

Diagnosis of Neonatal Sepsis Using Universal Primer Polymerase Chain Reaction Before and After Starting Antibiotic Drug Therapy

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Objective: To study universal primer 16S rRNA gene polymerase chain reaction (PCR) for diagnosis of blood culture–positive neonatal sepsis before and after starting antibiotic drug therapy.

Design: Prospective study of diagnostic tests.

Setting: Level III neonatal intensive care unit.

Patients: Neonates with a fresh episode of clinically suspected sepsis were enrolled; those with major malformations, life expectancy less than 12 hours, or contaminated blood cultures were excluded.

Interventions: Before starting antibiotic drug therapy, PCR (0 hour), blood culture, and sepsis screening (complete blood cell counts, micro–erythrocyte sedimentation rate, and C-reactive protein level) were performed. The PCR was repeated 12, 24, and 48 hours after starting antibiotic drug therapy.

Main Outcome Measures: The primary outcomes were the sensitivity and specificity of 0-hour PCR for diagnosing blood culture–positive sepsis, and the secondary

outcome was the proportion of 0-hour PCR–positive patients who remained positive after antibiotic drug therapy.

Results: Of 306 patients evaluated, 242 were included (mean [SD] gestation, 32.2 [3.1] weeks; and mean [SD] birth weight, 1529.2 [597.2] g). Blood culture was positive in 52 patients and 0-hour PCR in 57. The sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios of PCR were 96.2%, 96.3%, 87.7%, 98.8%, 26.1, and 0.04, respectively. Two patients were blood culture positive but 0-hour PCR negative, whereas 7 were 0-hour PCR positive but blood culture negative. Of the 0-hour PCR–positive patients, 7 remained positive at 12 hours and none at 24 and 48 hours after starting antibiotic drug therapy. In 0-hour PCR–positive patients, no predictors of positive 12-hour PCR were identified.

Conclusion: Universal primer PCR can accurately diagnose neonatal sepsis before but not after antibiotic drugs are given.

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THE IDEAL TEST FOR THE DIAGNOSIS of neonatal sepsis continues to be elusive.¹ Although blood culture is the currently accepted criterion standard, it may take 48 hours to get a report, and previous antibiotic drug exposure often interferes with growth on blood culture. Polymerase chain reaction (PCR) using universal bacterial primers targets conserved regions of the 16S rRNA gene that are common to all bacteria but not found in other organisms.²

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There are a few studies³⁻⁶ on the use of universal primer PCR on blood samples of neonates with suspected sepsis, and they have shown promising results. The authors of previous studies have not reported the antibiotic drug exposure sta-

tus of patients before sampling for PCR or the role of PCR after starting antibiotic therapy. Unlike blood culture, PCR does not depend on the viability of bacteria. We hypothesize that PCR results may remain positive in septicemic neonates even after antibiotic drug therapy. There are no clinical data on this issue, to our knowledge, but animal data⁷⁻⁹ suggest that a positive PCR result persists after starting antibiotic therapy. If this finding were validated for septicemic neonates, it would allow the diagnosis of sepsis in situations in which neonates receive antibiotic agents without a blood culture being performed. This study was conducted to determine the performance of universal primer PCR in the diagnosis of neonatal sepsis, whether this test remains positive after giving antibiotic agents, and, if so, the duration and predictors of positivity.

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The PCR protocol was standardized using known laboratory samples before embarking on the clinical study. The protocol of Jordan and Durso³ was adopted with modifications. For DNA extraction, 1 mL of various bacterial culture broths was added to 4 mL of tryptic soy broth (HiMedia Laboratories, Mumbai, India); the mixture was incubated for 5 hours at 37°C with shaking and was pelleted at 13 226g for 5 minutes at 4°C in a cold centrifuge (Heraeus-Biofuge Primo; Kendro Laboratory Products, Asheville, North Carolina). One milliliter of lysis buffer at 4°C (0.32M sucrose, 10mM Tris hydrochloride [pH, 7.5], 5mM magnesium chloride, and 1% Triton X-100) was added to the pellet, mixed, and left on ice for 5 minutes before being recentrifuged. To the remaining cell pellet, 50 U of mutanolysin (Sigma-Aldrich, St Louis, Missouri) in 100 µL of phosphate-buffered saline was added, and the pellet was incubated for 30 minutes at 37°C. Ten microliters of 10 mg/mL of proteinase K (Sigma-Aldrich) was added, the sample was incubated for 30 minutes at 70°C, and proteinase K was inactivated by boiling for 10 minutes.

The universal primer pair RW01 and DG74 (MWG Biotech AG, Ebersberg, Germany) was used for amplification.¹⁰ The PCR master mix (10mM Tris hydrochloride, 50mM potassium chloride, 1.5mM magnesium chloride, 200µM deoxyribonucleotide triphosphate mix, 25µM primers, and 2.5 U of Taq polymerase) was filtered through a centrifugal filter device (Centricon YM-100; Millipore, Billerica, Massachusetts) at 1000g for 1 hour at 4°C. Extracted DNA (2 µL) was added to 23 µL of the filtered PCR master mix. The PCR amplification was performed by heating to 95°C for 10 minutes followed by 30 cycles of 1 minute at 95°C, 2 minutes at 60°C, and 1 minute at 72°C in a thermal cycler (Mastercycler Gradient; Eppendorf AG, Hamburg, Germany). Ten microliters of the amplified product was analyzed by means of gel electrophoresis (Bangalore Genei, Bangalore, India) in 2% agarose gel (HiMedia) at 80 V. The desired amplicon of 380 base pairs was visualized under UV illumination after staining by ethidium bromide (ICN Biochemicals, Cleveland, Ohio). A 100-bp DNA ladder (catalog No. 1721933; New England Biolabs, Ipswich, Massachusetts) molecular weight marker was run alongside to identify the products.

The positive controls used for laboratory standardization were isolates of *Staphylococcus aureus*, *Enterococcus fecalis*, *Staphylococcus epidermidis*, *Streptococcus viridans*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter aeruginosa*. The negative controls were sterile water, sterile nutrient broth, sterile neonatal blood collected in EDTA-treated autoclaved tubes, and isolates of *Candida albicans*, nonalbicans *Candida* species, and *Aspergillus niger*.

The clinical phase of the study was conducted in a level III neonatal unit. Neonates who were clinically suspected by the treating neonatologist to have a fresh episode of sepsis meriting empirical antibiotic drug therapy were recruited. A fresh episode was defined as one whose onset was at least 72 hours after cessation of the antibiotic drug course for a previous episode of sepsis, if any. Patients with major malformations and those who were severely sick, with a life expectancy of less than 12 hours, were excluded. Parents who were approached for participation were provided an information sheet about the study. Patients were enrolled after written informed parental consent was provided. The study was approved by the Postgraduate Institute of Medical Education and Research ethics committee.

Just before use of antibiotics was started, blood was collected for PCR (0-hour sample), blood culture, and a sepsis screen in all the patients. A chest radiograph was performed only if clinically indicated. For PCR, 200 µL of blood was

aseptically collected in EDTA-treated, sterile, DNA-free PCR tubes and was either analyzed immediately or stored at -70°C until analysis. DNA was extracted and amplified from this sample, with positive and negative controls run alongside it, as described previously herein.³ For culture, blood samples were inoculated in brain heart infusion broth and bile broth, incubated, and subcultured using standard methods. Detection was manual, but the number of colonies was not quantified. Those whose blood cultures were contaminated were excluded after this information became available. A blood culture was defined as "contaminated" if growth occurred in only 1 culture bottle or if it grew a known nonpathogen, such as the aerobic spore bearers *Micrococcus* and *Propionibacterium*, or the onset of growth was after 72 hours of incubation.

The sepsis screen comprised C-reactive protein level, immature to total neutrophil ratio, absolute neutrophil count, and microerythrocyte sedimentation rate. C-reactive protein was analyzed using a semiquantitative latex agglutination kit (Rhelax; Tulip Diagnostics Private Limited, Goa, India). Agglutination at 1:2 dilutions (12 mg/L) or more was taken to be "positive C-reactive protein." An immature to total neutrophil ratio greater than 20% was considered "positive." The absolute neutrophil count was considered "positive" if the value fell outside the limits of normalcy as per the charts of Manroe et al¹¹ and Zipursky et al.¹² The microerythrocyte sedimentation rate was considered "positive" if it was above "age in days + 3 mm/h" in the first week of life or greater than 10 mm/h thereafter. The sepsis screen was considered "positive" if at least 2 of the 4 variables were positive.¹³

All enrolled patients received antibiotic agents. Samples for the PCR were collected again at 12 (±1) hours, 24 (±1) hours, and 48 (±1) hours after the start of antibiotic drug therapy. The details of antibiotic treatment and clinical signs of sepsis were recorded. Patients were categorized as having definite sepsis (a clinical diagnosis of sepsis by a neonatologist along with a blood culture positive result), probable sepsis (a clinical diagnosis of sepsis and sterile blood culture along with a positive sepsis screen or chest radiographic evidence of pneumonia), or no sepsis (only clinical signs present).

The key outcome was the sensitivity and specificity of the 0-hour PCR for the diagnosis of definite sepsis (ie, blood culture-positive sepsis). Secondary outcomes included the proportion of PCR-positive patients who remained positive at 12 hours and thereafter, factors that could predict persistently positive PCR, and the sensitivity and specificity of PCR for definite or probable sepsis (ie, blood culture or sepsis screen or chest radiograph positive). We analyzed certain factors for their association with persistently positive PCR. These factors were decided a priori as follows: birth weight, gestation, sex, day of onset of sepsis, small for gestational age status, infection by a gram-positive organism, and sensitivity of empirical antibiotic drug therapy. We compared 0-hour PCR as a diagnostic test for definite early-onset sepsis (EOS) (onset <72 hours of life) vs late-onset sepsis. Among patients with EOS, 0-hour PCR results were compared between those exposed to maternal antibiotic drugs within 72 hours before delivery and those who were not.

To detect a sensitivity and specificity of 95% each (with the 95% confidence interval being ±5%) in neonates with a 30% blood culture positive rate in clinically suspected sepsis,¹⁴ the required sample size was 242 (Epi Info 2000).

Sensitivity, specificity, predictive values, and likelihood ratios with their 95% confidence intervals (CIs) were calculated. Categorical variables were compared by means of the χ^2 test with continuity correction or the Fisher exact test.

Table 1. Polymerase Chain Reaction Status in Relation to Blood Culture and Sepsis Screen Status

PCR Status	Patients, No.			
	Blood Culture Positive	Blood Culture Negative	Blood Culture or Screen Positive	Blood Culture and Screen Negative
Positive	50	7	56	1
Negative	2	183	41	144
Total	52	190	97	145

Table 2. PCR and Sepsis Screen in the Diagnosis of Sepsis^a

Test Variable	0-h PCR		
	Definite Sepsis	Definite or Probable Sepsis	Sepsis Screen for Diagnosing Definite Sepsis
Sensitivity	96.2 (89.3-98.9)	57.7 (47.8-67.1)	81.6 (67.9-91.2)
Specificity	96.3 (94.4-97.1)	97.8 (88.4-99.9)	78.5 (71.9-84.1)
PPV	87.7 (81.5-90.2)	98.2 (90.7-99.9)	50.0 (38.6-61.4)
NPV	98.8 (97.0-99.7)	51.8 (41.3-62.1)	94.2 (89.3-97.3)
LR+	26.1 (16.0-33.6)	25.9 (3.7-181.8)	3.8 (2.9-4.6)
LR-	0.04 (0.01-0.11)	0.4 (0.3-0.5)	0.23 (0.13-0.39)

Abbreviations: LR, likelihood ratio; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value.

^aData are given as percentages (95% confidence intervals), except for LRs.

RESULTS

The known laboratory cultures of bacteria consistently yielded the desired 380–base pair amplicons, whereas the negative controls did not. Of 306 patients evaluated for suspected sepsis, 49 were excluded (34 for major malformations and 15 for having an estimated life expectancy of <12 hours). After enrollment, another 15 were excluded because they had contaminated blood cultures (8 had growth in only 1 bottle, 3 grew aerobic spore bearers, and 1 had onset of growth on the fifth day) and spillage of the PCR sample (n=3), leaving 242 patients eligible for analysis. The study population had a mean (SD) birth weight of 1529.2 (597.2) g and gestational age of 32.2 (3.1) weeks, a median age at enrollment of 3 days (interquartile range, 1-5 days), and a median 5-minute Apgar score of 8 (interquartile range, 8-9). Boys accounted for 65.3% of the participants. Eighty-two patients (33.9%) were small for gestational age.

Fifty-two patients (21.5%) had positive blood cultures and 57 (23.6%) had positive PCR results at 0 hours. Data on all the components of the sepsis screen were available for 235 patients. Among them, the screen was positive in 80 (34.0%). From the initial 242 patients, the number available at each scheduled sampling for PCR decreased due to deaths. At 12, 24, and 48 hours, 100%, 99.2%, and 95.5% of patients, respectively, were available.

Of the blood culture–positive patients, 50 were PCR positive (**Table 1**). Of the remainder, 183 were PCR negative. Of the subjects who were either blood culture or sepsis screen positive, 56 were PCR positive and, of those

who were neither blood culture nor screen positive, only 1 was PCR positive. When data from 235 patients with complete sepsis screen results were analyzed, 40 of the 49 blood culture–positive patients had positive screens, whereas 146 of the 186 blood culture–negative patients had negative screens. For the diagnosis of definite sepsis, 0-hour PCR had sensitivity and specificity greater than 95% each and a negative predictive value (NPV) approaching 99% (**Table 2**). For the diagnosis of definite or probable sepsis, 0-hour PCR had a higher specificity and positive predictive value (PPV) but a lower sensitivity and NPV. Sepsis screening was a poorer test than was PCR in the diagnosis of blood culture–positive sepsis. The sensitivity, specificity, and PPV of 0-hour PCR were significantly higher than those of sepsis screening ($P=.04$, $P<.001$, and $P<.001$, respectively). The NPV of PCR was higher than that of sepsis screening but was not significant ($P=.80$). There were 9 patients who were PCR positive but screen negative and who had blood culture–positive sepsis. There were 3 patients who were PCR positive and blood culture positive but whose full screen report was unavailable.

All 7 patients with negative blood culture and positive 0-hour PCR results had positive sepsis screens (**Table 3**). The clinical signs included shock, respiratory distress, and apnea, which were ascribed to sepsis by the treating neonatologists. There were 5 patients with respiratory distress, and all had radiologic pneumonia confirmed by a pediatric radiologist. There were 2 patients with positive blood culture and negative 0-hour PCR results (**Table 3**). The blood cultures grew *Acinetobacter anitratus* and *S aureus*. In both cases, the sepsis screen was positive, and clinical signs lasted more than 5 days. Patient 1 had radiologic pneumonia, and patient 2 had septic arthritis, corroborated on ultrasound and needle aspiration of the joint space. The mother of patient 1 had received intrapartum antibiotic agents, although the organism was resistant to the antibiotics given (ampicillin and gentamicin).

One hundred thirty-one patients (54.1%) had suspected EOS. Among them, 23 patients (17.6%) were blood culture positive; of these, 22 were PCR positive. Of 108 patients who had sterile blood cultures, 106 had negative PCR results. The sensitivity, specificity, PPV, and NPV of 0-hour PCR for the diagnosis of EOS were similar to those of late-onset sepsis (**Table 4**).

Of the patients with suspected EOS, mothers of 46 (35.1%) had received antibiotics within 72 hours before delivery. Ampicillin was administered to 100% of these mothers, gentamicin to 19.6%, and metronidazole to 18.3%. Ten patients had positive blood cultures despite maternal antibiotic drug therapy, and all were resistant to the antibiotics. Thirteen patients without prenatal antibiotic drug exposure had positive blood cultures. The sensitivity, specificity, PPV, and NPV of 0-hour PCR for the diagnosis of EOS in the subgroup with maternal antibiotic drug therapy were similar to the subgroup without maternal antibiotic drug therapy (**Table 4**). We analyzed patients who were completely “antibiotic naive,” that is, those who were not exposed to antibiotics in the previous 72 hours, prenatally or postnatally. The diagnostic performance of 0-hour PCR among them was no different from that of those exposed to maternal antibiotics.

Table 3. Profile of Patients With Discrepant Blood Culture and 0-h PCR Results

Patient No.	Body Weight, g	Gestation, wk	Sex	Onset, d	Screen Positive	CRP, Dilution	ANC, μ L	ITR	mESR, mm/h	Clinical Diagnosis, d	Clinical Signs
Blood culture negative and PCR positive											
1	1210	30	F	3	Yes	1:2	1500	ND	14	3	Lethargy, respiratory distress
2	1200	29	F	7	Yes	1:8	1400	4	3	≥ 5	Shock, apneas, feed intolerance
3	3713	38	M	4	Yes	1:4	6540	20	3	4	Respiratory distress
4	1039	32	M	4	Yes	ND	600	0	13	4	Respiratory distress, apneas
5	1225	34	F	4	Yes	1:8	4400	0	9	≥ 5	Shock, apneas
6	985	37	M	4	Yes	1:4	6300	0	10	2	Respiratory distress
7	1495	28	M	1	Yes	ND	800	0	6	2	Respiratory distress, apneas
Blood culture positive and PCR negative											
1	1100	31	M	1	Yes	1:4	3000	0	10	≥ 5	Respiratory distress
2	1400	36	M	11	Yes	1:2	19000	2	10	≥ 5	Septic arthritis

Abbreviations: ANC, absolute neutrophil count; CRP, C-reactive protein; ITR, immature to total neutrophil ratio; mESR, micro-erythrocyte sedimentation rate; ND, not done; PCR, polymerase chain reaction.

SI conversion factor: To convert ANC to $\times 10^9$ per liter, multiply by 0.001.

Table 4. Diagnosis of Culture-Positive Sepsis Using 0-h PCR in Patients With LOS and EOS With and Without Use of Antibiotic Therapy by the Mother^a

Test Variable	All Patients With LOS (n=111)	Patients With EOS			Antibiotic-Naive Patients Without Prenatal or Postnatal Exposure to Antibiotic Drugs (n=196)
		All (N=131)	With Prenatal Maternal Antibiotic Drug Exposure (n=46)	Without Prenatal Maternal Antibiotic Drug Exposure (n=85)	
Sensitivity	96.6 (82.8-99.8)	95.7 (79.0-99.8)	90.0 (59.6-99.5)	92.3 (66.7-99.6)	95.2 (84.2-98.7)
Specificity	93.9 (86.5-97.4)	98.1 (93.5-99.5)	97.2 (85.8-99.9)	98.6 (92.5-99.9)	96.1 (91.8-98.2)
PPV	84.8 (69.1-93.3)	91.7 (74.2-97.7)	90.0 (59.6-99.5)	92.3 (66.7-99.6)	87.0 (74.3-93.9)
NPV	98.7 (93.1-99.9)	99.1 (94.9-100)	97.2 (85.8-99.9)	98.6 (92.5-99.9)	98.7 (95.3-99.6)
LR+	15.8 (6.8-37.1)	51.7 (13.0-204.5)	32.4 (4.6-226.3)	66.5 (9.4-468.4)	24.4 (11.1-53.7)
LR-	0.04 (0.005-0.3)	0.04 (0.007-0.3)	0.1 (0.02-0.7)	0.08 (0.01-0.5)	0.05 (0.01-0.2)

Abbreviations: EOS, early-onset sepsis (<72 hours); LOS, late-onset sepsis; LR, likelihood ratio; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value.

^aData are given as percentages (95% confidence intervals), except for LRs.

Of 57 patients who were PCR positive at 0 hours, only 7 (12.3%) stayed positive at 12 hours, and none were positive at 24 and 48 hours of antibiotic drug therapy. No new patient developed a positive PCR result at 12 hours and beyond. Six of the 7 patients had positive blood cultures, and 5 received antibiotic agents to which the bacteria were sensitive in vitro. One had an inconclusive sensitivity report (organism not tested against the administered antibiotic agents) but improved clinically taking the same antibiotics. One patient died despite receiving antibiotics to which the etiologic organism was sensitive.

Of the patients who were 0-hour PCR positive and had data available for the 12-hour PCR, 7 were PCR positive and 47 were negative at 12 hours. A comparison of various predictive factors (as stated in the "Methods" section) between the 2 subgroups at 12 hours did not reveal any significant associations. The number who were PCR positive at 12 hours was too small to make these comparisons meaningful.

COMMENT

This study shows that universal primer PCR is a sensitive and specific method for diagnosing culture-positive

neonatal bacterial infections before starting antibiotic drug therapy but not 12 hours or more after starting therapy. The PCR positivity persists in only 12% of patients 12 hours after administration. Because we did not perform PCR tests earlier than 12 hours, it is not possible to comment on the utility of universal primer PCR during this period. Although PCR seemed to identify 7 "extra" patients with bacterial sepsis compared with blood culture, one cannot be certain that all were genuinely septic. The possibility of contamination of PCR cannot be excluded, given the sensitive nature of this test. Most of these discrepant cases in this study were premature patients who presented within the first week of life. It is difficult to distinguish respiratory distress syndrome, apnea of prematurity, and feed intolerance due to prematurity from true sepsis.

In the laboratory phase, the method was confirmed to be specific for bacteria. Because negative controls did not yield the amplified product, it proved that DNA of bacteria, whether viable or dead, was not present in the uninoculated broths, the sterile water, and the other reagents and sterile laboratoryware used in this study.

In this study, 1 of the inclusion criteria was the absence of postnatal exposure to antibiotic drugs in the pre-

ceding 72 hours. This was done to minimize the possibility of the criterion standard (blood culture) being false-negative because such a result would bias the interpretation of other diagnostic tests. We also excluded patients with contaminated blood cultures because if the criterion standard were itself false-positive, it would affect the interpretation of other diagnostic tests.

In this study, universal primer PCR met some of the criteria of an ideal diagnostic test for neonatal sepsis: an NPV approaching 100% and a PPV of approximately 85%.¹ However, it is still far from being an ideal marker because it does not provide information about antibiotic drug resistance, the assays are not yet standardized, it is costly, and it does not allow sufficient time for infants who may have received 1 or more doses of antibiotics for suspected sepsis. Only likelihood ratios greater than 10 or less than 0.1 can affect conclusive changes from pre-test to post-test probability.¹⁵ The likelihood ratios in this study showed that a positive PCR result increased the odds of having neonatal sepsis by 26 times over baseline odds and a negative result decreased it by 25 times.

The largest study performed to date was designed as a feasibility study.³ For PCR, the researchers took the discarded blood left over after complete blood cell count testing in neonates evaluated for sepsis. There are no data in their study about patient characteristics, C-reactive protein, hematologic variables, or hospital course. Other studies also lack similar information.^{4,5} The PCR by itself cannot distinguish between asymptomatic bacteremia, contamination, and true infection. The availability of clinical details and sepsis screening in this study served to better categorize the cases in which the PCR and blood culture reports were discrepant. In previous studies,³⁻⁶ a cumulative total of 18 discrepant patients (PCR positive and culture sterile) have been identified.

In the present study, 2 patients had negative PCR results but had positive blood cultures. The ancillary investigations and clinical course proved that these patients were definitely septicemic. Because the organism grown from patient 1 was resistant to maternal antibiotics, we do not have a satisfactory explanation for the false-negative PCR test in this patient. Patient 2 had a very high absolute neutrophil count (19,000/ μ L [to convert to $\times 10^9$ per liter, multiply by 0.001]). We cannot exclude the possibility that competition with genomic DNA from high white blood cell counts may have resulted in a false-negative 16S rRNA PCR assay.

With the exception of 1 patient, previous studies on neonatal sepsis have not reported any PCR-negative and blood culture-positive patients, thus yielding very high sensitivities.³ One reason for the apparent difference is that previous studies had few culture-positive patients, the combined total (n=46) being less than the number in the present study alone. If one were to calculate the 95% confidence intervals of the sensitivity in previous studies (from the published 2×2 tables),³⁻⁶ the lower bounds would be as low as 70.6%, 68.6%, 71.7%, and 47.3%.

A novel aspect of this study was the serial analysis of universal primer PCR positivity after administration of antibiotic drugs. The results were contrary to what we had hypothesized before starting the study based on ex-

perimental work.^{8,9} In this study, only 12% of PCR-positive patients remained positive at 12 hours and none after 24 hours of antibiotic drug therapy.⁷⁻⁹ The implication of the present study is that antibiotic drug administration not only rapidly kills bacteria but also enables rapid clearance of bacterial DNA from the bloodstream. Reasons for this may include cleavage by human or bacterial DNases, phagocytosis, or catalysis by antibodies. The turnover of free DNA in humans is rapid, with the half-life of fetal DNA in maternal blood being just 16 minutes.¹⁶ Also, the DNA extraction protocol was directed toward the centrifuged cellular pellet. With breakdown of bacteria, there was a theoretical possibility of the DNA floating free in the plasma and escaping detection. The negative result in most of these patients within 12 hours of starting antibiotic drug therapy is a drawback of universal primer PCR. Its sensitivity to antibiotics does not allow adequate time for infants who have been given 1 or more doses of antibiotic drugs for suspected sepsis.

The time of onset of suspected sepsis (early vs late) and maternal antibiotic drug use did not alter the performance of 0-hour PCR as a diagnostic test. There could be 2 explanations for this observation. The etiologic organisms that cause EOS have a high degree of resistance to the antibiotics used by our obstetrics unit; for this reason, their use would make no difference to the diagnostic accuracy of 0-hour PCR. These findings may not be generalizable to obstetric units in which resistance rates are lower. Second, antibiotics given to the mother could render the blood culture and the 0-hour PCR concordantly false-negative in cases in which sensitive antibiotics were used. This would give the impression that 0-hour PCR was unaffected by maternal antibiotic drug exposure.

In clinical situations, the use of universal primer PCR has been shown to significantly reduce the duration of antibiotic drug therapy and the hospital stay.¹⁷ Although universal primer PCR shows promise, one must exercise caution before introducing it into routine clinical practice for withholding antibiotic drugs in clinically suspected sepsis. In a study of 1223 near-term infants evaluated for EOS, PCR failed to detect 10 of 17 neonates who had positive blood cultures.¹⁸ At present, there is no single marker reliable enough to withhold antibiotic drugs in patients in the neonatal intensive care unit if clinical signs are consistent with septicemia.

The limitations of this study design were that the PCR test was not repeated at more frequent intervals (eg, 4, 6, and 8 hours after antibiotic drug administration) and that further molecular analysis with gram-specific or organism-specific probes was not performed. DNA sequencing could have enabled us to identify the organisms. This information might have helped distinguish pathogenic from commensal organisms in the 7 patients who had positive PCR results but sterile blood cultures.

We conclude that the universal bacterial primer PCR is a useful test for diagnosing a fresh case of culture-proven sepsis but that it should not be used for diagnosis if the patient has been exposed to 12 hours or more of antibiotic drug therapy. It is not possible for us to comment on its utility at less than 12 hours after starting antibiotic drug therapy. In the present setting, the 0-hour

PCR seems to perform well in patients with suspected EOS, irrespective of antibiotic drug use in the mother; however, this finding may not be applicable to other settings. Larger studies are required before the test can be recommended for routine clinical use.

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