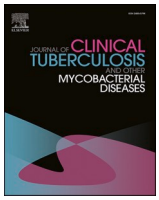
Journal of Clinical Tuberculosis and Other Mycobacterial Diseases 35 (2024) 100421

Contents lists available at ScienceDirect

Journal of Clinical Tuberculosis and Other Mycobacterial Diseases

journal homepage: www.elsevier.com/locate/jctube



Case Report

Cell-free DNA blood test for the diagnosis of pediatric tuberculous meningitis

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ARTICLE INFO

Keywords:
Tuberculous meningitis
Children
Cell-free DNA blood test
Molecular diagnostics

ABSTRACT

Pediatric tuberculous meningitis (TBM) is a severe form of tuberculosis that may present in children. The current diagnostic methods may have a limited impact on initial clinical decision-making. We present three children with tuberculous meningitis who had Mycobacterium tuberculosis complex cell-free DNA (cfDNA) detected in their blood within three days of sampling. Our cases described here illustrate for the first time the potential role of cfDNA blood tests in the rapid diagnosis of TBM.

1. Background

Tuberculous meningitis (TBM) is a rare and severe form of extrapulmonary tuberculosis caused by the Mycobacterium tuberculosis complex (MTBC). In the United States (US), 64 TBM cases were identified in 2020, which accounted for 4 % of the 1513 extrapulmonary tuberculosis cases reported [1]. Children younger than 2 years are at particularly high risk of disseminated tuberculosis, including TBM. Early diagnosis of pediatric TBM is important due to a high mortality rate of 19.3 % and the probability of survival without neurological sequelae of 36.7 % [2]. Although culture remains the gold standard for diagnosis, there are multiple barriers that impair its effectiveness, including the low sensitivity of acid-fast bacilli (AFB) culture isolation, the risk of failing to collect samples, and results that may take over 6 weeks, which lead to a limited impact of culture on the initial clinical decision-making for these children [3,4]. Therefore, the diagnosis and treatment decisions for pediatric TBM are often based on a combination of epidemiological history, clinical and cerebrospinal fluid (CSF) findings, and imaging studies. Novel diagnostic approaches that offer increased sensitivity and faster time to results are desired. We report on three pediatric cases of tuberculous meningitis that were promptly diagnosed using cell-free DNA (cfDNA) (Karius Test®) blood tests. To the best of our knowledge, this is the first case series of Mycobacterium tuberculosis complex cfDNA detection in blood in pediatric tuberculous meningitis.

2. Clinical Presentation

2.1. Case 1

A previously healthy 2-year-old male presented with a 3-week history of daily fever and lethargy. He was born in the US and recently traveled with his parents to Mexico for family visits. Two weeks after symptom onset, the child had progressive weakness and emesis, followed by left-eye ptosis and refusal to walk prior to being transferred to our hospital. Upon arrival, the child underwent a lumbar puncture and brain magnetic resonance imaging (MRI). CSF analysis was significant for 61 white blood cells/uL with 83% lymphocytes, a protein level of 123 mg/dL, and a glucose level of 40 mg/dL. Brain MRI revealed multiple restricted focal areas in the left basal ganglia and mild ventriculomegaly. Diagnostic laboratory results (Table 1) included negative T-SPOT.TB® and negative CSF MTB PCR, but a positive cfDNA blood test for Mycobacterium tuberculosis complex and cytomegalovirus two days after sampling and a positive CSF mycobacterial culture for MTBC at four weeks.

2.2. Case 2

A previously healthy 14-month-old female presented with a 3-week history of daily fever, progressive lethargy, and recurrent seizures. She was born in Mexico and immigrated to the US with her parents. The

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https://doi.org/10.1016/j.jctube.2024.100421

Available online 21 February 2024

Table 1
Initial laboratory diagnostic work-up and follow-up cultures.

	Case 1	Case 2	Case 3
Blood Test			
WBC (th/uL)	8.6	1.6	15.4
Neutrophil (%)	57	48	65
Blood culture	NP	No growth at 5 days	No growth at 5 days
Fungus culture	No growth at 4 weeks	No growth at 4 weeks	No growth at 4 weeks
Initial CSF Analysis			
WBC (uL)	61	30	51
Neutrophil (%)	1	14	42
Lymphocyte (%)	83	86	52
Total protein (mg/dL)	123	67	206
Glucose (mg/dL)	30	19	38
Meningoencephalitis PCR panel	Negative	HHV-6	Negative
MTBC Diagnostic tests			
PPD skin test (mm of induration)	0	1st result: 0 2nd result: 10	0
T-SPOT.TB®	Negative	Borderline	Positive
Turnaround time (day)	3.1	3.0	2.7
Blood AFB culture	NP	Negative at 6 weeks	NP
Gastric aspirate AFB culture	No AFB isolated after 6 weeks	No AFB isolated after 6 weeks	Positive at 6 weeks
Tracheal aspirate AFB culture	NP	No AFB isolated after 6 weeks	NP
CSF AFB culture	Positive at 4 weeks	No AFB isolated after 6 weeks	NP
CSF MTBC PCR	Negative	Negative	Negative
Cell-free DNA Testing (Karius Test ®)			
Day on test ordered during admission	Day 3	Day 2	Day 1
Turnaround time (day)	1.8	2.0	2.7
Initial result report	CMV (430 MPM)	Negative	Negative
Addended result report	*Mycobacterium tuberculosis complex* (at MPM level under test threshold)	*Mycobacterium tuberculosis complex* (at MPM level under test threshold)	*Mycobacterium tuberculosis complex* (at MPM level under test threshold)

child's brother and maternal grandmother, who visited her a few months prior, had recently tested positive for the tuberculin skin test (TST), indicating tuberculosis infection, but they had not received treatment. Upon transfer from the referring hospital, the child developed right-eye ptosis, and she required intubation and urgent extraventricular drainage at the bedside due to a deteriorating neurological status. The child's first TST test was 0 mm, but a repeated TST test showed a positive result with 10 mm of induration. CSF analysis demonstrated 76 white blood cells/uL with 44% lymphocytes, a protein concentration of 64 mg/dL, and a glucose level of 46 mg/dL. Brain MRI revealed a lacunar infarct in the right cerebral hemisphere, specifically in the right thalamus and frontal lobe. Empiric treatment for tuberculous meningitis was initiated while awaiting the results of the laboratory workup. Workup was significant for borderline T-SPOT.TB®, negative CSF MTB PCR and negative CSF mycobacterial culture, but positive cfDNA blood test for Mycobacterium tuberculosis complex after two days from sampling. In this case, the diagnosis of TBM was based on clinical, brain MRI, and CSF analysis findings without definitive microbiological confirmation.

2.3. Case 3

A previously healthy 4-year-old female presented with two weeks of fever, generalized seizures, and a disconjugate gaze. Prior to transfer to our hospital, she required intubation due to her deteriorating neurological status. The child had been exposed to active tuberculosis by her father, who recently died from complications of the disease. Brain MRI showed mild leptomeningeal enhancement in the midbrain and multiple punctate areas of restricted diffusion in the bilateral basal ganglia, inferior frontal lobes, bilateral hippocampal formations, midbrain, and cerebellar vermis. CSF analysis demonstrated 51 white blood cells/uL with 52% lymphocytes, a protein level of 206 mg/dL, and a glucose level of 39 mg/dL. Further laboratory workup yielded positive results for T-SPOT.TB® and the cfDNA blood test for Mycobacterium tuberculosis complex. Her gastric acid aspirate AFB culture was positive for MTBC at six weeks.

3. Discussion

Acid-fast bacilli staining, mycobacterial cultures, and conventional nucleic acid amplification tests (NAAT) of body fluids, including sputum, gastric aspirates, and CSF, are commonly utilized in the diagnosis of tuberculosis. However, it is often difficult to obtain sufficient respiratory tract specimens from nonintubated infants and children. In children with TBM, CSF mycobacterial culture is considered the gold standard for diagnosis, but specimen sampling can be delayed if lumbar puncture is contraindicated due to hemodynamic instability, elevated intracranial pressure, coagulopathy, or cardiovascular compromise. Furthermore, acid-fast bacilli staining only yields a sensitivity of 10%-20% for tuberculous meningitis, and the MTBC can be cultured from CSF in 32% of cases [4]. Therefore, a rapid diagnostic method with high sensitivity for MTBC from readily available specimens, such as blood is desired. Within 3 days of sampling, we confirmed the presence of the Mycobacterium tuberculosis complex using blood cfDNA via the Karius Test® in three cases of tuberculous meningitis in children. The consistent positive results among our three patients with a shorter turnaround time from ordering time to lab result compared with existing tests (approximately three days for T-SPOT.TB® with variable results) show the promise of a potential new diagnostic tool for TBM in children.

Multiple studies, conducted mostly in adults, have demonstrated the utility of detecting blood cfDNA for the identification of pulmonary tuberculosis. The sensitivities and specificities are highly variable, ranging from 29 % to 80 % and 67 % to 100 %, respectively [5–7]. A single retrospective study on pediatric pulmonary tuberculosis showed that cfDNA blood tests have a sensitivity of 75 % for detecting MTBC cfDNA in pediatric patients with smear-positive, culture-confirmed pulmonary tuberculosis [8]. However, no current studies specifically test the validity of using cfDNA blood tests to detect MTBC in extrapulmonary infections, such as TBM. In our case series, cfDNA blood tests detected MTBC cfDNA, assisting in the diagnosis of TBM in cases confirmed by positive gastric or CSF mycobacterial culture, or strong clinical suspicion.

For each of our patients, the medical director from Karius informed our hospital's pediatric infectious disease specialist that Mycobacterium tuberculosis complex DNA was present in the cfDNA blood test but was found at levels under their statistical threshold. The initial reports were updated to include the relevant findings. In a separate study involving children with tuberculosis, a relaxed research-use-only (RUO) statistical threshold was applied, allowing for the reporting of Mycobacterium tuberculosis if cfDNA derived from this microorganism was identified in three or more unique sequencing reads. Researchers found that when using the RUO threshold, detection was 75 % sensitive in smear-positive children, compared with 50 % when using the Karius standard threshold [8]. Further optimization of the statistical threshold is necessary to improve the sensitivity of Mycobacterium tuberculosis complex cfDNA detection in the blood.

In clinical practice, there are technological challenges associated with the use of MTBC as a potential biomarker for active tuberculosis. A primary concern is the uncertainty regarding whether the presence of MTBC-specific cfDNA in the blood is indicative of active tuberculosis or

latent TB infection (LTBI). One prospective study reported that 2 cases of LTBI tested positive for MTBC-cfDNA, and levels declined following anti-TB therapy. These observations suggest that plasma MTBC-cfDNA may also serve as a microbiological indicator in LTBI cases [9]. Another challenge is the differing sample preparation requirements for cfDNA blood tests, which presents barriers to integration into existing platforms commonly used in resource-limited regions. Compared with NAAT using PCR, such as Xpert MTB/RIF and Xpert MTB/RIF Ultra, the more costly techniques required for cfDNA blood tests, such as sequencing, further limit their applicability in low-resource settings [10].

In conclusion, the cfDNA blood test is a diagnostic tool that significantly shortens the time to diagnosis in suspected cases of pediatric TBM. Our findings necessitate further investigation into the validity and sensitivity of the cfDNA blood test for the rapid detection of the Mycobacterium tuberculosis complex, aiming to improve the diagnosis and treatment of patients with tuberculous meningitis.

4. Funding Source

No financial support or funding was received for this study.

5. Ethical Approval statement

Approval was not required for case series (3 cases). Please see details in the attachment from the Driscoll Children's Hospital IRB Office.

CRediT authorship contribution statement

Guyu Li: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Conceptualization. **Kendall Cannon:** Writing – original draft, Conceptualization. **Carlos Sisniega:** Writing – original draft. **Jaime Fergie:** Writing – original draft, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Jaime Fergie is a consultant for Karius, Inc. No financial support or funding was received for this work.

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