

Use of Ligase Chain Reaction in Early Diagnosis of Tuberculous Meningitis

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Abstract

Introduction: Nucleic acid-based amplification tests are currently licensed only for the detection of *Mycobacterium tuberculosis* in pulmonary specimens. There are insufficient data for extrapulmonary specimens. The aim of this study is to investigate the diagnostic value of these investigations in tuberculous meningitis. **Materials and Methods:** We performed a prospective study using a commercial ligase chain reaction DNA amplification technique [Ligase chain result (LCx) *M. tuberculosis*; Abbott Laboratories, Abbott Park, IL, USA] on cerebrospinal fluid (CSF) to diagnose tuberculous meningitis and compared the results with standard microbiological data. Conflicting cases were resolved according to the final clinical diagnosis. A total of 54 CSF specimens from 54 patients were tested. **Results:** Six (11.1%) specimens were culture-positive for *M. tuberculosis*; of these, only 1 (1.9%) was smear-positive. The sensitivity, specificity, positive predictive value and negative predictive value of the LCx assay, compared with culture results, were 66.7%, 100%, 100% and 96%, respectively. After resolution according to clinical data, the sensitivity, specificity, positive predictive value and negative predictive value were 33.3%, 100%, 100% and 75%, respectively. All controls had negative LCx results. There were no false positives. **Conclusion:** LCx assay is highly specific and complements conventional laboratory diagnostic methods (CSF smear and culture) in the diagnosis of tuberculous meningitis. In the appropriate clinical context, a positive result is strongly suggestive of tuberculosis and could enable antituberculous treatment to be started immediately.

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Introduction

Tuberculous meningitis (TBM) is a serious disease with 100% mortality in untreated patients. In patients in whom treatment is delayed, severe neurological deficits result.¹ Hence, rapid diagnosis of TBM is important but difficult by conventional diagnostic methods. Direct smears of cerebrospinal fluid (CSF) for acid-fast bacilli (AFB), although virtually diagnostic, are usually positive in fewer than 10% of cases of TBM.^{2,3} Culture on solid media takes up to 8 weeks.⁴ The radiometric BACTEC 460 TB system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) requires an average of 13 to 15 days to detect positive specimens.⁵

Technological advances in amplifying and detecting

specific regions of bacterial DNA have led to improvements in the laboratory diagnosis of tuberculosis.⁶⁻⁹ Ligase chain reaction (LCR) is based on the joining, catalysed sequentially by polymerase and ligase, of 2 oligonucleotide probes specific for adjacent sequences in the target DNA. LCR gives positive results within 24 hours. The target sequence of LCR is the chromosomal gene of *Mycobacterium tuberculosis*, which codes for protein antigen b.¹⁰ This gene sequence appears to be specific to the *M. tuberculosis* complex (MTBC) and has been detected in all MTBC strains examined to date. The ligase chain result (LCx) *M. tuberculosis* assay (Abbott Laboratories, Abbott Park, IL, USA) is based on LCR technology. This amplification assay uses a semi-automated system, which allows direct detection of *M. tuberculosis* in clinical specimens.

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To date, LCR is validated only for respiratory specimens. Publications of the LCx assay in extrapulmonary tuberculosis include normally sterile body fluids (pleural, pericardial, synovial, CSF, ascitic fluid), urine, faeces, gastric aspirate, pus and tissue biopsy specimens.^{3,6-9,11-13} The sensitivity and specificity of LCR in the diagnosis of extrapulmonary tuberculosis range from 50% to 90.4%, and 98.5% to 100%, respectively.^{3,6-9,11-13} Few data are available on LCR in TBM.

This study investigates the diagnostic value of CSF LCR in the early diagnosis of TBM and compares the results with final clinical diagnoses and standard microbiological data.

Materials and Methods

From January 1998 to April 2002, we prospectively investigated CSF specimens collected from 2 groups of patients admitted to the Department of Neurology at Tan Tock Seng Hospital. This study was approved by the Hospital Ethics Committee. Informed consent was obtained from all patients. For obtunded patients, consent was obtained from the next-of-kin. All patients with fever, headache and neck stiffness during the study period with a clinical diagnosis of meningitis were recruited. We excluded patients with fungal meningitis (positive CSF Indian ink preparation, CSF cryptococcal antigen and fungal cultures). The study group consisted of patients with TBM, whereas the control group comprised patients with bacterial meningitis, viral meningoencephalitis and normal pressure hydrocephalus. The following diagnostic criteria were used:

Tuberculous Meningitis

Positive CSF AFB smear and culture or subacute or chronic meningitis with CSF pleocytosis, CSF protein exceeding 40 mg/dL, negative bacterial and fungal culture, computed tomography (CT) scan brain consistent with TBM (hydrocephalus, oedema, basal meningeal enhancement with or without infarcts) and response to antituberculous treatment.¹⁴ When a positive CSF culture result was available, the diagnosis was not arbitrated by clinical parameters.

Bacterial Meningitis

Acute presentation, isolation of pathogenic bacteria from CSF, CSF pleocytosis (predominantly neutrophils), low CSF glucose (<50% of that in blood) and full recovery with appropriate antimicrobial treatment.

Viral Meningoencephalitis

Acute or subacute presentation, CSF pleocytosis (predominantly lymphocytes) and bacteriologically sterile cultures. Generally, clinical course is relatively benign and

recovery is the rule. Viral meningoencephalitis may mimic TBM in some aspects of its presentation and CSF findings. Differentiation of these 2 conditions depends on clinical presentation, diagnostic tests, disease progression and response to treatment.

Patients with viral meningoencephalitis and bacterial meningitis were recruited from the same hospital ward and in the same month as patients with TBM. During the study period, consecutive patients with normal pressure hydrocephalus who had CSF drainage as evaluation for shunt placement were also recruited as controls. Once LCR results were available, the ward doctors were immediately informed.

Smear Examination and Culture

Smears were stained by auramine-rhodamine fluorochrome and positive slides were confirmed to be positive by Ziehl-Neelsen stain. The processed sediment was inoculated onto solid slants containing Löwenstein-Jensen and Coletsos media and into BACTEC medium. A BACTEC growth index >100 was considered positive.

Detection of M. tuberculosis by the LCx M. tuberculosis Assay

The LCx assay consists of 3 steps: specimen preparation, amplification and detection. These were performed according to the manufacturer's recommendations.³ Specimens were prepared by the addition of pretreated (NaOH) specimen to LCx specimen tube and centrifuged. After vortexing, the suspension was placed into LCx-covered Dry Bath. Mycobacterial DNA was released by mechanical lysis in the LCx lysor. For the amplification reaction, the specimens and controls were placed in the LCx ThermiCycler and amplified. Amplified tubes were transferred unopened to the carousel of the LCx analyser. A sample rate/cutoff value ratio of >1.0 indicates an LCx *M. tuberculosis* assay-positive result.

Clinical Evaluation

After specimen collection, the medical records of all patients were obtained and reviewed. Diagnostic criteria defined earlier in the section were used. For TBM, evaluation included the patient's history, signs, symptoms, chest X-ray, cytological and histological results for specimens, CT scan of the brain, history of drugs administered, response to empirical antituberculous treatment and follow-up observations.¹⁴ All cases were reviewed by the senior staff of the Neurology Department and the final diagnosis of TBM was made by them. This enabled us to set the combination of culture and clinical diagnosis as the "gold standard". When CSF culture results are available, the diagnosis is not arbitrated by clinical parameters. LCR results were classified based on clinical diagnosis and culture results.

Statistical Analysis

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the LCx *M. tuberculosis* assay were calculated in comparison with the culture results, and with culture results plus clinical diagnosis.

Results

A clinical diagnosis of TBM was made in 18 patients. The controls included 20 patients with viral meningoencephalitis, 8 patients with bacterial meningitis and 8 patients with normal pressure hydrocephalus. A comparison of amplification results with CSF culture is summarised in Table 1. Six (11.1%) specimens were culture-positive for *M. tuberculosis*; of these, only 1 (1.9%) was smear-positive. LCR was positive in 4 out of 6 culture-positive specimens. The overall sensitivity, specificity,

PPV and NPV of LCR, compared with culture results, were 66.7%, 100%, 100% and 96%, respectively.

LCR was positive in 6 of 18 patients with a clinical diagnosis of TBM (Table 2). The sensitivity, specificity, PPV and NPV of LCR, compared with clinical data, were 33.3%, 100%, 100% and 75%, respectively.

The results of LCx assay, culture and smear were separately compared to the clinical diagnosis (Table 3). Positive LCR, smear and culture were only obtained from patients with TBM.

Discussion

In our study, the sensitivity of LCR in TBM was 66.7% as compared with culture (Table 1), which is consistent with the 50% to 90.4% for extrapulmonary tuberculosis cited in the literature (Table 4).^{3,6-9,11-13} This wide range is attributed to the varying proportion of smear-positive and

Table 1. Comparison of LCR with Culture Results for the Detection of *M. Tuberculosis*

Specimen (no.)	Culture-positive		Culture-negative		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	LCx-positive	LCx-negative	LCx-positive	LCx-negative				
Smear-positive (1)	1	0	0	0	100	0	100	0
Smear-negative (53)	3	2	0	48	60	100	100	96
All (54)	4	2	0	48	66.7	100	100	96

LCx: ligase chain result; NPV: negative predictive value; PPV: positive predictive value

Table 2. Comparison of LCR with Clinical Diagnosis of TB

Specimen (no.)	TBM-Positive		TBM-Negative		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	LCx-positive	LCx-negative	LCx-positive	LCx-negative				
Smear-positive (1)	1	0	0	0	100	0	100	0
Smear-negative (53)	5	12	0	36	29.4	100	100	75
All (54)	6	12	0	36	33.3	100	100	75

LCR: ligase chain reaction; LCx: ligase chain result; NPV: negative predictive value; PPV: positive predictive value; TB: tuberculosis

Table 3. Comparison of LCR, Culture and Microscopy Results with Clinical Diagnosis of TBM

Method and result (no. of samples)	No. of specimens		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	TBM (n = 18)	non-TBM (n = 36)				
LCx assay						
Positive (6)	6	0	33.3	100	100	75
Negative (48)	12	36				
Culture						
Positive (6)	6	0	33.3	100	100	75
Negative (48)	12	36				
Smear						
Positive (1)	1	0	5.6	100	100	67.9
Negative (53)	17	36				

LCR: ligase chain reaction; LCx: ligase chain result; NPV: negative predictive value; PPV: positive predictive value; TBM: tuberculous meningitis

Table 4. Sensitivity and Specificity of LCR in Diagnosis of Extrapulmonary Tuberculosis*

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Gamboa et al ³	78.5	100	100	93.1
Ruiz-Serrano et al ⁶	50	100	100	93
Palacios et al ⁷	90.4	98.5	86.3	99
Piersimoni et al ⁸	53.6	99.3	93.7	92.1
Tortoli et al ⁹	73.33	100	100	97.1
Garrino et al ¹¹	70	100	100	93.8
Lumb et al ¹²	67.6	100	100	95.3
Shetty et al ¹³	85.7	100	100	97.7
Our results	66.7	100	100	100

CSF: cerebrospinal fluid; NPV: negative predictive value; PPV: positive predictive value

*All studies were extrapulmonary from several sources combined: pleural, pericardial, synovial, CSF, ascitic fluid, urine, faeces, gastric aspirate, pus and tissue biopsy specimens. Our study worked on CSF only.

smear-negative specimens in different studies. The ability of LCx assay to detect *M. tuberculosis* is directly related to the concentration of bacilli in the specimen. Hence, studies with a high rate of smear-positive and culture-positive samples have a sensitivity of about 90%,^{9,12,15,16} while studies with a lower rate of positive samples have a sensitivity of about 78%.¹¹ Current literature include extrapulmonary tuberculosis from various sources: pleural, pericardial, synovial, CSF, ascitic fluid, urine, faeces, gastric aspirate, pus and tissue biopsy specimens. Our study is the only one that worked on CSF only. All studies found LCR useful as it is a rapid and specific test, but cautioned against replacing conventional diagnostic methods with LCR.

False negative LCR, despite positive CSF, culture is common and is attributed to 2 reasons. The first reason is non-uniform distribution or low number of micro-organisms in clinical specimens.^{11,12} Repeated samples should be tested, especially those that are smear-negative and culture-positive. We did not send repeat specimens in our study due to financial reasons. Secondly, the presence of inhibitors of the enzymatic amplification reaction can cause false negatives. Rates of occurrence of false negatives >20% have been reported.^{17,18} Inhibitors were present in 54.5% of CSF specimens using the Amplicor Sputum Preparation Kit (Hoffmann-LaRoche, Grenzach-Wyhlen, Germany)¹⁷ and 7.5% of nonrespiratory specimens using the Gen Probe Amplified *M. tuberculosis* Direct Test.¹⁸ The importance of proper sample preparation for amplification procedures to eliminate inhibitors has been demonstrated in several studies.^{19,20} Phosphate ions have been found to be potent inhibitors of LCR amplification. Leckie et al and other authors have described modifications to the original assay to reduce inhibitors, thereby reducing the false negatives.¹⁹

In conclusion, LCR cannot replace CSF smear and culture in the diagnosis of TBM. LCR complements these 2 processes with the aim of increasing yield and obtaining early clinical diagnosis. Because of the poor NPV, a negative result does not exclude TBM. Communication with the clinician is important as a clinical diagnosis of tuberculosis may be made even if LCR is negative and *M. tuberculosis* cannot be isolated from the CSF. In the clinical context of subacute or chronic meningitis with CSF lymphocytic pleocytosis, a positive result is strongly suggestive of TBM. Early institution of antituberculous therapy reduces the morbidity and mortality resulting from delayed treatment.

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REFERENCES

1. Kent SJ, Crowe SM, Yung A, Lucas CR, Mijch AM. Tuberculous meningitis: a 30-year review. *Clin Infect Dis* 1993;17:987-94.
2. Chapin K. Clinical microscopy. In: Murray PR, Baron EJ, Pfaller MA, Tenoer FC, Tenover RH, editors. *Manual of Clinical Microbiology*. 6th ed. Washington DC: American Society for Microbiology, 1995:33-51.
3. Gamboa F, Dominguez J, Padilla E, Manterola JM, Gazapo E, Lonca J, et al. Rapid diagnosis of extrapulmonary tuberculosis by ligase chain reaction amplification. *J Clin Microbiol* 1998;36:1324-9.
4. Abe C, Hosojima S, Fukasawa Y, Kazumi Y, Takahashi M, Hirano K, et al. Comparison of MB-Check, BACTEC, and egg-based media for recovery of mycobacteria. *J Clin Microbiol* 1992;30:878-81.
5. Siddiqi SH. BACTEC 460 TB SYSTEM: products and procedure manual, revision D. Becton Dickinson and Company, Sparks, MD 1995.
6. Ruiz-Serrano MJ, Albadalejo J, Martinez-Sanchez L, Bouza E. LCx: a diagnostic alternative for the early detection of *Mycobacterium tuberculosis* complex. *Diagn Microbiol Infect Dis* 1998;32:259-64.
7. Palacios JJ, Ferro J, Ruiz Palma N, Roces SG, Villar H, Rodriguez J, et al. Comparison of the ligase chain reaction with solid and liquid culture media for routine detection of *Mycobacterium tuberculosis* in nonrespiratory specimens. *Eur J Clin Microbiol Infect Dis* 1998;17:767-72.
8. Piersimoni C, Callegaro A, Scarparo C, Penati V, Nista D, Bornigia S, et al. Comparative evaluation of the new gen-probe *Mycobacterium tuberculosis* amplified direct test and the semiautomated Abbott LCx *Mycobacterium tuberculosis* assay for direct detection of *Mycobacterium tuberculosis* complex in respiratory and extrapulmonary specimens. *J Clin Microbiol* 1998;36:3601-4.
9. Tortoli E, Lavinia F, Simonetti MT. Evaluation of a commercial ligase chain reaction kit (Abbott LCx) for direct detection of *Mycobacterium tuberculosis* in pulmonary and extrapulmonary specimens. *J Clin Microbiol* 1997;35:2424-6.
10. Andersen AB, Hansen EB. Structure and mapping of antigenic domains of protein antigen b, a 38,000-molecular-weight protein of *Mycobacterium tuberculosis*. *Infect Immun* 1989;57:2481-8.
11. Garrino MG, Glupczynski Y, Degraux J, Nizet H, Delmee M. Evaluation of the Abbott LCx *Mycobacterium tuberculosis* assay for direct detection of *Mycobacterium tuberculosis* complex in human samples. *J Clin Microbiol* 1999;37:229-32.

12. Lumb R, Davies K, Dawson D, Gibb R, Gottlieb T, Kershaw C, et al. Multicenter evaluation of the Abbott LCx *Mycobacterium tuberculosis* ligase chain reaction assay. *J Clin Microbiol* 1999;37:3102-7.
 13. Shetty N, Shemko M, Holton J, Scott GM. Is the detection of *Mycobacterium tuberculosis* DNA by ligase chain reaction worth the cost: experiences from an inner London teaching hospital. *J Clin Pathol* 2000;53:924-8.
 14. Thwaites GE, Chau TT, Stepniewska K, Phu NH, Chuong LV, Sinh DX, et al. Diagnosis of adult tuberculous meningitis by use of clinical and laboratory features. *Lancet* 2002;360:1287-92.
 15. Ausina V, Gamboa F, Gazapo E, Manterola JM, Lonca J, Matas L, et al. Evaluation of the semiautomated Abbott LCx *Mycobacterium tuberculosis* assay for direct detection of *Mycobacterium tuberculosis* in respiratory specimens. *J Clin Microbiol* 1997;35:1996-2002.
 16. Lindbråthen A, Gaustad P, Hovig B, Tønjum T. Direct detection of *Mycobacterium tuberculosis* complex in clinical samples from patients in Norway by ligase chain reaction. *J Clin Microbiol* 1997;35:3248-53.
 17. Kirschner P, Rosenau J, Springer B, Teschner K, Feldmann K, Bottger EC. Diagnosis of mycobacterial infections by nucleic acid amplification: 18-month prospective study. *J Clin Microbiol* 1996;34:304-12.
 18. Ehlers S, Ignatius R, Regnath T, Hahn H. Diagnosis of extrapulmonary tuberculosis by Gen-Probe amplified *Mycobacterium tuberculosis* direct test. *J Clin Microbiol* 1996;34:2275-9.
 19. Leckie GW, Erickson DD, He Q, Facey IE, Lin BC, Cao J, et al. Method for reduction of inhibition in a *Mycobacterium tuberculosis*-specific ligase chain reaction DNA amplification assay. *J Clin Microbiol* 1998;36:764-7.
 20. Amicosante M, Richeldi L, Trenti G, Paone G, Campa M, Bisetti A, et al. Inactivation of polymerase inhibitors for *Mycobacterium tuberculosis* DNA amplification in sputum by using capture resin. *J Clin Microbiol* 1995;33:629-30.
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