

Neonatal Sepsis: Looking Beyond the Blood Culture

Evaluation of a Study of Universal Primer Polymerase Chain Reaction for Identification of Neonatal Sepsis

DIFFERENTIATING BACTERIAL SEPSIS FROM other conditions common in infants in the neonatal intensive care unit poses a number of challenges. First, clinical signs such as apnea, feeding intolerance, and need for increased respiratory support are nonspecific but still of concern for bacterial sepsis. These signs often prompt evaluation for sepsis that includes blood culture and antibiotic therapy for up to 48 hours while awaiting blood culture results. Second, blood culture accuracy may be decreased in neonates exposed to antibiotic agents during labor. Third, in some instances, a blood culture cannot be obtained before the initiation of antibiotic therapy. A test that could enable quick and accurate diagnosis of bacterial sepsis in these situations could avert unnecessary antibiotic therapy. To date, others tests such as white blood cell count, absolute neutrophil count, and C-reactive protein (CRP) level have not proved sufficiently accurate to justify withholding initial antibiotic therapy. In addition, no available test can enable accurate diagnosis of bacterial sepsis in neonates exposed to antibiotic agents before collection of blood for culture. Universal primer polymerase chain reaction (PCR) has been suggested as a potentially useful test in the diagnosis of neonatal sepsis in these settings.

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Universal PCR identifies regions of the 16S ribosomal RNA gene common to all bacteria but not present in other organisms. Because this assay does not depend on bacteria viability, both live and killed bacteria can be amplified to determine whether bacteria are present in the blood. In theory, this test could be used to identify sepsis in antibiotic-exposed patients who may have killed bacterial fragments present but in whom blood culture results could be falsely negative.

In this issue of the *Archives*, Dutta et al¹ present the results of a prospective cohort study that evaluated use of universal PCR for diagnosing neonatal sepsis both before and after treatment with antibiotic agents. The study included 242 neonates with a mean (SD) gestational age of 32.2 (3.1) weeks who were enrolled as part of a convenience sample obtained from a level III neonatal intensive care unit in India. Evaluation of universal PCR for diagnosing neonatal sepsis at the time of clinical decompensation demonstrated sensitivity of 96.2% and specificity of 96.3%, with a positive likelihood ratio (LR) of 26.1 and a negative LR of 0.04. However, universal PCR could not identify infants with bacterial sepsis after an-

tibiotics were administered. The investigators concluded that universal PCR could be used to diagnose bacterial sepsis in neonates before but not after the initiation of antibiotic therapy. We used the *Users' Guides to the Medical Literature*^{2,3} criteria to evaluate study validity and the applicability and clinical usefulness of universal PCR in diagnosing bacterial sepsis in neonates.

VALIDITY OF THE STUDY

Was there an independent, blind comparison with a reference (gold) standard? Dutta et al used blood culture as the gold standard. For all subjects, both a blood culture and universal PCR were performed at the time of clinical evaluation for sepsis, and additional PCR tests were performed at 12, 24, and 48 hours after the initial evaluation. A sepsis screen was also performed during the initial evaluation. This screening, which included complete blood cell count, micro-erythrocyte sedimentation rate, and CRP, seems to be a favored diagnostic adjunct for bacterial sepsis in the unit where the study was performed. The authors do not state whether study personnel performing universal PCR were blinded to the results of the blood culture or sepsis screening. However, the evaluation of the PCR assay result as described is unlikely to be influenced by interpretation of either of the other tests. Therefore, lack of blinding probably had a minimal effect on the validity of the results.

Was the test evaluated in an appropriate spectrum of patients? The study was performed at a level III neonatal intensive care unit in India and included neonates with clinical signs concerning for sepsis that merited antibiotic therapy but who had not received antibiotic agents in the previous 72 hours. The incidence of bacterial sepsis in the study population was 21.5%, which is within the range of rates cited in other studies.⁴ However, the selection criteria for study subjects are difficult to generalize to other populations because Dutta et al do not describe how the clinical determination for a suspected sepsis episode was made and whether this determination varied across neonatologists. In addition, only limited basic information was provided about the subjects (sex, age, birth weight, gestational age, and small for gestational age status), and it is not known whether the neonates in this unit were primarily medical, surgical, or cardiac patients. As a result, our ability to compare their risk for sepsis with that of neonates at other centers is limited.

Blood culture contamination was an exclusion criterion for the study. This exclusion decreases the applicability of the study in clinical practice where contamina-

tion occurs and affects clinical decision making. However, limited information is known about the test in comparison with the gold standard; therefore, it seems appropriate to limit evaluation of this assay to situations in which the gold standard can serve as a reliable reference.

A final important limitation of the study sample is the inclusion of neonates with both early-onset (<72 hours of life) and late-onset (\geq 72 hours of life) sepsis. Universal PCR performance may vary in these 2 neonatal intensive care unit subpopulations as a result of different underlying causes of infection. Infants with early-onset sepsis are likely to have acquired the infection perinatally and may have been exposed to antibiotic agents given to the mother (not an exclusion criterion), which could decrease the accuracy of postnatal blood culture. Infants with late-onset sepsis are at risk for nosocomial infection from exposure to staff, other neonates, and invasive equipment such as endotracheal tubes and vascular access catheters. The study compared performance of the test across several subpopulations including infants with early- and late-onset sepsis; however, the study was underpowered to provide precise test performance estimates in these situations.

Was the sample size adequate? In their study, Dutta et al chose to prospectively identify the sample size needed for precise test performance estimates (eg, sensitivity and specificity). They enrolled enough patients to obtain sensitivity and specificity estimates with 95% confidence intervals of $\pm 5\%$.

DETERMINING THE TEST'S IMPORTANCE

Are LRs for the test results presented or the data necessary for their calculation included? The study provides LR estimates for universal PCR at the time of evaluation for sepsis (0-hour PCR) for both neonates with definite and probable sepsis. For neonates to be categorized as having probable sepsis, a positive blood culture was not required, only sufficient clinical concern for sepsis and positive findings at sepsis screen or chest radiography. In these infants, study validity was compromised because the 0-hour PCR was not compared with the gold standard. As a result, we chose to limit our discussion to the findings in neonates with a positive blood culture.

An LR provides the likelihood of obtaining a certain test result in the presence of disease compared with the likelihood of obtaining that same result in the absence of disease. The LR reflects the sensitivity and specificity of the test in a single number but, unlike sensitivity and specificity, does not depend on disease prevalence. This is important for any study of a diagnostic test for neonatal sepsis because prevalence is variable across neonatal intensive care units.³ In general, an LR of 10 or higher or 0.1 or lower is clinically useful because it signifies a test whose result rules in or rules out disease, respectively, by producing significant changes in pretest and posttest probabilities of disease.

A positive 0-hour PCR resulted in a positive LR for 0-hour PCR of 26.1 (95% confidence interval, 16.0-33.6), whereas a negative 0-hour PCR resulted in a negative LR

of 0.04 (95% confidence interval, 0.01-0.11). The associated sensitivity and specificity were 96.2 (95% confidence interval, 89.3-98.9) and 96.3 (94.4-97.1), respectively. However, despite the low negative LR of the universal PCR in this study, 2 of 52 neonates with a positive blood culture had a negative 0-hour PCR. In these neonates, withholding antibiotic therapy on the basis of negative PCR results could have had devastating clinical consequences.

The secondary outcome of the study was performance of universal PCR after antibiotic administration. The first postantibiotic PCR was performed 12 hours after the initial evaluation. Only 12% of neonates with a positive blood culture and positive 0-hour PCR had a positive PCR at 12 hours, and no PCR was positive at 24 or 48 hours. Therefore, according to this study, universal PCR is not useful in diagnosing bacterial sepsis after antibiotic administration.

APPLYING THE DIAGNOSTIC TEST TO OUR PATIENTS

Is the diagnostic test available, affordable, accurate, and precise in our setting? Although PCR amplification is commonly performed in diagnostic laboratories, universal PCR is not widely available. Dutta et al state that the assay is not yet standardized and that performance has been variable across studies. The usefulness of the PCR also depends on cost and time to availability of test results. Through personal communication, the authors reported that their cost of performing universal PCR was 17 times that of CRP and that the assay takes 11 hours to perform. In contrast, another study of PCR estimated 18 hours for return of results to account for the custom of episodic performance of assays in clinical laboratories.⁵ In either case, results would not be available promptly enough to influence decisions about withholding initial antibiotic therapy.

Should this test be favored over other diagnostic tests in clinical use? In addition to universal PCR, other novel assays are undergoing evaluation. A systematic review of newer tests such as procalcitonin found few studies with appropriate methodologic rigor to make decisions about clinical usefulness despite some tests having greater accuracy than CRP.⁴ Although CRP results may still be used during initial evaluation for sepsis, recent studies have concluded that CRP is best used through serial measurements when deciding whether to continue antibiotic therapy after initiation.⁶ With this acknowledgment of the limitations of CRP, it is plausible that a more accurate test such as universal PCR to predict a positive blood culture during initial evaluation for sepsis could be incorporated into clinical practice. However, despite accuracy that is better than other diagnostic tests for neonatal sepsis in current use, universal PCR probably still does not reach a threshold high enough for this particular diagnostic question because sepsis was missed in some neonates in the present study. Withholding antibiotic therapy at the time of clinical evaluation for sepsis may require a test with perfect diagnostic accuracy so that no neonate with bacterial sepsis is missed.

In contrast, there continues to be a dilemma about antibiotic therapy in neonates for whom blood culture reli-

ability is impaired by antibiotic exposure. Because universal PCR can identify killed bacterial fragments, the test held great promise for clinical use. If the assay had performed well after antibiotic administration, neonates with previous antibiotic exposure or without blood culture potentially could be spared 7 to 14 days of antibiotic therapy that often necessitates central vascular access and its resultant increased costs and comorbidities. Unfortunately, universal PCR was unable to identify bacterial fragments in the blood of neonates after initiation of antibiotic therapy.

CONCLUSIONS

Compared with other diagnostic assays including the commonly used CRP assay, universal PCR is associated with higher accuracy and precision for predicting bacterial sepsis when performed before initiation of antibiotic therapy. However, universal PCR is still not accurate enough to justify withholding antibiotic therapy in neonates being evaluated for sepsis because some neonates would still be at risk of delay in treatment. Furthermore, universal PCR failed to identify bacterial sepsis after antibiotics had been administered, arguably the clinical situation for which a discriminating test would be most useful given the limitations of blood culture. Because universal PCR offers only modest improvement over other diagnostic assays for neonatal sepsis, it seems unlikely that clinicians will rely on universal PCR when making decisions about antibiotic therapy in the neonate with potential sepsis, regardless of previous exposure to antibiotic agents.

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Announcement

Take Our Online Quiz. The *Archives* is now offering its Picture of the Month ahead of print as an online quiz. To see what's in store and to test your clinical acumen, just go to the *Archives* home page at <http://archpediatrics.com> and follow the links to the Online Quiz. This is just one of several new features that the *Archives* will be offering online in coming months.

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Correction

Text Error. In the article titled "Neonatal Sepsis: Looking Beyond the Blood Culture: Evaluation of a Study of Universal Primer Polymerase Chain Reaction for Identification of Neonatal Sepsis," by DeCamp et al, published in the January issue of the *Archives* (2009;163[1]:12-14), a statement error occurred. On page 13, left column, "Determining the Test's Importance" section, paragraph 2, lines 4 through 6, should have read as follows: "The LR reflects the sensitivity and specificity of the test in a single number, but unlike positive and negative predictive value, does not depend on disease prevalence."