Detection of *Leptospira* DNA in Patients with Aseptic Meningitis by PCR

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Samples of cerebrospinal fluid from 103 patients with aseptic meningitis were tested by PCR for detection of *Leptospira* spp. and the results were compared with those of the microscopic agglutination test (MAT) and an enzyme-linked immunosorbent assay for detection of immunoglobulin M (ELISA-IgM). Of these samples, 39.80% were positive by PCR and 8.74 and 3.88% were positive by MAT and ELISA-IgM, respectively.

Leptospirosis is a worldwide disease which affects wild and domestic animals and humans (6). It is caused by the pathogenic *Leptospira* sp. strains that often are responsible for nervous system abnormalities (8). The disease varies from subclinical infection to a severe illness with multiorgan involvement. Because of the variety of clinical symptoms, leptospirosis is often misdiagnosed as influenza, hepatic disease, or fever of unknown origin (6). Signs of meningitis are common, and this form of leptospiral infection can be easily mistaken for other causes of benign aseptic meningitis.

The diagnosis is based on laboratory tests rather than on clinical symptoms alone. The currently used method is based on the serological response of the host to the infecting organism. Tests such as the microscopic agglutination test (MAT) and the enzyme-linked immunosorbent assay for detection of immunoglobulin M (ELISA-IgM) commonly detect titers of antibody to *Leptospira* spp. in cerebrospinal fluid (CSF) and serum. Leptospires can be demonstrated by dark-field microscopy or by the isolation of the agent by culture; however, the process is very laborious and can take up to 3 months (6, 15), with a low isolation rate (11).

The study of meningitis caused by *Leptospira* spp. not only is of epidemiological interest but also has important implications for the interpretation and use of currently available diagnostic tests. In this study, we used a PCR assay to help to establish a diagnosis of meningitis of unknown etiology and compared the results with those of the ELISA-IgM and MAT.

Clinical samples. Between January and December 1994, CSF samples were obtained from 103 patients with aseptic meningitis. The age of the patients ranged from 0 to 60 years. CSF samples from 10 patients with cerebral vascular accident and CSF samples from 4 patients with meningitis caused by *Streptococcus pneumoniae* (one sample), *Haemophilus influenzae* (one sample), and *Neisseria meningitidis* (two samples) served as negative controls. CSF samples were stored at −20°C for about 1 month before testing by ELISA-IgM, MAT, and PCR. In addition, CSF samples artificially contaminated with *Leptospira interrogans* serovar copenhageni served as a positive control for the PCR assays. Since leptospirosis was not suspected at the onset of the disease, no attempt to isolate leptospires was made.

ELISA-IgM. The ELISA-IgM was performed by the method described by Adler et al. (1), with some modifications (12). The technique was carried out as described by Camargo et al. (3).

MAT. The MAT was performed according to standard methodology (6) with the following *L. interrogans* serovars as live antigen: australis, autumnalis, bataviae, butembo, canicola, castellonis, copenhageni, cynthia, djasiman, grippotyphosa, hebdomadis, icterohaemorrhagiae, javanica, panama, pomona, pyrogenes, shermani, tarassovi, and wofi. These serovars represent the serogroups known to be prevalent in São Paulo, Brazil.

Sample preparation for PCR. CSF (500 μl of each sample) was centrifuged at 13,000 × g for 15 min at 4°C. The pellets, washed twice with 100 μl of distilled water, were suspended in 10 μl of TE 10-1 (10 mM Tris [pH 7.4], 1 mM EDTA [pH 8.0]) buffer and heated at 100°C for 10 min.

PCR. For the amplification, primers corresponding to nucleotides 38 to 57 and 348 to 368 in the primary structure of the *L. interrogans* rrS (16S) gene were used (9). Amplifications were carried out as described by Merien et al. (9) for 35 amplification cycles. The samples were subjected to a 1.5% agarose gel electrophoresis. After electrophoresis, the gel was stained with ethidium bromide, visualized, and photographed under UV light. Each specimen was tested in duplicate.

Hybridization. To increase detection sensitivity and to confirm the PCR identity, products were routinely subjected to hybridization with a probe specific for *Leptospira* 16S RNA. Primers corresponding to nucleotides 58 to 77 and 328 to 347 were used to synthesize the probe (289 bp) by PCR. The product of PCR was purified by the low-melting-point agarose gel technique and labeled with digoxigenin (Boehringer, Mannheim, Germany). Hybridization was performed by use of the Boehringer kit protocol. Briefly, the nylon membrane with the PCR products was incubated with denatured probe for 18 to 24 h at 65°C, followed by high-stringency washing for 1 h at 68°C. Digoxigenin-labeled probe was detected in accordance with the detection protocol from the Boehringer kit.

Specificity of the PCR assay. The specificity of the primers used in the PCR was tested with the microorganisms generally involved in meningitis in our country, namely, *H. influenzae*, *N. meningitidis*, *Escherichia coli*, *S. pneumoniae*, and *Mycobacterium tuberculosis*.

The CSF samples used as negative controls were all negative.

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by PCR, hybridization with a specific probe, MAT, and ELISA-IgM.

The results for 103 CSF samples tested by PCR, MAT, and ELISA-IgM are shown in Tables 1 and 2. We found 41 of the 103 CSF samples (39.80%) to be positive by PCR. In 41 CSF samples, a 330-bp product was detected by agarose gel electrophoresis band visualization and confirmed by hybridization. One CSF sample that was PCR negative by gel electrophoresis analysis showed a positive sign by hybridization. This sample was negative by MAT and ELISA-IgM.

Among 103 CSF samples, 9 (8.74%) were positive by MAT (Table 1) and 4 (3.88%) were positive by ELISA-IgM (Table 2), although some of these samples were negative by PCR (Tables 1 and 2). One sample was positive by MAT and ELISA-IgM, two were positive only by MAT, and one was positive only by ELISA-IgM.

The patients ranged in age from 0 to 60 years, but 19 (46.34%) of the 41 patients with positive results were under 10 years of age (Table 3). This study demonstrates that a *Leptospira* sp. was present in the CSF of a large proportion of the patients studied. The rapid detection of leptospires at an early stage may favorably influence the course of the disease. Depending on the method of detection, diagnosis of meningitis caused by leptospires is difficult. The detection of production of specific antibodies toward *Leptospira* spp. in the CSF is a standard laboratory procedure for establishing the diagnosis (6), although during the early stages of leptospirosis, serological tests of CSF may be negative. A sensitive, specific, and rapid method for the diagnosis of leptospirosis is important for the clinician, the patient, and effective epidemiological surveillance. Because of the problems associated with detecting and interpreting immune responses, the use of PCR to detect DNA of *Leptospira* spp. seems promising. We have explored the use of PCR analysis of CSF as a diagnostic tool for leptospirosis. Our study showed that of 103 patients with meningitis of unknown origin, 39.80% were positive by PCR whereas only 3.88 and 8.74% were positive by ELISA-IgM and MAT, respectively. Among the PCR-negative patient samples, two were positive by MAT, one was positive by ELISA-IgM, and one was positive by both ELISA-IgM and MAT (Tables 1 and 2). The failure of PCR in these cases could be due to the absence of the organism from the CSF, to degradation of DNA during prolonged sample storage (1 month at −20°C), or to microbial counts that were below the detection limit of the assay, which was about 5 cells in our study.

The results obtained raise several questions regarding the diagnosis of meningitis; these data should indicate caution in making clinical decisions based on immune responses because some cases may be misdiagnosed if based only on routine laboratory tests.

The manifestations of leptospirosis are variable and generally include signs of meningitis. Furthermore, some authors draw attention to an important point in cases where jaundice is absent, i.e., the meningeal signs may completely dominate the clinical picture (5). Farr (7) stated that leptospirosis may account for ~10% of cases of aseptic meningitis. Since our study showed results as high as 39.80%, *Leptospira* should be strongly considered as the agent in this kind of meningitis.

The age distribution of the 41 patients positive by PCR assay indicates that leptospiral meningitis is a disease of young people, as described by other authors (2). One of these patients was a 32-day-old infant, probably contaminated by the mother.

The MAT is sensitive but because of the antigenic heterogeneity of *Leptospira* spp. requires a large number of serovars as antigens. In addition, it would not be useful at the early stages of the disease when the antibody to *Leptospira* spp. is not present or, if present, is at a low level in the CSF. In Brazil, urban leptospirosis is an endemic disease, with a well-defined seasonal pattern with most cases occurring during heavy rain-fall months. Serological evidence of the widespread occurrence of leptospirosis has been reported (4, 10), although because of the low level of suspicion among clinicians and the difficulty of laboratory diagnosis of *Leptospira* infections, the number of cases reported does not represent the total number.

Our results show that PCR has advantages over MAT and ELISA-IgM in the early diagnosis of some cases of leptospirosis, but further studies with other patients including culture, serology, and CSF analysis are needed to confirm the present findings.

We cannot affirm that all cases of acute aseptic meningitis are due to leptospires, but for any case of meningitis of unknown origin, it would seem useful to consider the possibility of leptospiral infection.

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