnionitis be used to identify infants at increased risk of EOS. Clinical chorioamnionitis is an intrapartum clinical diagnosis based on presence of maternal fever and two or more clinical signs including maternal tachycardia, uterine tenderness, fetal tachycardia or amniotic fluid purulence (Figure 1). Identified in 3-6% of all deliveries, this diagnosis correlates strongly with placental inflammation and EOS in neonates.(Alexander, McIntire, & Leveno, 1999; Fassett, Wing, & Getahun, 2013; Jan, Ramanathan, & Cayabyab, 2017) Importantly, administration of intrapartum antibiotics among women with clinical chorioamnionitis reduces incidence of EOS from 80 to 200 per 1000 live-births(Gibbs, Dinsmoor, Newton, & Ramamurthy, 1988; Gilstrap et

al., 1988; Sperling, Ramamurthy, & Gibbs, 1987; Yoder, Gibbs, Blanco, Castaneda, & St Clair, 1983) to as low as 4 per 1000 live-births. (Braun, Bromberger, Ho, & Getahun, 2016; Gibbs et al., 1988; Gilstrap et al., 1988; Sperling et al., 1987; Yoder et al., 1983) However, infants exposed to clinical chorioamnionitis still remain at higher risk of EOS compared to non-exposed infants. Consequently, until 2018, the American Academy of Pediatrics (AAP) and the Centers for Disease Control (CDC) recommended that all chorioamnionitis-exposed infants receive a suspected EOS laboratory evaluation (blood culture, completed blood count (CBC) and/or C-reactive protein (CRP)), as well as empiric broad-spectrum intravenous antibiotics until blood culture results became available (referred to as AAP guidelines throughout).(R. A. Polin & Newborn, 2012; Verani et al., 2010) Typically, this approach results in antimicrobial treatment for all exposed infants for a minimum of 36-48 hours but may extend to five or more days for infants with positive blood culture, abnormal laboratory values, or clinical symptoms concerning for EOS.

1.2 Risks of Empiric Early Onset Sepsis Evaluations and Antimicrobial Therapy

Evaluating and treating uninfected infants for EOS poses significant risks. Among well-appearing infants exposed to clinical chorioamnionitis, the number needed to treat to identify one confirmed case of EOS is 249;(Braun et al., 2016) but ranges from 60 to 1400 infants depending on the prevalence of clinical chorioamnionitis.(Puopolo, Benitz, & Zaoutis, 2018; Wortham et al., 2016) This highlights the poor specificity of this clinical diagnosis for neonatal EOS, particularly among well-appearing infants. Thus, the risks associated with diagnostic evaluations and antibiotic therapy for neonatal EOS must be considered among this group. For example, many of these uninfected, well-appearing infants are cared for in intensive care units, which are increasingly

recognized for their colonization with anti-microbial resistant bacteria.(Cantey & Sánchez, 2011; Cantey, Vora, & Sunkara, 2016; Cantey, Wozniak, Pruszynski, & Sánchez, 2016; Patel & Saiman, 2010; Tripathi, Cotten, & Smith, 2012) Meanwhile, broad-spectrum antibiotics during early life can compromise the neonatal microbiome, which may lead to short-term consequences such as diarrhea and dermatitis or long-term negative health-outcomes such as increased asthma, allergy, and obesity.(Alm et al., 2008; Chang & Neu, 2015; Madan, Farzan, Hibberd, & Karagas, 2012) Neonatal EOS evaluations also result in increased hospital costs and longer lengths of stay in intensive care units.(Mukherjee, Davidson, Anguvaa, Duffy, & Kennea, 2015; Mukhopadhyay et al., 2017) Further, they can negatively impact maternal/infant bonding and breastfeeding due to early mother-infant separation resulting in increased formula supplementation, delayed breast-feeding initiation and overall lower uptake of breast-feeding.(Jan et al., 2017; Mukhopadhyay, Lieberman, Puopolo, Riley, & Johnson, 2015) Consequently, the low incidence of EOS and the poor specificity of clinical chorioamnionitis among asymptomatic infants prompted both pediatric and obstetric experts to revise national guidelines in the past two years.

1.3 Revised National Pediatric Screening Guidelines for Neonatal Early Onset Sepsis

In 2018, the Committee on the Fetus and Newborn (COFN) for the AAP revised national guidelines to recommend three approaches to identify infants ≥ 35 weeks gestational age at increased risk of EOS. These approaches include: 1) categorical risk factor assessment; 2) multivariate risk assessment; and 3) risk assessment based on newborn clinical condition.(Puopolo, Benitz, & Zaoutis, 2018) A categorical risk assessment is an identified threshold at which point an infant is considered high-risk for EOS and should receive diagnostic evaluation and treatment.

This refers to the approach used in the previous AAP guidelines where all infants who were critically ill or ≥37 weeks gestational age and exposed to clinical chorioamnionitis received diagnostic evaluation and antibiotics for suspected infection.(R. A. Polin & Newborn, 2012) Multivariate risk assessment refers to a web-based Neonatal EOS Risk Calculator that uses an infant's individual risk factors (such as gestational age, maternal temperature, duration of membrane rupture, antibiotic exposure, and clinical appearance) to identify the probability of EOS and to provide more tailored clinical management ranging from enhanced observation to neonatal intensive care unit (NICU) transfer, blood culture and empiric antibiotics.(Escobar et al., 2014; Kuzniewicz et al., 2017; Puopolo et al., 2011) Prospective validation of this approach demonstrates significant reductions in blood cultures and antibiotic administration without an increase in adverse events when compared to the AAP's approach.(Dhudasia, Mukhopadhyay, & Puopolo, 2018; Kuzniewicz et al., 2017) A risk assessment based on the newborn clinical condition typically uses either a categorical or multivariate risk assessment to first identify infants at higher risk of infection, then uses serial clinical exams to follow those infants to determine which ones warrant diagnostic evaluation and antibiotics. This approach has also been shown to significantly reduce blood culture and antibiotic use while identifying infants with underlying EOS who develop clinical symptoms(Joshi et al., 2019; Joshi et al., 2018). The AAP guidelines acknowledge that each of these approaches has merits and limitations for identifying EOS, which must be recognized to use safely and effectively at individual institutions.(Puopolo, Benitz, & Zaoutis, 2018)

1.4 Revised National Obstetric Guidelines for Diagnosis of Intraamniotic Infection

In 2017, the American College of Obstetrics and Gynecologists (ACOG) revised national guidelines to change the terminology for clinical chorioamnionitis, the criteria used for diagnosis, and the recommended maternal management. Adapting recommendations from a multidisciplinary consensus panel hosted by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, ACOG revised the clinical chorioamnionitis definition in order to: 1) simplify diagnosis criteria; 2) improve identification of intraamniotic infection; and 3) improve identification of infants with EOS.(ACOG, 2017; Higgins et al., 2016) This revised definition, known as **Triple I - Intrauterine Inflammation** or **Infection** or both – includes three defined clinical diagnoses – *isolated maternal fever*, *suspected intraamniotic infection*, and *confirmed intraamniotic infection* (Table 1).(ACOG, 2017) Most notably, these diagnoses differ from clinical chorioamnionitis in their fever criteria, their inclusion of fewer qualifying clinical signs, and the decreased number of signs required for diagnosis.

Table 1. Criteria for maternal diagnosis of isolated maternal fever, suspected or confirmed intraamniotic infection or clinical chorioamnionitis.

Diagnosis	Isolated Maternal Fever	Suspected Intraamniotic Infection	Confirmed Intraamniotic Infection	Clinical Chorioamnionitis
Criteria	Temperature ≥38.0°C-38.9°C[1]	Temperature ≥39.0°C OR Temperature ≥38.0°C-38.9°C[1] AND ≥1 clinical sign (*Maternal WBC >15,000 cells/cubic millimeter; fetal heart rate >160 bpm[2]; purulent amniotic fluid*)	Suspected intraamniotic infection AND ≥1 amniotic or placenta sign (*Amniotic fluid with low glucose, positive gram stain or bacterial culture; placenta with inflammation[3] or positive bacterial culture*)	Temperature ≥38.0°C AND ≥2 clinical signs (*Maternal heart rate >100 bpm; WBC >15,000 cells/cubic millimeter or neutrophilic bands >9%; fetal heart rate >160 bpm; fundal tenderness; purulent amniotic flui*d)

°C: Celsius; WBC: white blood cell; bpm: beats per minute. [1]Elevated maternal temperature should be sustained over thirty minutes to be considered fever. [2]Defined as two elevated heart rates at least ten minutes apart or clinician documentation of presence. [3]Defined as histopathologic inflammation of the placenta, fetal membrane or umbilical cord.

Furthermore, they allow an opportunity for amniotic fluid or placenta histopathology data to be incorporated into a *confirmed intraamniotic infection* diagnosis. Under these recommendations, providers first categorize maternal intrapartum fever as either *isolated maternal fever* or *suspected intraamniotic infection*. If criteria for suspected intraamniotic infection are met, then criteria for confirmed intraamniotic infection are evaluated. It is recommended that all mothers with a *suspected* or *confirmed intraamniotic infection* diagnosis receive intrapartum antibiotics, and that women with *isolated maternal fever* may be observed if there is a likely alternative source of fever.

1.5 Validation of Obstetric Diagnoses for Intraamniotic Infection for their Ability to Identify Neonatal EOS is Needed

In a recent national survey of newborn nurseries, 97.5% of nurseries identified that maternal diagnosis of clinical chorioamnionitis was used to identify infants at high risk of EOS.(Mukhopadhyay et al., 2017) Once identified, this would trigger institutional algorithms for EOS evaluation. Over 60% of nurseries identify that their institution uses a categorical risk assessment based on the previous AAP guidelines (diagnostic evaluation and antibiotics for all infants exposed to maternal clinical chorioamnionitis); 14% use the Neonatal EOS Risk Calculator and 3% use clinical exam. This study highlights the continued dependency of neonatal EOS algorithms on an obstetric diagnosis. It also emphasizes a critical gap: it is unknown how ACOG's guideline changes for the identification and management of intraamniotic infection impact the identification of EOS among infants. Furthermore, in the context of the three clinical diagnoses now being used in obstetrics for intraamniotic infection (isolated maternal fever, suspected

intraamniotic infection, and confirmed intraamniotic infection), it is unknown which diagnosis is optimal as a threshold to initiate a diagnostic evaluation and antibiotics for institutions using a categorical approach. Finally, it is unknown how using these new diagnoses compares to other evidence-based approaches to EOS screening. These gaps in knowledge may have serious implications for infants as it may impact their management and for institutions as they reevaluate their approach to EOS screening in light of the recent AAP guideline changes.

To address these gaps, this dissertation evaluates the new ACOG criteria and diagnoses for the identification of intraamniotic infection (Triple I) for their ability to identify infants with EOS First, we evaluate the test characteristics of isolated maternal fever, suspected intraamniotic infection and confirmed intraamniotic infection for the identification of neonatal culture-confirmed EOS in infants ≥ 35 weeks gestational age adding to its validity. We will also compare those test characteristics to those of clinical chorioamnionitis to determine if these changes improve EOS screening that relies on a maternal diagnosis for intraamniotic infection. Next, we will directly compare two approaches supported by the new AAP guidelines for the identification of infants ≥ 35 weeks gestational age at increased risk of EOS - a categorical risk assessment using suspected intraamniotic infection and infant clinical appearance and a multivariate risk assessment using the Neonatal EOS Risk Calculator. Finally, we will evaluate the potential of placenta histopathology and culture data to enhance specificity of the categorical approach using suspected intraamniotic infection at two important clinical decision points for well-appearing infants ≥ 35 weeks gestational age: 1) when deciding whether to initiate empiric laboratory evaluation and antimicrobial therapy and 2) when deciding whether to stop antimicrobials for infants with negative blood culture at 36-48 hours. The results of this work will inform the validity of using a

categorical approach that incorporates the new obstetric diagnoses for intraamniotic infection. This will better inform clinicians as they consider these guideline changes in their own clinical practice.

2.0 Comparison of Test Characteristics of Triple I and Clinical Chorioamnionitis for Neonatal Early Onset Sepsis

2.1 Introduction

Neonatal EOS affects less than 0.5 per 1000 live-births of late preterm/term infants but can result in devasting morbidity and mortality.(Brocklehurst et al., 2011; Puopolo, Benitz, & Zaoutis, 2018; Verani et al., 2010) Clinical chorioamnionitis, which is diagnosed in 3-6% of deliveries, is a major risk factor for EOS and is often used to identify infants who should receive empiric blood culture and antibiotics.(ACOG, 2017; R. A. Polin & Newborn, 2012; Verani et al., 2010) Among late preterm/term infants, however, this approach results in empiric treatment of 60 to 1400 uninfected infants for each asymptomatic infant at birth who is later identified with infection.(Braun et al., 2016; Gibbs et al., 1988; Gilstrap et al., 1988; Wortham et al., 2016) Consequently, screening that uses clinical chorioamnionitis is met with increasing scrutiny as the risks of broad-spectrum antibiotics, disruption in maternal-infant bonding, and prolonged hospital length of stay for uninfected infants become more apparent.(Kiser, Nawab, McKenna, & Aghai, 2014; Mukherjee et al., 2015; Mukhopadhyay, Dukhovny, Mao, Eichenwald, & Puopolo, 2014)

In 2016, an obstetric and neonatology expert panel hosted by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) proposed new recommendations for the intrapartum diagnosis and management of intraamniotic infection with an aim to improve identification of infants with EOS.(Higgins et al., 2016) These recommendations replace the term clinical chorioamnionitis with the term "Triple I" or "intrauterine inflammation, infection or both. They also provide criteria based on the maternal

presentation, which help clinicians to classify the level of concern for infection and subsequently guide maternal and neonatal management. These classifications in order of increasing concern for infection include *isolated maternal fever*, *suspected intraamniotic infection* and *confirmed intraamniotic infection*. These NICHD recommendations were then adapted and disseminated by the American College of Obstetrics and Gynecology (ACOG) for clinical practice in 2017 **(Table 1).**(ACOG, 2017) Then, in 2018, the American Academy of Pediatrics (AAP) provided pediatric guidance on using Triple I to identify infants ≥35 weeks gestational age at risk of EOS.(Puopolo, Benitz, & Zaoutis, 2018) Despite its obstetric and pediatric dissemination, it is unknown whether using Triple I actually improves the identification of late preterm/term infants with EOS compared to clinical chorioamnionitis.

In this study, we sought to identify the test characteristics (sensitivity, specificity, area under the receiver operating curve (AUC), and number needed to treat to benefit (NNTb)) of the Triple I classifications *isolated maternal fever*, *suspected intraamniotic infection* and *confirmed intraamniotic infection* for EOS in late pre-term/term infants using results of bacterial cultures of infant blood or cerebral spinal fluid (CSF) as the gold standard. We then compared these characteristics to that of clinical chorioamnionitis. We hypothesized that the diagnosis of suspected intraamniotic infection would have better discrimination for EOS and lower NNTb compared to clinical chorioamnionitis.

2.2 Methods

2.2.1 Study Population and Setting

This was a retrospective nested case control study of mother-infant pairs delivered at ≥35 weeks gestational age at a single academic tertiary urban hospital. We identified mother-infant pairs born from June 1, 2008 to December 31, 2017 through the Magee Obstetric Medical and Infant (MOMI) database, which collects real-time data on hospital births from the electronic health record (EHR) at our institution from January 1, 1995 to present. After excluding infants <35 weeks gestational age, we identified infants with culture-confirmed early onset bacterial infections (cerebral spinal fluid, peripheral or cord blood) within the first 72 hours of life through MOMI and the hospital microbiology laboratory databases. We excluded infants with positive cultures for likely contaminants including *Aerococcus* and any *Staphylococcus* species other than *Staphylococcus aureus*.(Schrag et al., 2016) We also excluded infants who were born outside of the hospital, readmitted or with significant anomalies as defined by the Vermont-Oxford Neonatal Network (www.vtoxford.org). Remaining infants with positive cultures were considered cases. We then randomly selected three controls for each case from the MOMI database who were frequency matched by birth year, ≥35 weeks gestational age, and met other inclusion/exclusion criteria. In addition, we excluded infants who were treated for suspected early onset bacterial infection (received seven or more days of antibiotics in the absence of a positive culture) as potential controls. To be included, mothers needed at least one set of vital signs recorded prior to delivery.

2.2.2 Data Collection

We abstracted detailed maternal and infant demographic, prenatal and peripartum hospitalization data from the EHR using a structured protocol and trained research assistants for all cases and controls. Maternal intrapartum antibiotics were categorized as: none; received antibiotic less than two hours prior to delivery; group B *Streptococcus* (GBS) intrapartum antibiotic prophylaxis (IAP) (including penicillin, ampicillin, amoxicillin, clindamycin, cefazolin, vancomycin) received at least two hours prior to delivery; broad-spectrum antibiotic (other cephalosporins, fluoroquinolone, or any antibiotic from IAP antibiotic plus aminoglycoside) between 2 to 3.99 hours prior to delivery; and broad-spectrum antibiotic at least 4 hours prior to delivery.(Escobar et al., 2014; Kuzniewicz et al., 2017; Puopolo et al., 2011) We identified infants as having clinical illness within 12 hours of delivery if they had a 5 minute Apgar < 5; required oxygen or respiratory support for two or more hours; had seizure activity; or received vasopressor support.(Kuzniewicz et al., 2017; Wortham et al., 2016)

2.2.3 Statistical Analysis

Descriptive statistics. Baseline characteristics were described using means and proportions and compared using Student's t-test and Chi-square or Fisher's exact with odds ratios reported, respectively. An alpha of less than 0.05 was considered significant.

Assessment of test characteristics. We assessed the test characteristics of Triple I for EOS using the three Triple I classifications: 1) isolated maternal fever; 2) suspected intraamniotic infection; 3) confirmed intraamniotic infection; as well as the test characteristics of clinical chorioamnionitis for EOS. For each, we first identified cases and controls who met criteria for

each classification (**Table 1**; additional details on data abstraction in Appendix A).(ACOG, 2017; Gibbs, 1977)

We then determined the sensitivity, specificity, and AUC with 95% confidence intervals for each diagnosis for EOS using infant blood or CSF culture results as the gold standard. Bacterial growth on blood or CSF culture indicated early onset infection, while no growth on blood or CSF cultures or absence of a culture indicated no infection. The assumption that absence of a culture indicated no infection is necessary and reasonable as cultures and antibiotics are not routine in well-appearing, low-risk infants and untreated, infected infants would typically become critically ill within 72 hours of birth, at which time a culture would be obtained.(Puopolo, Benitz, & Zaoutis, 2018; Verani et al., 2010)

Next, we compared the sensitivity, specificity and AUC for each Triple I classification (*isolated maternal fever, suspected intraamniotic infection,* or *confirmed intraamniotic infection)* to the characteristics of clinical chorioamnionitis using a McNemar or DeLong chi-squared test, respectively. We used a Bonferroni correction to account for multiple comparisons, where an alpha less than 0.02 was considered significant. To calculate the number needed to treat to benefit (NNTb) for each definition, we used the odds ratio for each definition from a logistic regression analysis and the incidence of bacterial infections in our base population (0.62 per 1,000 live-births).(Mendes, Alves, & Batel-Marques, 2017) Finally, we conducted a sensitivity analysis excluding all positive cord blood cultures to evaluate impact on our estimates. All analyses were completed using Stata 15.0 (Stata Corp, College Station, Texas). This study was approved by the University of Pittsburgh institutional review board (PRO17110548).

2.3 Results

2.3.1 Study Population

During the study period, 85,786 neonates ≥35 weeks gestational age were born at our institution **(Figure 1).** Fifty-three cases of neonatal early onset culture-confirmed bacterial infections (0.62 cases per 1,000 live-births) and 159 controls were included. Fifty-one (96%) cases had bacterial growth on blood cultures from peripheral (N=39) or cord blood specimens (N=12), and 2 (4%) cases had bacterial growth on cultures of cerebral spinal fluid. GBS (34%, N=18) and *Escherichia coli* (19%, N=10) accounted for the majority of the infections **(Figure 2).** Placenta pathology reports were available for 121 (57%) mother-infant pairs. Thirty-one (15%) mother-infant pairs had placenta cultures, of which 18 (58%) had bacterial growth, of which 16 (89%) grew the same bacterial species as was identified in the infant's blood culture.

Demographics and gestational age were similar between cases and controls **(Table 2).** Mothers of cases were more likely to have null parity, rupture of membranes for 18 or more hours and receipt of broad-spectrum antibiotics four or more hours prior to delivery than mothers of controls. Infants with culture-confirmed EOS were more likely to be critically ill within 12 hours of delivery. Individual criteria used to make a Triple I or clinical chorioamnionitis diagnosis are described by case or control status in **Table 3.**

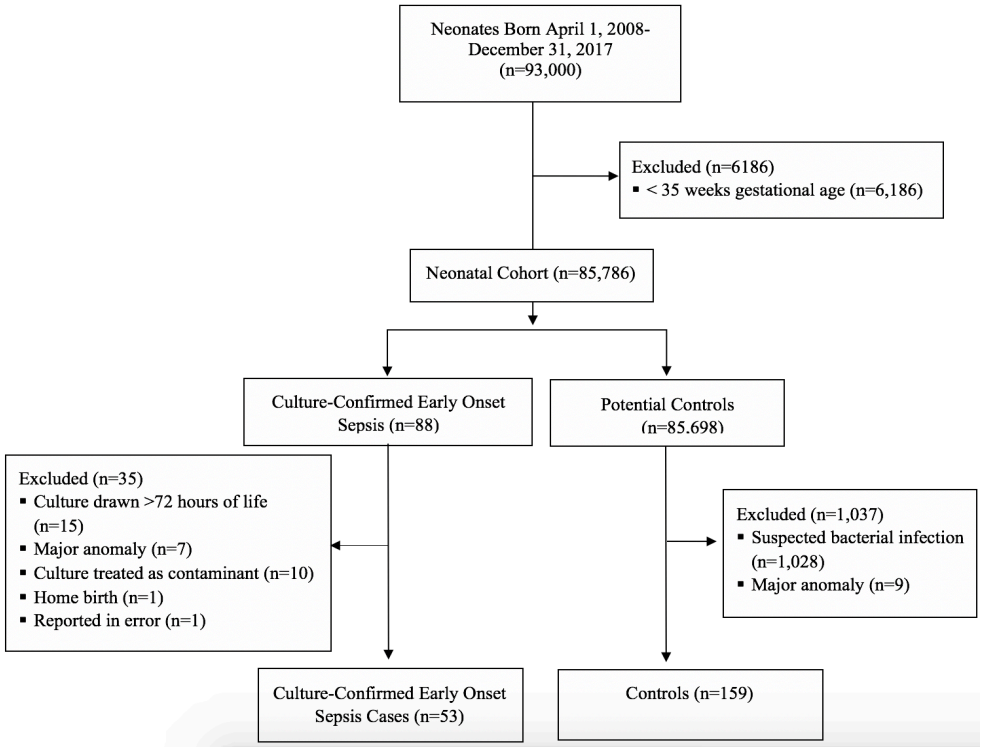

Figure 1. Flow diagram of neonates included in study.

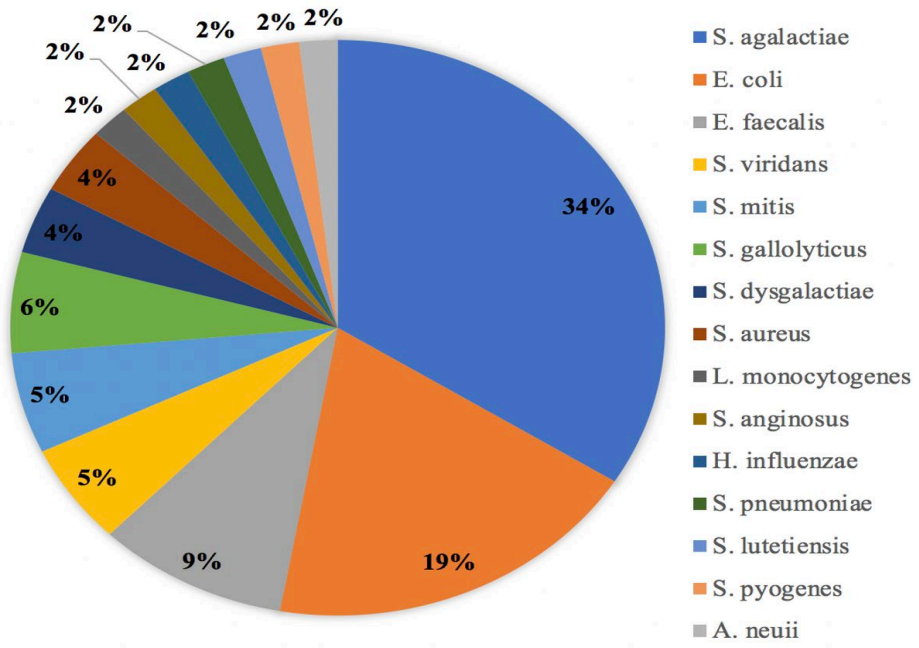

Figure 2. Bacteria isolates identified on infant blood or cerebral spinal fluid culture.

Table 2. Demographic, labor and delivery characteristics of culture-confirmed early onset bacterial infection cases and controls.

Characteristic	Cases (n=53)	Controls (n=159)	OR (95%CI) or Student T-test P-value
Maternal Age, years, mean (SD)	28.4 (±4.9)	29.3 (±5.4)	0.306
Maternal Ethnicity			
White	30 (57)	108 (68)	Ref
Black	17 (32)	42 (26)	1.5 (0.7-2.9)
Other	6 (11)	9 (6)	2.4 (0.8-7.4)
Marital Status			
Married	30 (57)	91 (57)	Ref
Not Married	23 (43)	68 (43)	0.9 (0.5-1.7)
Education	N=53	N=156	
< 12^{th} grade	6 (11)	13 (8)	1.2 (0.4-3.8)
Highschool or GED	14 (26)	36 (23)	Ref
Some College/Associate	14 (26)	33 (21)	1.1 (0.5-2.6)
Bachelor's Degree	10 (19)	49 (31)	0.5 (0.2-1.3)
Graduate Degree	9 (17)	25 (16)	0.9 (0.3-2.5)
Parity			
0	41 (77)	61 (38)	**5.5 (2.6-11.8)**
≥ 1	12 (23)	98 (62)	**Ref**

Table 2 (continued).

Maternal GBS status	N=53	N=154	
Negative	41 (77)	122 (79)	Ref
Positive	12 (21)	32 (21)	1.1 (0.5-2.4)
Intrapartum Antibiotics			
None or < 2 hours	39 (74)	124 (78)	**Ref**
GBS IAP ≥ 2 hours	8 (15)	31 (20)	**0.8 (0.3-2.0)**
Broad Spectrum 2-4 hours	1 (2)	0 (0)	**--**
Broad Spectrum > 4 hours	5 (9)	4 (7)	**6.6 (1.5-28.6)**
Duration of Rupture of	N=52	N=153	
<18 hours	33 (64)	140 (91)	**Ref**
≥ 18 hours	19 (36)	13 (9)	**6.2 (2.6-14.5)**
Anesthesia			
Epidural	43 (81)	115 (72)	Ref
Spinal	4 (8)	24 (15)	0.4 (0.1-1.4)
Other	3 (6)	3 (2)	2.7 (0.5-13.9)
None	3 (6)	17 (11)	0.5 (0.1-1.7)
Mode of Delivery			
Vaginal	32 (60)	114 (72)	Ref
Caesarian	21 (40)	45 (28)	1.7 (0.9-3.2)

Table 2 (continued).

Gestational Age, weeks			
35-36	7 (13)	12 (8)	1.9 (0.7-5.3)
37-38	9 (17)	31 (20)	0.9 (0.4-2.2)
39-40	30 (57)	98 (61)	Ref
≥41	7 (13)	18 (11)	1.3 (0.5-3.3)
Gestation			
Single	52 (98)	155 (97)	Ref
Multiple	1 (2)	4 (3)	0.7 (0.1-6.9)
Sex			
Female	29 (55)	69 (43)	Ref
Male	24 (45)	90 (57)	0.6 (0.3-1.2)
Birth Weight, grams, mean (SD)	3241 (±523)	3315 (±517)	0.368
Birth Weight			
< 2500 g	3 (6)	7 (4)	1.3 (0.3-5.2)
≥ 2500 g	50 (94)	152 (96)	Ref
Critically Ill < 12 hours from Delivery			
No	35 (66)	154 (97)	**Ref**
Yes	18 (34)	5 (3)	**15.8 (4.9-51.2)**

OR: Odds ratio; 95%CI: 95% confidence interval; SD: standard deviation; ref: reference group; GBS: group B *Streptococcus*. IAP: Intrapartum antibiotic prophylaxis.

Data are N (%) unless otherwise specified. Bolded data indicate $p<0.05$.

Table 3. Proportion of women with peripartum signs concerning for intraamniotic infection.

	Cases (N=53)	Controls (N=159)
Maternal Temperature ≥ 38.0-38.9°C	33 (62)	7 (4)
Maternal Temperature ≥ 39°C	9 (17)	0 (0)
Maternal Tachycardia (> 100 bpm)	30 (57)	37 (23)
Fundal Tenderness	1 (2)	0 (0)
WBC >15,000 cells/mm^3	17/43 (40)	12/113 (11)
Bands >9%	5/32 (16)	0/76 (0)
Fetal Tachycardia (> 160 bpm)	30 (57)	4 (3)
Foul/purulent odor	0 (0)	0 (0)
Acute Placenta Histology	N=47	N=73
Deciduitis	19 (40)	14 (19)
Chorioamnionitis	41 (87)	19 (26)
Chorionic plate fetal vasculitis	37 (79)	11 (15)
Funisitis or Umbilical cord vasculitis	29 (62)	6 (8)
Bacteria Visible in Membranes	4 (9)	0 (0)
Bacteria Growth on Placenta Culture	18/28 (64)	0/3 (0)

C: Celsius; bpm: beats per minute; mm^3: cubic millimeters. Data are N (%) unless otherwise specified.

2.3.2 Test Characteristics of Triple I and Clinical Chorioamnionitis

More than 40% of cases did not meet criteria for any definition **(Table 4)**. Of those, 15 (65%) infants had clinical illness at time of delivery. Suspected intraamniotic infection had higher numerical sensitivity for EOS at 52.8% (95%CI: 39.7-65.6) **(Table 4)**, compared to clinical chorioamnionitis at 43.4% (95%CI 31.0-56.7%) but was not significant when using a Bonferroni correction (p=0.025). Among the mothers with suspected intraamniotic infection, there were nine cases and zero controls who had a temperature of 39.0°C or higher. All nine mothers also had other signs besides fever consistent with suspected intraamniotic infection**.** All mother-infant pairs with EOS identified by clinical chorioamnionitis (N=23) were also identified by suspected intraamniotic infection.

One control mother-infant pair who met criteria for suspected intraamniotic infection did not have placenta pathology or placenta culture results available and thus could not be assessed for confirmed intraamniotic infection. Notably, four infants with EOS who met criteria for suspected intraamniotic infection were ruled-out by normal placenta histopathology and cultures. This reduced the sensitivity of confirmed intraamniotic infection for EOS, which was similar to clinical chorioamnionitis (p=0.739). Isolated maternal fever had lower sensitivity for EOS than clinical chorioamnionitis (p<0.0001). Confirmed intraamniotic infection had the highest numerical specificity for EOS (99.4%, 95%CI:96.5-99.9%) but was not significantly different from clinical chorioamnionitis (p=0.157).

The AUC for suspected intraamniotic infection (0.752; 95%CI:0.683-0.821) was significantly higher than AUC for clinical chorioamnionitis (0.704; 95%CI: 0.636-0.773; p=0.02); while AUC for isolated maternal fever was significantly lower (0.513; 95%CI:0.485-0.540; p<0.001) **(Table 4)**. The NNTb was lowest for confirmed and suspected intraamniotic infection.

Table 4. Classification of cases and controls by clinical diagnosis with sensitivity, specificity, AUC, and NNTb for culture-confirmed EOS.

Definition	Cases N=53 N (%)	Controls N=159 N (%)	Sensitivity % (95%CI)	Specificity % (95%CI)	AUC # (95%CI)	NNTb # (95%CI)
Did Not Meet Any Definition	23 (43)	153 (96)	NA	NA	NA	NA
Isolated Maternal Fever	2 (4)	2 (1)	**4 (1-13)**	99 (96-100)	**0.513 (0.485-0.540)**	770 (39-2042)
Suspected Intraamniotic Infection	28 (53)	4 (3)	53 (40-66)	98 (94-99)	**0.752 (0.683-0.821)**	39 (10-133)
Confirmed Intraamniotic Infection	24 (45)	1 (1)[†]	45 (33-59)	99 (97-100)	0.723 (0.655-0.791)	14 (1-102)
Clinical Chorioamnionitis	23 (43)	4 (3)	43 (31-57)	98 (94-99)	0.704 (0.636-0.773)	57 (14-203)

EOS: early onset sepsis; 95%CI: 95% confidence interval, AUC: area under the receiver operating curve; NNTb: number needed to treat to benefit; NA: Not applicable. Bolded data indicate $p<0.02$ on DeLong chi-square test comparing AUC of each definition to AUC of clinical chorioamnionitis. [†]Pathology report missing for one control with suspected intraamniotic infection.

Sensitivity analysis. After excluding infants with bacterial growth on cultures from cord blood specimens, 41 cases and 159 controls remained. Similar test characteristics for Triple I and clinical chorioamnionitis were observed **(Appendix Table 1).**

2.4 Discussion

2.4.1 Principal Findings

Our study demonstrates that the Triple I diagnosis of suspected intraamniotic infection modestly improves the identification of late preterm/term infants with culture-confirmed EOS compared to clinical chorioamnionitis. If using a categorial approach based on obstetric diagnosis for EOS screening, this informs which categorical threshold should be used by clinicians based on current obstetric practice. It also reinforces the importance of obstetric providers utilizing the new Triple I criteria when making a diagnosis of intraamniotic infection, as it has downstream effects on newborn care. However, with a sensitivity of only 53% and AUC less than 0.90, there are significant limitations in using suspected intraamniotic infection, and Triple I as a whole, as a screening tool for EOS. This is highlighted by the finding that over 40% of infants with culture-confirmed EOS did not meet criteria for any of the Triple I classifications. While two-thirds of those infants had clinical illness within 12 hours of delivery and would likely receive empiric therapy, the other third were initially well-appearing and could potentially be missed if they were not tested or treated.

Despite this, our results suggest that using suspected intraamniotic infection instead of clinical chorioamnionitis to empirically treat infants for EOS may reduce the NNTb by 30-45%, which may still be clinically significant. Interestingly, confirmed intraamniotic infection may reduce the NNTb by approximately 80%. This is driven by the small but clinically meaningful increase in specificity. While not statistically significant in our study likely due to low power, in a larger population this may have important implications for "ruling-out" non-infected infants. Finally, our study demonstrates that isolated maternal fever is not common among infants with EOS and by itself, it discriminates poorly between infected and uninfected infants. We interpret this to mean that infants exposed to isolated maternal fever should not be empirically treated for EOS but instead warrant close monitoring.

2.4.2 Our Findings in the Context of Existing Literature

The test characteristics for suspected intraamniotic infection suggest that using this approach will modestly improve the ability to identify late preterm/term infants with and without EOS compared to the previous standard. However, other EOS screening tools, which are also supported by the AAP, may further improve identification of these infants. The Early Onset Sepsis Risk Calculator is a user-friendly, web-based tool based on predictive models for early onset sepsis derived from a cohort of 608,000 newborns ≥34 weeks' gestation.(Escobar et al., 2014; Kuzniewicz et al., 2017; Puopolo et al., 2011) It provides a probability of EOS and clinical recommendations based on objective data such as gestational age, highest maternal intrapartum temperature, maternal group B *Streptococcus* colonization status, duration of rupture of membranes, and type and duration of intrapartum antibiotic therapies that is then adjusted based on the newborn's clinical appearance through 12 hours of life. Prospective validation of this tool

in 204,685 late preterm/term newborns demonstrated a 66% reduction in blood cultures, 48% reduction in empiric antibiotics, and no difference in readmission rates or adverse events compared to using chorioamnionitis to guide management.(Dhudasia et al., 2018; Kuzniewicz et al., 2017) This tool has a reported AUC of greater than 0.9 and the number needed to treat is less than 22.(Deshmukh, Mehta, & Patole, 2019; Dhudasia et al., 2018; Kuzniewicz et al., 2017; Puopolo et al., 2011) While this compelling data makes screening with the calculator across institutions seem logical, implementation of this tool requires numerous practice and system-wide changes, including a centralized way to assess risk and more frequent vital sign and physical exam monitoring of higher-risk infants. These changes can significantly limit the ability of some settings to implement this tool. On the other hand, a recent survey of newborn nurseries identified that over 60% of clinicians still use maternal diagnosis of clinical chorioamnionitis to identify infants at increased risk of early onset bacterial infection.(Mukhopadhyay et al., 2017) Therefore, modifying obstetric practice to use Triple I criteria and pursuing empiric treatment for infants born to mothers with suspected intraamniotic infection may be better accepted and practical in institutions currently using clinical chorioamnionitis or where resources limit use of the calculator. Thus, each institution must be aware of the strengths and limitations of each strategy and identify what may work best in their setting.(Puopolo, Benitz, Zaoutis, NEWBORN, & DISEASES, 2018)

Few studies have examined the test characteristics of Triple I diagnoses for neonatal EOS. In a study by Ona et al, the accuracy of the ACOG recommendations for identifying confirmed intraamniotic infection was examined among a cohort of women with a diagnosis of intrapartum fever.(Ona et al., 2019) They found that suspected intraamniotic infection had a sensitivity of 71.4% (95% CI 61.4–80.1%) and specificity 40.5% (95% CI 33.6–47.8%) for confirmed intraamniotic infection. However, they were unable to assess the test characteristics of these

definitions for EOS as they only identified one case of infant bacteremia. A retrospective study by Coleman et al, which examined test characteristics of suspected intraamniotic infection among infants exposed to clinical chorioamnionitis, found that all three infants with culture-confirmed EOS met criteria for suspected intraamniotic infection, concluding 100% sensitivity for EOS.(Coleman et al., 2020) This is consistent with our finding that 100% of the 23 infants with EOS who met criteria for clinical chorioamnionitis also met criteria for suspected intraamniotic infection. However, our study uses a broader population, including EOS infants who were not exposed to clinical chorioamnionitis, making our findings more generalizable and providing broader insight into Triple I as a screening tool for EOS.

2.4.3 Implications for Clinical Practice and Research

Our data demonstrates that using a maternal diagnosis of suspected intraamniotic infection to identify late preterm/term infants at increased risk of infection can modestly improve the early identification of infected infants and potentially reduce treatment of uninfected infants compared to clinical chorioamnionitis. This should reinforce implementation of Triple I criteria for obstetric diagnosis and management of intraamniotic infection across institutions. By adhering to these diagnostic criteria, obstetricians will influence pediatric diagnostic and management practices for EOS in institutions that use maternal diagnosis to identify infants that need empiric therapy. Our data should also direct pediatricians who use a categorical approach to EOS screening to use maternal diagnosis of suspected intraamniotic infection as their threshold for empiric treatment.

Furthermore, our data reinforces the ACOG and the NICHD panel recommendations that empirically treating mothers and infants for infection who have isolated maternal fever will result in overtreatment of uninfected infants.(ACOG, 2017)·(Mukhopadhyay et al., 2017; Puopolo,

Benitz, & Zaoutis, 2018) This is important as isolated maternal fever occurs in 2-4% of deliveries.(Braun et al., 2016; Towers et al., 2017) Thus, practice modifications around this diagnosis could have substantial impact on empiric treatment practices. Yet, as indicated by our data, EOS can be associated with isolated maternal fever. Therefore, it is essential that obstetric and pediatric teams communicate this risk and the degree of suspicion for infection so that these infants can be appropriately monitored with more frequent vital sign and physical exam assessments to permit early identification of symptom progression.(Dhudasia et al., 2018; Kuzniewicz et al., 2017)

The potential for confirmed intraamniotic infection to substantially reduce the number of uninfected infants empirically treated with antibiotics is important but unlikely to be useful in the current practice setting. In most institutions, placenta pathology data is not available until 24-96 hours after delivery and amniotic fluid testing is uncommon. Thus, these results are typically unavailable to guide provider decision-making for maternal and initial neonatal management. Therefore, to effectively utilize pathology data to augment initial decision-making, development of rapid placental pathology techniques should be considered.(Mahe et al., 2014) Incorporating pathology results at a later time-point, however, may still be beneficial in reducing laboratory evaluations or determining the duration of treatment with antibiotics for infants.

2.4.4 Limitations

There are several important limitations in this study. The retrospective case-control nature of this study subjects it to selection, information, confirmation and surveillance bias. For example, mothers with fever tend to have more frequent monitoring of vital signs, as well as additional laboratory and pathology testing. We attempted to minimize these biases by only including cases

and controls included in the MOMI database and by completing a robust EHR abstraction. Furthermore, other studies have found similar sensitivity of clinical chorioamnionitis for neonatal bacterial infections, suggesting comparability of our results.(Romero, Chaemsaithong, Korzeniewski, et al., 2016; Tita & Andrews, 2010) Repeat measurements of elevated temperatures were not consistently available. Consequently, we modified the ACOG Triple I criteria to require only a single elevated temperature. This could lead to overestimation of the sensitivity of each Triple I classification. However, it is unlikely that this would significantly impact the overall interpretation of results and conclusions. Finally, we were unable to obtain estimates for negative and positive predictive value, as these rely on disease prevalence in the underlying population, which is artificially inflated in a case control study.(Steinberg, Fine, & Chappell, 2009)

2.4.5 Conclusion

Our study overall supports implementation of the 2017 ACOG and 2018 AAP recommendations to use Triple I diagnoses in the identification and management of mothers and infants at risk of bacterial infection, while also highlighting their limitations. Use of suspected intraamniotic infection will modestly improve the distinction of late preterm/term infants with and without EOS compared to clinical chorioamnionitis. However, its performance as a screening tool remains suboptimal. Yet, its familiarity to obstetric and pediatric practitioners due to its diagnostic similarity to clinical chorioamnionitis is likely to facilitate its implementation compared to screening methods that require substantial changes in practice. Thus, use of suspected intraamniotic infection to identify late preterm/term infants who require empiric treatment will represent an incremental but important improvement in the diagnosis and management of neonatal EOS.

3.0 Comparison of a Categorical Approach using Suspected Intraamniotic Infection and Infant Appearance and the Multivariate EOS Calculator for Identification of Early Onset Sepsis

3.1 Introduction

In 2018, the American Academy of Pediatrics (AAP) revised national guidelines to recommend three approaches to identify infants ≥35 weeks gestational age at increased risk of EOS. These approaches include: 1) categorical risk factor assessment; 2) multivariate risk assessment; and 3) risk assessment based on newborn clinical condition.(Puopolo, Benitz, & Zaoutis, 2018) Each approach aims to identify infants with EOS quickly to allow rapid intervention and treatment while minimizing the identification of uninfected infants to avoid unnecessary antibiotic exposure, laboratory evaluations and disruption of maternal/infant bonding.

The AAP guidelines suggest that maternal diagnosis of suspected intraamniotic infection, as defined by the recent ACOG guidelines, may be used as a threshold when employing a categorical approach for neonatal EOS screening. However, evidence to support use of this threshold is based primarily on that for clinical chorioamnionitis, which uses different diagnostic criteria and has poor sensitivity for EOS. (Table 1).(R. A. Polin & Newborn, 2012; Tita & Andrews, 2010) Furthermore, AAP guidelines do not provide clear guidance on how to incorporate a categorical approach with infant clinical appearance. Conversely, multivariate risk assessment refers to a web-based Neonatal EOS Risk Calculator (hereon referred to as calculator) that uses an infant's individual risk factors (such as gestational age, maternal temperature, duration of membrane rupture, antibiotic exposure, and clinical appearance) to identify the probability of EOS

and to provide more tailored clinical management ranging from enhanced observation to NICU transfer, blood culture and antibiotics.(Escobar et al., 2014; Kuzniewicz et al., 2017; Puopolo et al., 2011). Increasing evidence suggests this approach can safely and effectively identify infants with EOS, while reducing antibiotics and laboratory evaluations.(Deshmukh et al., 2019; Dhudasia et al., 2018; Kuzniewicz et al., 2017; Puopolo et al., 2011)

Since the introduction of these guidelines, there is increasing heterogeneity in diagnostic approaches used for EOS screening across U.S. hospital centers. However, uncertainty about the best approach remains as each has its merits and limitations. We sought to directly compare the multivariate EOS calculator and a categorical approach using a combination of maternal diagnosis of suspected intraamniotic infection and infant clinical appearance for their sensitivity, specificity, area under the curve (AUC), and the number needed to treat to benefit (NNTb) for culture-confirmed EOS in infants ≥ 35 weeks gestational age. We hypothesized that the multivariate EOS calculator would have better discrimination between infants with and without culture-confirmed EOS compared to the categorical approach.

3.2 Methods

A detailed description of the study population and data collection procedures are available in Chapter 2 with procedures and modifications reviewed here in brief.

3.2.1 Study Population and Setting

This was a single-institution, retrospective nested case-control study of mother-infant pairs who delivered at ≥ 35 weeks gestational age from June 1, 2008 to December 31, 2017. Mother-infant pairs were first identified through the Magee Obstetric Medical and Infant (MOMI) electronic database, which collects real-time data on >95% of hospital births from the electronic health record (EHR). We identified infants with bacterial growth on blood (peripheral or cord) culture or cerebral spinal fluid culture obtained within 72 hours of life using an automated continuous detection culture system. We then excluded infants with cultures that were likely contaminants, such as coagulase negative *Staphylococcus* or *Aerococcus* (Schrag et al., 2016), who were born outside of the hospital, readmitted or with significant anomalies as defined by the Vermont-Oxford Neonatal Network (www.vtoxford.org). For this study, we also excluded infants if time of maternal membrane rupture was not available. Remaining infants were included as cases of culture-confirmed early onset bacterial infection. For the previous study, three controls were randomly selected for each case among infants with the same birth year from the MOMI database. We applied similar inclusion/exclusion criteria, in addition to excluding infants with culture negative EOS, which we defined as those who received 5 or more days of antibiotics in the absence of a positive culture. We additionally excluded infants where time of maternal membrane rupture was not available and replaced them with randomly selected controls from the same birth year.

3.2.2 Data Collection

As described previously, we abstracted all demographic, prenatal and peripartum data necessary for the multivariate EOS calculator and categorical risk assessment including maternal

and infant vital signs, laboratory data, and antibiotics from the EHR into a REDCap database.(Escobar et al., 2014) Duration of membrane rupture was calculated based on documented time of membrane rupture and time of delivery. Maternal group B Streptococcus (GBS) colonization status was primarily obtained from laboratory reports and secondarily obtained from obstetric admission notes if laboratory reports were unavailable. Maternal intrapartum antibiotics were categorized as: none; received antibiotic less than two hours prior to delivery; Group B Streptococcus (GBS) intrapartum antibiotic prophylaxis (IAP) (including penicillin, ampicillin, amoxicillin, clindamycin, cefazolin, vancomycin) received at least two hours prior to delivery; broad-spectrum antibiotic (other cephalosporins, fluoroquinolone, or any antibiotic from IAP antibiotic plus aminoglycoside) between 2 to 3.99 hours prior to delivery; and broad-spectrum antibiotic at least 4 hours prior to delivery.(Escobar et al., 2014; Kuzniewicz et al., 2017; Puopolo et al., 2011) White blood cells (WBC) were abstracted as exact counts in thousands per cubic milliliter. Fetal tachycardia was recorded if fetal heart rate >160 beats per minute was recorded in labor vital sign flowsheets or if documented in obstetric or neonatology notes. Purulence of amniotic fluid was identified exclusively from obstetric and neonatology notes. For infant clinical appearance, data on Apgar score at 5 minutes of life as well as any oxygen or vasopressor support, respiratory rate and breathing description, or presence of seizures during the first twelve hours of life were recorded from vital sign and medication flowsheets and neonatology notes.

3.2.3 Risk Stratification for EOS

Neonatal EOS Risk Calculator: The multivariate calculator is available for public use at https://neonatalsepsiscalculator.kaiserpermanente.org. To establish each infant's probability of EOS at birth and to obtain the calculator's management recommendations, we utilized our cohort's

incidence of EOS and entered peripartum factors including gestational age, highest maternal temperature in Celsius, duration of membrane rupture, GBS status, antibiotic exposure for each infant into the online calculator. Infant clinical appearance for the first 12 hours of life was categorized as well-appearing, equivocal or clinical illness based on vital sign patterns and clinical presentation as defined by the calculator.(Kuzniewicz et al., 2017; Wortham et al., 2016) **(Table 5)**. The calculator's estimated probability of EOS, clinical and vital sign monitoring recommendations were recorded. Clinical recommendations include no blood culture or antibiotics, blood culture only, strongly consider blood culture and empiric antibiotics, and blood culture and empiric antibiotic. Vital sign recommendations include routine vitals, vitals every 4 hours for 24 hours, or vitals per the neonatal intensive care unit (NICU).

Categorical EOS Risk Assessment: Categorical risk was determined using the criteria for suspected intraamniotic infection in the mother based on ACOG's 2018 guidelines or if an infant developed equivocal or clinical illness as defined by the multivariate EOS risk calculator during the first 12 hours of life (**Table 1 and 5).** Suspected intraamniotic infection is diagnosed based on a maternal temperature of ≥39.0˚C or maternal temperature of 38.0-38.9˚C with either presence of maternal WBC >15,000 cells/cubic millimeter (mm^3), fetal heart rate >160 beats per minute or amniotic purulence. We assumed that infants who were exposed to suspected intraamniotic infection or had equivocal or clinical illness presentation would be managed with a blood culture and empiric antibiotics, while all other infants would receive routine care.

3.2.4 Statistical Analysis

Descriptive statistics. Baseline characteristics of cases and controls were described using means and proportions.

Test characteristics of risk stratification approaches. We examined sensitivity and specificity with Wilson's 95% confidence intervals for the multivariate EOS risk calculator and the categorical approach for EOS using results from infant blood or cerebral spinal fluid (CSF) cultures as the gold standard. Bacterial growth on blood or CSF culture indicated early onset infection, while no growth on blood or CSF cultures or absence of a culture indicated no infection. This is a necessary but reasonable assumption as cultures and antibiotics are not routine in well-appearing, low-risk infants but untreated, infected infants would typically progress to clinical illness within 48-72 hours of birth, at which time a culture would be obtained.(Puopolo, Benitz, & Zaoutis, 2018; Verani et al., 2010) For both approaches, we considered a recommendation for obtaining a blood culture as a "positive" test.

Table 5. Categorization of infant clinical appearance using critiera from the multivariate EOS risk calculator.

Clinical Illness	Equivocal	Well Appearing

• Persistent need for ventilation outside of the delivery room
• Need for O2 ≥ 2 hours
• Hemodynamic instability requiring vasoactive drugs
• Neonatal encephalopathy/ perinatal depression characterized by seizures or 5-minute Apgar score < 5
	• Persistent physiologic abnormality ≥ 4 hours
	  ○ HR ≥160 beats/minute
	  ○ RR ≥160 breaths/minute
	  ○ ≥ 38.0°C or ≤ 36.4°C
	  ○ Respiratory distress not requiring O2
	• ≥ 2 of the above for ≥ 2 hours
		Not clinical illness or equivocal

EOS: early onset sepsis; O2: oxygen; HR: heart rate; RR: respiratory rate; C: Celsius

We compared sensitivity and specificity for the two approaches using a McNemar test and the area under the receiver operating curve (AUC) using DeLong chi-squared test. To calculate the number needed to treat to benefit (NNTb) for each definition, we used the odds ratio for each definition from a logistic regression analysis and the incidence of bacterial infections in our base population (0.6 per 1,000 live-births). We then conducted two secondary analyses. First, for the categorical approach, we excluded infants with equivocal clinical appearance to assess impact on results. Second, for the multivariate EOS calculator, we assessed test characteristics where we considered a vital sign recommendation of vitals at least every four hours for 24 hours as a positive test. All analyses were completed using Stata 15.0 (Stata Corp, College Station, Texas). An alpha of less than 0.05 was utilized to indicate significance on all statistical tests. This study was approved by the University of Pittsburgh institutional review board (PRO17110548).

3.3 Results

Fifty cases of blood culture-confirmed EOS and 2 cases of CSF culture-confirmed EOS and 156 controls were included **(Figure 3)**. This gives a local EOS incidence of 0.60 cases per 1,000 live-births. Bacteria species on cultures were previously reported (Chapter 2, **Figure 2).** Demographic, peripartum and screening tool characteristics are reported in **Table 6.**

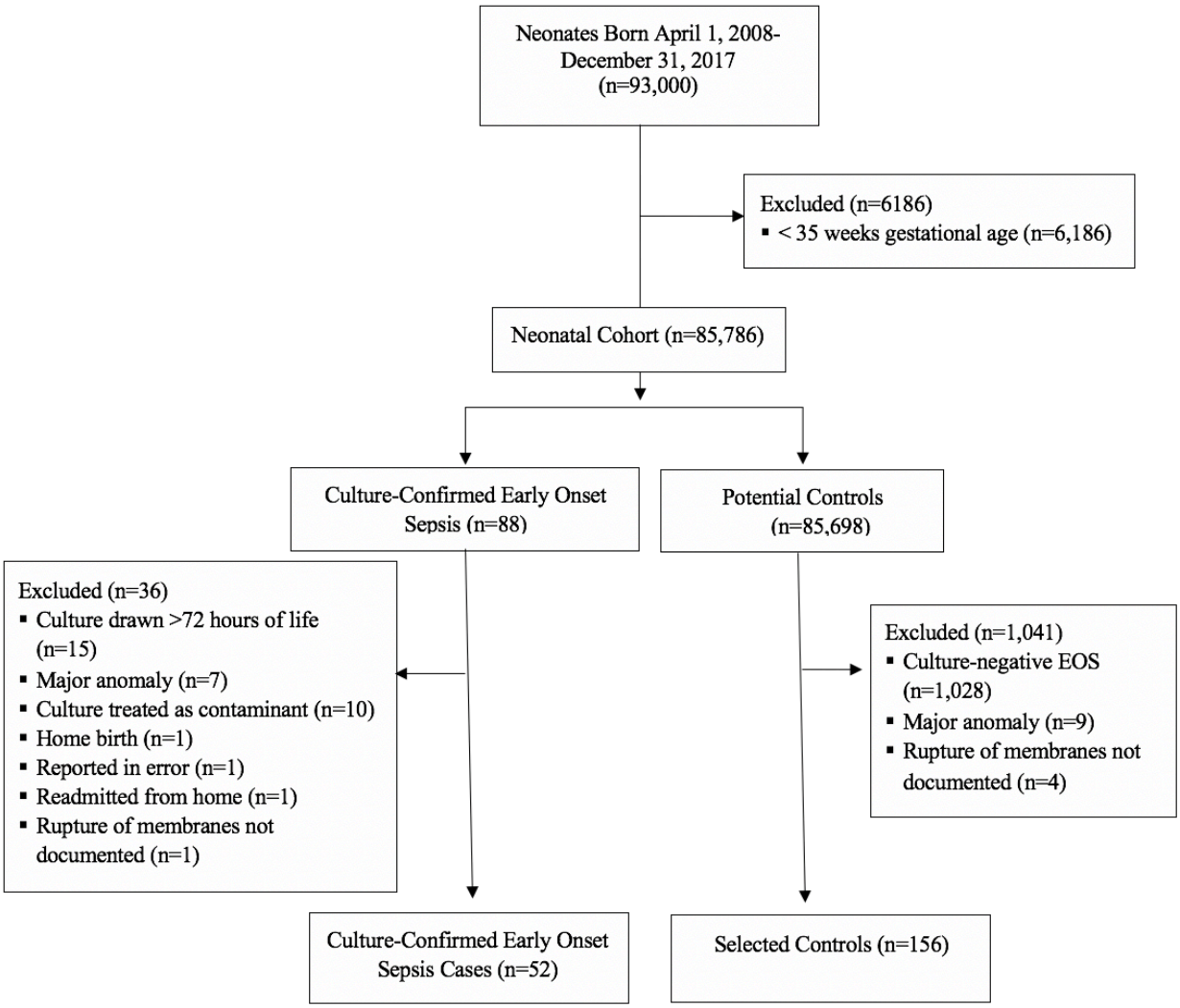

Figure 3. Flow diagram of neonates included in the study.

Table 6. Demographic, peripartum and screening tool characteristics for infants ≥ 35 weeks gestational age with culture-confirmed eary onset sepsis and their controls.

Characteristic	Cases (n=52)	Controls (n=156)
Demographic and Peripartum Characteristics		
Maternal Age, years, mean (SD)	28.5 (±4.9)	29.3 (±5.3)
Maternal Ethnicity		
White	30 (58)	106 (68)
Black	16 (31)	41 (26)
Other	6 (11)	9 (6)
Parity		
0	40 (77)	60 (38)
≥ 1	12 (23)	96 (62)
Anesthesia		
Epidural	43 (82)	112 (72)
Other	7 (14)	27 (17)
None	2 (4)	17 (11)
Mode of Delivery		
Vaginal	31 (60)	111 (71)
Caesarian	21 (40)	45 (29)

Table 6 (continued).

Characteristics Used by Screening Tools		
Highest Maternal Temperature		
< 38.0°C	21 (41)	150 (96)
38.0-38.9°C	22 (42)	6 (4)
≥ 39.0°C	9 (17)	0 (0)
Maternal GBS status		
Negative	41 (79)	119 (76)
Positive	11 (21)	32 (21)
Unknown	0 (0)	5 (3)
Maternal Intrapartum Antibiotics		
None or < 2 hours prior to birth	38 (73)	121 (78)
GBS IAP ≥ 2 hours prior to birth	8 (15)	31 (20)
Broad spectrum 2-3.9 hours prior to birth	1 (2)	0 (0)
Broad spectrum ≥ 4 hours prior to birth	5 (10)	4 (2)
Rupture of Membranes		
< 12 hours	20 (38)	129 (83)
12 to < 18 hours	13 (25)	14 (9)
18 to < 24 hours	6 (12)	9 (6)
≥ 24 hours	13 (25)	4 (2)

Table 6 (continued).

Gestational Age, weeks		
35-36	6 (12)	13 (8)
37-38	9 (17)	30 (19)
39-40	30 (58)	96 (62)
≥41	7 (13)	17 (11)
Maternal white blood cell count		
< 15,000 cells/mm^3	26 (54)	101 (66)
≥ 15,000 cells/mm^3	17 (35)	11 (7)
Unknown	9 (17)	44 (35)
Fetal Tachycardia (≥ 160 beats per minute)		
No	22 (42)	152 (97)
Yes	30 (58)	4 (3)
Mother with Suspected Intraamniotic Infection		
No	24 (46)	152 (97)
Yes	28 (54)	4 (3)
Infant Appearance 0-12 hours of birth		
Well-Appearing	26 (50)	136 (87)
Equivocal	9 (17)	14 (9)
Clinical Illness	17 (33)	6 (4)

°C: Celsius; GBS: group B *Streptococcus;* IAP: Intrapartum antibiotic prophylaxis.

Data are N (%) unless otherwise specified.

3.3.1 Test Characteristics of the Multivariate EOS Calculator and Categorical Approaches

The categorical approach using maternal diagnosis of suspected intraamniotic infection and infant clinical appearance identified a total of 47 (90%) infants with culture-confirmed EOS and 24 (16%) controls. The multivariate EOS risk calculator identified 35 (67%) infants with culture-confirmed EOS and 12 (8%) controls **(Table 7).** Thus, the multivariate EOS calculator had lower sensitivity for EOS (67% (95%CI: 54-79%) compared to the categorical approach (90%; 95%CI:79-96%; p<0.001) **(Table 8).** Five (10%) cases of EOS were not identified by either approach. Twelve (23%) cases were identified by the categorical approach only **(Table 7).** Of these, eight (67%) were positive for bacteria on peripheral blood culture and four (33%) were positive on cord blood culture. For these cases, the multivariate EOS calculator recommended vital signs every four hours for 24 hours for seven infants and routine vitals for five infants. Two patterns of risk factors emerged among cases of EOS not identified by the multivariate EOS calculator **(Table 9).** The first was infants ≥ 37 weeks gestational age with lower grade maternal fevers (38.0°C-39.1°C) and well appearing (N=11) and the second was an infant 35-36 weeks gestational age with no maternal fever, and with equivocal appearance (N=1).

Specificity was higher for the multivariate EOS calculator (92% (95%CI:87-96%) compared to the categorical approach (85%; 95%CI:78-89%; p=0.003) **(Table 8).** The two approaches were 90% concordant in ruling out controls. The AUC for the categorical approach (0.875; 95%CI:0.825-0.924) was significantly higher than AUC for the multivariate EOS calculator (0.798 (95%CI: 0.730-0.865; p=0.016) **(Figure 4).** The NNTb was 34 for the categorical approach and 71 for the multivariate EOS calculator.

Table 7. Infants identified by the categorical approach using suspected intraamniotic infection and clinical appearance and the multivariate EOS risk calculator.

		Categorical Risk Assessment		
	Cases	No blood culture	Blood culture	**Total cases**
Multivariate EOS Calculator	No blood culture	5 (10)	12 (23)	17 (33)
	Blood culture	0 (0)	35 (67)	33 (67)
	Total cases	5 (10)	47 (90)	52 (100)
	Controls	No blood culture	Blood culture	**Total controls**
	No blood culture	130 (83)	14 (9)	144 (92)
	Blood culture	2 (1)	10 (7)	12 (8)
	Total controls	132 (84)	24 (16)	156 (100)

EOS: early onset sepsis; Data are N (%).

On secondary analysis, where we considered infants with an equivocal exam as negative for the categorical approach, a total of 43 (83%) cases and 12 (8%) controls were identified **(Appendix Table 2).** The resulting sensitivity of 83% (95%CI:70-91%) was still significantly higher than the multivariate EOS calculator (p=0.021) but specificity of the categorical approach improved to 94% (95%CI: 89-97%) making it similar to the calculator (p=0.527) **(Table 8).** For the multivariate EOS calculator, when we considered the recommendation of vitals signs at least every four hours for 24 hours as a positive test, the calculator identified a total of 45 (87%) cases and 16 (8%) controls **(Appendix Table 3).** This made the test characteristics for this approach

similar to those of the categorical approach, whereby sensitivity increased to 87% (95%CI: 75-93; p=0.414); specificity decreased to 90% (95%CI: 84-94%) but was still higher than the categorical approach (p=0.033) **(Table 8).**

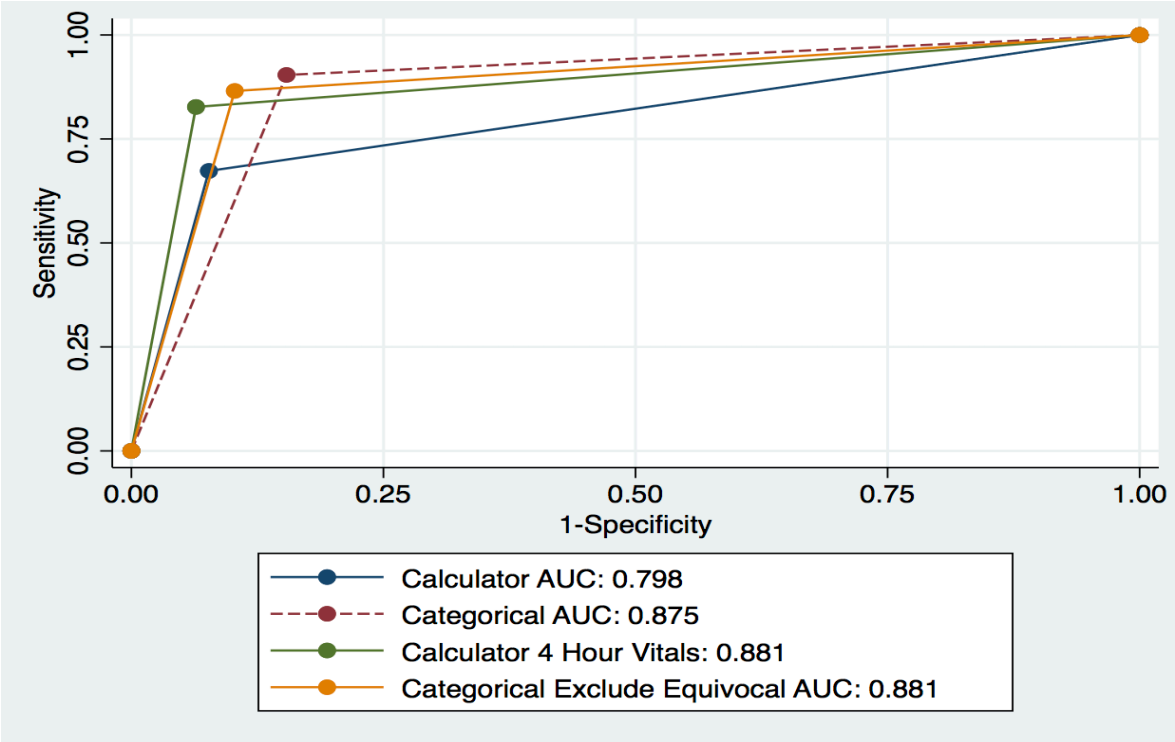

Figure 4. Area under the receivor operating curve for the categorical and multivariate EOS risk calculator for culture-confirmed EOS among infants ≥35 weeks gestational age.

3.4 Discussion

3.4.1 Principal Finings

In this analysis, we examined two EOS screening approaches supported by current AAP guidelines for their ability to identify culture-confirmed EOS through recommendation of

Table 8. Sensitivity, specificity, AUC, and NNTb for culture-confirmed EOS by the categorical approach and multivariate EOS risk calculator.

Definition	Sensitivity % (95%CI)	Specificity % (95%CI)	AUC N (95%CI)	NNTb N (95%CI)
Primary Analysis				
Categorical	90 (79-96)	85 (78-89)	0.875 (0.825-0.924)	34 (13-95)
Multivariate EOS Calculator	67 (54-79)	92 (87-96)	0.798 (0.730-0.865)	71 (31-171)
Secondary Analysis				
Categorical: Exclude Equivocal Exams as Positives	82 (70-91)	94 (89-97)	0.881 (0.727-0.863)	25 (10-66)
Multivariate EOS Calculator: Vitals at Least Every 4 Hours is Positive	87 (75-93)	90 (84-94)	0.881 (0.805-0.919)	31 (13-81)

AUC: area under the receiver operating curve; NNTb: number needed to treat to benefit; EOS: early onset sepsis; 95%CI: 95% confidence interval.

Table 9. Characteristics of infants with culture-confirmed EOS identified by the categorical approach but not identified by the multivariate EOS calculator.

ID	GA (w, d)	Max. Maternal Temp. (°C)	ROM (hrs)	GBS Status	Maternal Antibiotics	Infant Exam	Infant Culture Pathogen	Probability of EOS	Calculator Recommendation
1	37, 2	38.3	7.6	Neg	Broad >4hrs	Well	*L. monocytogenes*	0.22	Routine VS
2	39, 1	38.2	9.2	Pos	GBS IAP	Well	*A. neurii*	0.27	Routine VS
3	40, 6	38.1	3.3	Neg	None	Well	*S. gallolyticus*	0.30	Routine VS
4	39, 5	38.3	14.4	Pos	GBS IAP	Well	*S. mitis*	0.37	Routine VS
5	39, 0	38.1	14.3	Neg	None	Well	*S. mitis*	0.44	VS Q4hrs x24hrs
6	39, 1	38.4	15.6	Pos	GBS IAP	Well	*E. coli*	0.45	VS Q4hrs x24hrs
7	40, 2	38.1	17.1	Neg	None	Well	*E. faecalis*	0.48	VS Q4hrs x24hrs
8	39, 6	38.0	26.7	Pos	None	Well	*S. agalactiae*	0.49	VS Q4hrs x24hrs
9	41, 5	38.7	21.5	Neg	Broad >4hrs	Well	*S. gallolyticus*	0.61	VS Q4hrs x24hrs
10	39, 6	38.1	38.0	Neg	None	Well	*S. viridans*	0.68	VS Q4hrs x24hrs
11	39, 1	39.1	24.0	Neg	Broad >4hrs	Well	*S. anginosus*	0.80	VS Q4hrs x24hrs
12	35, 3	36.6	0.0	Pos	None	Equi.	*S. viridans*	0.98	Routine VS

EOS: early onset sepsis; GA: gestational age; w: weeks; d: days; Max: maximum; Temp: temperature; ROM: rupture of membranes; hrs: hours; GBS: Group B Streptococcus; Neg: Negative; Pos: Positive; Broad: broad spectrum antibiotics administered more than 4 hours before delivery; IAP: intrapartum antibiotic prophylaxis; Equi: equivocal; VS: vital signs; Q4hrs x 24 hrs: every 4 hours for 24 hours

obtaining a blood culture. Our data suggest that a categorical approach that combines maternal diagnosis of suspected intraamniotic infection and infant clinical exam during the first 12 hours of life may identify infants with culture-confirmed EOS superiorly to the multivariate EOS calculator when considering recommendation of blood culture as a positive test. Importantly, the higher sensitivity and AUC for the categorical approach identified on our primary analysis is eliminated when factoring in the multivariate EOS calculator's vital sign recommendations, making both approaches comparable. The multivariate EOS calculator generally maintains higher specificity than the categorical approach, which would significantly reduce the number of uninfected infants from whom a blood culture would be obtained. Specificity of the categorical approach can be improved, however, by adjusting how the infant's clinical appearance is incorporated (i.e. considering infants with equivocal findings as negative). While we expected the multivariate EOS calculator to have better diagnostic performance compared to a categorical approach on all measures, our data instead highlights how each approach has different strengths. Our study also illuminates how the infant's clinical appearance can and should be incorporated into a categorical approach using suspected intraamniotic infection. Finally, our data illustrate that some infants with EOS will be missed, regardless of screening approach. Thus, monitoring of all infants is critical during the first 24-72 hours of life.

3.4.2 Our Findings in the Context of Existing Literature

The multivariate EOS calculator in our study consistently demonstrated high specificity ranging from 90-92%. Similar to other studies, this would reduce laboratory evaluations and ultimately empiric antibiotics among well-appearing infants.(Achten et al., 2019; Kuzniewicz et al., 2017) Furthermore, implementation can be done safely, whereby a systematic review identified

no adverse events or delayed antibiotics for infants with EOS, and reduce costs.(Achten et al., 2019; Achten et al., 2020) However, concern about missing EOS remains.

In our analysis, we found that the multivariate EOS calculator did not recommend blood culture in 17 infants with EOS, 12 of whom were identified by the categorical approach. This gives it relatively poor sensitivity for EOS (67%, 95%CI: 54-79). In another recent metanalysis of EOS cases among studies examining the calculator, it was estimated that 19-29% of infants with EOS would receive delayed or missed treatment.(Pettinger, Mayers, McKechnie, & Phillips, 2020) Also, similar to our study, the calculator was more likely to miss EOS among those with chorioamnionitis.(Pettinger et al., 2020) It is possible that underlying differences in patient populations/settings compared to the population from which it was derived may limit the calculator's generalizability and reduce its overall diagnostic ability.(Good & Hooven, 2019; Kuzniewicz et al., 2017) Yet, the calculator did recommend vital signs every four hours for the majority of those infants. Under these circumstances, an infant would receive serial exams from a clinician, typically the bedside nurse. This makes it likely than an infant who is developing clinical illness would be rapidly identified and receive escalated care. Thus, it may not be accurate to consider these infants as "missed". We accounted for this in our secondary analysis, which indeed enhanced the sensitivity of the multivariate EOS calculator for culture-confirmed EOS. Under these circumstances, only 13% of EOS cases would be "missed", with recommendation for routine care. While in some facilities, all infants undergo vital signs every 4 hours for 24 hours as part of routine care, in others, routine care may include vital signs every 8-12 hours. Thus, it is possible that under routine care an infant with EOS may progress to clinical illness without being rapidly identified and triaged. This highlights that no matter which screening approach is used, some infants with EOS will be missed and that all infants warrant close monitoring in the first 24-72

hours of life. To this end, the multivariate EOS calculator could further mitigate risk of a "missed" case of EOS by specifying that vital signs every four hours for 24 hours is recommended for all low-risk infants.

3.4.3 Implications for Clinical Practice and Research

The categorial approach to EOS screening using a maternal diagnosis of clinical chorioamnionitis was still being used by greater than 60% of nurseries in 2017.(Mukhopadhyay et al., 2017) While this has likely decreased since then with increasing popularity of the multivariate EOS calculator, our study supports that implementation of a categorical approach using a new threshold of suspected intraamniotic infection and the clinical appearance of the child can be a powerful tool in EOS screening. It also has the added advantage of familiarity and established clinical pathways, which may enhance compliance and implementation. We previously identified that criteria for maternal suspected intraamniotic infection incrementally improves discrimination of infants with EOS compared to clinical chorioamnionitis (Chapter 2). However, sensitivity was still low overall at only 53% (95%CI: 40-66%). Incorporating the evolving physical exam for the infant into EOS screening has proven to be of great importance for both the multivariate EOS calculator and the serial exams approaches. Thus, incorporating it into the categorical approach makes it more directly comparable. It is also more reflective of clinical practice where a sick infant, regardless of maternal risk factors, will be evaluated for EOS. Yet, how illness is defined significantly impacts the test characteristics of this approach. Including infants with an equivocal exam enhances sensitivity, while excluding them enhances specificity. Deciding which threshold to use can therefore vary depending on institutional and provider comfort with the risks associated with EOS and the risks from empiric treatment.

An inherent weakness of the categorical approach as we define it is its reliance on a maternal clinical diagnosis of suspected intraamniotic infection. Prior to ACOG's guidelines establishing the criteria for suspected intraamniotic infection, there was significant variation in how and when clinical chorioamnionitis was diagnosed. While there are currently no studies evaluating how this has changed under the current guidelines, it is reasonable to assume that variation in diagnosis persists. The multivariate EOS calculator offers a distinct advantage of calculating probability based on risk factors that are readily and reliably obtainable in the EHR independent of a maternal clinical diagnosis. In fact, the calculator can be incorporated into some EHRs so that an EOS probability is obtained on every infant without specific provider input.(Fowler, Garcia, & Hankins, 2019; Stipelman et al., 2019) Furthermore, the multivariate EOS calculator can tailor probability of EOS based on local incidence of EOS, which allows a more personalized risk assessment.

3.4.4 Limitations

It is important to recognize the limitations of this data including its case control design at a single institution. While the case control design can be used to obtain direct estimates of sensitivity and specificity, it only provides indirect estimates for negative and positive predictive value, as these rely on disease prevalence in the underlying population, which is artificially inflated in a case control study.(Steinberg et al., 2009) Additionally, as an urban, tertiary referral care center, which cares for many high-risk pregnancies and infants, it is unclear if our results are generalizable to other populations. Finally, the retrospective nature of our study subjects data to information and selection bias. For example, maternal fevers may only be based on one measured temperature as repeat temperatures are not standard practice in our institution. Furthermore,

clinical exam may be obscured for some infants based on what was documented and if they were treated empirically for EOS. For these reasons, prospective validation studies to further examine our findings are critical. Our institution also obtains cord blood cultures for infants exposed to maternal chorioamnionitis/suspected intraamniotic infection. Cord blood cultures can expand identification of bacteria in EOS, particularly in high-risk infants.(Kalathia, Shingala, Parmar, Parikh, & Kalathia, 2013; Meena et al., 2020; J. I. Polin et al., 1981) However, the multivariate calculator was validated among infants with EOS on peripheral blood cultures, thus it is unclear how this would impact its results. Importantly, though, this only affected a small proportion of the infants with EOS.

3.4.5 Conclusion

The results of our study suggest that a categorical approach using the combination of maternal suspected intraamniotic infection and infant clinical appearance and the multivariate EOS calculator are comparable for identification of infants with EOS. However, the increased specificity of the multivariate EOS calculator makes it less likely for this approach to result in empiric treatment of uninfected infants. Nevertheless, each approach has limitations that will result in some missed cases of EOS. Therefore, it is critical that vigilance through vital sign and physical exam monitoring is utilized for all infants regardless of maternal or peripartum risk factors.

4.0 Diagnostic Value of Placenta Histopathology and Culture for Neonatal Early Onset Sepsis

4.1 Introduction

Neonatal early onset sepsis (EOS) with a culture-confirmed infection is rare but can have high morbidity and mortality.(Puopolo, Benitz, Zaoutis, et al., 2018) The American Academy of Pediatrics supports using a maternal diagnosis of suspected intraamniotic infection as a categorical approach to identify infants who warrant empiric laboratory evaluations and antimicrobial therapy for potential EOS.(Puopolo, Benitz, Zaoutis, et al., 2018) This intrapartum diagnosis, which is based on maternal fever, leukocytosis, fetal tachycardia, and amniotic fluid purulence, uses revised criteria set by the American College of Obstetrics and Gynecologists (ACOG) in 2017 that replaced the maternal diagnosis of clinical chorioamnionitis.(ACOG, 2017) We have previously shown that infant exposure to maternal suspected intraamniotic infection combined with clinical appearance identifies between 82-90% of infants with culture-confirmed EOS. However, this approach has moderate specificity at 85-94%. This limits its ability to rule-out infants who are not infected and could result in unnecessary exposure of these infants to broad-spectrum antibiotics, mother-infant separation, and prolonged length of hospital stay. As over 60% of newborn nurseries report continued use of a categorical approach for EOS screening, there is a need to improve its diagnostic specificity.(Mukhopadhyay et al., 2017)

The placenta, which can be reliably accessed in all hospital deliveries, can provide important information about fetal *in utero* exposure to inflammation and infection. Acute histologic chorioamnionitis, which reflects maternally derived neutrophilic invasion of the

chorion, and acute histologic funisitis, which reflects fetally derived neutrophilic invasion of the umbilical cord, are present in 71% and 79% of cases with culture-positive amniotic fluid, respectively.(Kim et al., 2015; Romero et al., 1992) Consequently, the revised ACOG criteria use inflammation of the placenta on histopathology or evidence of infection on placenta or amniotic fluid gram-stain/culture as the gold standard to diagnose maternal intraamniotic infection.(ACOG, 2017) However, it is unknown whether using placenta inflammation or culture among infants exposed to suspected intraamniotic infection can enhance discrimination between infants with and without culture-confirmed EOS. Such findings could be used by clinicians to decide whether to empirically evaluate and treat or when to stop empiric treatment for infants exposed to suspected intraamniotic infection *in utero*.

Our aim with this study was to evaluate whether inflammation on placenta histopathology or bacteria growth on placenta culture can be used to distinguish infants with culture-confirmed EOS from culture-negative infants among a cohort of well-appearing infants ≥ 35 weeks gestational age who were exposed to suspected intraamniotic infection *in utero*. We hypothesized that presence of funisitis or umbilical cord vasculitis, which result from a fetal inflammatory response, would best discriminate between infants with and without culture-confirmed EOS.

4.2 Methods

4.2.1 Study Population

To identify a cohort of well-appearing infants exposed to suspected intraamniotic infection, we first used the electronic health record (EHR) to identify infants ≥35 weeks gestational age from

10/1/2015 to 11/30/2017 with ICD-10 diagnosis codes of P027 (exposure to maternal chorioamnionitis) and P36 (bacterial sepsis) from a single-tertiary academic birth hospital. After abstracting maternal and infant vital signs, laboratory data, and antibiotics from the EHR into a REDCap database, we then excluded infants who did not have a blood culture drawn within 72 hours of birth, were born outside of the hospital or with significant anomalies as defined by the Vermont-Oxford Neonatal Network (www.vtoxford.org). We also excluded infants whose mother did not have a documented fever of 38.0C or higher at least once during her pre-delivery course or a placenta pathology report available. We then categorized maternal symptoms during delivery as either isolated maternal fever (documented fever of 38.0-38.9C only) or suspected intraamniotic infection (documented fever of ≥39.0C or 38.0-38.9C and either fetal tachycardia ≥160 beats per minute, purulent amniotic fluid, or white blood cell count ≥15,000 cells/mm^3). We then excluded infants with isolated maternal fever only. Next, we excluded infants who were critically ill within 12 hours of life, which we defined as infants with 5 minute Apgar less than 5, requiring respiratory support (nasal cannula, CPAP, intubation), vasopressor support or with on-going seizures or apnea.

4.2.2 Placenta Pathology

We evaluated exposure of infants to intraamniotic inflammation and infection using placenta histopathology reports and culture results collected at time of delivery and available in the EHR. Pathologists consistently used the widely accepted Redline criteria to categorize placental lesions.(Redline, 2015) Amniotic fluid was not obtained during any included deliveries so could not be evaluated. We categorized intraamniotic inflammation as either maternal or fetal in origin based on anatomic positioning and origin of inflammatory cells.(Kim et al., 2015; Redline, 2012, 2015) Inflammation was characterized as maternal if described as deciduitis,

subchorionitis or mild (Stage I), moderate (Stage II) or severe (Stage III) chorioamnionitis. Inflammation was characterized as fetal if described as chorionic plate vasculitis, umbilical cord vasculitis (venous or arterial) or funisitis. Any bacteria growth on placenta culture that was not classified as "vaginal flora" was considered a positive culture.

4.2.3 Outcome

We defined our outcome of culture-confirmed EOS as infants with bacteria growth on their blood culture obtained peripherally or with cord blood. Obtaining cord blood culture, in addition to peripheral culture, for infants exposed to clinical chorioamnionitis or suspected intraamniotic infection was standard practice in our institution throughout the study time-frame. Cultures with ≥ 2 organisms or single organisms with *Aerococcus* or *Staphylococcus* species other than *Staphylococcus aureus* were considered contaminants.(Schrag et al., 2016) All other infants were considered culture-negative for EOS.

4.2.4 Statistical Analysis

Descriptive statistics. Baseline characteristics of the cohort were described using means and proportions. Distinct placenta features were compared between culture-confirmed EOS and culture negative infants using Fisher's exact tests. A Bonferroni correction was used to adjust for multiple comparisons where an alpha of less than 0.005 indicated significance.

Test characteristics of placenta histopathology and culture. We used the results of the univariate analysis to guide selection of specific placenta features for further analysis. This included umbilical cord vasculitis or funisitis, which were combined into a new variable umbilical

cord inflammation, and bacteria growth on placenta culture. We then evaluated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) with Wilson's 95% confidence intervals and area under the receiver operating curve (AUC) of these variables for culture-confirmed EOS independently. We compared sensitivity and specificity for these two variables using a McNemar test and their AUC using DeLong chi-squared test. Alpha of <0.05 was considered significant. We then examined how these two features could be used in combination as serial tests. In serial testing, placenta culture results were only considered if umbilical cord inflammation was present and the patient had to be positive for both to be considered positive. We then calculated net sensitivity, specificity, PPV, NPV, AUC with Wilson's 95% CIs. All analyses were completed using Stata 15.0 (Stata Corp, College Station, Texas). This study was approved by the University of Pittsburgh institutional review board (PRO17110548).

4.3 Results

Of the 513 infants we identified in the EHR, 145 met inclusion criteria **(Figure 5).** Cohort demographic and peripartum characteristics are described in **Table 10**. Fourteen (9.7%) infants had culture-confirmed EOS. Eight (57.1%) positives resulted from cord blood cultures, six (42.9%) were from peripheral cultures. Group B *Streptococcus* (N=5, 35.7%) and *Streptococcus mitis* (N=3, 21.4%) were the most common pathogens. 131 (90.3%) infants had negative blood cultures. 41 (31.3%) of those infants received antibiotics for ≥ 5 days based on abnormal laboratory values. No infants developed new symptoms consistent with critical illness 24 hours post-delivery.

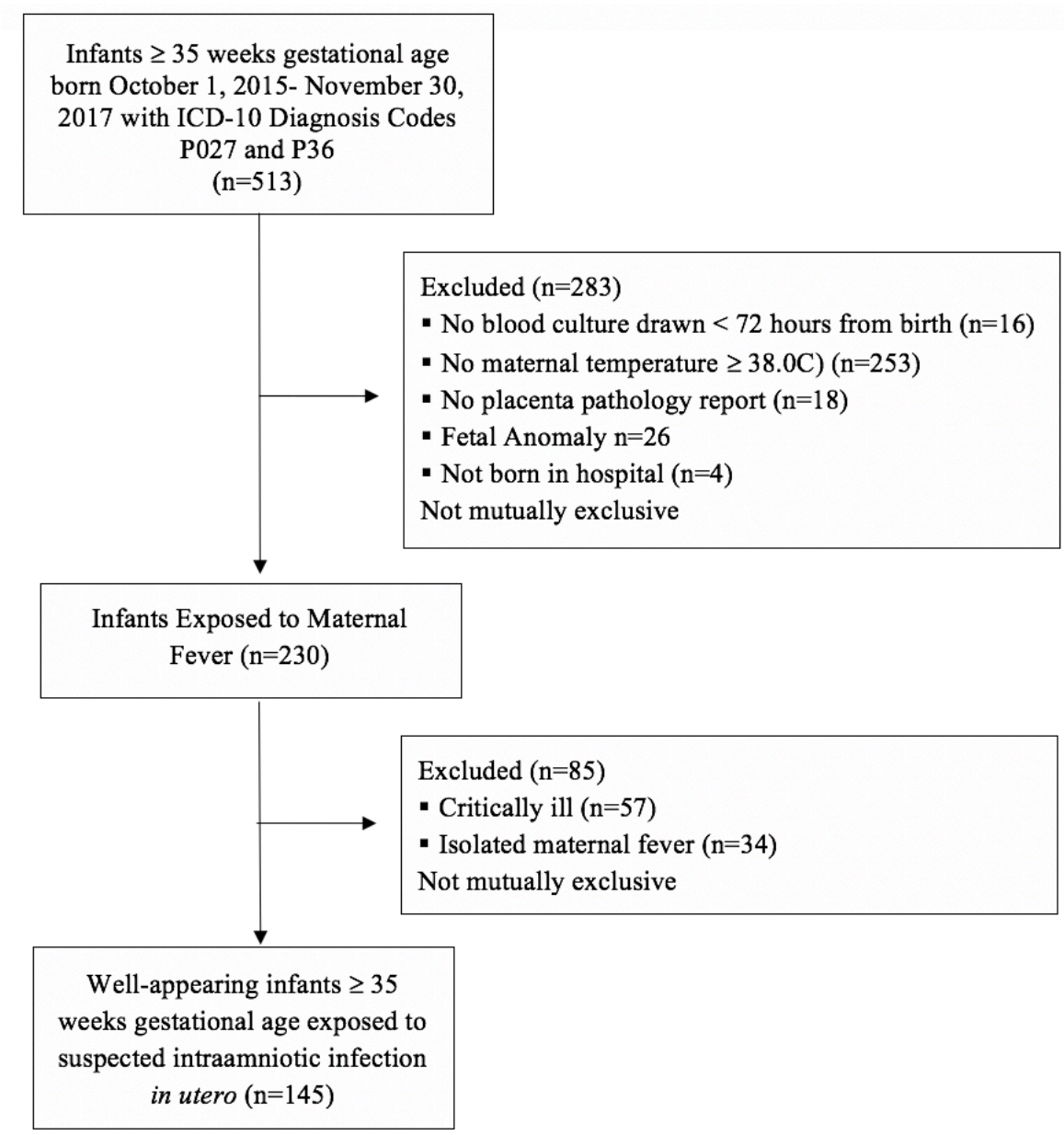

Figure 5. Flow diagram of infants included in the study.

Table 10. Demographic and Peripartum Characteristics of Well-Appearing Infants ≥ 35 weeks Gestational Age Exposed to Suspected Intraamniotic Infection *in utero*.

Characteristic	**Overall Cohort N=145**
Maternal Race	
Caucasian	79 (54.5)
African American	37 (25.5)
Asian	21 (14.5)
Other	2 (1.4)
Missing/Unknown	6 (4.1)
Maternal Age (years)	
<20	10 (6.9)
≥20-34	115 (79.3)
≥35	20 (13.8)
GBS Status	
Negative	117 (80.7)
Positive	27 (18.6)
Unknown	1 (0.7)

Table 10 (continued).

Maternal Antibiotics (hours prior to delivery)	**13 (9.0)**
None	
GBS Specific	**11 (7.6)**
< 4	**6 (4.1)**
≥ 4	
Broad Spectrum	
< 4	**82 (56.6)**
≥ 4	**33 (22.8)**
Mode of Delivery	
Vaginal	67 (46.2)
Caesarian	78 (53.8)
ROM ≥ 18 hours	
No	83 (61.0)
Yes	53 (39.0)
Highest Maternal Temperature	
38.0-38.9 °C	105 (72.4)
≥ 39.0 °C	40 (27.6)

Table 10 (continued).

Gestational Age (weeks)	
≥ 35-36	6 (4.1)
37-38	29 (20.0)
39-40	80 (55.2)
≥ 41	30 (20.7)
Low Birth Weight (grams)	
< 2500	3 (2.1)
≥ 2500	142 (97.9)
Infant Sex	
Male	63 (42.5)
Female	82 (56.5)

N (%). GBS: group B *Streptococcus;* ROM: Rupture of Membranes If received both broad and GBS antibiotics, classified based on broad antibiotics

Table 11. Comparison of Placenta Features in Women with Suspected Intraamniotic Infection during Labor by Infant Blood Culture Status using Fisher's Exact Tests.

Placenta Feature	Culture- Confirmed EOS (N=14)	Culture Negative (N=131)	P-value
≥ 1 Feature of Maternal Inflammation	14 (100.0)	114 (87.0)	0.374
Acute deciduitis	6 (42.9)	44 (33.6)	0.558
Acute subchorionitis	0 (0.0)	15 (11.5)	0.362
Acute chorioamnionitis	14 (100.0)	104 (79.4)	0.073
Unknown	0 (0.0)	1 (0.8)	0.130
Mild	2 (14.3)	28 (21.4)	
Moderate	8 (57.1)	67 (51.1)	
Severe	4 (28.6)	8 (6.1)	
≥ 1 Feature of Fetal Inflammatory Response	14 (100.0)	95 (72.5)	0.022
Chorionic plate acute fetal vasculitis	13 (92.9)	91 (69.5)	0.114
Umbilical cord with acute vasculitis	11 (78.6)	61 (46.6)	0.026
Acute funisitis	12 (85.7)	50 (38.2)	**0.001**

Table 11 (continued).

Bacteria Growth on Placenta Culture	**9/13 (69.2)**	**11/111 (9.9)**	<0.001

N (%) unless otherwise specified EOS: early onset sepsis. Bolded p-values indicates significance using a Bonferroni correction where p-value less than 0.005 is significant.

Three pathologists interpreted 90% of the placenta histopathology slides. The median time from delivery to pathology report in the EHR was 69 hours (interquartile range 43 to 93 hours). Acute histologic chorioamnionitis and acute chorionic plate vasculitis were the most common histologic findings, occurring in over 80% and 70% of all placentas, respectively **(Table 11**). Placenta fetal inflammation consistent with funisitis and bacteria growth on placenta culture occurred more frequently among infants with culture-confirmed EOS than for infants who were culture negative ($p=0.001$ and $p<0.001$ respectively).

Umbilical cord inflammation demonstrated higher sensitivity than bacteria growth on placenta culture, identifying 100% of infants with culture-confirmed EOS ($p=0.046$**, Table 12).** Conversely, bacteria growth on placenta culture demonstrated significantly higher specificity (90.1% versus 45.8%; $p=<0.001$). However, there was no difference in AUC ($p=0.350$, **Figure 6).** Combining umbilical cord inflammation and bacteria growth on placenta culture demonstrated AUC of 0.813 (95%CI: 0.680-0.945) with a NPV of 96.6% (91.5-98.7). Importantly, four infants with culture-confirmed EOS who had umbilical cord inflammation became false negatives on serial testing due to negative placenta cultures.

Table 12. Test characteristics of umbilical cord inflammation and placenta culture for culture-confirmed EOS among infants ≥ 35 weeks gestational age exposed to suspected intraamniotic infection in utero with Wilson's 95% confidence intervals.

Feature	AUC N (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Umbilical Cord Inflammation	0.729 (0.686-0.772)	100.0 (78.5-100.0)	45.8 (37.5-54.3)	16.5 (10.1-25.8)	100.0 (94.0-100.0)
Bacteria Growth on Placenta Culture	0.797 (0.663-0.930)	69.2 (42.4-87.3)	90.1 (83.1-94.4)	45.0 (25.8-65.8)	96.2 (90.5-98.5)
Umbilical Cord Inflammation AND Bacteria Growth on Placenta Culture	0.813 (0.680-0.945)	69.2 (42.4-87.3)	93.3 (87.4-96.6)	52.9 (31.0-73.8)	96.6 (91.5-98.7)

EOS: early onset sepsis; AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value; umbilical cord inflammation: acute umbilical vasculitis or funisitis.

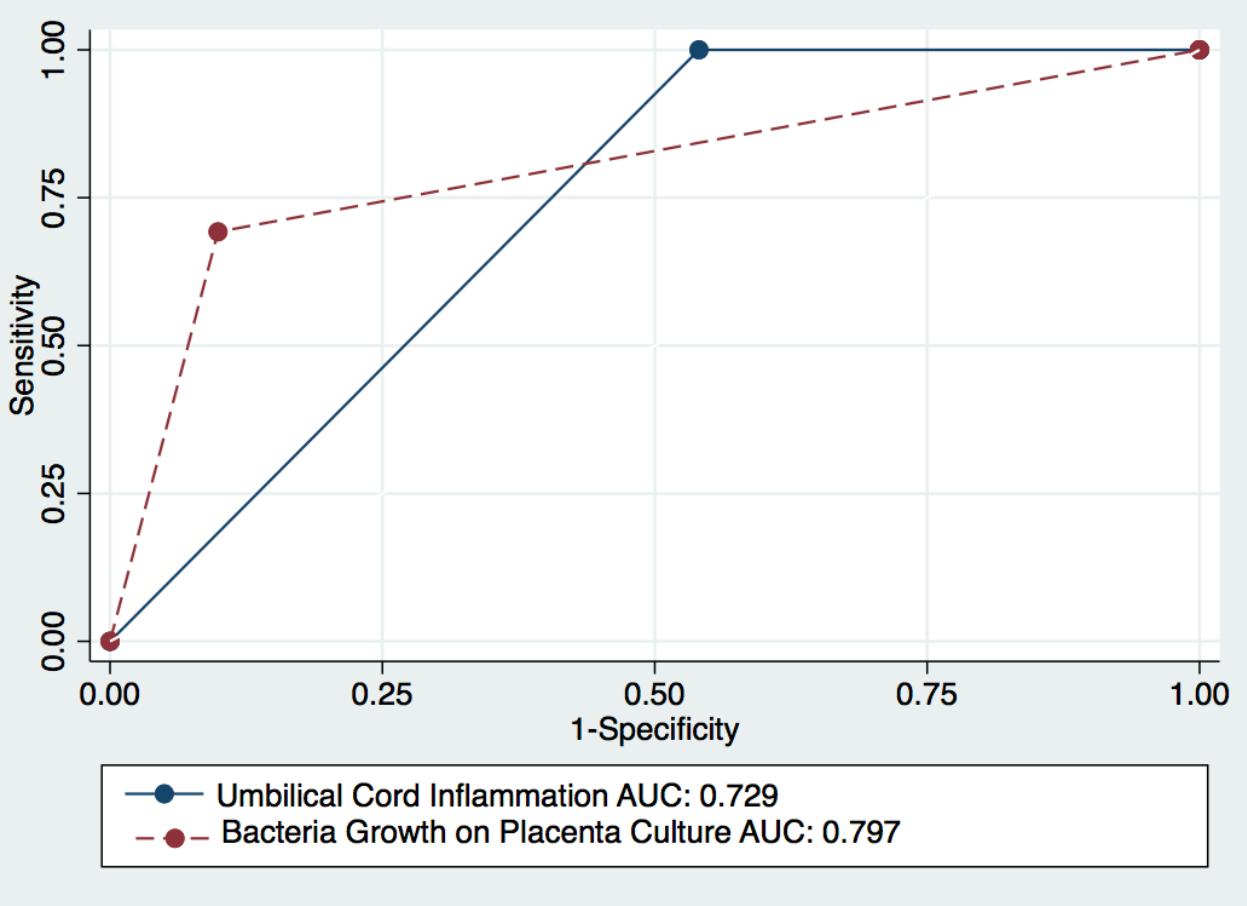

Figure 6. Comparison of AUC for Umbilical Cord Inflammation and Placenta Culture

4.4 Discussion

4.4.1 Principal Findings

Our findings demonstrate that a two-step approach using presence of fetal inflammation of the umbilical cord and bacteria growth on placenta culture can effectively distinguish well-appearing infants exposed to suspected intraamniotic infection *in utero* who have culture-confirmed EOS from infants who are culture negative. Thus, incorporating the results of placenta

histopathology and culture into the algorithm for evaluating infants exposed to suspected intraamniotic infection can improve the specificity of this categorical approach for neonatal EOS evaluations at two important clinical decision-points.

4.4.2 Implications for Clinical Practice and Research

First, is its potential to guide the initial decision to evaluate and treat an infant exposed to maternal suspected intraamniotic infection. In our cohort, umbilical cord inflammation identified all exposed infants with culture-confirmed EOS and ruled-out 45% of culture negative infants. The advantage of this approach is that it utilizes the placenta, an organ that is readily available in all deliveries, and can avoid invasive evaluations of many infants. The disadvantage is that for histopathology to be useful in the initial triage of an infant, it requires rapid evaluation of the placenta. In most institutions, including our own, this is not routinely done. In this cohort, median time to histopathology report was nearly three days from delivery but can take up to one week in some institutions.(Mahe et al., 2014) Frozen sections, however, can be completed in as little as twenty minutes and demonstrate reasonable accuracy compared to formalin-fixed paraffin-embedded methods.(Mahe et al., 2014; Mendilcioglu et al., 2003; Novis & Zarbo, 1997) Therefore, strong consideration of expanding and further evaluating this technique for its potential to augment pediatric clinical decision-making for EOS at time of delivery is needed.

The second opportunity to utilize these findings is when deciding duration of empiric antibiotic treatment in culture-negative infants exposed to suspected intraamniotic infection. While a positive blood culture for a bacterial pathogen typically confirms infection in infants and thus continuation of antibiotics, a negative blood culture does not necessarily rule-out bacterial infection. This phenomenon, known as culture-negative EOS, may account for 14-60% of infants

treated for EOS.(Garges et al., 2006; Squire, Favara, & Todd, 1979) As negative cultures may be secondary to insufficient blood volume or maternal intrapartum antibiotic exposure, these infants are typically diagnosed based on symptoms or abnormal laboratory values including elevated white blood cell count, C-reactive protein, and procalcitonin.(Benitz, Wynn, & Polin, 2015; Connell, Rele, Cowley, Buttery, & Curtis, 2007; Neal et al., 1986; Ottolini et al., 2003; J. I. Polin et al., 1981; Schelonka et al., 1996; Wynn et al., 2014) However, while abnormal values demonstrate good sensitivity for culture-confirmed EOS, they have poor specificity, resulting in poor positive predictive value.(Wynn et al., 2014) Consequently, among well-appearing infants exposed to suspected intraamniotic infection with a negative blood culture at 36-48 hours, these are inadequate markers to decide whether treatment should continue or not and will result in over-treatment. Our two-step approach among our cohort, however, resulted in a net NPV of 96.6% (95%CI: 91.5-98.7%) and a PPV of 52.9% (95%CI: 31.0-73.8%). While this has similar NPV to other laboratory values, PPV is substantially higher than single or serial CRP and similar to that of procalcitonin.(Auriti et al., 2012; Benitz, Han, Madan, & Ramachandra, 1998; Lacaze-Masmonteil, Rosychuk, & Robinson, 2014; Wynn et al., 2014) Consequently, this approach in a well-appearing but exposed neonatal population could reduce the reliance on invasive inflammatory markers with poor predictive value for neonatal EOS and reduce prolonged antibiotic exposure.

Importantly, negative placenta culture “ruled-out” 4 infants with culture-confirmed EOS. However, in an algorithm where infants who have umbilical cord inflammation still receive a blood culture, these infants would still have been identified by their blood culture, regardless of placenta culture. Therefore, to reduce the risk of false negatives or “missing” an EOS case, a blood culture should still be obtained when umbilical cord inflammation is present.

4.4.3 Our Findings in the Context of Existing Literature

Our findings that placenta inflammation and bacterial growth among 70-80% of all placentas, which confirms the diagnosis of suspected intraamniotic infection according to ACOG's criteria, is consistent with previous studies.(Ona et al., 2019; Romero, Chaemsaithong, Korzeniewski, et al., 2016) However, our study uniquely evaluates how confirmed intraamniotic infection can be utilized to distinguish infants with and without culture-confirmed EOS.

We identified that fetal inflammation and positive culture of the placenta is strongly associated with culture-confirmed EOS, which is supported by previous literature.(Du et al., 2017; Lau et al., 2005; Tita & Andrews, 2010; Yoder et al., 1983) Funisitis is strongly associated with Fetal Inflammatory Response Syndrome (FIRS), which is characterized by elevated IL-6 in cord blood.(Kim et al., 2015; Romero, Chaemsaithong, Docheva, et al., 2016; Tita & Andrews, 2010) This systemic response propagates the infiltration of the umbilical cord with leukocytes. FIRS due to infection results in substantially increased risk of perinatal morbidity, including multiorgan injury and cerebral palsy, and mortality.(Lau et al., 2005; Tita & Andrews, 2010; Yoder et al., 1983) Thus, funisitis among infants exposed to suspected intraamniotic infection helps identify those infants who had an *in utero* systemic response to a provocation, which in the context of maternal fever, is most concerning for sepsis.(Romero, Chaemsaithong, Docheva, et al., 2016; Tita & Andrews, 2010) Conversely, absence of funisitis suggests that the fetus either did not have or was early in a systemic response making the overall risk for neonatal EOS much lower. However, while umbilical cord inflammation was 100% sensitive in this study for culture-confirmed EOS, making its NPV 100%, this is unlikely to be true for all EOS cases. For example, in our previous work (Chapter 2), we determined that 4 infants with culture-confirmed EOS who were exposed to suspected intraamniotic infection *in utero* had no evidence of placenta

inflammation or placenta culture growth. Thus, further validation of using umbilical cord inflammation in different populations or through prospective studies is warranted to confirm its utility. Additionally, algorithms that incorporate placenta histopathology should still include a blood culture to reduce false negatives.

Previous studies have demonstrated that culture-confirmed intraamniotic infection using amniotic fluid is not synonymous with culture-confirmed EOS using neonatal blood culture.(Kim et al., 2015; Romero, Chaemsaithong, Docheva, et al., 2016) In one study, 0 of 25 infants had positive blood cultures.(Romero, Chaemsaithong, Docheva, et al., 2016) Similarly, in our data, 10% of infants who were culture negative had positive bacteria growth on placenta culture. Notably, however, use of cord blood culture at our institution increased the total number of positive blood cultures among our cohort. Increased positivity of cord blood cultures compared to peripheral cultures has also been demonstrated elsewhere.(Kalathia et al., 2013; Meena et al., 2020) This would consequently improve the overall specificity and PPV of umbilical cord inflammation and placenta culture for neonatal EOS, as these would have been classified as negative if only a peripheral culture had been obtained. Thus, caution should be used in applying our results to setting where only a peripheral culture is obtained.

4.4.4 Limitations

Our study is limited by the retrospective nature of the data, which subjects it to surveillance and confirmation bias. Furthermore, while three experienced pathologists interpreted the majority of the placenta histopathology, they were not blinded to the clinical history and there is no way to assess inter-rater reliability and thus interpretations, particularly of inflammation severity, may vary.

4.4.5 Conclusion

Our overall findings suggest that incorporating placenta histopathology and culture results into the algorithm for screening and subsequent management of well-appearing infants ≥ 35 weeks gestational age, exposed to suspected intraamniotic infection *in utero* for EOS can substantially improve the specificity and NPV of this approach. This has important implications for clinicians who are utilizing a categorical approach to neonatal EOS screening but would like to reduce the exposure of uninfected infants to invasive laboratory evaluations and systemic antibiotics. Development and expansion of rapid pathology techniques should be considered to enhance real-time clinical decision making for clinicians.

5.0 Conclusion

5.1 Summary of Major Findings

Through these analyses, we aimed to validate the new obstetric diagnoses for intraamniotic infection, known as Triple I, for their ability to identify early onset sepsis among infants ≥ 35 weeks gestational age. Through the series of studies presented, we first determined that suspected intraamniotic infection modestly improves the identification of infants with and without EOS compared to clinical chorioamnionitis as demonstrated by a numerically higher sensitivity and significantly higher AUC. This supports its use in obstetric and pediatric practice over the traditional clinical chorioamnionitis criteria. However, with a sensitivity of only 53% (95%CI: 40-66) and an AUC of 0.752 (95%CI: 0.682-0.821), we determined that by itself, suspected intraamniotic infection remains a suboptimal screening tool for EOS as it would still miss nearly half of the cases of EOS and result in over-treatment of many uninfected infants.

However, given its familiarity to obstetric and pediatric practitioners due to its similarity to clinical chorioamnionitis, we sought to determine if incorporating the infant's clinical appearance into a categorical approach with suspected intraamniotic infection would enhance its diagnostic utility at time of delivery. This is reasonable as clinicians inherently use physical exam in their initial assessments for EOS; and physical exam is a critical element of both the multivariate EOS calculator and serial exams. We then sought to compare this approach to the multivariate EOS calculator. Here, we identified that using a categorical approach of either exposure to suspected intraamniotic infection or evidence of clinical illness increased the test characteristics of this approach dramatically. Sensitivity went from 53% (95%CI: 40-66) to a maximum of 90%

(95%CI: 79-96%) and AUC increased to 0.875 (0.825-0.924). Furthermore, when comparing it to the multivariate EOS calculator, we were surprised to learn that the categorical approach recommended blood culture in a significantly higher number of infants with EOS compared to the calculator. However, this difference nearly disappeared if close observation through frequent vital signs was considered a positive test for the calculator. Regardless, specificity of the categorical approach remained lower than that of the calculator. Thus, while a categorical approach using exposure to suspected intraamniotic infection and infant clinical exam would identify most infants with EOS, it would continue to identify many infants who are not infected compared to the multivariate EOS calculator. This would unnecessarily expose them to laboratory evaluations and broad-spectrum antibiotics.

Consequently, we sought to determine if criteria for confirmed intraamniotic infection could be useful in enhancing specificity of this categorical approach. Among well-appearing infants ≥ 35 weeks gestational age exposed to suspected intraamniotic infection *in utero*, we identified that incorporating placenta histopathology, specifically umbilical cord inflammation, and placenta culture results into the algorithm for EOS screening could substantially improve its specificity and NPV. In this approach, nearly half of well-appearing infants exposed to suspected intraamniotic infection could be ruled-out through absence of umbilical cord inflammation and up to 90% could be ruled-out by combining that with placenta culture results. This could significantly reduce exposure of uninfected infants to laboratory evaluations and antibiotics if the results could be available to clinicians at critical decision points including initial treatment determination and at 36-48 hours when treatment continuation is decided. However, to garner the maximum benefit from placenta data, development and expansion of rapid pathology techniques are needed and thus a major limitation at this time.

5.2 Proposed Algorithm for Categorical Approach

For institutions who are currently or intending to use a categorical approached based on the new ACOG diagnoses for intraamniotic infection, we have developed a clinical algorithm for infants ≥ 35 weeks gestational age who are exposed to maternal fever ≥38 °C that incorporates our findings **(Figure 7)** and may be used by clinicians to guide treatment decisions. Of note, while this algorithm will still reduce empiric evaluations and treatment of infants exposed to isolated maternal fever, and could reduce overall laboratory evaluations, reducing empiric treatment of well-appearing infants exposed to suspected intraamniotic infection is predicated on placenta pathology results being rapidly available. In the absence of this service, this algorithm will optimize identification of infants with EOS and guide clinicians in their treatment decisions at 36-48 hours of life.

5.2.1 Categorizing Maternal Fever

After identifying an infant who is ≥ 35 weeks gestational age and exposed to maternal fever ≥38 °C prior to delivery, the clinician should categorize maternal fever as being either isolated maternal fever or suspected intraamniotic infection based on the ACOG criteria (Table 1). If a diagnosis has been made by the obstetric team, the pediatric provider should confirm this whenever possible through review of the medical record to ensure accurate use of ACOG criteria for these diagnoses.

5.2.2 Isolated Maternal Fever

For infants exposed to isolated maternal fever who are well-appearing, we propose close observation for 48 hours but no empiric laboratory evaluations or antimicrobial therapy. This is based on our data that isolated maternal fever occurred in only 4% of infants with EOS (Chapter 2). However, isolated maternal fever due to epidural use, dehydration or other non-infectious etiologies is common.(Greenwell et al., 2012; Koerner et al., 2018; Riley et al., 2011; Yancey, Zhang, Schwarz, Dietrich, & Klebanoff, 2001) Close observation of the physical exam and vitals every four hours by an experienced provider should rapidly identify any progression of underlying illness that warrants further evaluation.

5.2.3 Suspected Intraamniotic Infection

5.2.3.1 Infant Clinical Appearance

For infants exposed to suspected intraamniotic infection, the decision to empirically treat is initially based on the infant's clinical appearance. While infants with clinical illness should be empirically evaluated and treated, those who are well-appearing may receive more nuanced care. If at any time an infant goes on to develop clinical symptoms, then a blood culture should be obtained and treatment with antibiotics started. Care of those with equivocal findings should be tailored based on an institution's underlying EOS incidence, ability to closely monitor such infants, and overall comfort with risk. As our studies defined clinical appearance using the same criteria as used by the multivariate EOS risk calculator, we suggest utilizing these criteria to distinguish infants as well-appearing, equivocal or having clinical illness.(Kuzniewicz et al., 2017)

5.2.3.2 Umbilical Cord Inflammation

Among well-appearing infants, umbilical cord inflammation should be evaluated using either frozen section or traditional histopathology techniques if they can be rapidly obtained within 12 hours after delivery. This would allow clinicians to incorporate the results into the clinical algorithm without significantly delaying treatment decisions.

If there is no umbilical cord inflammation present on frozen section or histopathology, then a blood culture should be obtained, and the infant observed for 48 hours. This is based on our finding that 100% of infants with culture-confirmed EOS had evidence of umbilical cord inflammation in Chapter 4. Thus, if it is absent, it is very unlikely that an infant has EOS. However, our findings from Chapter 2, which showed that 4 infants with culture-confirmed EOS who were exposed to suspected intraamniotic infection had negative placenta histopathology and cultures, give caution to fully "ruling-out" these infants who do not have umbilical cord inflammation. By obtaining a blood culture and continuing close observation, it is expected that the rare infant who has EOS and no umbilical cord inflammation will still be rapidly identified and receive appropriate care.

If umbilical cord inflammation is present or if histopathology cannot be evaluated within 12 hours, then blood and placenta cultures should be obtained, and empiric antibiotics started. If histopathology and placenta culture can be reliably obtained within 36-48 hours of delivery and the infant is well-appearing, then we do not believe that obtaining other inflammatory markers is necessary at this time given the high negative predictive value of these two placenta markers.

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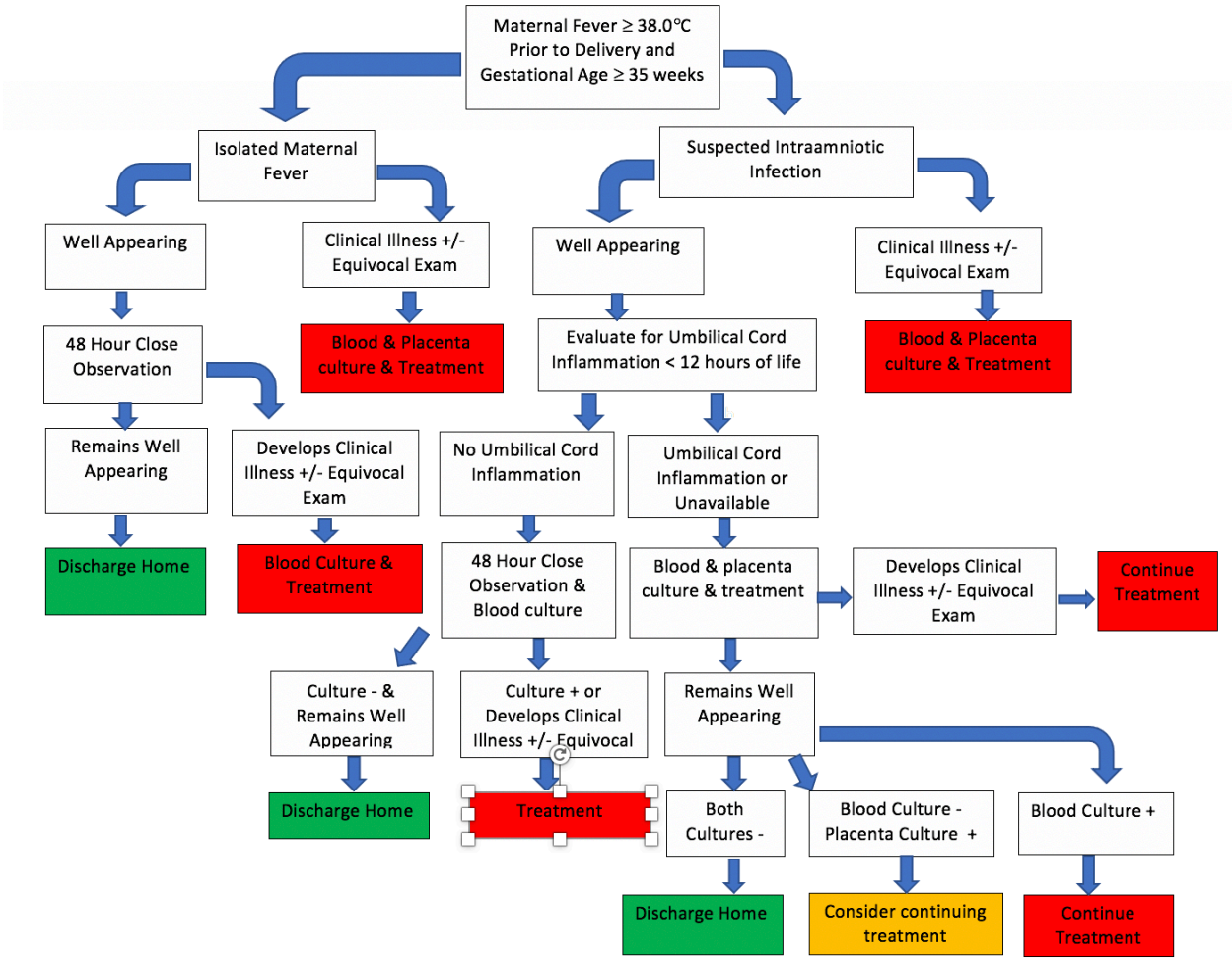

Figure 7. Proposed Algorithim for Categorical Approach using Maternal Diagnosis of Suspected Intraamniotic Infection and Infant Clinical Appearance

5.2.3.3 Blood and Placenta Cultures

The combination of blood and placenta cultures can be used to guide management of well-appearing infants with umbilical cord inflammation or if placenta histopathology is unknown. If both the placenta and blood culture are negative at 36-48 hours, then antibiotics may be discontinued and the infant discharged home. If the blood culture is negative and the placenta culture is positive, then the infant's individual clinical situation and presentation should be carefully considered in deciding whether to continue or stop antibiotics. Obtaining additional inflammatory markers may be useful in this situation. Finally, among well-appearing infants with a positive blood culture for bacteria pathogen, antibiotics should be continued.

5.2.4 Impact of Proposed Categorical Approach Algorithm on Laboratory Evaluations and Empiric Antibiotics

If we apply this algorithm to the 230 infants ≥ 35 weeks gestational age exposed to maternal fever at time of delivery from Chapter 3 **(Figure 8),** who all received blood culture and empiric antibiotics, we estimate there would be a 12% reduction in blood cultures obtained, 38% reduction in empiric antibiotics, 64% reduction in obtaining other inflammatory markers, 15% reduction in prolonged antibiotics (≥ 5 days) among well-appearing infants, and only 1 case of EOS born to a mother with isolated maternal fever that we presumed would have been identified through close observation. These are clinically meaningful reductions that will impact the early exposures of these infants.

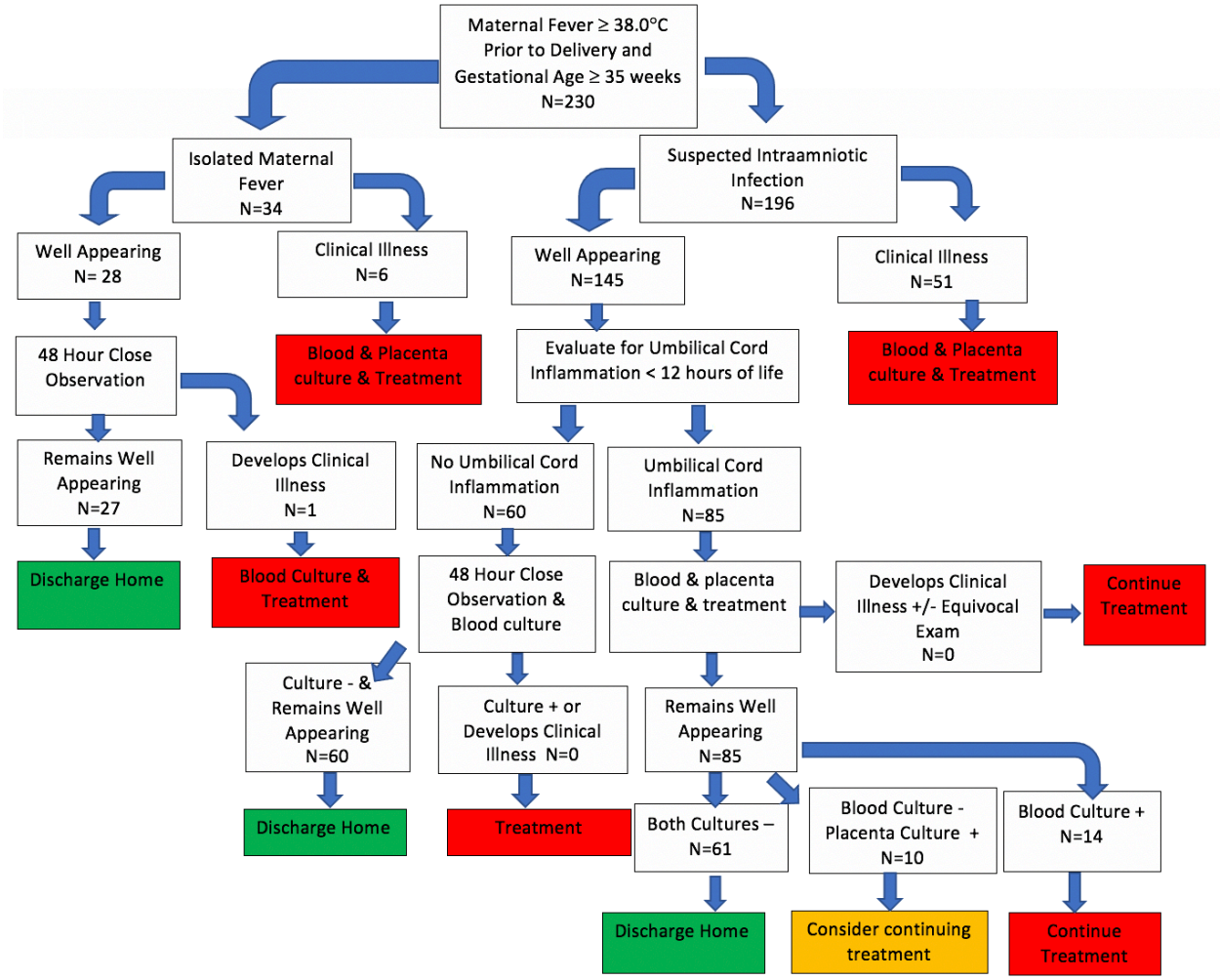

Figure 8. Application of Proposed Categorical Algorithm to Cohort of Infants ≥35 Weeks Gestational Age Exposed to Maternal Fever at Time of Delivery.

5.2.5 Important Considerations for Algorithm Implementation

For clinicians, this algorithm holds the advantage of being familiar by using similar criteria to clinical chorioamnionitis and existing protocols can be adapted. The maximum benefit of this algorithm in reducing empiric antibiotics, however, is contingent on placenta histopathology being a more rapidly available tool that can be accessed by clinicians for clinical decision making.

Currently, this is not widespread and thus would be a major limitation that should be addressed through future research. Regardless, it would still be useful in reducing evaluations among infants with isolated maternal fever, reducing laboratory evaluations with inflammatory markers in most infants, and discontinuing antibiotics earlier in some infants.

Critically, we based this algorithm on data derived from a single institution experience. Furthermore, given the overall low incidence of EOS, our studies have small sample size. This may limit the generalizability of these findings and it is essential that additional validation of this approach is obtained prior to wide-spread implementation. This could be completed with multi-institutional retrospective data and/or through prospective implementation of this approach in different settings and populations with careful monitoring of outcomes.

5.3 Final Considerations

It is apparent from this work and that of many others, that non-invasive screening for EOS using maternal and peripartum risk factors will always have some limitations in identifying cases of EOS. These limitations can be mitigated through close observation of all infants during the first 48 hours of life. Yet, implementation of screening approaches that accurately identify asymptomatic infants shortly after delivery reduces the risk and potential consequences of either missing or delaying treatment of an EOS case. The new ACOG criteria used to identify intraamniotic infection at time of delivery offer a small improvement in identifying EOS compared to the diagnosis of clinical chorioamnionitis. This is further improved by combining it with the infant's clinical appearance. In this approach, blood culture and empiric treatment would be initiated in a significantly higher number of infants with EOS compared to other screening

approaches such as the multivariate EOS calculator. But the trade-off of this higher sensitivity is lower specificity, whereby this approach identifies more uninfected infants who would be exposed to laboratory evaluations and empiric treatment. The potential for short and long-term harms related to these exposures for these infants are not inconsequential and must be weighed against the risks of delayed treatment for EOS. Particularly as dozens of infants may be affected for every one infant with EOS that is identified with the categorical approach. However, as there is no national consensus on acceptable risk for missing or delaying treatment for EOS, each institution must evaluate which approach is most appropriate for them based on their risk tolerance, feasibility, resources, EOS incidence, and patient population.

Thus, there is no right or wrong answer. This is critically important to recognize as there is an underlying current within the EOS literature that the categorical approach is suboptimal. While this may be true in many settings such as large academic centers with on-site newborn hospitalists and robust NICU coverage who see larger volumes of high-risk infants and have adequate resources, training and experience to utilize alternative approaches, this may not be true in smaller settings who see single-digits of high-risk infants each month and therefore have less bedside experience and opportunity to identify disease progression and/or have limited on-site physician coverage to rapidly escalate care. In these settings, an approach that prioritizes sensitivity may be optimal and preferred. Thus, refining this approach using current practice parameters/maternal diagnoses is just as important as exploring new evidence-based approaches such as the multivariate EOS calculator or serial exams.

Yet, additional investigation is still needed to further refine the categorical approach to allow more nuanced care. Our data suggest that placenta pathology and culture could improve specificity and could be implemented in a way that significantly reduces invasive evaluations and

antibiotics while not compromising identification of EOS. However, feasibility and validation studies are needed to make such an approach realistic.

In conclusion, categorical screening for EOS using the new ACOG criteria for suspected intraamniotic infection in combination with infant physical exam is a reasonable approach to identifying infants at high-risk for EOS with the understanding that it prioritizes sensitivity and will identify more uninfected infants compared to other screening approaches. Placenta pathology and culture can enhance specificity of this approach but requires expansion of rapid evaluation techniques and additional validation.

Appendix Detailed description of criteria used for Triple I classifications

For this study, we used the criteria specified by ACOG for each of the Triple I classifications: isolated maternal fever, suspected and confirmed intraamniotic infection.(ACOG, 2017) Although these criteria emphasize that elevated maternal temperature should be sustained over thirty minutes to be considered fever, for purposes of this study, we did not require a repeat temperature as this was not standard practice across our institution. Thus, we defined isolated maternal fever as maternal temperature between 38.0°C to 38.9°C in the absence of other signs and suspected intraamniotic infection as temperature of 39.0°C or higher or a temperature of 38.0°C or higher and one clinical sign. Fetal tachycardia was defined as two sequential heart rates ≥160 beats per minute recorded at least ten minutes apart or clinician documentation of presence. Absence of documented cervical purulence or malodor was considered negative. Confirmed intraamniotic infection was defined as a mother-infant pair who meets criteria for suspected intraamniotic infection plus a pathology diagnosis consistent with histopathologic inflammation of the placenta, fetal membrane or umbilical cord or bacterial growth on placenta culture. While amniotic fluid glucose, gram stain and bacterial culture could also be used to confirm intraamniotic infection, these studies were not obtained for any women in our study.

All maternal temperatures and heart rates from time of admission through delivery were included. Maternal temperatures were obtained orally per institutional practice and reported in Celsius (°C). Length of membrane rupture was calculated based on documented time of rupture of membranes and time of delivery or reported as missing. Timing, name, and frequency of maternal intrapartum antibiotics were recorded from the EHR and were categorized as: none; received

antibiotic less than two hours prior to delivery; GBS intrapartum antibiotic prophylaxis (IAP) (including penicillin, ampicillin, amoxicillin, clindamycin, cefazolin, vancomycin) received at least two hours prior to delivery; broad-spectrum antibiotic (other cephalosporins, fluoroquinolone, or any antibiotic from IAP antibiotic plus aminoglycoside) between 2 to 3.99 hours prior to delivery; and broad-spectrum antibiotic at least 4 hours prior to delivery.(Escobar et al., 2014; Kuzniewicz et al., 2017; Puopolo et al., 2011) White blood cells were abstracted as exact counts in thousands per cubic milliliter. Neutrophilic bands were abstracted as a percentage. All pathology diagnoses were manually abstracted from pathology reports, which were consistently completed by three pathologists including J.B. during the study time period. Full reports were reviewed by A.M.R. for all women with suspected intraamniotic infection. All placenta cultures obtained were assessed for growth with details recorded. For infants, Apgar score at 5 minutes of life as well as any oxygen or vasopressor support, respiratory rate and breathing description, or presence of seizures during the first six hours of life were included.

Appendix Table 1. Sensitivity, specificity, AUC and NNTb of diagnoses for culture-confirmed EOS excluding cord blood cultures.

	Cases N=41 N (%)	Controls N=159 N (%)	Sensitivity % (95%CI)	Specificity % (95%CI)	AUC N (95%CI)	NNTb N (95%CI)
Isolated maternal fever	0 (0)	2 (1)	0 (0-0)	99 (96-100)	--	--
Suspected intraamniotic infection	18 (44)	4 (3)	44 (30-59)	98 (94-99)	**0.707 (0.629-0.785)**	56 (14-211)
Confirmed intraamniotic infection	17 (42)	1 (1)*	42 (28-57)	99 (97-100)	0.704 (0.628-0.781)	16 (1-112)
Clinical chorioamnionitis	13 (32)	4 (3)	32 (20-47)	98 (94-99)	0.646 (0.573-0.719)	96 (22-407)

AUC: area under the receiver operating curve; NNTb: number needed to treat to benefit; EOS: early onset sepsis; 95%CI: 95% confidence interval. Bolded data indicate p<0.02. *One control with suspected intraamniotic infection missing pathology data.

Appendix Table 2. Sensitivity analysis of the proportion of cases and controls identified by the categorical risk assessment excluding infants with an equivocal exam and the multivariate EOS calculator approaches.

		Categorical Risk Assessment		
	Cases	No blood culture	Blood culture	Total cases
Multivariate EOS Calculator	No blood culture	7 (14)	10 (19)	19 (33)
	Blood culture	2 (4)	33 (63)	33 (67)
	Total cases	9 (18)	43 (82)	52 (100)
	Controls	No blood culture	Blood culture	Total controls
	No blood culture	140 (90)	4 (2)	144 (92)
	Blood culture	6 (4)	6 (4)	12 (8)
	Total controls	146 (94)	10 (6)	156 (100)

Data are N (%). EOS: Early onset sepsis.

Appendix Table 3. Sensitivity analysis of the proportion of cases and controls identified by the categorical risk assessment and the multivariate EOS calculator including those with Q4 vitals.

		Categorical Risk Assessment		
	Cases	No blood culture	Blood culture	Total cases
Multivariate EOS Calculator	Routine Vitals	3 (6)	4 (8)	7 (13)
	Vitals Q4	2 (4)	43 (83)	45 (87)
	Total cases	6 (12)	46 (88)	52 (100)
	Controls	No blood culture	Blood culture	Total controls
	Routine Vitals	129 (83)	11 (9)	140 (92)
	Vitals Q4	3 (1)	13 (7)	16 (8)
	Total controls	132 (84)	24 (16)	156 (100)

Data are N (%).EOS: early onset sepsis; Q4: every 4 hours.

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