

A Case Report of Tuberculous Peritonitis with Negative GeneXpert and Positive Metagenomic Sequencing Results

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Abstract: A 65-year-old male patient undergoing peritoneal dialysis was diagnosed with *Mycobacterium tuberculosis*-related peritonitis. Initial diagnostic tests, including acid-fast smear of ascites, GeneXpert test, and *Mycobacterium tuberculosis* culture, yielded negative results. However, metagenomic capture sequencing (metaCAP) and metagenomic next-generation sequencing (mNGS) identified the presence of *Mycobacterium tuberculosis*. The patient was subsequently treated with standard anti-tuberculosis therapy, leading to clinical improvement. This case highlights the utility of advanced molecular diagnostics in identifying atypical pathogens in peritoneal dialysis-related infections.

Keywords: tuberculous peritonitis, *Mycobacterium tuberculosis*, next-generation sequencing, GeneXpert

Introduction

Smear-negative pulmonary and extrapulmonary tuberculosis significantly contributes to the underdiagnosis of TB, resulting in delayed diagnosis, inappropriate antibiotic use, and higher mortality rates, particularly among critically ill patients.¹ Traditional detection methods, such as culture-based techniques, demonstrate relatively low overall sensitivity. Additionally, conventional PCR-based methods, including GeneXpert and GeneXpert Ultra, are restricted to detecting known pathogenic microbial gene sequences. In contrast, metagenomic next-generation sequencing (mNGS) enables comprehensive DNA sequencing of the entire microbial community genome within a specific sample without requiring isolation or culture, thereby providing enhanced potential for diagnosing smear-negative tuberculosis.²

However, mNGS has significant limitations, including longed turnaround times, high costs, complex bioinformatics requirements, and difficulties in standardization, which together impede its widespread clinical application.³ To tackle these challenges, metagenomic capture sequencing (metaCAP) has emerged as a complementary approach. MetaCAP captures target nucleic acids through probe hybridization and has a wide detection range covering more than 3000 pathogens of key concern, including both DNA and RNA pathogens. By removing human host nucleic acids and using probes to capture pathogen nucleic acids, it has high sensitivity and is particularly suitable for samples with extremely low pathogen content. For tissues and sterile body fluids, it effectively solves the problem of low amplification efficiency of cfDNA or fragmented DNA in these samples. Moreover, the cost is as low as 30% of that of mNGS, and it has a turnaround time of only 24 h.⁴

Herein, we report a typical case of a patient who was undergoing peritoneal dialysis and was diagnosed with *Mycobacterium tuberculosis*-related peritonitis with negative results for acid-fast smear of ascites, GeneXpert test, and *Mycobacterium tuberculosis* culture. However, metaCAP and mNGS identified the presence of gene fragments of *Mycobacterium tuberculosis*.

Case Presentation

A 65-year-old male with a history of stage 5 chronic kidney disease, chronic nephritis, and renal anemia was diagnosed in the nephrology department four years prior due to chest tightness. He underwent renal replacement therapy via peritoneal dialysis (PD) since January 2019. Six days before admission, the patient experienced right lower abdominal pain, frequent urination, and urgency. Three days later, his PD fluid became turbid, leading to a diagnosis of PD-related peritonitis with an unidentified pathogen. Laboratory findings included: nucleated cell count in PD fluid of $503 \times 10^6/L$ (69% polymorphonuclear cells), WBC $5.44 \times 10^9/L$, NEU 66.8%, Hs-CRP 22.77 mg/L, PCT 0.25 ng/mL, IL-6 27.20 pg/mL. Cultures for bacteria, fungi, and anaerobes were negative, as were acid-fast staining and GeneXpert for tuberculosis. Empirical antibiotic therapy with ceftazidime and cefazolin was initiated upon admission, but symptoms persisted. Physical examination revealed abdominal distension, tenderness in the right lower abdomen, and bilateral lower extremity edema. Ascitic fluid was collected for metagenomic capture sequencing (metaCAP). Concurrently, prior to availability of the results, meropenem, vancomycin, and fluconazole were administered to control infections caused by most pathogens. MetaCAP identified the *Mycobacterium tuberculosis* complex (*Mtb*, 142 reads). The interferon-gamma release assay (IGRA) was positive (30.33 IU/mL), and metagenomic next-generation sequencing (mNGS) confirmed *Mtb* (7 reads). The diagnosis of tuberculous peritonitis was established. Antibiotics treatment was switched to anti-tuberculosis treatment: levofloxacin 0.1 g per dialysis session (four times daily), isoniazid 0.3 g daily, and rifampicin 0.45 g daily. Abdominal pain significantly improved. Ten days later, the patient was discharged with no reported abdominal pain and continued anti-tuberculosis treatment. The patient underwent outpatient follow-up for three months and remained in stable condition.

The diagnosis and treatment of tuberculous peritonitis in this patient were extremely difficult and went through three stages. In the first stage, based on the patient's clinical symptoms, as well as the results of blood routine tests and inflammation-related tests for hs-CRP, PCT and IL-6, the clinical diagnosis was dialysis-related peritonitis. The common pathogens of peritoneal dialysis-associated peritonitis include *Escherichia coli*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.⁵ Therefore, upon admission, empirical antibiotic therapy with ceftazidime and cefazolin was initiated. However, the patient's symptoms persisted. In the second stage, after the cultures for bacteria, fungi, anaerobes and even GeneXpert tests yielded negative results, meropenem, vancomycin, and fluconazole were administered to cover potential pathogens that were not detected by routine tests. Consistently, the patients showed no response to these multiple antibiotic treatments. In the final stage, with the detection of *Mtb* nucleic acid fragments, a positive IGRA test result, and the favorable response to subsequent anti-tuberculosis antibiotic treatment, this patient was ultimately diagnosed with dialysis-related tuberculous peritonitis.

The patient's ascites acid-fast smear and GeneXpert test were both negative, but 142 reads of *Mtb* gene fragments were detected by metaCAP 20M test data. Further mNGS 20M test data detected 7 reads of specific *Mtb* gene fragments, confirming the presence of *Mtb*. However, why was the GeneXpert test negative? The reasons are analyzed as follows:

GeneXpert detects the *rpoB* gene, characterized by a single gene and low copy number within the *Mtb* genome. It relies on multiple probes to detect mutations at key sites responsible for RIF resistance. The newly improved GeneXpert Ultra detects three genes: *rpoB*, *IS6110*, and *IS1081*. Increased sensitivity is achieved by combining two new PCR targets that multi-copy genes, *IS6110* and *IS1081*. Notably, *IS6110* exhibits significant copy number variations among strains, and its sole use may pose a risk of missed detection. The combination with *IS1081* significantly improves detection efficiency.⁶ In the metaCAP 20M test data, 142 reads of *Mtb* gene fragments were detected, whereas in the mNGS 20M test data, only 7 reads of *Mtb* gene fragments were detected. The results demonstrated effective detection of *Mtb* nucleic acid traces. The distribution of fragments detected by metaCAP was compared with the reference strain of *Mtb* H37Rv for the target fragments *rpoB*, *IS6110*, and *IS1081* used in GeneXpert and GeneXpert Ultra (Figure 1A). However, interestingly, the *Mtb rpoB* gene fragments detected by metaCAP did not align completely with the amplification fragments of GeneXpert and GeneXpert Ultra, and the upstream fragment of the *rpoB* target amplification fragment region was absent (Figure 1B). Consequently, GeneXpert and GeneXpert Ultra were unable to detect the *rpoB* gene. Additionally, while metaCAP detected 6 copies of *IS6110* gene fragments, it remains unclear whether these sequences correspond to the target amplification fragments of GeneXpert Ultra. Therefore, the effectiveness of GeneXpert Ultra in this case requires further technical evaluation. Overall, the ascites sample analyzed in this case demonstrated effective detection of *Mtb* traces using both metaCAP and mNGS. Due to the sequence-specific capture

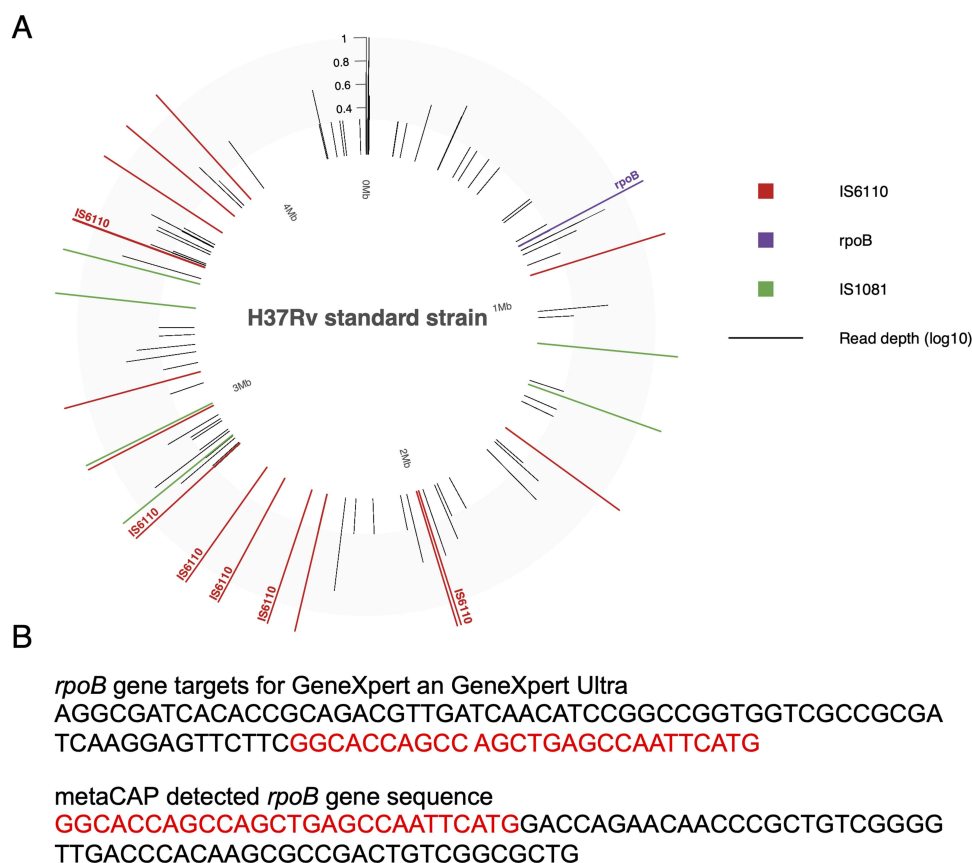


Figure 1 Distribution and comparison of metaCAP-detected fragments relative to the *Mycobacterium tuberculosis* genome - reference strain H37Rv (NC_000962.3). **(A)** A total of 142 reads corresponding to *Mtb* gene fragments identified by metaCAP were aligned to the reference H37Rv genome. The alignment positions and read depth are represented black lines; the locations of *rpoB*, *IS6110* and *IS1081* genes are indicated on the H37Rv reference genome, with the gene fragments detected by metaCAP labeled by their respective names at the end of each line. **(B)** Comparison between the *rpoB* target sequence used by GeneXpert and GeneXpert Ultra and the *rpoB* gene sequence detected by metaCAP. Regions showing complete alignment are highlighted in red.

enrichment procedure of metaCAP, the number of detected fragments was substantially increased, demonstrating its higher efficiency compared to mNGS.

Discussion and Conclusions

In clinical practice, it is commonly to encounter cases where patients are smear-negative but exhibit a significant response to anti-tuberculosis treatment.⁷ When multiple tests yield negative results and sequencing data reveal only a small number of sequences, such findings should be carefully interpreted. This is particularly important in cases of extrapulmonary tuberculosis infection, where nucleic acid fragmentation may occur. Therefore, it is essential to perform a comprehensive sequence analysis in conjugation with other test results and exercise caution when ruling out tuberculosis as a diagnosis.

Based on the analysis of the original data presented above, the following comparisons can be drawn regarding existing molecular diagnostic techniques for tuberculosis: Firstly, when comparing tuberculosis gene detection technologies such as GeneXpert, GeneXpert Ultra, metaCAP, and mNGS, GeneXpert demonstrates relatively lower detection efficiency compared to other methods. Additionally, GeneXpert requires high genomic integrity for accurate detection; for example, the target region of the *rpoB* gene fragment must remain intact.⁸ Secondly, GeneXpert Ultra significantly improves the sensitivity of tuberculosis detection by incorporating high-copy-number *IS6110* and *IS1081* genes.⁹ However, due to the substantial variation in *IS6110* copy numbers among clinical isolates, its actual detection efficiency is closely associated with the *IS6110* copy number of prevalent tuberculosis strains in different regions and the bacterial load in clinical samples. Although 6 copies of *IS6110* gene fragments have been detected by metaCAP, the detection efficiency of GeneXpert Ultra remains constrained by the integrity of the *IS6110* gene. Thirdly, metaCAP demonstrates a significant advantage in detecting

fragmented tuberculosis genomes, markedly improving the detection efficiency of clinical samples that either lack viable *Mtb* or have incomplete genomes. Fourthly, regarding the large number of smear-negative tuberculosis patients currently encountered in clinical practice (where no pathogenic evidence is detected in various body fluids using existing technologies but who respond effectively to anti-tuberculosis treatment), it is plausible that tuberculosis infection in different tissues generates tissue-specific genomic fragments due to intracellular or interstitial nuclease degradation. The application of metaCAP deep sequencing may therefore potentially reveal the genomic fragmentation characteristics of tissue-derived *Mtb*, which could, in turn, aid in the development of more efficient detection and analysis techniques based on these genomic fragmentation patterns.

Given that tuberculous peritonitis is a rare and tissue-specific manifestation of *Mycobacterium tuberculosis* infection, only one case has been reported here. The proportion of patients with tuberculous peritonitis having a fragmented *rpoB* gene that cannot be detected by GeneXpert has not been widely studied. Moreover, it remains unclear whether the *IS6110* and *IS1081* gene fragments can be detected by GeneXpert Ultra due to the absence of reference targeted gene sequences. Additionally, advanced molecular diagnostics such as metaCAP and mNGS are not incorporated into the standard diagnostic workflows of tuberculosis. Currently, relevant technical services are only used when infected individuals are seeking rare or unknown pathogens.

In this study, we present a case of a patient diagnosed with tuberculous peritonitis. The conventional acid-fast smear of ascites, the GeneXpert test, and *Mycobacterium tuberculosis* culture all yielded negative results. However, both metaCAP and mNGS detected the presence of nucleic acid fragments of *Mycobacterium tuberculosis*. A more in-depth analysis of the gene fragments indicated that the *Mtb rpoB* gene detected by metaCAP did not fully align with the amplification fragments of GeneXpert, which implies that GeneXpert was unable to detect this *rpoB* gene fragment. Notably, this case underscores the potential value of advanced molecular diagnostics in identifying atypical pathogens in peritoneal dialysis-related infections.

Ethics Approval and Consent to Participate

This was a retrospective case report study conducted in a tertiary hospital and was approved by the Clinical Research and Ethics Committee of Peking University Shenzhen Hospital (No. [2020] 023-2). The patient provided consent for the publication of the case details.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors report no potential conflicts of interest in this work.

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