Tuberculosis (TB) is a major cause of morbidity and mortality throughout the world. Tuberculous meningitis (TBM) is uncommon and accounts for approximately 1% of all cases of tuberculosis (21), but TBM remains the focus of research because of its high mortality and severe neurological sequelae (19, 20, 24). The early diagnosis of TBM is fundamental to the clinical outcome. However, it is always difficult to confirm the clinical suspicion of TBM. Conventional gold standards, i.e., acid-fast bacillus (AFB) staining and TB bacillus culture, are insensitive and slow. Only 10 to 20% of cerebrospinal fluid (CSF) smears of those with TBM can detect acid-fast bacilli (4, 25).

Some rapid diagnostic techniques, such as anti-TB antibody, adenosine deaminase (ADA), and PCR, have been developed. The detection of anti-TB antibody in CSF has poor sensitivity (16 to 57%) (2, 18) and unsatisfactory specificity due to cross-reactivity (13, 14). ADA is considered to be a marker of cell-mediated immunity (12). The elevation of ADA represents the proliferation and differentiation of lymphocytes (10). However, some studies have demonstrated that ADA is of limited value because it is also elevated in other types of meningitis and encephalitis (5, 9, 11). PCR is a promising method to identify genetic materials of TB bacilli. PCR has an accepted role in the detection of TB bacilli in pulmonary specimens (6). For other body fluids, such as pleural effusion and CSF, the sensitivity of PCR was almost uniformly poor (8, 15–17). More importantly, some studies have demonstrated that ADA is of limited value because it is also elevated in other types of meningitis and encephalitis (5, 9, 11). PCR is a promising method to identify genetic materials of TB bacilli. PCR has an accepted role in the detection of TB bacilli in pulmonary specimens (6). For other body fluids, such as pleural effusion and CSF, the sensitivity of PCR was almost uniformly poor (8, 15–17). Moreover, PCR has high false-positive results due to cross-contamination in high-volume clinical laboratories (3). In fact, in Zhejiang province, an eastern province of China that is relatively developed, PCR is limited for research only but not for clinical diagnosis of TB.

Sumi et al. (23) reported a new method using immunocytochemical staining of intracellular mycobacterial antigens (TBAg) found in macrophages in CSF. This method has a theoretical advantage over other assays because of the characteristics of the host immune response to the tubercle bacilli. The initial stage of infection is the ingestion of the bacilli by the macrophage. During the second stage, bacilli grow logarithmically within newly recruited macrophages (7). Thus, the cytoplasm of the macrophages in active TBM patients contains TBAg (23). Theoretically, the positive immunostaining of cytoplasm of CSF macrophages means that viable tubercle bacilli are present. Correspondingly, Sumi et al. (23) reported that the method had promising sensitivity and specificity (72.5% and 100%, respectively). Nevertheless, the sample size in their study was relatively small (22 cases). Moreover, only 3 of the 22 patients definitely had TBM, and the diagnoses of the remaining 19 patients were not well categorized. In other words, the specificity of the test would be overestimated.

Because the test is a pathological diagnostic technique, and it has solid theoretical fundaments and good clinical outcomes, we administered the same method to demonstrate intracellular TBAg of macrophages from 456 consecutive CSF samples from November 2006 to June 2009. These 456 CSF specimens came from 393 suspected TBM patients (35 patients provided CSF specimens twice, 11 patients provided them thrice, and two patients provided them four times). Only 393 CSF samples received before antimicrobial treatment were eligible for this study. These 393 patients’ ages ranged from 18 months to 88 years, with a mean of 42 years. There were 238 males and 155 females. All patients or their families signed informed consent according to the Declaration of Helsinki. All of the patients

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The sensitivity and specificity of immunocytochemical staining of mycobacterial antigens in the cytoplasm of cerebrospinal fluid (CSF) macrophages for diagnosis of tuberculous meningitis (TBM) was prospectively compared with Ahuja criteria from 393 consecutive CSF specimens. The assay can play an important role for the diagnosis of TBM, with sensitivity of 73.5% and specificity of 90.7%.

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had a headache and at least one sign of meningeal irritation. A total of 347 patients had fever, and 32 patients had diffuse encephalopathy with mental status changes that ranged from confusion and delirium to coma. Forty-five patients had focal signs such as focal seizures, cranial nerve deficit, weakness, or dysesthesia of limbs. All of the patients were hospitalized, and their final diagnoses were reviewed. The diagnosis of TBM was based on the criteria laid down by Ahuja et al. (1).

CSF samples were obtained by lumbar puncture. Three cytological slides for each CSF specimen were prepared using sedimentation chambers. A maximum of 0.5 ml of fresh CSF was added to each chamber. These chambers were put into a 4°C refrigerator and were air dried for 12 h at a minimum. One slide was stained with May-Grunwald-Giemsa (MGG) for microscopy. The second slide was prepared for immunocytological staining (for details of this method, see reference 23). One slide was left unstained for possible additional studies as needed. To establish a diagnosis of meningitis, CSF was subjected to routine biochemical and bacteriological analyses, including Gram staining, India ink preparation, and special stains and cultures for AFB.

According to the Ahuja criteria (1), 6 patients were diagnosed with definitive TBM, 17 patients with highly probable TBM, 23 patients with probable TBM, and 31 patients with possible TBM. Viral meningitis was diagnosed in 172 patients. Because we were unable to detect the specific pathogen, the diagnosis of viral meningitis was made based on its self-limited clinical course (less than 2 weeks without specific antibiotic treatment), milder symptoms and signs, and less marked CSF findings. A total of 37 patients were diagnosed with bacterial meningitis; 35 patients were diagnosed with viral encephalitis. The number of patients with other diagnoses was as follows: immune-mediated encephalitis in 14, multiple sclerosis in 12, carcinomatous meningitis in 8, intracranial hypotension in 7, other functional headache in 5, cryptococcal meningitis in 4, subarachnoid hemorrhage in 4, multiple cranial neuropathies in 4, Japanese encephalitis in 3, CNS syphilis in 3, cerebral venous sinus thrombosis in 3, stroke in 3, and normal pressure hydrocephalus in 2.

Of the 393 CSF samples, 68 showed positive immunostaining for TBAg in the cytoplasm of CSF macrophages (Fig. 1), while 325 had negative results. Of the 68 positive results, 6 were samples from definite TBM with positive culture; 45 were from the other three categories of Ahuja criteria, i.e., highly probable, probable, and possible TBM; and 17 were from patients with non-TBM infections (9 were from the group of 172 viral meningitis patients, 2 were from the group of 35 viral encephalitis patients, 5 were from the group of 37 bacterial meningitis patients, and 1 was from the group of 3 Japanese encephalitis patients).

The positive predictive values were 91.7%, 66.7%, and 38.5% in the highly probable, probable, and possible TBM groups, respectively (1). The patients suspected of having TBM could be simply categorized into TBM and non-TBM groups, and the numbers of TBM and non-TBM patients could be calculated (Table 1). Therefore, these 393 patients could be
categorized into four groups, i.e., TBM patients with positive and negative TBAg and non-TBM patients with positive and negative TBAg (Table 2). The results shown in Table 2 were used to determine the percent sensitivity, percent specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR−), and percentages of false-negative and false-positive results of immunocytochemical staining of TBAg (Table 3).

The results of our study showed similar sensitivity (73.5% versus 72.5%) and lower specificity (90.7% versus 100%) compared to those reported by Sumi et al. (23). The probable reasons for higher specificity in the report by Sumi et al. (23) are as follows: their study was retrospective, it had a small sample size, and they assumed that all clinically diagnosed TBM patients were definite TBM patients (only 3 of 22 patients were definite TBM patients). On the other hand, our prospective study has a larger sample size, and we modified the number of definite TBM patients according to the positive predictive value (PPV) based on Ahuja’s diagnostic criteria. Therefore, some positive immunostained cases that were diagnosed as highly probable, probable, and possible TBM will be assigned to the non-TBM group. As a result, the specificity will decrease. Nevertheless, immunocytochemical staining of intracellular TBAg is a promising assay for diagnosing TBM. Furthermore, the high values of the positive likelihood ratio (LR+) (7.9) and the negative predictive value (NPV) (96%) are also helpful for diagnosing TBM.

Of 344 non-TBM patients, 32 had positive immunostaining (9.5% false positive). Of those 32 cases, 15 cases were the deduced number from the suspicious TBM based on PPV using the Ahuja criteria. Of the non-TBM diagnoses, 9 cases of 172 were viral meningitis (5.2%), 2 cases of 35 were viral encephalitis (5.7%), 5 cases of 37 were bacterial meningitis (13.5%), and 1 case of 3 was Japanese encephalitis (33.3%). The reason for this is not likely contamination, because the positive immunostaining is well defined within the cytoplasm whereas negative immunostaining presents in lymphocytes (Fig. 1). When we administered polyclonal antibodies to Mycobacterium bovis, there are two possible explanations. One is that there are cross-antigens between tubercle bacilli and other microorganisms such as pyogenic bacteria and viruses. Administering specific monoclonal antibody to mycobacterium bacilli would then help to lower the false-positive rate. Another explanation is that some cases might be caused by nontuberculous mycobacteria and that there are common antigens between tuberculous mycobacteria and nontuberculous mycobacteria (22).

In summary, we demonstrated that immunocytochemical staining of mycobacterial antigens in the CSF macrophages has a sensitivity of 73.5% and a specificity of 90.7%. This outcome is consistent with that of previous reports (23). The high positive likelihood ratio (LR+) (7.9) can moderately increase the pretest probability to a higher posttest probability. The high negative predictive value (NPV) (96%) can rule out TBM under conditions in which the proportion of disease is similar to that in our lab. Moreover, the technique of the assay is relatively simple and the result of the assay can be easily observed by microscopy. Therefore, this assay can be adopted for, and has an important role in diagnosing, TBM.

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