Determination of cerebrospinal fluid adenosine deaminase activity cut-off for the diagnosis of tuberculous meningitis in Hong Kong

Toby Chun Hei Chan ,[1,2] Sammy Pak Lam Chen ,[2,3] Chloe Miu Mak,[1,2] Chor Kwan Ching,[2] Kristine Shik Luk,[4] Yat Ming Tsang,[4] Daniel Cheuk Wa Leung[2]

[1]Chemical Pathology Laboratory, Department of Pathology, Hong Kong Children's Hospital, Kowloon, Hong Kong
[2]Chemical Pathology Laboratory, Department of Pathology, Princess Margaret Hospital, Kowloon, Hong Kong
[3]Chemical Pathology Laboratory, Department of Pathology, Queen Elizabeth Hospital, Kowloon, Hong Kong
[4]Microbiology Laboratory, Department of Pathology, Princess Margaret Hospital, Kowloon, Hong Kong

Correspondence to
Dr Sammy Pak Lam Chen, Chemical Pathology Laboratory, Department of Pathology, Queen Elizabeth Hospital, Kowloon, Hong Kong; chenpls@ha.org.hk

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ABSTRACT

Aims Tuberculous meningitis (TBM) is a severe infection which may lead to serious complication and mortality. Prompt diagnosis and treatment are essential. There is a need for a simple and fast laboratory test to differentiate TBM from other causes.

Methods Retrospective review was conducted for cerebrospinal fluid adenosine deaminase (CSF-ADA) activity which was measured at the Chemical Pathology Laboratory of Princess Margaret Hospital, the sole centre providing such service in Hong Kong, for 51 patients with suspected meningitis from nine local hospitals between June 2014 and July 2017. TBM diagnosis was defined by positive culture and/or nucleic acid amplification test result of *Mycobacterium tuberculosis* complex in CSF.

Results CSF-ADA activity was significantly higher in the TBM group (8.6±2.1 IU/L, n=8) than that of the non-TBM group (2.8±5.9 IU/L, n=43). The optimal clinical cut-off of 5.1 U/L for TBM diagnosis in our laboratory yielded 100% sensitivity, 91% specificity, positive likelihood ratio of 10.8 and negative likelihood ratio of 0. In rare circumstance, false elevation may be seen in non-tuberculous cause, such as central nervous system lymphoma and fungal infection.

Conclusions We recommend the use of CSF-ADA activity, which is a simple, fast and robust test for early differentiation of TBM from other causes, to facilitate timely initiation of antituberculous treatment and potentially improve patients' outcome.

INTRODUCTION

Mycobacterium tuberculosis infection is a major healthcare problem accounting for significant morbidity and mortality, and has been a notifiable disease in Hong Kong since 1939. Tuberculous meningitis (TBM) is notorious for its poor prognosis. In 2015, TBM accounted for 0.9% of all forms of tuberculosis (38 cases) and constituted 3.6% of all tuberculosis deaths in Hong Kong.[1]

Timely diagnosis and treatment are important, but are often challenging to clinicians because of the ambiguous clinical characteristics and the lack of a simple and rapid diagnostic test with good sensitivity and specificity. The gold standard test of culturing mycobacteria is time-consuming. Nucleic acid amplification test (NAAT), on the other hand, may give false-negative results for small volume cerebrospinal (CSF) samples or in the presence of inhibitors (eg, CSF protein). The determination of cerebrospinal fluid adenosine deaminase (CSF-ADA) activity has been suggested to be a simple, robust and reliable diagnostic tool for TBM. The 2017 clinical practice guideline for the diagnosis of tuberculosis joint-published by the American Thoracic Society, Centers for Disease Control and Prevention, and Infectious Diseases Society of America suggested the use of CSF-ADA activity in patients with suspected TBM. However, the lack of consensus for threshold to define an elevated CSF-ADA activity, which could range from 10 to 71 IU/L in the literature, has been a major obstacle in its clinical application.[2]

We undertook this retrospective study to evaluate the usefulness of CSF-ADA activity and to determine a practical cut-off value to differentiate TBM from other causes in Hong Kong.

MATERIALS AND METHODS

We retrospectively reviewed the electronic medical records of patients with suspected meningitis from nine local hospitals in Hong Kong with CSF-ADA activity measured at our Chemical Pathology Laboratory of Princess Margaret Hospital, the sole laboratory providing such service to all local public and private healthcare sectors, between 1 June 2014 and 31 July 2017. Only the CSF-ADA activity measured for the earliest specimen was included for individual patients. Patients less than 18 years old or whose CSF-ADA activity were measured after commencement of antituberculous therapy were excluded.

TBM diagnosis was defined by positive culture and/or NAAT result of *M. tuberculosis* complex in CSF. Patients with negative results were grouped as the non-TBM group, which their final diagnoses were confirmed by further microbiological, serological and histological investigations.

The CSF-ADA activity was measured by the Diazyme adenosine deaminase assay on the automated Beckman Coulter AU681 clinical chemistry analyser (Beckman Coulter, Brea, California, USA). The assay principle replies on the enzymatic deamination of adenosine to inosine and Trinder reaction, with subsequent colorimetric detection. One unit of ADA activity is defined as the amount required to generate 1 μmol of inosine from adenosine per minute at 37°C.

The CSF-ADA activity of the TBM group was compared against that of the non-TBM group by two sample t test. Optimal cut-off value was determined by receptor-operating characteristic (ROC)

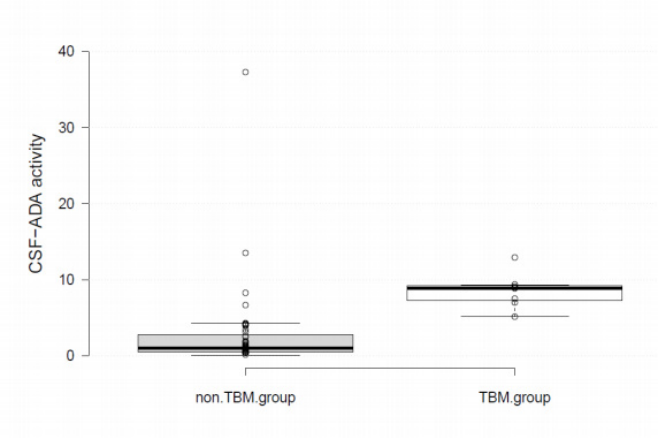

Figure 1 Box and whisker plot of CSF-ADA activity in the TBM group and non-TBM group

curve analysis. Data analyses, including two-tailed t test of heteroscedastic type, were performed using Microsoft Excel V.2016 (Microsoft).

RESULTS

Fifty-one Chinese patients, 29 male and 22 female, of age from 18 to 89 years old with a median age of 54 years old were included in the study. There were 8 HIV-positive patients, with 1 in the TBM group (total number=8) and the rest in the non-TBM group (total number=43). The HIV status of the others were unknown.

There were eight patients in the TBM group. The diagnoses in the non-TBM group were as follows: aseptic meningitis (n=12), delirium (n=6), viral meningoencephalitis (n=4), sepsis (n=4), bacterial meningitis (n=2), TB lymphadenopathy (n=2), epilepsy (n=2), fungal meningitis (n=1), central nervous system (CNS) lymphoma (n=1), transverse myelitis (n=1), lupus cerebritis (n=1), rheumatoid meningitis (n=1), sarcoidosis (n=1) and unknown (n=1).

The mean CSF-ADA activity in the TBM group (8.6±2.1 IU/L) was statistically higher than that of the non-TBM group (2.8±5.9 IU/L) with p value<0.01 (figure 1). ROC curve analysis (figure 2) demonstrated the area under curve (AUC) to be 0.91. The optimal cut-off value of 5.1 U/L showed a sensitivity of 100% and specificity of 91%. The positive likelihood ratio was 10.8 and the negative likelihood ratio was 0, suggesting the test to be a good clinical tool for TBM diagnosis.

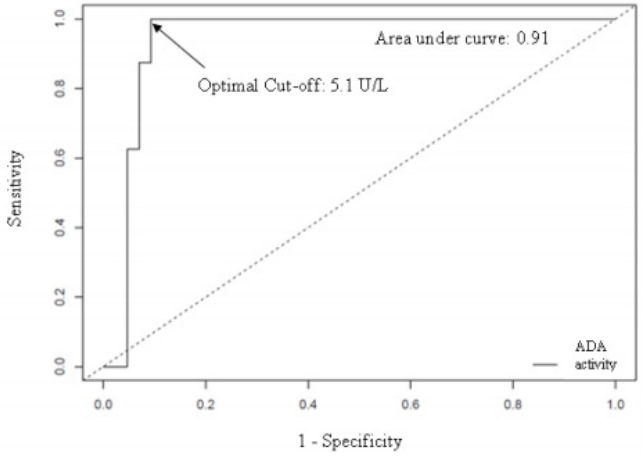

Figure 2 ROC curve of CSF-ADA activity in the diagnosis of TBM

DISCUSSION

Early diagnosis and commencement of antituberculosis treatment is probably the most important factor to reduce TBM patients' mortality and morbidity.[3] Diagnostic tools that could effectively differentiate TBM from other causes during the initial presentation would certainly improve patients' prognosis.

Conventional methods such as acid-fast bacilli smear microscopy and mycobacterial cultures have excellent specificity of >90% and >97%, respectively; however, both suffer from poor sensitivity of <5% and 45%–70%, respectively, when performed on CSF.[2] Furthermore, positivity of culture results often takes around 6 weeks. Newer technique, such as NAAT performed on CSF, has remarkable specificity of 98% but only fair sensitivity of 62%.[2]

CSF-ADA activity has been suggested to be a useful marker for differentiating TBM from other causes.[2 4–7] Xu *et al*, in their meta-analysis, had selected 10 studies and showed the sensitivity and specificity of CSF-ADA activity for TBM diagnosis to be 79% and 91%, respectively.[4] The meta-analysis of 13 studies by Tuon *et al* demonstrated that a cut-off of 4 IU/L could achieve 93% sensitivity and 80% specificity, while a lower cut-off of 3 IU/L would practically exclude TBM with 98% sensitivity.[5] Lately in 2017, Pormohammad *et al* further summarised the usefulness of CSF-ADA activity with overall 89% sensitivity, 91% specificity and AUC of 0.96 in TBM diagnosis.[6] However, some had questioned its clinical value because cut-off levels across studies were heterogeneous and ranged from 5 to 20 IU/L, likely resulted from suboptimal assay standardisation.[8] This highlighted the importance that regional laboratory should provide an appropriate and optimal cut-off value for clinical interpretation.

In the present study, CSF-ADA activity was statistically higher in the TBM group than that of the non-TBM group (TBM: 8.6±2.1 IU/L, non-TBM: 2.8±5.9 IU/L, p<0.01). A small degree of overlap was observed, in keeping with past studies.[4–7] The optimal cut-off of 5.1 U/L with 100% sensitivity and 90.7% specificity confirmed CSF-ADA activity to be a good diagnostic tool for TBM. Our findings were comparable with that of the prospective study by Raviraj *et al* in 2017, in which the mean CSF-ADA activity in the TBM group and non-TBM group were found to be 10.97 U/L and 5.09 U/L, respectively; the cut-off value of 6.65 U/L provided 85% sensitivity and 84% specificity.[7]

In the non-TBM group, a case with an exceptionally high CSF-ADA activity (37.2 U/L) was encountered. The patient was subsequently diagnosed of primary CNS lymphoma with CSF showing lymphocytosis (178 cell/mm^3), high protein (2457 mg/L) and very low glucose (<0.2 mmol/L). ADA activity could be found in all cells, with highest activity in monocyte and lymphocytes. Therefore, elevated CSF-ADA activity may sometimes be seen in other neurological conditions, such as lymphoma, sarcoidosis, neurobrucellosis, neurological **cytomegalovirus** disease, cryptococcal meningitis, candidal meningitis and subarachnoid haemorrhage, but the elevations were generally to a lesser degree as compared with TBM.[9 10] In the non-TBM group, another HIV-positive patient with CNS *Penicillium marneffei* infection also had modestly elevated CSF-ADA activity (8.2 U/L). Therefore, confirmatory tests for TBM, for example, neuroimaging, mycobacterial cultures and NAAT should be performed for patients with elevated CSF-ADA activity.

Selection bias is one of the limitations of this retrospective study. The necessities of requesting for CSF-ADA activity testing

were decided by the attending clinicians. Therefore, the cases included in this study would represent a certain proportion of all TBM and meningitis of other cause in Hong Kong. The low number of CSF-ADA requested during the study period highlighted the underutilisation of this simple and useful test in the workup of TBM. Another limitation is that the HIV statuses of most patients were unknown. Apart from the case of *P. marneffei* infection mentioned above, there was as well another HIV-positive patient with TBM who had elevated CSF-ADA activity (8.8 U/L). The relationship between HIV status and CSF-ADA activity had been a continuing debate. CSF-ADA activity was most often assumed to be lower in patients in immunocompromised state; however, this assumption had been repeatedly challenged. Some had reported that HIV statuses do not significantly affect CSF-ADA activity in patient with TBM.[11 12] A recent review by Ekermans *et al* had unexpectedly showed that CSF-ADA activities were even higher in HIV-positive patients, the underlying reason was unknown, and may be related to comorbidities in HIV-positive patients.[8] Corral *et al* suggested that the diagnostic utility of CSF-ADA activity was relatively limited for HIV-positive patient; using a cut-off of 8.5 IU/L, the sensitivity and specificity were 57% and 87%, respectively.[10] Furthermore, conditions that may give rise to false elevation of CSF-ADA activity, for example, cryptococcal meningitis, candidal meningitis and cerebral toxoplasmosis, were considerably more prevalent in HIV-positive patient. Overall, the role of CSF-ADA activity in TBM diagnosis for HIV-positive patient is unclear. It should also be noted that assays for CSF-ADA activity are not well standardised across laboratories; the cut-off value derived in this study would be only applicable to laboratories employing similar methodology.

We recommend the clinical use of CSF-ADA activity for earlier differentiation of TBM from other causes. In Hong Kong, the mean lag time between admission and initiation of antituberculosis treatment in Hong Kong was found to be 5.22 days and could be up to 30 days.[3] The measurement of CSF-ADA activity is simple, fully automated and reproducible, with turnaround time within 1–2 working days. Early measurement of CSF-ADA activity would facilitate timely initiation of antituberculous treatment and potentially improve patients' outcome.

Take home messages

- Cerebrospinal fluid adenosine deaminase (CSF-ADA) activity is a useful marker of tuberculous meningitis (TBM), which is significantly elevated in patients with TBM compared with other neurological conditions.
- CSF-ADA activity measurements were simple and fully automated; results were available within 1–2 working days, providing clinically invaluable information in a timely manner.
- The optimal cut-off of CSF-ADA activity was determined to be 5.1 U/L with excellent sensitivity and specificity distinguishing TBM from other neurological conditions.

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ORCID iDs
Toby Chun Hei Chan http://orcid.org/0000-0001-9099-4720
Sammy Pak Lam Chen http://orcid.org/0000-0003-0767-3163

REFERENCES

1 Tuberculosis and Chest Service DoH, the Government of the HOng Kong Special Adminstrative Region. Annual report 2016 Tuberculosis & Chest Service of the,Department of Health 2016.
2 Lewinsohn DM, Leonard MK, LoBue PA, *et al*. Official American thoracic Society/Infectious diseases Society of America/Centers for disease control and prevention clinical practice guidelines: diagnosis of tuberculosis in adults and children. *Clin Infect Dis* 2017;64:111–5.
3 Lau KK, Yu ITS, Chan ACK, *et al*. A Registry of tuberculous meningitis in Hong Kong. *Int J Tuberc Lung Dis* 2005;9:1391–7.
4 Xu H-B, Jiang R-H, Li L, *et al*. Diagnostic value of adenosine deaminase in cerebrospinal fluid for tuberculous meningitis: a meta-analysis. *Int J Tuberc Lung Dis* 2010;14:1382–7.
5 Tuon FF, Higashino HR, Lopes MIBF, *et al*. Adenosine deaminase and tuberculous meningitis--a systematic review with meta-analysis. *Scand J Infect Dis* 2010;42:198–207.
6 Pormohammad A, Riahi S-M, Nasiri MJ, *et al*. Diagnostic test accuracy of adenosine deaminase for tuberculous meningitis: A systematic review and meta-analysis. *J Infect* 2017;74:545–54.
7 Henry RA, Rao GG. Determination and validation of a lower cut off value of cerebrospinal fluid adenosine deaminase (CSF-ADA) activity in diagnosis of tuberculous meningitis. *J Clin Diagn Res* 2017;11:OC22–4.
8 Ekermans P, Dusé A, George J. The dubious value of cerebrospinal fluid adenosine deaminase measurement for the diagnosis of tuberculous meningitis. *BMC Infect Dis* 2017;17:104.
9 Katti MK, Pathogenesis KMK. Pathogenesis, diagnosis, treatment, and outcome aspects of cerebral tuberculosis. *Med Sci Monit* 2004;10:RA215–29.
10 Corral I, Quereda C, Navas E, *et al*. Adenosine deaminase activity in cerebrospinal fluid of HIV-infected patients: limited value for diagnosis of tuberculous meningitis. *Eur J Clin Microbiol Infect Dis* 2004;23:471–6.
11 Ena J, Crespo MJ, Valls V, *et al*. Adenosine deaminase activity in cerebrospinal fluid: a useful test for meningeal tuberculosis, even in patients with AIDS. *J Infect Dis* 1988;158:896.
12 López-Cortés LF, Cruz-Ruiz M, Gómez-Mateos J, *et al*. Adenosine deaminase activity in the CSF of patients with aseptic meningitis: utility in the diagnosis of tuberculous meningitis or neurobrucellosis. *Clin Infect Dis* 1995;20:525–30.