RESEARCH PAPER

Cerebrospinal Fluid Polymerase Chain Reaction in the Diagnosis of Neonatal Bacterial Meningitis: A Single-Center Experience From Vietnam

THI QUYNH NGA NGUYEN,[1] THI VAN NGUYEN,[1] MINH DIEN TRAN,[2] ALYSON SKINNER,[3,4] HASSIB NARCHI[4]

From [1]Department of Pediatrics, Hanoi Medical University, and Neonatal Centre, Vietnam National Children's Hospital, Hanoi, Vietnam; [2]Surgical Intensive Care Unit, Vietnam National Children's Hospital, Hanoi, Vietnam; [3]Department of Paediatrics, Manor Hospital, Walsall Healthcare NHS Trust, Walsall, United Kingdom; [4]Department of Paediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates.

Correspondence to: Prof Hassib Narchi, Department of Pediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates. hassib.narchi@uaeu.ac.ae
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Objective: To compare the performance of cerebrospinal fluid (CSF) polymerase chain reaction (PCR) with bacterial culture for the diagnosis of neonatal bacterial meningitis (NBM). **Method:** The CSF analysis of neonate with confirmed bacterial meningitis was performed with PCR and bacterial culture, and results were compared. **Result:** Among 24 neonates, the pathogens Identified included *E. coli K1*, GBS, *Streptococcus pneumoniae* and *Listeria*. PCR identified 20 (83.3%) pathogens, and culture 4 (16.7%) pathogens. Prior antibiotics were administered to 20 (83.3%) neonates in whom PCR identified 17 (85%) and culture 3 (15%) pathogens. **Conclusion:** CSF PCR had a higher yield of pathogens than CSF culture in confirmed neonatal bacterial meningitis with a high rate of prior antibiotic therapy.

Keywords: *Bacterial culture, Molecular assay, Neonatal ICU, Sepsis.*

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Neonatal bacterial meningitis is a devastating infection in neonates, with a mortality rate of up to 58%. The most common causative pathogens in developed countries are Group B *Streptococcus* (GBS) and *Escherichia coli*. The incidence of neonatal meningitis is significantly higher in developing countries where the common causative pathogens are Gram-negative bacteria such as *Klebsiella pneumoniae* and *Escherichia coli* [1,2]. Neonatal bacterial meningitis is confirmed by microscopic, chemical, and bacteriological analyses of the cerebrospinal fluid (CSF), with culture as the gold standard. However, CSF analysis is unreliable in case of exposure to antibiotics during the birthing process [3], or before a lumbar puncture is performed [4]. A sterile CSF culture does not rule out neonatal bacterial meningitis and, precludes the identification of the causative pathogen.

Bacterial nucleic acid-based polymerase chain reaction (PCR) assays have been developed for the detection of common pathogens with a rapid turnaround time compared to conventional culture [5,6]. There are limited studied on comparison between CSF PCR testing and bacterial culture in developing countries, where antibiotics are often adminis-tered before the diagnosis is made and bacterial profile is different. We evaluated the diagnostic value of CSF PCR in neonatal bacterial meningitis in this study.

METHODS

This was a cross-sectional study on neonates aged between 0 and 28 days of life, who were admitted to the Neonatal Centre, National Children's Hospital, Hanoi, Vietnam, the main pediatric referral center between July, 2019 and June, 2020.

Neonatal bacterial meningitis was suspected in the presence of suggestive clinical features with or without risk factors for sepsis. Neonates with confirmed meningitis, where the organism was identified in the CSF by culture or by PCR, were included. Probable cases of meningitis were not included. Diagnosis made in the first three days of life or between days 4 and 28 of life was defined as early-onset and late-onset neonatal meningitis, respectively.

The study was approved by the Institutional Ethics Committee. Written informed consent to participate in the study was obtained from parents or legal guardians. The following data were extracted from the medical records: gestational age at birth, birth weight, gender, prior administration of antibiotics before lumbar puncture, maternal risk factors for infection, clinical features, peripheral total leukocyte count, platelet count, and serum C-reactive protein (CRP) concentration. The CSF was collected and sent for biochemical analysis (protein and glucose content), cell count, culture, and PCR testing.

While the results of the CSF culture were available within three days, the PCR testing result was reported within 24 hours.

CSF culture was performed by inoculating 100 µL of CSF onto each bacterial culture plate containing blood agar, chocolate agar, Mac Conkey agar, and brain heart infusion agar. A negative result was defined by the absence of bacterial growth after 5 days. DNA extraction was performed using MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Inc). Multiplex real-time PCR was accomplished using the Allplex Meningitis-B Assay kit for *H. influenzae, S. pneumoniae, L. monocytogenes, N. meningitidis,* Group B *Streptococcus,* and *E. coli K1*.

Statistical analysis: SPSS software version 22.0 (IBM) was used. Categorical variables were expressed as numbers (percentages), and median (interquartile range) for continuous variables with a skewed distribution. The Fisher exact test was used for the univariate comparison of the frequency of variables. A two-tailed *P* value < 0.05 was considered statistically significant.

Table I Clinical and Laboratory Findings of Neonates With Confirmed Bacterial Meningitis (*N*=24)

Parameter	*Value*
Males	13 (54.0)
Gestational age <37 wk	12 (50.0)
Birth weight <2500g	10 (42.0)
Maternal infection	6 (25.0)
Premature rupture of membranes	6 (25.0)
Temperature instability	12 (50.0)
Poor perfusion	14 (58.3)
Jaundice	14 (58.3)
Lethargy	12 (50.0)
Irritability	8 (33.3)
Hypertonia	3 (12.5)
Convulsion	1 (4.2)
Bulging fontanelle	9 (37.5)
Poor feeding	23 (95.8)
Feeding refusal	18 (75.0)
Abdominal distension	13 (54.2)
Hepatomegaly	3 (12.5)
Vomiting	8 (33.3)
Blood[a]	
White cell count ($\times 10^9$/L)	18.5 (9.9, 26.7)
Platelet count ($\times 10^9$/L)	292 (98, 387)
Serum C-reactive protein (mg/L)	33.8 (6.9, 123.9)
Cerebrospinal fluid[a]	
Leukocytes	445 (96, 2000)
Protein (g/L)	2.0 (1.1, 2.9)
Glucose (mmol/L)	1.8 (0.9, 2.8)

Values expressed as no. (%) or [a]median (IQR).

RESULTS

During the study period, 4318 neonates were hospitalized, of whom 324 underwent lumbar puncture with suspected neonatal meningitis. Twenty four neonates met the diagnostic criteria for confirmed neonatal bacterial meningitis. The clinical and laboratory findings in blood and CSF are shown in **Table I**.

A total of 20 (83.3%) pathogens were identified by PCR, and 4 (16.7%) by culture. Prior antibiotics had been administered to 20 (83.3%) neonates (**Table II**). Five of these neonates had early onset and 15 had late-onset infection (*P*=0.54).

DISCUSSION

The present study demonstrated a higher rate of bacterial identification by PCR than conventional bacterial culture in CSF samples in neonatal meningitis. Most infections were late onset with non-specific clinical manifestations, conforming to an earlier report [7].

CSF culture is traditionally considered to be the gold standard for diagnosis. However, the yield of positive culture was very low in this study, similar to an earlier study [8]. The low yield of CSF culture was related to the high rate of prior exposure to antibiotics (83.3%) at the local hospital before transfer to referral hospital. Prior antibiotics were administered in early-onset infections that commonly occur in preterm neonates where the risk of sepsis is high. In addition, performing a lumbar puncture before initiating antibiotic therapy may have been contraindicated in clinically unstable neonates.

PCR has been used for pathogen detection in neonatal sepsis and meningitis, allowing rapid bacterial identi-

Table II Pathogens Identified by Cerebrospinal Fluid (CSF) Culture and/or Polymerase Chain Reaction (PCR) in Neonatal Meningitis (*N*=24)

Organism	*CSF PCR*	*CSF Culture*
All (n=24)		
E. coli	8	2
GBS	5	1
Listeria	3	0
S. pneumoniae	4	1
Prior antibiotics (n=20)		
E. coli	7	1
GBS	4	1
Listeria	3	0
S. pneumoniae	3	1

Values expressed as no. (%). GBS-group B streptococcus.

WHAT THIS STUDY ADDS?

- Cerebrospinal fluid (CSF) polymerase chain reaction (PCR) outperformed CSF culture to identify bacterial pathogens causing neonatal bacterial meningitis, even in babies with prior antibiotic exposure.

fication with a higher detection rate (58%) compared with culture (29%) [9]. The present study also reported a higher percentage of positive PCR than culture, as described earlier [8]. The higher prevalence of GBS meningitis in this study than in other reports may be attributed to the absence of routine GBS screening during pregnancy and inadequate intrapartum antibiotic prophylaxis.

The study had few limitations. The bacterial coverage of the PCR kit was narrow and included only six bacteria. Neonates with suspected neonatal meningitis who did not undergo lumbar puncture were not included..

CSF PCR is useful in suspected neonatal bacterial meningitis who have prior exposure to antibiotics before lumbar puncture, or when it is contraindicated, and with sterile CSF cultures. PCR allows identification of the causative pathogen, thus permitting appropriate antibiotic selection and duration [10].

In conclusion, CSF PCR had a higher yield of patho-gens than culture even with prior antibiotic therapy. How-ever, unlike culture, PCR does not allow testing antibiotic sensitivity of identified pathogens that is a limitation of this test.

Ethics clearance: IEC, National Children's Hospital, Hanoi; No. 1565/BVNTW-VNCSKTE.
Contributors: TQNN,AS,HN: conceptualized and designed the study; TQNN,TVN,MDT: collected the data and performed laboratory analyses; HN,TQNN: analyzed the data and composed the first manuscript. The document was critically reviewed and approved all authors who are accountable for the totality of the work.
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