Culture-Negative Neonatal Meningitis and Endocarditis Caused by *Streptococcus agalactiae*

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We describe a case of culture-negative meningitis and endocarditis caused by *Streptococcus agalactiae* in a 27-day-old boy. *S. agalactiae* was detected in cerebrospinal fluid and serum by broad-spectrum PCR amplification.

**CASE REPORT**

A previously healthy neonate aged 27 days was admitted to the pediatric department of Robert Ballanger Hospital (Aulnay sous bois, France) because of fever onset the day before. On initial evaluation he was pale and continuously crying, and he had a temperature of 40.8°C. Neurologic examination was normal, except for an anterior bulging fontanelle. Cardiologic examination showed a 2-3/6 systolic murmur at the apex. The ears, lungs, and abdomen appeared normal. A chest radiograph was normal. Blood, cerebrospinal fluid (CSF), and urine samples were immediately taken for culture. Laboratory findings included a white blood cell (WBC) count of 21,000/mm³ (44% polymorphonuclear neutrophils) and a C-reactive protein (CRP) level of 157 mg/liter. Urine laboratory examination was normal. Lumbar puncture yielded cloudy CSF with 50 red blood cells/mm³ and 7,800 WBC/mm³ (89% polymorphonuclear neutrophils), an elevated protein level (1.75 g/liter; serum concentration, 3.6 mmol/liter). Direct examination with Gram staining was negative. Tests for *Streptococcus agalactiae* and *Escherichia coli* K1 soluble antigens using the Pastorex Meningitis kit (Bio-Rad, Marne-La-Coquette, France) were negative in urine and CSF.

Empirical intravenous antibacterial chemotherapy for neonatal meningitis was immediately started, with cefotaxime (200 mg/kg of body weight/24 h), amoxicillin (200 mg/kg/24 h), and amikacin (15 mg/kg/24 h). Blood and CSF cultures remained sterile after 5 days. A second lumbar puncture performed 48 h after admission yielded sterile bloody CSF. The temperature and CRP normalized after 2 and 5 days of treatment, respectively. Amikacin and amoxicillin were withdrawn, and cefotaxime (200 mg/kg/24 h) was continued. The boy’s clinical status improved, and neurological examination remained normal. Because the systolic murmur was still present at 15 days of hospitalization, the infant was transferred to the neonatal cardiology unit of Robert-Debré Hospital for further investigations. An echocardiography showed grade 2/4 mitral regurgitation and a 3 mm vegetation on the mitral valve. The other valves were normal.

Because of sterile cultures, an admission CSF sample and a day 1 serum sample, both stored at −20°C, were sent to Robert-Debré Hospital for molecular diagnosis by universal 16S rRNA PCR amplification. DNA was extracted with the QIAamp DNA Mini kit (QIAGEN, Courtaboeuf, France). The initial PCR mixture (final volume, 50 μl) contained 0.4 μM each universal primer, 200 μM each deoxynucleoside triphosphate, and 1 U of HotStarTaq DNA polymerase (QIAGEN) in 1× amplification buffer supplied by the manufacturer; the final concentration of MgCl₂ was 3 mM. The universal primers 779F (5′-CAACAGGATTAGATACCC-T3′) and 1191R (5′-CGTCATCCACCTTCCCTC-3′) correspond to highly conserved nucleotide sequences of the 16S rRNA gene, located at nucleotides 779 and 1191, respectively, in the *E. coli* 16S rRNA gene (accession no. NC004431). To prevent amplification of contaminant bacterial DNA present in the reagents, the initial mixture was submitted to DNase decontamination as described by Hilali et al. (4). Two units of DNase (Roche, Meylan, France) was added to the mixture, and the mixture was incubated at 37°C for 20 min. The DNase was then inactivated by heating at 90°C for 50 min. Five microliters of extracted DNA was added to the decontaminated mixture, and a PCR was performed for 15 min at 95°C followed by 35 cycles of 15 s at 95°C, 30 s at 56°C, and 30 s at 72°C, with a final extension step of 10 min at 72°C. A negative control consisting of all PCR components except for the template DNA was included in each PCR run. The PCR product was sequenced by Genome Express (Meylan, France). GenBank sequence comparison yielded a 100% match with the 16S rRNA sequence of *S. agalactiae* (accession no. AB002480). To confirm this result, *S. agalactiae*-specific PCR was also performed on both samples as previously described (5) and was positive.
Treatment with cefotaxime was continued for a total 5 weeks. Three months later the boy had normal physical findings, with no neurological sequelae. The echocardiography showed a subnormal mitral valve with moderate thickening but no regurgitation or vegetation.

**Discussion.** *S. agalactiae* is the chief cause of bacterial infection in neonates, 5 to 25% of whom develop meningitis (7). *S. agalactiae* can also cause bacteremia in adults, and this is complicated by endocarditis in 2 to 6% of cases (3). *S. agalactiae* neonatal endocarditis is very rare (8), and *S. agalactiae* meningitis associated with endocarditis is exceptional in adults (1). The case described here appears to be the first report of *S. agalactiae* meningitis associated with endocarditis in a neonate. It is noteworthy that most pediatric patients with endocarditis have an underlying heart disease or other risk factors (2), which did not appear to be the case of our patient.

Contrary to the negativity of soluble antigen, which could be explained by the poor sensitivity of this diagnosis test, the CSF and blood culture negativity is intriguing given the severity of the infectious syndrome and the fact that the infant had not received antibacterial agents before admission. However, cases of meningitis due to *S. agalactiae* have been described in which CSF PCR was positive and culture was negative (6). Moreover, the small blood volume that can be taken for culture and the impossibility of repeating this exam with newborns may also explain the blood culture negativity. PCR false positivity was unlikely, as blood and CSF were extracted and amplified in two separate runs and *S. agalactiae*-specific PCR was positive in a third run.

This observation shows that although exceptional, *S. agalactiae* endocarditis may occur in neonates with no underlying heart disease. When culture is negative, broad-range PCR amplification is a powerful tool for diagnosing neonatal bacterial infection and for guiding treatment.

**REFERENCES**