Received: 21 December 2020 | Revised: 15 May 2021 | Accepted: 3 June 2021

DOI: 10.1002/ccr3.4526

CASE REPORT

Clinical Case Reports Open Access WILEY

Use of alternative cerebrospinal fluid-based biomarkers to help diagnose Xpert-negative tuberculous meningitis: A case report

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Abstract

The widely used Xpert MTB/RIF assay, limited by its suboptimal sensitivity, does not perform well as a rule-out test for tuberculous meningitis. Alternative CSF-based biomarkers can help determine the microbial etiology of clinical meningitis when standard diagnostic modalities fail.

KEYWORDS

adenosine deaminase, CSF-based biomarkers, negative Xpert assay, tuberculous meningitis

1 | INTRODUCTION

Tuberculosis is one of the leading causes of death in the world, and about 98% of its global burden is accounted for by low- and middle-income countries.[1] It is a raging public health problem in the Nepalese context as well, with estimates of prevalence and incidence as high as 416 per 100,000 and 245 per 100,000, respectively.[2] A TB mortality rate that ranks 18th globally suggests an even graver status of the disease in the country.[1]

Tuberculous meningitis (TBM) is the most devastating form of extrapulmonary TB associated with significant neurological morbidity and mortality, in large part due to the difficulty in its diagnosis. The clinical presentation of TBM is non-specific; thus, in practice, different diagnostic tools are sought to initiate chemotherapy. However, many such tools are limited by their subpar diagnostic accuracy or are not suited to the resource settings of a country like Nepal. Furthermore, patients often present late to health facilities with a dreadful clinical picture, allowing physicians only a small window of time to diagnose and treat the disease. Hence, this warrants the need for a diagnostic modality that not only holds a high sensitivity and specificity but also is feasible enough on the grounds of time and resources.

Herein, we report a case of a 5-year-old boy with suspected meningitis who, despite having negative microscopy and Xpert test, was treated with empiric antituberculous therapy (ATT). The decision was aided by the assessment of alternative CSF-based markers, namely adenosine deaminase (ADA), lactate dehydrogenase (LDH), and C-reactive protein (CRP). We believe this case reflects the limitations of standard diagnostic modalities for TBM and emphasizes the use of alternative diagnostic tools, especially in resource-poor settings like Nepal, for the timely intervention of the disease.

2 | CASE PRESENTATION

A 5-year-old boy was brought to the pediatric emergency unit with a 2-week history of low-grade intermittent fever—usually occurring in the evening—and persistent throbbing frontal headache, along with a 1-week history of vomiting. He had first presented to his local hospital after the onset of vomiting, where he underwent empiric antibiotic therapy for having been suspected of acute bacterial meningitis. He was reportedly responding well to the antibiotics; however, on the seventh day of the treatment, he suffered from an episode of generalized tonic-clonic seizure and was then referred to our

center. He described having weakness in his left limbs after the seizure episode but reported no complaints of nuchal rigidity, photophobia, numbness, or paresthesia. Furthermore, he had no history of TB in the past or contact with known cases of TB.

On examination, the patient was febrile with an oral temperature of 38.2℃. His pulse rate was 95 beats/min, respiratory rate was 28 breaths/min, and blood pressure was 80/60 mm Hg. He had altered consciousness with a Glasgow Coma Scale score of 11:E4V2M5. His left pupil was dilated and non-reactive, but other functions of the left third cranial nerve (CN III) were intact. Further, there were brisk tendon reflexes and increased muscle tone in all four extremities, and both plantar responses were upwards. Both the left limbs had an MRC muscle power grade of 3/5, while the right limbs had normal power. No abnormal breath sounds were heard on auscultation of the chest, and there were no signs and symptoms of respiratory infection.

Complete blood count revealed leukocytosis (16.8×10^9/L; normal range for the 2–6 age group: $5.0–15.0 \times 10^9$/L) with neutrophilia (13.1×10^9/L; normal: $1.5–8.0 \times 10^9$/L) and monocytosis (1.52×10^9/L; normal: $0.2–1.0 \times 10^9$/L). RBC count, platelet count, and hemoglobin level were normal, whereas erythrocyte sedimentation rate was elevated (30 mm/hr). Serum sodium, potassium, and glucose levels were within normal limits. VDRL, HIVAb, HBsAg, and HCVAb tests were negative, and liver function tests (LFTs) and renal panel were unremarkable.

CSF of the patient was clear in appearance and had elevated protein (1.6 g/L; normal: 0.15–0.45 g/L) and low glucose (1.0 mmol/L; normal: 2.5–4.4 mmol/L) levels. There was marked pleocytosis (1.0×10^8/L; normal: $<5.0 \times 10^6$/L) with lymphocytic predominance (75%), and no organisms were observed on the Gram stain, Ziehl-Neelsen (ZN) stain, and India ink stain. A sample was sent for culture in chocolate and blood agar media. Likewise, the Xpert MTB/RIF assay was negative.

The patient had a normal chest X-ray. An axial non-contrast CT scan of his head revealed a hypodense lesion in the area of the right basal ganglia and internal capsule suggestive of an infarct, along with a mildly dilated entire ventricular system suggestive of extraventricular obstructive hydrocephalus. A subsequent CT scan obtained after contrast administration showed enhancement of the basal meninges (Figure 1). All in all, the history of seizure and the findings of altered consciousness, left-sided hemiparesis, partial left CN III palsy, hydrocephalus, and cerebral infarction indicated that the patient was already in an advanced disease stage.

The history of failed antibiotic therapy and the findings of CSF examination—clear appearance, lymphocytic pleocytosis, and negative Gram stain—established that this was a case of aseptic meningitis. However, the underlying cause for it was not apparent. Low glucose in CSF and the increasing disease severity were inconsistent with the usually self-limiting viral meningitis. Negative India ink stain and negative VDRL test ruled out cryptococcal and syphilitic meningitis, respectively, while negative ZN stain and Xpert test were inconsistent with TBM. There was no history suggestive of drug-induced aseptic meningitis, and CSF cytology showed no evidence of malignant cells that could indicate the diagnosis of neoplastic meningitis. Parasitic meningitis was ruled out due to the lack of CSF eosinophilia and any relevant CT findings, and autoimmune causes of meningitis were dismissed because the patient had no extra-neurological signs and symptoms indicative of diseases like Behçet's and SLE.

Given the lack of any definitive evidence to establish a diagnosis, three additional markers were evaluated in the CSF of the patient, namely ADA, LDH, and CRP. The former two were elevated (29 IU/L and 99 IU/L, respectively), and the qualitative latex agglutination test for the latter was negative. The elevated ADA level suggested the diagnosis of TBM, which was corroborated by the negative CRP result (indicative of non-pyogenic meningitis) and the elevated LDH level

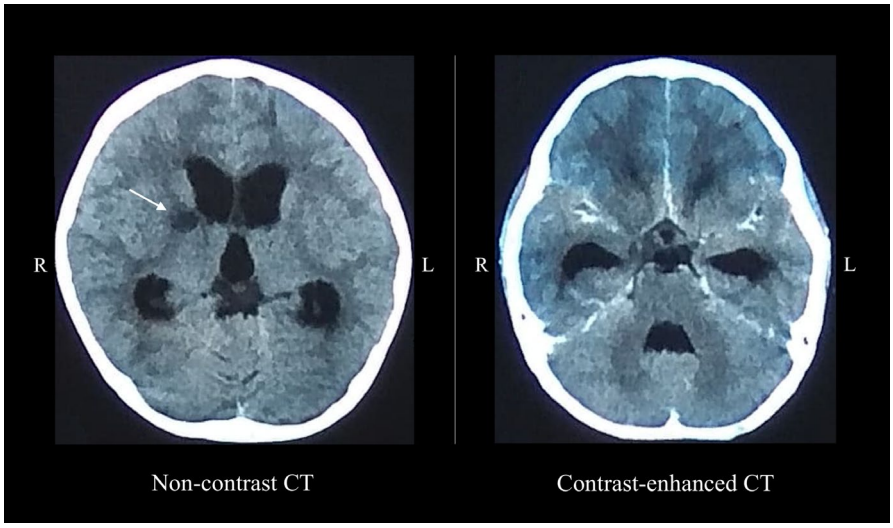

FIGURE 1 Axial non-contrast CT scan of the head (left) revealing a hypodense lesion suggestive of an infarct. Subsequent CT scan obtained after contrast administration (right) showing basal meningeal enhancement

(indicative of non-viral meningitis). Therefore, despite having a negative Xpert test, the patient was promptly started on ATT within a day of presentation. Understanding the limitations of Xpert assay and the fatality and national prevalence of TBM also helped decide on the treatment. Moreover, the CSF culture results returned negative after 48 hours of incubation and did not affect the treatment strategy.

The ATT was to consist of a 2-month intensive phase (isoniazid, rifampicin, pyrazinamide, and ethambutol) followed by a 10-month maintenance phase (isoniazid and rifampicin). Furthermore, the patient was also commenced on pyridoxine supplementation, phenytoin therapy, and adjunctive corticosteroid therapy (prednisone gradually tapered over 4 weeks). An external ventricular drain was placed to manage hydrocephalus, which was converted into a ventriculoperitoneal shunt after a week.

The patient had a delay of about two weeks between the onset of fever and the start of ATT. Although the delay was indicative of a poor prognosis, significant clinical improvement was observed during the therapy. His temperature dropped to normal by the 10th day, he recovered full consciousness by the 18th day, and power in his left limbs improved to 4/5 by the 28th day. CSF analysis revealed normal leukocyte and glucose levels with a slightly elevated protein level on the 5th day, and ADA level was down to 4.8 IU/L on the 21st day. The positive treatment response thus confirmed the diagnosis of TBM. The patient was regularly monitored for any adverse drug reactions, with LFTs being performed every 2 weeks until he was discharged. Slight elevations in bilirubin, alanine transaminase, and aspartate transaminase levels were noted, but they were well below the cutoff levels for liver dysfunction.

The patient was discharged 6 weeks after the start of treatment, with an arrangement of directly observed therapy in his local health center, and was called for a follow-up 3 months later. Upon follow-up, there were no signs of neurological deficit, findings of LFTs and complete blood count were unremarkable, and no adverse drug reactions were noted.

3 | DISCUSSION

Aseptic meningitis is the inflammation of meninges not caused by pus-forming bacteria. Etiologically, it can be viral (enterovirus, human parechovirus, paramyxovirus), tuberculous, bacterial (*Treponema*, *Brucella*, *Borrelia*, *Leptospira*), fungal (*Candida*, *Histoplasma*, *Cryptococcus*), parasitic (*Angiostrongylus*, *Taenia*), drug-induced, neoplastic, autoimmune, etc. Viruses are the most common cause of meningitis in the pediatric population, and enteroviruses are the most frequently detected ones.[3] TBM is another common type of meningitis in children, especially in the third world, and is far more fatal than viral meningitis. The pathogenesis of TBM involves the seeding of bacilli in meninges or brain parenchyma during the bacillemia of primary infection or disseminated disease, forming caseous lesions called Rich foci, the rupture of which into the subarachnoid space heralds the onset of meningitis.[4]

The clinical features of TBM are non-specific, with fever (frequency: 60%–95%) and headache (50–80%) being the cardinal symptoms. Vomiting (30%–60%), nuchal rigidity (40%–80%), and photophobia (5%–10%) are usually absent in the initial stages of the disease. Many patients present late with focal neurological signs like hemiparesis (10–20%) and cranial nerve palsy (CN VI: 30%–40%, CN:VII: 10–20%, and CN III: 5%–15%), seizures (50% among children), altered consciousness (10%–30%), and coma (30%–50%).[5]

The recommended CSF criteria for the diagnosis of TBM include clear appearance, elevated protein (> 1g/L), low glucose (absolute concentration <2.2 mmol/L or CSF-to-plasma glucose ratio <0.5), and pleocytosis (10–500/μl) with lymphocytic predominance (> 50%).[6] However, the findings noted above can also be seen in fungal, carcinomatous, and non-tuberculous bacterial meningitis. Atypical CSF findings like normal cell counts, neutrophilic predominance, and normal glucose levels have also been reported in TBM patients co-infected with HIV, indicating that the above CSF criteria do not hold a high diagnostic value.[7]

The sighting of acid-fast bacilli in the CSF allows for a definitive diagnosis of TBM; however, its sensitivity is less than 15%. Culture has a higher sensitivity (50–60%) but is too slow to be feasible for clinical use.[8] The Xpert MTB/RIF assay is a novel diagnostic tool based on polymerase chain reaction that simultaneously detects DNA sequences specific for *Mycobacterium tuberculosis* and rifampicin resistance. A recent Cochrane review estimated its sensitivity and specificity against culture to be 71.1% and 96.9%, respectively. However, against a composite reference standard, its sensitivity dropped to just 42.3%.[9] Although feasible on the grounds of time, the assay's suboptimal sensitivity and several other limitations such as constant power demand, high cost, and short shelf life of consumables make it difficult for it to be used in resource-limited settings.[10]

The common radiological features of TBM are hydrocephalus, basal meningeal enhancement, and infarction. Hydrocephalus could be either of the communicating type or the obstructive type, the former being more common than the latter. Infarcts arise due to obliterative vasculitis and are most frequent in the middle cerebral artery territory. Although the above radiological features are believed to be of significant diagnostic utility for TBM, other types of meningitis such as bacterial, fungal, and carcinomatous meningitis can also result in similar radiological findings.[11]

The diagnostic modalities for TBM described above are not without limitations, necessitating the search for better diagnostic approaches, one of which could be the use

of CSF-based biomarkers discussed hereafter. ADA is an enzyme catalyzing the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. It aids in the proliferation of lymphocytes and maturation of monocytes and is a significant indicator of active cellular immunity. Elevated CSF ADA level is highly suggestive of TBM, with estimated sensitivity and specificity of 89% and 91%, respectively.[12] Likewise, CRP is an acute-phase protein synthesized in the liver in response to inflammation. It binds to polysaccharides on the surface of microorganisms and triggers the complement pathway. In one study, a CSF CRP level of 4 mg/L or higher had a sensitivity of 96.9% to diagnose pyogenic meningitis, while 90% of TBM patients and 79% of viral meningitis patients had a CRP level below the cutoff.[13]

Similarly, LDH is a fermentative enzyme present in many tissues and body fluids. It is elevated in the CSF in non-viral meningitis and is believed to be sourced from polymorphonuclear leukocytes, neural tissue, and bacteria.[14,15] Other CSF markers of relative diagnostic importance are the enzymes gamma-glutamyl transpeptidase (GGTP) and creatine kinase (CK). GGTP is present in the neuronal cell membrane and is responsible for transferring amino acids across it, and CK, which is located intracellularly, is responsible for energy homeostasis. They are indicative of neuronal injury and are found to be elevated in pyogenic and tuberculous meningitis.[14]

The markers discussed above are cheap and easy to assess and, when used together, can be helpful to determine the etiology of meningitis. However, further research is necessary to better evaluate their diagnostic accuracy and standardize them for clinical use.

4 | CONCLUSION

Tuberculous meningitis is one of the most severe infections of the central nervous system afflicting the developing world. Early institution of chemotherapy is crucial for the outcome of the disease; however, in practice, it is severely impeded by the limitations of current diagnostic approaches, resulting in morbid complications and even death. A high index of clinical suspicion is thus an essential guide for the early intervention of the disease. In conclusion, physicians, especially those who practice in the third world and come across tuberculosis frequently in various forms, should consider empiric antituberculous treatment for patients with clinical meningitis in whom available diagnostic measures fail to identify the underlying etiology.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Deechit Poudel wrote the original draft of the manuscript. Diptee Poudel and Dhiraj Poudel contributed to the review and editing of the manuscript. All authors read and approved the final version of the manuscript.

ETHICAL STATEMENT

Written informed consent was obtained from the patient's father for the publication of this case report and any accompanying images.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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How to cite this article: Poudel D, Poudel D, Poudel D. Use of alternative cerebrospinal fluid-based biomarkers to help diagnose Xpert-negative tuberculous meningitis: A case report. *Clin Case Rep*. 2021;9:e04526. https://doi.org/10.1002/ccr3.4526