Early Detection of Central Nervous System Tuberculosis with the Gen-Probe Nucleic Acid Amplification Assay: Utility in an Inner City Hospital

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Central nervous system tuberculosis is a serious clinical problem, the treatment of which is sometimes hampered by delayed diagnosis. We investigated the utility of the Gen-Probe nucleic acid amplification assay for the rapid diagnosis of tuberculous meningitis and as a noninvasive method of identifying intracranial tuberculoma.

CNS tuberculosis, particularly tuberculous meningitis (TBM), remains a serious clinical problem. Missed diagnosis and delayed treatment result in significant mortality and morbidity. The signs and symptoms, results of routine analysis of CSF, and radiographic findings for patients with CNS tuberculosis are often inadequate in making a definitive diagnosis. Clearly, prompt laboratory diagnosis is of vital importance. Acid-fast staining of CSF sediment is the most rapid method for detection of mycobacteria, but this method lacks sensitivity. The diagnostic reference standard, isolation of *Mycobacterium tuberculosis* from CSF samples, is insufficiently timely (it requires 2–6 weeks) to aid clinical judgment with respect to treatment, and this method is insensitive if large amounts of CSF are not tested [1]. PCR and molecular analysis techniques show promise as tools for rapid diagnosis of pulmonary and CNS tuberculosis [2–6]. However, the accuracy and reproducibility of these molecular analysis techniques for the detection of *M. tuberculosis* in CSF and in brain tissue has not been clearly defined.

Despite the fact that the total number of cases of tuberculosis in the United States has decreased by 39% since 1992, the case rate among foreign-born persons remains at least 7 times higher than that among persons born in the United States, and the number of cases among foreign-born persons per 100,000 population continues to rise (7270 cases in 1992 and 7554 cases in 2000) [7]. In addition, the increasing number of persons living with AIDS has created an expanding population of patients who are at risk for active *M. tuberculosis* infection. The incidence of extrapulmonary tuberculosis, particularly CNS tuberculosis, in these high-risk groups presents an increased challenge to the clinician with regard to prompt diagnosis and treatment.

At our institution, an inner-city hospital caring for many immigrants and refugees from Africa, Latin America, and Southeast Asia, we have seen a marked increase in the prevalence of extrapulmonary tuberculosis. The growing number of cases of CNS tuberculosis, particularly in the large population of Somali refugees at our hospital [8], led us to conduct this study and to review the literature addressing the use of PCR as a tool for detection of CNS tuberculosis. Specifically, we examined whether the use of the Gen-Probe Amplified *Mycobacterium tuberculosis* Direct Test (MTD), a nucleic acid amplification assay intended for use on respiratory specimens, would allow for rapid (within 24 h) and accurate diagnosis of TBM and intracranial tuberculoma.

Methods. The medical records of 29 patients who were admitted to Hennepin County Medical Center (Minneapolis) during a 6-year period (1996–2001) were reviewed. The study was approved by the institutional review board of Hennepin County Medical Center. All patients had clinical signs and symptoms of CNS infection and were considered to be at risk for tuberculosis. Thus, in addition to obtaining CSF samples (1–2 mL) for culture for *M. tuberculosis*, we used the Gen-Probe MTD to detect *M. tuberculosis* in CSF samples. The data collected included risk factors (immigrant status, HIV infection, and injection drug abuse history), documented non-CNS *M. tuberculosis* infection, and the final diagnosis. TBM was defined by results of CSF culture that were positive for *M. tuberculosis*. The diagnosis of tuberculoma was made if results of culture of a brain biopsy specimen were positive for *M. tuberculosis* or if a ring enhancing lesion was seen on MRI for a patient with positive results of a culture of a sample from another body site. "Final diagnosis" was defined as the discharge diagnosis estab-

Received 4 January 2002; revised 19 March 2002; electronically published 8 July 2002.

Financial support: Gen-Probe, Inc.

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Clinical Infectious Diseases 2002; 35:339–42

1058-4838/2002/3503-0021$15.00

lished by the patient's team of physicians. The accuracy of the MTD was evaluated using the result of CSF culture as the reference standard.

In total, 29 CSF samples were submitted for both MTD and culture for *M. tuberculosis*. The MTD was performed using a minimum of 2 mL of CSF. Nucleic acid was extracted from aliquots (450 μL) of the processed CSF sediment according to the manufacturer's instructions for analyzing respiratory specimens. In addition, an amplification inhibition control was prepared from each sample. The MTD amplification assay was performed in duplicate on each patient sample and once on the inhibition control. The specimen was deemed to be positive for *M. tuberculosis* RNA if at least 1 of the 2 assays resulted in a signal stronger than the cutoff point for a negative sample (i.e., $\geqslant$30,000 relative light units [RLU]). A negative result was considered to be valid if the inhibition control assay resulted in a signal stronger than the cutoff point for a positive sample (i.e., $\geqslant$500,000 RLU). Results were released to clinicians within 1–2 days of the time the laboratory received the samples. In each of the 29 cases, the MTD was done before results of culture were obtained.

Results. Of the 29 patients whose samples were tested by Gen-Probe MTD, 9 had CNS tuberculosis, 5 had culture-proven TBM (time to positive results of culture ranged from 11–17 days), and 4 had intracranial tuberculoma (table 1). The remaining 20 patients had a variety of other neurological diseases that clinically mimic TBM (table 1). Of the 9 patients with CNS tuberculosis, 5 (56%) were immigrants from areas in which tuberculosis is endemic, 7 (78%) had coexisting pulmonary or miliary tuberculosis, 3 (33%) were HIV positive, and 3 (33%) were intravenous drug users.

The Gen-Probe MTD detected *M. tuberculosis* in CSF from the 5 patients with culture-confirmed cases of TBM. There were no false-positive results. However, the results of the Gen-Probe MTD and CSF culture were negative for all patients with CNS tuberculoma. MTD was done on samples of fresh brain tissue from 2 of these 4 patients and yielded positive results in both cases.

Discussion. Despite many published reports on the rapid detection of *M. tuberculosis* in CSF by means of PCR, little is known about the reproducibility and reliability of the technique. Although PCR has been found to be relatively specific in most reports (82%–100%), its sensitivity has ranged from 32% to 100%. In addition, PCR has been found to have a high rate of false-positive results, which has been attributed to cross-contamination [2–6]. A benefit of the Gen-Probe Amplified MTD over conventional PCR is that MTD uses a single-tube format, which reduces the possibility of cross-contamination and false-positive readings.

Use of the Gen-Probe MTD as a tool for the early detection of TBM and intracranial tuberculoma has not been fully explored. Vlaspolder et al. [9] were the first to publish data on the utility of the MTD in testing CSF. Pfyffer et al. [10] modified the processing recommendations initially intended for use on sputum specimens and enhanced the sensitivity of the MTD for detection of *M. tuberculosis* in CSF. In total, 54 CSF samples were examined in their series. Of these, 6 were from patients in whom TBM was suspected. The MTD was 100% sensitive and 96% specific for the 6 high-probability samples [10]. Using the modifications of Pfyffer et al. [10] and a similar combination of clinical assessment and mycobacterial culture results as a diagnostic reference standard (only 5 cases were confirmed by culture; the diagnosis was assigned in the remainder on clinical grounds), Lang et al. [11] tested 84 CSF samples. They found the MTD to be 33% sensitive and 100% specific when the manufacturer's cutoff point of $\geqslant$30,000 RLU was used and 83% sensitive and 100% specific when a cutoff point of $\geqslant$11,000 RLU was used. Lowering the threshold for positivity is problematic because of the possibility that nonspecific signals in the 10,000–20,000-RLU range will be found, leading to false-positive results. This may not have been a problem in the population studied by Lang et al. [11], in which the prevalence of disease was high (Santo Domingo, Dominican Republic), but it could drastically affect the positive predictive value of the test in our clinical population, in which the incidence of TBM is lower.

Our data support the value of the Gen-Probe MTD as a promising tool for the detection of *M. tuberculosis* in CSF. We chose to use culture alone as the diagnostic reference standard and a cutoff point of $\geqslant$30,000 RLU. Our aim was to determine whether the probe was able to provide the same information as culture but in a considerably more rapid fashion. In addition, we tested a larger sample of CSF (2 mL, compared with 50 μL [9, 10] or 500 μL [11] in other studies). The larger quantity of CSF increased the likelihood that the sample contained mycobacteria and may explain why the test appeared to be very accurate for patients with culture-confirmed TBM. Further studies that use 2-mL aliquots of CSF are warranted, to determine whether the MTD may be more sensitive than culture.

We were unable to assess the sensitivity and specificity of the test because of the limited size of the group of patients in this study. Nonetheless, the MTD appears to be specific, and positive results of MTD would support a diagnosis of TBM. However, one cannot assume that a negative test result would rule out TBM, because culture of CSF, our reference standard, has a suboptimal yield, according to the literature [1]. However, empiric treatment based on a positive MTD result may prevent some of the devastating consequences of delayed therapy and is not likely to jeopardize subsequent diagnostic confirmation [1].

In our study, the Gen-Probe assay did not identify *M. tuberculosis* in the CSF of 4 patients with intracranial tuberculoma

Table 1. Results of CSF culture and Gen-Probe Amplified *Mycobacterium tuberculosis* Direct Test (MTD), risk factors for CNS tuberculosis, and final diagnosis among inner city patients considered to be at risk for tuberculosis.

Patient	Results of CSF culture/MTD[a]	Risk factor(s)	Final diagnosis
1	+/+	Immigrant (Mexico), IDU, pulmonary tuberculosis	TBM
2	+/+	IDU	TBM
3	+/+	HIV positive, IDU, pulmonary tuberculosis	TBM
4	+/+	Immigrant (Ethiopia), pulmonary tuberculosis	TBM
5	+/+	Immigrant (Kenya), Hodgkin lymphoma	TBM
6	−/−	HIV positive, pulmonary tuberculosis	Intracranial tuberculoma
7	−/−	Immigrant (Liberia), HIV positive, miliary tuberculosis	Intracranial tuberculoma
8	−/−	Pulmonary tuberculosis	Intracranial tuberculoma
9	−/−	Immigrant (Ecuador), miliary tuberculosis	Intracranial tuberculoma
10	−/−	None	Neurosarcoid
11	−/−	HIV positive, IDU	Cryptococcal meningitis
12	−/−	HIV positive, IDU	Endocarditis/cerebral infarct
13	−/−	Immigrant (Mexico)	Aseptic meningitis
14	−/−	Immigrant (Somalia)	Post-mumps meningitis
15	−/−	Immigrant (Somalia)	Typhus
16	−/−	Known tuberculosis exposure	Seizure disorder
17	−/−	None	Varicella-zoster virus encephalitis
18	−/−	Immigrant (Ecuador)	Probable viral meningitis
19	−/−	HIV positive	HIV encephalitis
20	−/−	Pulmonary tuberculosis	Wernicke encephalopathy
21	−/−	Immigrant (Mexico)	Unknown encephalopathy
22	−/−	None	Probable viral meningoencephalitis
23	−/−	Immigrant (Thailand)	Probable viral meningitis
24	−/−	Immigrant (Somalia)	Aseptic meningitis
25	−/−	Immigrant (Ethiopia)	Depression with psychotic features
26	−/−	Immigrant (Ethiopia), HIV positive, pulmonary tuberculosis	Herpes simplex virus meningitis
27	−/−	Travel to Latin America	Multiple enhancing brain lesions (unknown etiology)
28	−/−	Immigrant (Ethiopia)	Bacterial meningitis/CNS hemorrhage
29	−/−	HIV positive, pulmonary tuberculosis	Aseptic meningitis

NOTE. IDU, injection drug use; TBM, tuberculosis meningitis.

[a] A positive culture result was indicated by growth of *M. tuberculosis* on culture of 0.5–1-mL samples of CSF inoculated into Bactec 12B bottles (Becton Dickinson) and solid culture medium (Middlebrook 7H11); a negative culture result was indicated by no growth after 6 weeks of incubation. A positive MTD result was indicated by a result in at least 1 of 2 assays of signal stronger than the cutoff value for a negative result (i.e., ⩾30,000 relative light units); a negative result was indicated by a result in the inhibition control assay of a signal stronger than the cutoff value for a positive result (i.e., ⩾500,000 relative light units).

who also had negative results of culture of CSF for *M. tuberculosis*. However, PCR has been reported to be useful in establishing the diagnosis of CNS tuberculoma. A case report of a patient with en plaque tuberculoma of the brain described the use of PCR to detect *M. tuberculosis* in a sample of CSF [12]. Also, Monno et al. [13] found that PCR identified *M. tuberculosis* in the CSF of 5 AIDS patients who had tuberculous brain lesions without meningeal involvement. In our study, the Gen-Probe MTD did detect *M. tuberculosis* in 2 biopsy specimens of brain tissue. Further investigation of the utility of the Gen-Probe MTD in diagnosis of intracranial tuberculoma from biopsy specimens is warranted.

In conclusion, we have found that the Gen-Probe MTD is a valuable tool in the rapid diagnosis of TBM at our hospital. For hospitals that serve large populations of immigrants from areas in which tuberculosis is endemic and of immunosuppressed persons who are at high risk for TBM, the MTD has the potential to expedite the diagnosis of TBM and minimize the devastating consequences of delayed treatment of the disease. In our hospital, the test was useful in differentiating TBM from other causes of aseptic meningitis with a mononuclear pleocytosis. Unfortunately, it appears that this test cannot be relied on as a noninvasive method to identify intracranial tuberculoma. Until PCR can be further evaluated as a means of

identifying tuberculoma, one must maintain a heightened index of suspicion in treating high-risk patients with intracranial mass lesions.

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