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NOTES

Broad-Range 16S rRNA PCR with Cerebrospinal Fluid May Be Unreliable for Management of Postoperative Aseptic Meningitis[▽]

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We previously demonstrated that discontinuing presumptive antibiotic treatment in cases of negative conventional cultures is safe and effective for patients with postoperative aseptic meningitis (PAM). Here, we prospectively investigated 32 patients with postoperative meningitis. All 26 patients with PAM diagnosed on the basis of conventional cultures demonstrated negative 16S rRNA PCR results. Our results suggest that the PCR technique does not change PAM management.

Postoperative meningitis is a rare but life-threatening infection (6, 9, 13). Aseptic postoperative meningitis may account for up to 70% of all cases of postoperative meningitis (13, 15). Aseptic meningitis may be due to a local inflammatory reaction to blood breakdown products, sutures, tissue breakdown products, chemicals, etc. (7, 8), or to a small bacterial inoculum acquired perioperatively (5). Distinction between the two entities is clinically very difficult, and the results for direct bacteriological examination are often negative (1). Rapid diagnosis of postoperative bacterial meningitis is crucial, as the mortality rate can exceed 20%. The clinical outcome of aseptic postoperative meningitis is favorable, although recovery may be long. Whatever the mechanism of postoperative meningitis, the British Society for Antimicrobial Chemotherapy (2) recommends presumptive antibiotic therapy for all patients with signs of postoperative meningitis, based on local microbial ecology. Therapy is discontinued after a 48- or 72-h regimen in the case of negative cerebrospinal fluid (CSF) culture. We previously validated the safety and effectiveness of this approach (15).

Several studies have examined the use of broad-range 16S rRNA PCR analysis of CSF in patients suffering from meningitis in different settings. This analysis has been found to be of little accuracy in the case of small-bacterial-inoculum meningitis (3, 12, 14). A single study specifically assessed the accuracy of this analysis for patients with aseptic postoperative meningitis. Surprisingly, the CSF PCR results were positive in all cases (5). The aim of the present study was therefore to prospectively investigate the accuracy of broad-range 16S rRNA PCR analysis for CSF samples drawn from patients with aseptic postoperative meningitis and to assess the help provided to clinicians in discriminating etiology and improving management.

We studied all patients demonstrating postoperative meningitis diagnosed between October 2004 and October 2008 in our teaching hospital. Patients with external or internal CSF shunts were excluded. All consecutive patients who had undergone neurosurgery or ear, nose, and throat (ENT) surgery in the previous 3 months, who had a clinical indication for lumbar puncture, or who met the criteria for meningitis on the basis of the CSF analytical results (see below) were prospectively enrolled. Meningitis was considered to be of bacterial origin if (i) the results obtained from direct examination or culture of CSF were positive or (ii) a microbiological sample (blood, CSF leakage fluid, or surgical wound infection) obtained during the same episode was positive and the CSF contained more than 100 leukocytes/mm^3 (8, 15). Meningitis was considered aseptic if the CSF contained more than 100 leukocytes/mm^3 and showed negative results for direct examination and culture after 72 h (2, 11, 15). All patients diagnosed with postoperative meningitis received empirical antibiotic therapy combining vancomycin, ceftazidime, and ciprofloxacin on the basis of bacterial species and the resistance pattern encountered in our institution (15). During the first 3 days of treatment, the patient's condition was reassessed. If the meningitis was proved to be of bacterial origin, the antimicrobial therapy was adapted to the bacterial isolate and was continued for 2 weeks (15). If the meningitis was found to be aseptic, the antimicrobial therapy was discontinued on the third day. PCR results were not available at the time the decision was made.

CSF samples from all the patients with postoperative meningitis were sent for microscopic examination and bacterial culture. On arrival at the laboratory, 400 μl of each CSF sample was taken under sterile conditions and stored at −80°C until further processing. DNA extraction and PCR were car-

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TABLE 1. Clinical and biological features of patients with postoperative meningitis according to their etiology

Clinical or biological feature	Value for:	
	Bacterial meningitis (n = 6)	Aseptic meningitis (n = 26)
Median age (yr)	48	46
No. of male patients/no. of female patients	3/3	12/14
No. of patients with indicated disease		
Vestibular schwannoma	5	18
Other	1	8
No. of patients treated with indicated surgical approach		
Transpetrosal	4	20
Craniotomy	2	6
No. of patients treated perioperatively with the prophylactic antibiotic cefamandole	4	22
Duration of surgery (h)	3.3 (1–6)	4.2 (1–11)
No. of patients with CSF leakage	5	13
Time between surgery and onset of meningitis (days)	9 (4–18)	6 (1–31)
No. of patients with indicated symptom		
Temperature >38°C	6	24
Headache	4	16
Vomiting	3	6
Meningeal stiffness	1	3
Median (range) CSF leukocyte count (no. of leukocytes/mm^3)	1,345 (340–1,400)	1,027 (105–13,100)
Median (range) CSF glycorrhachia concn (mmol/liter)	3.2 (2.6–4.2)	2.65 (0.28–6.9)
Median (range) proteinorrachia concn (g/liter)	0.68 (0.35–2.16)	1.3 (0.49–8.53)
No. of patients with positive CSF culture	3	0
No. of patients with positive wound or CSF leakage fluid culture	3	0

ried out in separate areas. PCR was performed in a blinded manner, and cultures and PCR sequencing results were compared in retrospect. For each CSF sample, DNA of two aliquots (200 μl each) was extracted with a QIAamp DNA minikit in accordance with the manufacturer's instructions (Qiagen, Courtaboeuf, France). For each batch of extraction, a negative control containing all reagents minus CSF was processed.

PCR was done using universal primers (91E [5′-TCAAAK GAATTGACGGGGGC-3′] and 13BS [5′-GCCCGGGAACG TATTCAC-3′]) designed on the conserved sequences of the *rrn* gene coding for 16S rRNA and generating a 479-bp fragment as previously described (10). Concurrently, a 268-bp fragment of the human β-globin gene was amplified to ensure DNA extraction efficiency and the absence of inhibitors. Sequence analysis of the positive 16S rRNA amplicons was carried out with a 3130 XL genetic analyzer (Applied Biosystems, Courtaboeuf, France). The 16S rRNA sequences were compared with those available in the BIBI database and in the GenBank database with the BLASTN program (http://umr5558-sud-str1.univ-lyon1.fr/lebibi/lebibi.cgi and http://blast.ncbi.nlm.nih.gov/Blast.cgi). Identification at the genus or species level was defined according the recommendations of Drancourt et al. (4). The detection levels for the extraction method combined with 16S rRNA PCR for Gram-positive (*Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Enterococcus faecalis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae*) bacteria in CSF were determined as follows. Strains were cultured and diluted 10-fold in 0.15 M NaCl and the viable counts determined. Bacterial suspensions were mixed with culture- and PCR-negative CSF samples in order to obtain inocula of 10^6 to 10^1 CFU/ml. The detection levels were 10 to 10^2 CFU/ml for *E. coli*, *P. aeruginosa*, *H. influenzae*, and *E. faecalis* and 5×10^3 CFU/ml for *S. aureus* and *S. pneumoniae*.

For every patient, demographic and clinical data (indication for surgery, surgical approach, duration of surgery, antibiotic prophylaxis, clinical manifestations on the day of diagnosis, CSF leakage, delay between surgery and onset of meningitis, and surgical wound aspect) and biological data (CSF cell count and differential; protein and glucose levels; and results for bacteriological examination, blood culture, and CSF leakage fluid culture) involving the antimicrobial treatment and outcome were collected prospectively. PCR results were available after the clinical episode of meningitis and were compared with clinical and bacterial data. We used Student's t test when comparing means and the chi-square test when comparing proportions. The sensitivity, specificity, positive predictive value, and negative predictive value of 16S rRNA PCR were determined with exact confidence intervals (CIs).

Thirty-two patients were included. Twenty-six patients fulfilled the definition of aseptic postoperative meningitis and six of bacterial meningitis (Table 1). No patient was pretreated with antibiotics except for surgical prophylaxis, and none received selective decontamination of the digestive tract. As expected, no clinical or biological feature in CSF was significantly different between the two groups (Table 1). No patient had a positive blood culture or other focus of infection. The outcome was favorable in all cases, without any neurological sequelae. Among the 26 patients with aseptic meningitis, the results for all 16S rRNA PCRs were negative (Table 2). Among the six

TABLE 2. Overall results obtained by 16S rRNA PCR compared to those for culture from 32 patients with postoperative meningitis

Finding	No. of CSF samples with indicated result for culture		Total no. of specimens
	Positive	Negative	
Positive	2	0	2
Negative	4	26	30
Total	6	26	32

patients with bacterial meningitis, CSF samples had been drawn by lumbar puncture in 3 cases and from wound or CSF leakage in 3 other cases. In the first three cases (involving lumbar puncture), the CSF samples grew *Streptococcus pneumoniae*, *Bacteroides thetaiotaomicron*, and *Staphylococcus capitis*. The 16S rRNA PCR results were positive in the first two cases and negative in the case involving CSF growing *S. capitis*. In the 3 other cases (involving wound or leakage samples), the CSF samples grew a *Corynebacterium* sp., *Propionibacterium avidum*, and *Enterobacter cloacae*. The PCR results were negative for all three patients.

Overall, the PCR assay sensitivity was 33% (95% CI, 4 to 78%), with a specificity of 100% (95% CI, 89 to 100%), a positive predictive value of 100% (95% CI, 22 to 100%), and a negative predictive value of 87% (95% CI, 69 to 96%) (Table 2). Compared to lumbar-drawn CSF culture, the PCR assay sensitivity was 67% (95% CI, 9%), with a specificity of 100% (95% CI, 89 to 100%), a positive predictive value of 100% (95% CI, 22 to 100%), and a negative predictive value of 96% (95% CI, 81 to 99%).

The main result of this study is that all 26 patients diagnosed with postoperative aseptic meningitis on the basis of sterile 72-h CSF culture demonstrated negative PCR results. From a physiopathological point of view, this result may appear a strong argument against the hypothesis of a bacterial pathogenic mechanism in postoperative aseptic meningitis and more in favor of a chemical mechanism.

However, one must be cautious in interpretation, for we also noticed, as did other authors, the low sensitivity of PCR, compared to the level for conventional CSF culture, for patients suffering from meningitis in various settings (12, 14). The hypothesis of a bacterial pathogenic mechanism in postoperative aseptic meningitis relies on an intraoperatively acquired small bacterial inoculum (13). This would explain why a short, 3-day antibiotic course could be sufficient for a cure (15). In this situation, the sensitivity of PCR may be too low to improve the diagnostic performance of conventional bacterial CSF culture. For instance, in our study, the patient with CSF growing *S. capitis* showed a negative PCR result.

From a practical point of view, we failed to demonstrate a benefit in using the PCR technique in helping a clinical decision, compared to what was found for the conventional culture technique, even if the PCR technique were available in a routine practice. 16S rRNA PCR-based microbial identification always corresponded to CSF culture-based microbial identification. PCR analysis does not enable antibiotic susceptibility testing but may help species identification in positive culture samples when phenotypic characterization has failed (4). When recommendations are followed (2), postoperative bacterial meningitis may be overdiagnosed. This means diagnosing a patient with postoperative bacterial meningitis when in fact he has only surgical wound infection and/or microbial contaminated CSF leakage fluid. All patients with such clinical presentation and lumber-drawn CSF showed negative CSF 16S rRNA PCR results. On the other hand, the low sensitivity of PCR in the case of the small bacterial inoculum may lead to underdiagnosis of postoperative bacterial meningitis. This means that a patient with a negative CSF PCR result cannot be considered free of postoperative bacterial meningitis.

In summary, diagnosis and management of postoperative meningitis on the basis of conventional cultures should remain the gold standard until more-efficient microbiological tools are available.

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