JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2003, p. 3851–3853 Vol. 41, No. 8
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.8.3851–3853.2003

NOTES

Evaluation of a Rapid PCR Assay for Diagnosis of Meningococcal Meningitis

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Received 6 February 2003/Returned for modification 16 March 2003/Accepted 12 May 2003

We compared the results of Gram staining and culture of cerebrospinal fluid to results obtained with a rapid PCR assay for the diagnosis of meningococcal meningitis in 281 cases of suspected bacterial meningitis. PCR had a sensitivity of 97% compared to a sensitivity of 55% for culture, and the PCR specificity was 99.6%. PCR results were available within 2 h of the start of the assay.

Neisseria meningitidis is the second most common cause of bacterial meningitis in North America, with an incidence of 0.6 per 100,000 population reported in the United States in 1995 (14). In Canada, approximately 300 cases of invasive meningococcal disease are reported annually for an incidence of 1 per 100,000 population (11, 15). The outcome of invasive meningococcal disease can be devastating, with case fatality rates of 10 to 15% (15, 16); the case fatality rate of meningococcal meningitis was recently reported to be 3% (14). Delays in diagnosis and treatment of meningococcal disease may contribute to its high morbidity and mortality. For this reason, administration of preadmission antibiotics to patients with suspected invasive meningococcal disease has been advocated by some (3, 13). However, this approach results in a decreased ability to identify the etiologic agent by culture of cerebrospinal fluid (CSF) (13). Investigators in the United Kingdom have dramatically increased the sensitivity of diagnosis with the routine use of a PCR assay. They have been able to confirm 56% more cases of invasive meningococcal disease with PCR than with culture (8, 10, 13). We report here the test characteristics of our rapid PCR assay in comparison with those of Gram staining and culture of CSF for the diagnosis of meningococcal meningitis in patients with suspected bacterial meningitis.

CSF specimens were obtained from 281 patients with suspected bacterial meningitis between February 1998 and June 2002. The samples were stored refrigerated at 4°C for up to 72 h prior to performing the PCR assay. For DNA extraction, 100 μl of the CSF was centrifuged at 16,000 × *g* for 5 min. After centrifugation, the supernatant was discarded and the pellet was resuspended in 25 μl of Triton X-100 lysis buffer (100 mM NaCl, 10 mM Tris-HCl [pH 8] 1 mM EDTA [pH 9] 1% Triton X-100) (Sigma Chemical Co., St. Louis, Mo.). The specimens were boiled for 10 min, cooled to room temperature, and then centrifuged for 1 min at 16,000 × *g*. One microliter of the supernatant was used as the template in each PCR.

The PCR assay was performed with a 25-μl volume containing 1× buffer (Roche Molecular Systems, Inc., Mississauga, Ontario, Canada), a 200 μM concentration of each deoxynucleoside triphosphate, 2.5 U of Taq polymerase (Roche Molecular Systems), a 0.2 μM concentration of each primer (Life Technologies/Invitrogen, Burlington, Ontario, Canada), and 1 μl of template DNA. Thermocycling conditions in a GeneAmp 9600 thermocycler (Applied Biosystems, Foster City, Calif.) were as follows: 94°C for 2 min, followed by 30 cycles of 94°C for 1 s and 55°C for 15 s, with a final extension at 72°C for 10 min. The primer set targeting the meningococcus-specific insertion sequence IS *1106* (12) and the expected amplicon size are listed in Table 1. The primer set targeting the universal bacterial 16S rRNA coding region (ribosomal DNA) was used as an internal control with all samples. PCR amplicons were visualized on a 1% agarose gel following staining with ethidium bromide and photographed under UV illumination. Samples positive for IS *1106* but culture negative and Gram stain negative were confirmed with two different PCR assays, one targeting the meningococcal capsular transfer gene (*ctrA*) and the second targeting the sialyltransferase gene (*siaD*) (Table 1). These assays were performed as previously described (7). Prior studies using this method demonstrated the ability to detect IS *1106* and 16S rRNA amplicons at a level of 10^2 CFU of *N. meningitidis* (S. O. Matsumura, L. Louie, M. Louie, and A. E. Simor, Abstr. 38th Int. Sci. Conf. Antimicrob. Agents Chemother., abstr. D25, 1998).

Upon receipt in the laboratory, CSF samples submitted for bacterial culture were centrifuged at 3,000 × *g* for 15 min. The pellet was used to prepare a Gram-stained smear and to inoculate culture media (blood agar and chocolate agar), which were incubated in CO_2 for up to 72 h. A diagnosis of bacterial meningitis due to *N. meningitidis* required the presence of clinical signs and symptoms of meningitis and pleocytosis (>10 cells/mm^3). In addition, one of the following criteria had to be met: (i) positive CSF or blood culture for *N. meningitidis;* (ii) Gram staining of CSF sample with gram-negative diplococci;

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TABLE 1. Primer sets used for detection of the meningococcus-specific insertion sequence IS *1106,* the meningococcal capsular transfer gene (*ctrA*), and the meningococcal sialyltransferase gene (*siaD*)

Primer (reference)	Primer sequence (5′ -3′)	Amplicon size(s) (bp)
IS1106-F (13)	ATT ATT CAG ACC GCC GGC AG	
IS1106-R	TGC CGT CCT GCA ACT GAT GT	331
ctrA-F (11)	GCT GCG GTA GGT GGT TCA A	
ctrA-R	TTG TCG CGG ATT TGC AAC TA	111
siaD B-F (11)	CTC TCA CCC TCA ACC CAA TGT C	
siaD B-R	TGT CGG CGG AAT AGT AAT AAT GTT	457
siaD C-F (11)	GCA CAT TCA GGC GGG ATT AG	
siaD C-R	TCT CTT GTT GGG CTG TAT GGT GTA	442
siaD W-135/Y-F (11)	CAA ACG GTA TCT GAT GAA ATG CTG GAA G	698[a]
siaD W-135/Y-R	TTA AAG CTG CGC GGA AGA ATA GTG AAA T	438, 260[b]
16S-F	AGA GTT TGA TCA TGG CTC AG	
16S-R	GGA CTA CCA GGG TAT CTA AT	798

[a] Following restriction with *Xba*I. Size given is for primer W-135.
[b] Following restriction with *Xba*I. Sizes given are for primer Y.

or (iii) a positive PCR assay of CSF sample for meningococcal IS *1106* confirmed with a second positive PCR assay.

Of the 281 patients with suspected bacterial meningitis, 38 met the criteria for meningococcal infection. The median age of these 38 patients was 16 years (range, 6 weeks to 63 years). Two-thirds of the patients were female. The mean CSF white blood cell count, protein level, and glucose level, were 9,880/mm^3 (range, 13 to 59,000/mm^3), 4.17 g/liter (range, < 0.4 to 13.5 g/liter), and 1.7 mmol/liter (range, < 1.1 to 5.2 mmol/liter), respectively. Thirty-seven (97%) of the CSF samples from patients with meningococcal meningitis were positive by PCR, whereas CSF Gram staining revealed organisms in 25 (66%) patients and CSF culture was positive for only 21 (55%) patients (Table 2). The patients whose CSF cultures failed to grow an organism had generally received one or more doses of an antibiotic prior to undergoing a lumbar puncture. The one CSF sample that was negative by PCR had gram-negative cocci visible by Gram staining but yielded no bacterial growth.

There were five specimens that were positive by PCR for meningococcal IS *1106* but had negative CSF culture and Gram staining results. These were all confirmed to be positive by PCR for *ctrA* and four were also positive by PCR for *siaD.* These five CSF specimens had a mean white blood cell count of 5,300/mm^3 (range, 197 to 16,800/mm^3), and mean protein and glucose levels of 6.20 g/liter (range, 1.81 to 9 g/liter) and < 1.1 mmol/liter (range, < 1.1 to 1.4 mmol/liter), respectively. There was a sixth specimen that was positive by PCR for meningococcal IS *1106* but that was negative by culture and Gram staining. Unfortunately, there was no sample available for a confirmatory second PCR with the *ctrA* primers, and therefore this case did not meet our criteria for meningococcal meningitis. With a prevalence of meningococcal meningitis of 14% in our series, the positive predictive value of the PCR assay for the diagnosis of meningococcal meningitis was 97% and the negative predictive value was greater than 99% (Table 2).

Of the remaining 242 cases of suspected bacterial meningitis, the following etiologic agents were identified in 65: *Streptococcus pneumoniae* (45 cases), *Haemophilus influenzae* (5 cases), *Staphylococcus aureus* (4 cases), group B streptococcus (3 cases), *Candida albicans* (3 cases), and one each of group G streptococcus, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Enterobacter cloacae*, and *Acinetobacter baumannii.* None of these CSF specimens were positive by PCR for *N. meningitidis* IS *1106.*

The sensitivities of CSF Gram staining and culture for the diagnosis of bacterial meningitis are suboptimal. The CSF Gram staining procedure is expected to reveal the presence of organisms in no more than 60 to 90% of cases (5, 6). The sensitivity of Gram stain examination is even lower in patients who have received antimicrobial therapy prior to lumbar puncture (4, 6, 9). Prior antimicrobial therapy may also result in negative CSF and blood cultures. PCR-based assays have demonstrated greater sensitivity than culture, with excellent specificity for the diagnosis of meningococcal meningitis (2, 8, 13). Consequently, they should be considered the diagnostic "gold standard." In the United Kingdom, where PCR has been used for the diagnosis of meningococcal disease since late 1996, there has been a 56% increase in laboratory-confirmed cases of meningococcal disease with PCR over that with culture (8, 10).

TABLE 2. Test characteristics of Gram staining, culture, and PCR of CSF for diagnosis of meningococcal meningitis in 38 infected patients

Test	No. of samples		Sensitivity (%)	Specificity (%)	NPV[a] (%)	PPV[b] (%)
	Positive	Negative				
Gram staining	25	13	66	100	95	100
Culture	21	17	55	100	93	100
Gram staining culture	33	5	87	100	98	100
PCR	37	1	97	99.6	99.6	97

[a] Negative predictive value.
[b] Positive predictive value.

For the diagnosis of meningococcal meningitis, the PCR assay described in this report had a sensitivity of 97%, compared to a CSF culture sensitivity of only 55%. This increase in sensitivity is similar to that reported by other investigators (8, 10). It is reasonable to assume that the five specimens in this evaluation that were positive by PCR for both IS *1106* and the *ctrA* gene were true positives despite being culture and Gram stain negative. One of these five specimens was negative by PCR for the *siaD* gene, possibly because this assay is less sensitive than the other two assays (13). Although less likely, another possible explanation for this result would be infection due to *N. meningitidis* serogroup A, for which *siaD* primers were not available. Based on these results, CSF Gram staining and culture would have missed 5 (13%) of the 38 cases of meningococcal meningitis in our series. These missed cases might have had several important implications, including misdiagnosis of meningococcal meningitis as aseptic meningitis. Public health implications might have included a lost opportunity for contact tracing and administration of appropriate chemoprophylaxis to household contacts. Accurate surveillance for meningococcal meningitis and invasive disease is particularly important now, with the availability of the protein conjugate meningococcal vaccines.

The PCR assay described in this report can be performed rapidly with a turnaround time of 2 h from initiation of DNA extraction to the issuing of a report. With this rapid turnaround time and the high positive and negative predictive values of the test, the clinician can rapidly and accurately establish or exclude a diagnosis of meningococcal meningitis. While we had only one possible false-positive result with our assay during the study and in prior testing with multiple different organisms (data not shown), it has been found by other investigators that the IS *1106* PCR assay may result in false-positive results with organisms other than *N. meningitidis* (1, 8). This outcome may occur because of the inherent genetic mobility of insertion sequences, which may result in their transfer among bacterial species and genera. As a result, we now perform PCR assays to detect the meningococcal capsular transfer gene (*ctrA*) and the sialyltransferase gene (*siaD*) on all IS *1106*-positive samples for verification of a true-positive test result and to allow determination of the meningococcal serogroup. The PCR assay detecting *ctrA* has been found to be more specific than the IS *1106* assay (8). However, in a study by Ragunathan et al. (13), the PCR assay based on the detection of IS *1106* had a greater sensitivity than assays detecting either the meningococcal capsular transfer gene (*ctrA*) or the sialyltransferase gene (*siaD*). Since a false-negative test result would be less desirable than a false-positive result in the diagnosis of bacterial meningitis, the use of the IS *1106* PCR assay for detection of *N. meningitidis* would be preferable.

The management of patients with meningococcal meningitis and the public health implications of this disease require that the diagnosis be rapid and accurate. In this report, we have described a PCR-based assay that meets both of these criteria. The assay could be used effectively for testing all CSF samples from patients assessed in an emergency department for bacterial meningitis when there is pleocytosis but no stainable organisms. The assay is likely to be particularly helpful in patients who had received antibiotics before the lumbar puncture was done.

Funds from the Orly Watkin Fund for Meningitis Research supported this study.

We thank laboratories in the province of Ontario for providing aliquots of CSF samples from patients with suspected bacterial meningitis for PCR testing. We thank S. O. Matsumura, K. Nikitas, and C. Watt for excellent technical assistance.

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