Use of Leukocyte Counts in Evaluation of Early-onset Neonatal Sepsis

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Background: Early-onset sepsis is a common diagnosis in neonatal intensive care units. Because of the low incidence, overtreatment is also common.

Objective: To measure the sensitivity and negative predictive value of 2 serial white blood cell counts and a negative blood culture at 24 hours in predicting a noninfected neonate in the evaluation of early-onset sepsis.

Methods: We performed a historical cohort study of neonates in the University of Massachusetts Newborn Nursery and neonatal intensive care unit born between 1999 and 2008 who had sepsis evaluations within the first 24 hours of life.

Results: Three thousand two hundred thirteen patients were identified; 59 were excluded due to missing data. Of the 3154 included neonates, 1539 (49%) had 2 normal immature to total neutrophil (I:T) ratios and a negative blood culture at 24 hours. Two of these blood cultures showed growth of bacteria after 24 hours but were considered contaminants, and antibiotics were stopped at 48 hours. None of the 1539 neonates with normal I:T ratios was subsequently diagnosed with sepsis (negative predictive value 100%; [95% confidence interval: 99.905%–100%]).

Conclusions: In this study, the combination of 2 serial normal I:T ratios and a negative blood culture at 24 hours in the evaluation of early-onset sepsis shortly after birth is indicative of a noninfected neonate. This suggests that antibiotics can safely be stopped at 24 hours in these neonates, which comprises approximately 50% of our study population.

Key Words: early-onset sepsis, antibiotics, white blood cell count, I:T ratio

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Early-onset sepsis is a fulminant multisystem infection that most frequently presents in neonates within the first 72 hours of life but can occur as late as day 6 of life. Although the incidence is low (1.4%–3.2%, increasing with decreasing gestational age),[1,2] the severity of the disease and nonspecific nature of early symptoms support evaluating and initially treating many more infants than those that have the disease. However, this results in many healthy neonates being exposed to unneeded broad-spectrum antibiotics and potential adverse effects. In the study by Lieberman et al[3] on intrapartum fever and neonatal sepsis evaluations, 104 neonates were ruled out for everyone with infection.

Multiple studies have attempted to define ways to identify babies with infection at birth.[4–9] Most of these studies have focused on various laboratory tests, including white blood counts and differentials, acute phase reactants (C-reactive protein, erythrocyte sedimentation rate, cytokines), and clinical symptoms to select infants at high risk for sepsis. Although studies have shown that screening white blood cell (WBC) are poorly predictive of infection in neonates,[10–12] there is the suggestion that serial normal values can be reassuring for noninfection.[13] Presently, unequivocal evidence regarding conclusive identification of infected newborns remains a challenge.

Our hypothesis was that 2 serial normal WBC performed 8 to 12 hours apart and a negative blood culture at 24 hours is predictive of healthy newborns in the evaluation of early-onset sepsis in the first 24 hours after birth. We specifically sought to measure the sensitivity and negative predictive value (NPV) of these tests in predicting a healthy neonate in the work-up of early-onset sepsis at birth. If a sufficiently high sensitivity and NPV were found, it would suggest that discontinuing antibiotics at 24 hours in these neonates is safe; by decreasing their exposure to unnecessary antibiotics, we could potentially decrease their risks of adverse effects.

METHODS

A historical cohort study of neonates in the UMass Newborn Nursery and Neonatal Intensive Care Unit born between 1999 and 2008 who had sepsis evaluations within the first 24 hours of life was performed. The neonatal intensive care unit (NICU) at the UMass Memorial Children's Medical Center is a level IIIb referral center in Massachusetts. The catchment area includes Central Massachusetts and Northern Connecticut, a population of approximately 1 million people with approximately 11,000 deliveries and with approximately 550 to 600 admissions to the NICU yearly.

During the study period, criteria for evaluating neonates for early-onset sepsis were consistent. All neonates born at less than 35 weeks' gestation were evaluated and given antibiotics, except when born strictly for maternal indications (eg, maternal preeclampsia or bleeding without premature labor or rupture of membranes). Neonates born at ≥35 weeks' gestation were evaluated for sepsis based on a risk factor assessment and signs (eg, respiratory distress, unexplained hypoglycemia). Risk factors included maternal Group B *streptococcus* (GBS) positive status without appropriate treatment, prolonged rupture of membranes for ≥18 hours, maternal fever of ≥100.4, and premature labor. If ≥2 risk factors were present, an evaluation was initiated. In addition, neonates with a prior sibling treated for early-onset sepsis and those born to mothers with presumed chorioamnionitis were evaluated for early-onset sepsis.

Evaluations for early-onset sepsis were performed by obtaining a blood culture at hour 0, and serial WBC with manual differentials at hour 0 and at hour 8 to 12. Blood cultures were analyzed using the BacT/ALERT 3D System. Cultures were placed at the time of arrival in the laboratory and read every 15 minutes for 5 days. A positive result was immediately reported to the medical provider. Ampicillin and gentamicin were started intravenously at hour 0. Antibiotics were stopped after 48 hours if the WBC had been normal or normalized, and the blood culture was negative. A normal WBC was defined as an immature to total neutrophil (I:T) ratio of less than 0.2 (20%) and a total WBC between 6000 and 30,000.

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Neonates were considered eligible if they had a blood culture drawn in the first 24 hours of life and had at least 2 serial WBC with manual differentials; one drawn at initiation and another drawn 8 to 12 hours later. Neonates who had missing data were excluded. Eligible neonates were identified through our electronic laboratory database (Meditech). Through the program, birth weight, sex of neonate, WBC, and blood culture data were collected. Before use for cohort identification, the program was checked against charts and Meditech to make sure the information was accurate. Data on antibiotic course were collected through chart review. Demographic data, WBC, blood culture results, and antibiotic course were reviewed, and I:T ratios were calculated. For positive blood cultures, data on the organism and time of growth were collected.

A negative blood culture was defined as no growth of a pathogenic organism. A diagnosis of true sepsis was defined as a blood culture positive for a pathogenic organism and treatment of the neonate with antibiotics for at least 7 days. A diagnosis of presumed sepsis was defined as a persistently abnormal I:T ratio ($\geq$0.2) and a negative blood culture with antibiotic treatment of neonate for at least 7 days. A diagnosis of a noninfected neonate was defined as a blood culture negative for a true pathogen and antibiotics discontinued at 48 to 72 hours. After the collection of data, the sensitivity, specificity, NPV, positive predictive value, and confidence intervals were calculated.

In addition, all medical records of infants admitted to the NICU between 1989 and 1999 were reviewed, and those with proven infection that were evaluated in the first 24 hours of life were further analyzed. Specific organisms were identified, time of onset to positivity (when available) was determined, and white blood counts were reviewed.

This study was approved by the University of Massachusetts Medical School Institutional Review Board and qualified for exempt status.

TABLE 1. Organisms Yielded in Blood Cultures

Organism	1999–2008	1989–1999
Escherichia coli	9	21
Group B *Streptococcus*	7	47
Haemophilus influenzae	2	5
Viridans streptococci*	3	3
Klebsiella pneumoniae	1	1
Listeria monocytogenes	1	1
Candida species	1	3
Micrococcus species*	3	—
Staphylococcus, non-*aureus**	2	—
Aerobic diphtheroids*	1	—
*Corynebacterium** spp	1	—
Other	—	10

*Contaminants.

RESULTS

A total of 3213 patients were identified through our electronic database as having a blood culture drawn at less than 24 hours of age. Fifty-nine neonates were excluded because of missing second WBC data. WBC data were missing due to death or transfer of neonates to another institution before obtaining the 8 to 12 hour WBC. All excluded neonates had negative cultures. Of the 3154 newborns who were included in the cohort, 300 were <1000 g, 330 were between 1000 and 1499 g, 1059 were between 1500 and 2499 g, 1273 were between 2500 and 4000 g, and 191 were >4000 g.

Of the 3154 neonates in the study cohort, 31 (0.98%) had positive cultures. Twenty-three (0.73%) were diagnosed with true sepsis and 8 (0.25%) were considered contaminants. Of the neonates with true sepsis, the majority of positive cultures were GBS and *Escherichia coli* (*E. coli*). Other organisms recovered in neonates with sepsis were *Streptococcus*, *Haemophilus*, *Klebsiella*, *Listeria,* and *Candida* (Table 1). All of the neonates with true sepsis had at least one abnormal WBC except one of the patients with *E. coli* who had 2 normal WBC with a positive culture at less than 24 hours. The organisms recovered in the contaminated blood cultures were *Micrococcus, Staphylococcus* non-*aureus,* aerobic diphtheroids*, Corynebacterium,* and viridans streptococci (Table 1). All of these neonates had 2 normal WBC and were not treated for sepsis.

Of the study cohort, 1615 (51%) had at least one abnormal I:T ratio and/or a positive blood culture at 24 hours (Table 2). Of these neonates with a positive test, 142 were treated for sepsis (23 for true sepsis and 119 for presumed sepsis), and 1473 were not infected. Of the 3154 neonates in the cohort, 1539 neonates (49%) had 2 normal I:T ratios and a negative blood culture at 24 hours. Two of these blood cultures became positive after 24 hours but were considered contaminants by the NICU care team, and antibiotics were stopped at 48 hours. One culture showed growth of *Corynebacterium* at day 4. The other culture showed growth of *Micrococcus* on day 3. None of the 1539 neonates with 2 normal I:T ratios and a negative blood culture at 24 hours was diagnosed with sepsis. The sensitivity of 2 normal WBC and a negative blood culture at 24 hours was 100%, as was the NPV (Table 2). The specificity was 51%, and the positive predictive value was 8.8% (Table 2).

In addition, of the 23 neonates with true sepsis, 4 (17%) had an initial normal I:T ratio, which was then abnormal on the 8- to 12-hour follow-up. Two neonates had culture-positive GBS sepsis, and 2 had culture-positive *E. coli* sepsis.

Ninety-one infants were identified from the chart review of all NICU admissions with documented early-onset sepsis between 1989 and 1999. All of these neonates had at least one abnormal I:T ratio and/or a positive blood culture by 24 hours. Approximately two-thirds of these infections were attributed to GBS and *E. coli* (Table 1). Of the 91 babies, 88 (97%) had 1 or 2 initially abnormal WBC. The 3 babies with 2 normal sequential WBC had positive blood cultures within 24 hours of life. All 3 infants were asymptomatic, most likely indicating bacteremia rather than septicemia.

TABLE 2. Sepsis Rule Out Data and Statistical Calculations

	True Infection + Presumed Sepsis	No Infection	Total	
At least 1 abnormal I:T ratio and/or (+) blood culture at 24 h	142 (23 + 119)	1473	1615	PPV = 8.8% (95% CI: 0%–8.805%)
2 normal I:T ratios and (−) blood culture at 24 h	0	1539	1539	NPV = 100% (95% CI: 99.905%–100%)
	Sensitivity = 100% (95% CI: 99.905%–100%)	Specificity = 51% (95% CI: 50.992%–51.016%)		

DISCUSSION

Antibiotic use in NICUs is widespread. Although there is justification behind it, exposure to antibiotics is not without potential side effects. Gentamicin has potential renal toxicity at high levels as well as ototoxicity associated with an inherited genetic susceptibility (1/1161 newborns in United States)[14] that may occur with any level of exposure. Any antibiotic use affects the bacterial balance and often selects out resistant bacteria. In neonates treated with ampicillin and gentamicin, there are lower counts of aerobic and anaerobic intestinal bacteria,[15] and these changes can occur as early as day 3 of life.[16] While one study examining intestinal bacterial colonization in preterm neonates suggested an association between decreased diversity and antibiotic exposure in neonates with necrotizing enterocolitis (NEC),[17] another found that the risk of NEC was actually increased by 7% for each day in which antibiotics were given to extremely low birth weight neonates.[18] In addition, neonatal antibiotic treatment is a risk factor for early wheezing requiring corticosteroids, likely on the basis of altering intestinal flora.[19]

Findings from our study show that, in this cohort of infants who were evaluated for sepsis in the first 24 hours of life, the combination of 2 serial normal I:T ratios 8 to 12 hours apart and a negative blood culture at 24 hours is indicative of a noninfected neonate. In our cohort from 1999 to 2008, 49% of neonates had 2 normal I:T ratios and a negative blood culture, but none had sepsis. The sensitivity and NPV of this test are both 100% (95% CI: 99.905%–100%). This finding suggests that antibiotics could safely be stopped in almost 50% of the cohort at 24 hours. In addition, of the 91 babies with culture-positive early-onset sepsis between 1989 and 2000, none had 2 normal I:T ratios and a negative blood culture at 24 hours, which supports our hypothesis. In early-onset sepsis evaluations in which ampicillin is administered every 12 hours and gentamicin is administered every 24 to 48 hours (depending on gestational age), the number of doses effectively decreases to 2 of ampicillin and 1 of gentamicin.

Of the neonates with true sepsis in our 1999–2008 cohort, all had at least one abnormal I:T ratio, except for one neonate. This neonate had 2 normal I:T ratios, and a blood culture that showed growth of *E. coli* at 12 hours. C-reactive protein was <2.5. Although this neonate was treated for sepsis based on the blood culture results, the symptoms were minimal, and it is likely that this infant was bacteremic and not septic.

A previous attempt at identifying neonates for whom discontinuing antibiotics at 24 hours would be safe was undertaken by Escobar et al.[20] A methodologically rigorous multicenter retrospective review of neonates who underwent sepsis evaluations was performed to identify criteria for the decision rule. The criteria, consisting of clinical assessments, demographic variables, maternal risk factors, and laboratory studies, accurately identified all neonates with a positive blood culture (100% sensitivity and NPV) and persistently symptomatic neonates with negative cultures. Our proposed practice of discontinuing antibiotics after 24 hours of treatment based on serial WBC data and blood culture results at 24 hours would serve to simplify these criteria to 3 universal laboratory studies.

In addition to supporting our hypothesis, our study also suggests that a single normal WBC at birth is not predictive of a healthy neonate. In our original cohort, of the 23 neonates who had true (culture positive) infection, 4 (17%) had an initial normal I:T ratio that became abnormal on subsequent WBCs. Additionally, in our chart review of NICU admissions, 5 (5.5%) of the 91 neonates with sepsis had an initial normal WBC that became abnormal with serial testing. In these 9 infants, a normal screening WBC would be falsely reassuring for noninfection. These results support previously cited studies that suggest that a single-screening WBC is not predictive of a healthy neonate. The Red Book algorithm,[21] which suggests using a WBC as a screening tool for evaluation of possible sepsis in the newborn, could not only lead to overtreatment because of multiple noninfectious factors that cause abnormal WBC (eg, maternal pitocin, maternal fever, prolonged newborn crying, hypoglycemia) but also to undertreatment due to false reassurance from a normal initial WBC. The CDC Statement published in November 2010 recognizes the low sensitivity of a WBC at birth in an asymptomatic neonate and thus suggests a WBC at 6 to 12 hours of life in addition to or in lieu of one at birth.[22] A single normal WBC at 6 to 12 hours may have the same applicability as 2 normal serial WBC, although further studies would be indicated to verify this.

In conclusion, 2 normal WBC screens separated by 8 to 12 hours in infants started on antibiotics at birth along with a negative blood culture by 24 hours of age would appear to safely allow for discontinuation of antibiotics after the first 24 hours of coverage. In the study cohort, almost half of the neonates (49%) would have met the criteria for early antibiotic discontinuation. If the cohort can be generalized to the United States and 10% is a conservative estimate of all newborns evaluated for sepsis and started on antibiotics at birth per year, approximately 200,000 neonates would potentially meet these criteria and qualify for antibiotic discontinuation at 24 hours. Thus, more than half a million fewer doses of antibiotics would need to be administered. This could significantly decrease the effect of antibiotic exposure on gastrointestinal flora, allow for fewer painful procedures, decrease hospital costs, lower the risk of NEC, and potentially allow for shorter hospital stays in selected infants. It could also allow the return of neonates to the well-baby nursery sooner, encouraging the establishment of breast-feeding and promoting family bonding. Overuse of antibiotics is a problem that affects all aspects of medicine with major implications for health and disease. An approach that would allow for decreasing such exposure safely to neonates could be of significant benefit.

The limitations of this study include its retrospective nature as well as an inability to apply the data to infants undergoing sepsis evaluations at greater than 24 hours of age. It is not possible to know whether the same results would apply to these infants. The strengths of the study include the large database and the lack of any false-negative results strongly supporting the conclusions.

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