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ORIGINAL RESEARCH

Diagnostic Utility of Nanopore Sequencing for Tuberculous Serous Effusions

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Objective: Early and precise diagnosis of tuberculous serous effusions is a huge challenge. Nanopore sequencing is a potentially efficient assay. The objective of the current study was to evaluate the diagnostic accuracy of nanopore sequencing for tuberculous serous effusions using clinical specimens directly, and to provide a new pathway for the early and precise diagnosis of tuberculous serous effusions.

Methods: This was a retrospective analysis of the effectiveness of nanopore sequencing as a diagnostic method for tuberculous serous effusions using clinical specimens (pleural fluid, pericardial effusion, and ascitic fluid). Using clinical diagnosis as reference standard, the diagnostic accuracy indicators such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) for the tests in question were evaluated.

Results: In total, 132 patients were eligible for inclusion. Nanopore sequencing showed sensitivity of 93.3%, specificity of 85.2%, PPV of 96.1%, NPV of 76.7%, and AUC of 0.89 for tuberculous serous effusions. The diagnostic accuracy of nanopore sequencing was significantly superior than that of Xpert MTB/RIF and culture. Similar results were observed in different types of tuberculous serous effusions (pleural tuberculosis, pericardial tuberculosis, and peritoneal tuberculosis).

Conclusion: Nanopore sequencing was efficient for the rapid diagnosis of tuberculous serous effusions and had a very positive effect. For paucibacillary tuberculous serous effusions, nanopore sequencing might become an effective method for detecting pathogenic bacteria.

Keywords: nanopore sequencing, tuberculosis, pleural effusion, pericardial effusion, abdominal effusion, diagnostic accuracy

Introduction

Tuberculosis (TB) is an ancient contagious disease due to infection of the human body by *Mycobacterium tuberculosis* (MTB), which continues to pose a major health risk to humans to this day.[1] About 10.6 million new TB diagnosed cases and 1.3 million TB related cases of death globally in 2022.[2] Thus, TB was ranked as one of the ten leading contributors to deaths from an infectious disease.[3] TB is mainly categorized into pulmonary TB and extrapulmonary TB (EPTB).[4] The sites of infection of EPTB are diverse, and the serous cavities are common sites of infection of MTB.[5] The serous cavities mainly include the pleural, pericardial and abdominal cavities, and early infection of these places with MTB usually manifests as serous effusions.[6] Tuberculous serous effusions will produce severe symptoms such as chest tightness and shortness of breath, abdominal distension, and edema, etc.[7] If the diagnosis is not timely and appropriate treatment is not given at an early stage, it will easily lead to serious complications such as septic thorax, constrictive pericarditis, and enteric obstruction, and even life-threatening,[8,9] which will seriously affect the prognosis. Moreover, because of the closed nature of the serous cavities, invasive operations are required to obtain specimens of serous effusions, which also increases the associated risks and diagnostic difficulties. How to reduce the number of invasive procedures and improve the diagnostic ability of tuberculous serous effusions is an urgent clinical problem to be solved.

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The acid-fast bacilli (AFB) smear is the most classical tool in the diagnosis of TB, but it plays a minimal role in less-bacterial serous effusions, and it is difficult to detect MTB and thus establish the diagnosis of tuberculous serous effusions solely by this method.[10,11] MTB culture is the gold standard for the diagnosis of TB, but in tuberculous serous effusions, the efficacy of this method is extremely challenging.[12,13] The low sensitivity and time-consuming nature of culture in serous effusions does not meet the need for early and precise diagnosis.[14]

Advances in molecular diagnostic tests have made early and precise diagnosis of TB possible.[15] Molecular diagnostic tests (such as Xpert MTB/RIF) have shown bright results in the diagnosis of TB,[16] but the results in the diagnosis of tuberculous serous effusions are still limited.[6] Gene sequencing has advanced the accurate diagnosis of infectious diseases even further, and next-generation sequencing (NGS) has shown good diagnostic efficacy in infectious diseases including TB.[17,18] Nanopore sequencing is a new generation of gene sequencing method, which has the advantages of long read lengths, real-time sequencing, and portable equipment over NGS.[19] These features make it particularly advantageous in detecting MTB in paucibacillary samples like serous effusions. However, no large-scale or comparative studies exist specifically in tuberculous serous effusions. Nanopore sequencing has been applied in the diagnosis of various infections, including bacterial meningitis and pulmonary TB,[20–23] with promising results. However, evidence regarding its use in extrapulmonary TB, particularly in serous effusions, is limited. Given the unique diagnostic challenges posed by these forms of TB, further evaluation of this technology in real-world clinical settings is warranted. The objective of the current study was to evaluate the diagnostic accuracy of nanopore sequencing for tuberculous serous effusions using clinical specimens (pleural fluid, pericardial effusion, and ascitic fluid) directly, and to provide a new pathway for the early and precise diagnosis of tuberculous serous effusions. If validated, this approach may reduce the need for invasive biopsies or expedite treatment initiation.

Materials and Methods

Study Design

This was a retrospective analysis of the effectiveness of nanopore sequencing as a diagnostic method for tuberculous serous effusions at the Zhejiang Provincial Tuberculosis Diagnostic and Treatment Center during the period July 2021 to October 2023. Patients with serous effusions whose cause of effusion was considered to be due to TB were participants in this study. Nanopore sequencing was the index test, and the comparison tests were Xpert MTB/RIF and culture. The outcome metrics were diagnostic accuracy indicators such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) for the tests in question.

The criteria for suspected tuberculous serous effusion were TB-related symptoms such as fever, night sweats, etc.; ultrasound or CT findings of serous effusions with a predominantly lymphocytic cellular composition and elevated ADA; a positive gamma interferon release assay (GIRA) or tuberculin purified protein derivative (PPD); comorbidities with pulmonary tuberculosis or tuberculosis at other sites; and the absence of any other pathogenic bacilli infections. Patients with suspected tuberculous serous effusions who had undergone nanopore sequencing using serous effusions were included in the study without regard to age or sex. Patients with low effusions who did not use effusions for nanopore sequencing and patients with incomplete clinical data were excluded. Serous effusions were obtained by thoracentesis, pericardiocentesis, or peritoneal puncture. Written informed consent was obtained from the patient or family before performing the relevant puncture. The study was approved and consented by the Ethics Committee of Hangzhou Red Cross Hospital and exempted from informed consent of patients or guardians due to its retrospective nature (2024-YS -015). The study was in accordance with the Declaration of Helsinki.

Based on the eventual clinical diagnosis (a composite reference standard), the patients were divided into three groups for the next step of statistical analysis.

Confirmed tuberculous serous effusion: Positive MTB smear and/or culture in effusions.

Probable tuberculous serous effusion: Cases with typical TB symptoms, imaging changes such as pleural, pericardial calcifications, positive PPD and/or GIRA, biochemical examination of effusion suggestive of elevated ADA, positive results of other molecular diagnostic tests (such as TB-RNA), positive histopathologic TB findings in pleural tissue, pericardial tissue, and peritoneal tissue, and positive response to anti-TB treatment.

Non-tuberculous serous effusion (non-TB group): No evidence of TB on effusion testing, diagnosis of tumor or other pathogenic bacterial infection, ineffective anti-TB treatment or cure of disease without anti-TB treatment.

Confirmed and probable tuberculous serous effusion was considered as the eventual clinical diagnosis of tuberculous serous effusion (TB group).

Diagnostic Specimen Collection and Handling

Fresh serous effusions obtained by puncturing the serous cavity were utilized for the assay in question. Fresh effusion specimens were sent directly to the laboratory for testing, or if that was not possible, they were placed in a 4°C refrigerator to be tested for correlative tests within 6 hours.

MTB Culture

5 mL of fresh effusion was used for culture. The supernatant was discarded by centrifugation, and the precipitate was digested and decontaminated by the addition of N-acetyl-l-cysteine–NaOH. After centrifugation again the precipitate was obtained, and the precipitate was inoculated on medium for mycobacterial culture. We used both solid (Lowenstein–Jensen solid medium) and liquid media (BACTEC MGIT 960 liquid medium; BD Diagnostic Systems in Sparks, MD) for the culture and seeing growth of MTB on any of the media was considered as positive culture.

Xpert MTB/RIF

At least 3 mL of effusion from the serous cavity was used for the Xpert MTB/RIF assay. The effusion specimen was first centrifuged, and the supernatant was discarded to obtain a sediment. 2 mL of the specimen preparation solution was added to the sediment and shaken to mix. 2 mL of the prepared specimen was placed into the Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) reaction cassette, which was then inserted into the Xpert MTB/RIF device, which automatically completed the assay and produced a report in less than 2 hours.

Nanopore Sequencing

The process of nanopore sequencing has been reported in detail in our previous articles.[24,25] 5 mL of serous cavity effusion was centrifuged to obtain a precipitate, which was washed using phosphate-buffered saline and then resuspended by adding lysis solution. After resuspension, the lysates were further lysed by adding grinding beads for physical grinding. Subsequent to lysis, DNA was extracted using the QIAamp DNA Microbiome Kit (Qiagen, Cat. No. 51707, Hilden, Germany). Polymerase chain reaction (PCR) amplification targeted the rpoB gene of Mycobacterium spp. utilizing specific primers (Rpo5′: 5′-TCAAGGAGAAGCGCTACGA-3′; Rpo3′: 5′-GGATGTTGATCAGGGTCTGC -3′). Amplification employed a touchdown protocol: initial denaturation at 98°C for 3 min; followed by 6 cycles of denaturation (95°C, 15 s), annealing (starting at 66°C, decreasing by 1°C per cycle, 60 s), and extension (72°C, 30 s); then 29 cycles with annealing at 61°C; concluding with a final extension at 72°C for 5 min. The amplified product underwent purification and barcode labeling. Sequencing was performed on the GridION platform (Oxford Nanopore Technologies). Following platform instructions, labeled amplicons were loaded into a flow cell and inserted into the GridION for automated sequencing. Real-time data acquisition utilized Oxford Nanopore's MinKnow v3.6.5 software. Raw sequencing reads were subjected to initial quality filtering to remove low-quality data, assessed based on read length, target gene coverage, and sequencing depth. Further analysis utilized only high-quality reads; fragments shorter than 200 base pairs (bp) were excluded to ensure result reliability. Sequence alignment against reference genomes for Mycobacterium tuberculosis (NC_000962.3) and the genus Mycobacterium (Taxonomy ID 1763) was performed using Minimap2 (v2.17). Host DNA contamination was mitigated by alignment against the human reference genome (GRCh38) and subsequent removal of matching reads. This entire workflow yielded results within a 48-hour timeframe. The threshold for MTB detection is 100 copies/mL.

Data Processing and Statistical Analysis

The collected data was first recorded in Excel 2019. Using these data we calculated descriptive values such as mean, quartiles, and standard deviation using SPSS (version 24.0, IBM Corp). The four values of true positive (TP), false

positive (FP), false negative (FN), and true negative (TN) in the diagnostic cross-tabulation were also calculated using SPSS. Sensitivity, specificity, PPV, NPV, and AUC and their 95% confidence intervals were calculated through these four values using MedCalc Statistical Software version 15.2.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2015). The receiver operating characteristic (ROC) curves were plotted using R 4.2.1 (R Core Team 2024) with pROC and ggplot2 packages. Differences between two different ratios were compared using the chi-square or Fisher's exact tests. Differences between two different AUC were compared using the Z-test. A statistical value of p less than 0.05 was considered statistically significant for the difference between the two.

Results

During the study period, 139 patients underwent nanopore sequencing of serous effusion specimens. Seven patients were lost to follow-up, yielding 132 patients in the final cohort. Each patient contributed one specimen, totaling 132 effusions: 107 pleural, 16 peritoneal, and 9 pericardial Based on final clinical diagnosis, 105 cases were tuberculous serous effusions: 87 pleural, 13 peritoneal, and 5 pericardial. Twenty-seven cases were nontuberculous serous effusions: 20 pleural, 3 peritoneal, and 4 pericardial. Figure 1 illustrated the patient screening process, specimen categorization, and diagnostic classification. Table 1 presented patient baseline characteristics including age and sex.

All specimens from all patients underwent nanopore sequencing; in addition, culture was performed in 104 cases and Xpert MTB/RIF testing in 78 cases. Nanopore sequencing was positive in 102 cases (98 TB group, 4 non-TB group) and negative in 30 cases (7 TB group, 23 non-TB group). Culture was positive in 23 cases (all TB group) and negative in 81 cases (59 TB group, 22 non-TB group). Xpert MTB/RIF was positive in 16 cases (all TB group) and negative in 62 cases (46 TB group, 16 non-TB group) (Figure 1). AFB smear, performed on only 20 specimens, was positive in 1 case. Figure 1 also detailed test results by effusion type. The distribution of sequence reads for nanopore sequencing positive specimens in all specimens ranged from 2 to 25539, with a median number of reads of 5. The distribution of sequence reads for positive specimens in the TB group ranged from 2 to 25539, with a median number of reads of 5. The distribution of sequence reads for positive specimens in the non-TB group ranged from 2 to 3, with a median number of

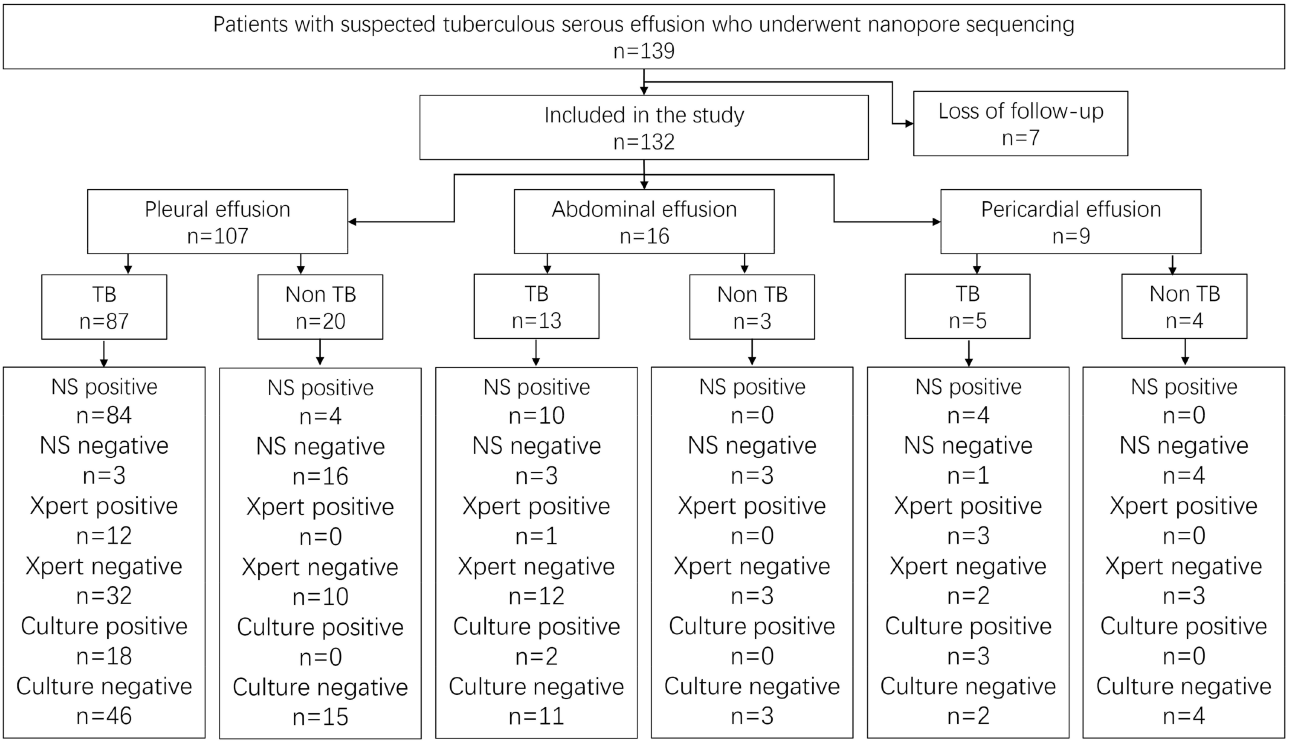

Figure 1 The patient screening process, specimen categorization and diagnostic classification.
Abbreviations: TB, tuberculosis; NS, nanopore sequencing.

Table 1 Clinical Characteristics of the Included Patients

Characteristics	All (132)	TB (105)	Non TB (27)
Age (year, IQR)	59 (30–73)	47 (26–71)	67 (59–80)
Male (n, %)	86 (65.2%)	66 (62.9)	20 (74.1)
Female (n, %)	46 (34.8%)	39 (37.1)	7 (25.9)
HIV positive (n, %)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Concomitant diseases			
Hypertension, n (%)	31 (23.5%)	24 (22.9)	7 (25.9)
T2DM, n (%)	16 (12.1%)	13 (12.4)	3 (11.1)
Pulmonary tuberculosis	58 (43.9%)	51 (48.6)	7 (25.9)
Coronary artery disease	9 (6.8%)	7 (6.7)	2 (7.4)
Nanopore sequencing	132 (100.0%)	105 (100.0)	27 (100.0)
Xpert MTB/RIF	78 (59.1%)	62 (59.0)	18 (66.7)
MTB Culture	104 (78.8%)	82 (78.1)	22 (81.5)
Characteristics of fluid			
Lymphocyte ratio (%, Mean ± SD)	74.9 ± 28.1	76.4 ± 29.3	70.0 ± 22.6
Adenosine deaminase (U/ L, Mean ± SD)	36.6 ± 22.7	41.8 ± 22.6	18.9 ± 13.2
Lactate dehydrogenase (U/L, Mean ± SD)	525.8 ± 793.8	588.4 ± 894.2	360.9 ± 324.9
Erythrocyte sedimentation rate	38.9 ± 23.4	40.2 ± 24.2	34.9 ± 20.4
C-reactive protein	42.5 ± 44.8	44.5 ± 43.3	34.9 ± 49.3

Abbreviations: IQR, Interquartile Range; SD, standard deviation; T2DM, type 2 diabetes; MTB, *Mycobacterium tuberculosis*.

reads of 2.5. The distribution of sequence reads for positive specimens in the pleural effusions ranged from 2 to 25539, with a median number of reads of 5. The distribution of sequence reads for positive specimens in the abdominal effusions ranged from 2 to 126, with a median number of reads of 2.5. The distribution of sequence reads for positive specimens in pericardial effusions ranged from 3 to 19 with a median reading of 3 (Figure 2). No statistically significant differences ($P > 0.05$) were observed in read counts between TB and non-TB groups or across effusion types (Figure 2).

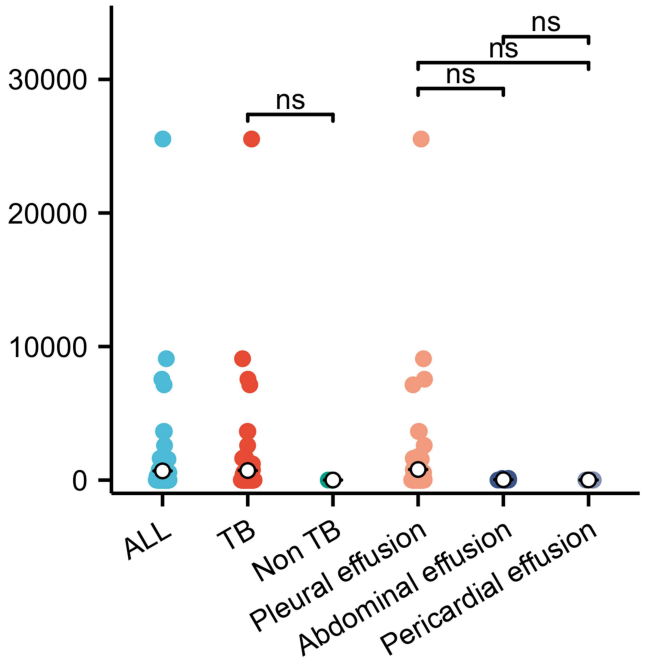

Figure 2 The distribution of sequence reads for positive specimens in different group. The difference in reads between nanopore sequencing positive specimens in the TB and non-TB groups was not statistically significant ($P > 0.05$). The difference in the number of reads of positive nanopore sequencing in different types of effusion specimens was also not statistically significant ($P > 0.05$). ns, $P > 0.05$.
Abbreviation: TB, tuberculosis.

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Table 2 Diagnostic Accuracy of Nanopore Sequencing, Xpert MTB/RIF, and Culture for Tuberculous Serous Effusions

Assay	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	PPV (%, 95% CI)	NPV (%, 95% CI)	AUC (95% CI)
Nanopore sequencing	93.3 (86.8–97.3)	85.2 (66.3–95.8)	96.1 (90.3–98.9)	76.7 (57.7–90.1)	0.89 (0.83–0.94)[*&]
Xpert MTB/RIF	25.8 (15.5–38.5)	100.0 (79.4–100.0)	100.0 (68.8–100.0)	25.8 (15.5–38.5)	0.63 (0.51–0.74)[#]
Culture	28.1 (18.7–39.1)	100.0 (84.6–100.0)	100.0 (85.2–100.0)	27.2 (17.9–38.2)	0.64 (0.54–0.73)

Notes: Comparison between the nanopore sequencing and Xpert MTB/RIF, *P < 0.001, comparison between the nanopore sequencing and culture, [&]P < 0.001, comparison between the Xpert MTB/RIF and culture, [#]P = 0.765.
Abbreviations: PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve.

Diagnostic Accuracy of Nanopore Sequencing for Tuberculous Serous Effusions

The diagnostic accuracy of nanopore sequencing for tuberculous serous effusions was demonstrated in Table 2, as were the results associated with Xpert MTB/RIF and culture. The diagnostic accuracy of nanopore sequencing for tuberculous serous effusions was significantly better than that of Xpert MTB/RIF and culture (P < 0.001, Table 2). The diagnostic accuracy of Xpert MTB/RIF and culture for tuberculous serous effusions was similar (P > 0.05, Table 2). The ROC curves were displayed in Figure 3.

Diagnostic Accuracy of Nanopore Sequencing in Different Types of Serous Effusions

The diagnostic accuracy of nanopore sequencing in different types of serous effusions is demonstrated in Table 3. The diagnostic accuracy of nanopore sequencing using pleural effusion, abdominal effusion and pericardial effusion was similar and not statistically significant (P > 0.05, Table 3). Nanopore sequencing in each type of serous effusions demonstrated significantly better accuracy than Xpert MTB/RIF and culture (P < 0.001, Table 3), which is consistent with the overall picture. The accuracy of Xpert MTB/RIF and culture was consistent with the overall profile, and there were no significant differences between the two, regardless of the specimen type.

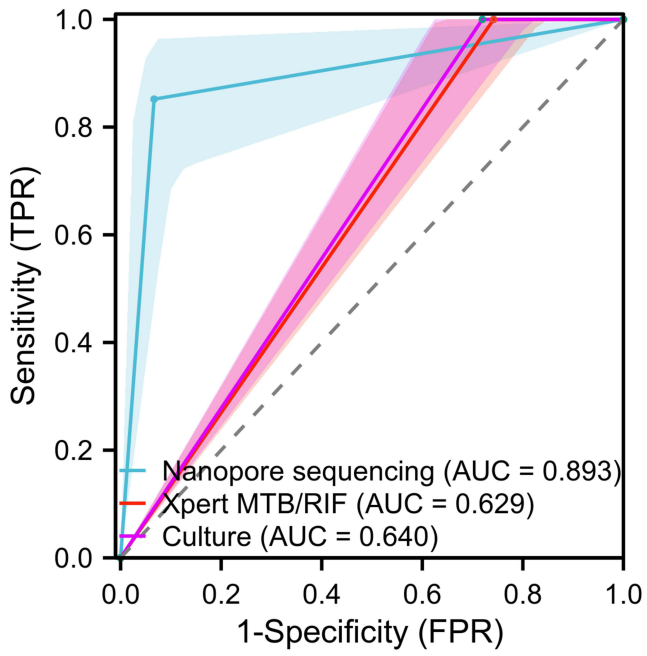

Figure 3 The ROC curves for nanopore sequencing, Xpert MTB/RIF, and culture. The area under the curve of nanopore sequencing was significantly better than that of Xpert MTB/RIF and culture. The area under the curve of Xpert MTB/RIF and culture was similar.
Abbreviation: AUC, area under the curve.

Table 3 Diagnostic Accuracy of Nanopore Sequencing, Xpert MTB/RIF, and Culture for Different Type of Tuberculous Serous Effusion

Sample Type	Assay	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	PPV (%, 95% CI)	NPV (%, 95% CI)	AUC (95% CI)
Pleural effusion	Nanopore sequencing	96.6 (90.3–99.3)	80.0 (56.3–94.7)	95.5 (88.8–98.8)	84.2 (60.4–96.6)	0.88 (0.81–0.94)*#
	Xpert MTB/RIF	27.3 (15.0–42.8)	100.0 (69.2–100.0)	100.0 (73.5–100.0)	23.8 (12.1–39.5)	0.64 (0.49–0.76)&
	Culture	28.1 (17.6–40.8)	100.0 (78.2–100.0)	100.0 (81.5–100.0)	24.6 (14.5–37.3)	0.64 (0.52–0.75)
Abdominal effusion	Nanopore sequencing	76.9 (46.2–95.0)	100.0 (29.2–100.0)	100.0 (69.2–100.0)	50.0 (11.8–88.2)	0.88 (0.63–0.99)*
	Xpert MTB/RIF	7.7 (0.2–36.0)	100.0 (29.2–100.0)	100.0 (2.5–100.0)	20.0 (4.3–48.1)	0.54 (0.28–0.78) &
	Culture	15.4 (1.9–45.5)	100.0 (29.2–100.0)	100.0 (15.8–100.0)	21.4 (4.7–50.8)	0.58 (0.31–0.81)
Pericardial effusion	Nanopore sequencing	80.0 (28.4–99.5)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	80.0 (28.4–99.5)	0.90 (0.53–1.00)*
	Xpert MTB/RIF	60.0 (14.7–94.7)	100.0 (29.2–100.0)	100.0 (29.2–100.0)	60.0 (14.7–94.7)	0.80 (0.40–0.98) &
	Culture	60.0 (14.7–94.7)	100.0 (39.8–100.0)	100.0 (29.2–100.0)	66.7 (22.3–95.7)	0.80 (0.42–0.98)

Notes: Comparison between the nanopore sequencing and Xpert MTB/RIF and culture, *$P < 0.001$, comparison between the nanopore sequencing using pleural effusion, abdominal effusion and pericardial effusion, #$P > 0.05$, comparison between the Xpert MTB/RIF and culture, &$P > 0.05$.
Abbreviations: PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; BALF, bronchoalveolar lavage fluid.

Discussion

Previous studies have largely concluded that MTB levels in tuberculous serous effusions are very low,[7] which makes early and precise pathogenetic diagnosis of tuberculous serous effusions extremely challenging. The performance of AFB smear in the diagnosis of tuberculous serous effusions is unsatisfactory and is often not preferred in cases of limited effusion. In our study, only 20 specimens were subjected to AFB smear with a positive rate of only 5%. This result was discouraging, but was roughly the same as that of previous studies. This was the reason we did not compare this test with others. There was no need to suspect that the diagnostic efficacy of the AFB smear for tuberculous serous effusions was minimal.

MTB culture is not as useful in tuberculous serous effusions as it is in pulmonary TB, and its diagnostic accuracy in tuberculous serous effusions is limited in its ability to effectively differentiate between TB and non-TB.[26] The sensitivity of MTB culture in this study was only 28.1% in all effusion specimens, which was an unsatisfactory figure, and most of the TB failed to be detected by this test. The reason for this is still the paucibacillary nature of the effusion. Culture takes up to several weeks, during which time it may lead to complications such as pericardial constriction and pyothorax formation, which are detrimental to the treatment of the patient, therefore, the contribution of culture to early and precise diagnosis is very small.

Clinicians have been exploring ways to improve the accuracy of the diagnosis of tuberculous serous effusions. The advent of molecular diagnostic tests has shown us the light. Molecular diagnostic tests such as Xpert MTB/RIF are good in pulmonary TB and have greatly improved the ability to precisely diagnose TB at an early stage.[27] But these tests are not as useful in tuberculous serous effusions as they are in pulmonary TB.[14,28] Most studies have shown that while molecular tests have improved early and precise diagnosis to some extent, the room for improvement is still enormous.[28] Our study showed that the sensitivity of Xpert MTB/RIF for tuberculous serous effusions was only 25.1%, which was similarly to the result of MTB culture and also to the results of previous related studies.[6] This suggested that although Xpert MTB/RIF can be used for rapid diagnosis, its diagnostic accuracy for tuberculous serous effusions still needs to be improved.

Gene sequencing technologies are increasingly utilized in TB diagnostics.[29] While NGS demonstrated improved sensitivity over Xpert MTB/RIF for tuberculous pleurisy, its performance remains suboptimal.[30] Nanopore sequencing is gaining prominence in TB diagnostics due to unique technical advantages,[21] with established efficacy in pulmonary TB that warrants investigation in paucibacillary disease.[22,24,31] This study suggested that the diagnostic accuracy of nanopore sequencing in tuberculous serous effusions was very high with excellent sensitivity to detect most of the TB. However, the specificity was relatively low with false positives (4 cases), which may be related to the following reasons: first, the PCR amplification before performing nanopore sequencing as errors may be generated in PCR amplification; second, the error rate of long-read nanopore sequencing and potential for off-target reads; third, the contamination risks of samples; Fourth, the number of patients in the non-TB group was limited. Specificity still needs to be confirmed by studies with larger sample sizes. In the group of non-tuberculous serous effusions, four false-positive effusions were eventually diagnosed as tumor and cardiac insufficiency related and were treated accordingly, and the effusions improved after no anti-TB treatment was given. In positive nanopore sequencing specimens, reads were not as high as in pulmonary TB

specimen testing. In pulmonary TB specimens, previous studies have observed that the reads in the TB group were significantly higher than those in the non-TB group,[24,32] but we did not observe this in the tuberculous serous effusions. Nanopore sequencing in tuberculous serous effusions was mostly low, which on the other hand proved that the MTB content in serous effusions was very low. The results of nanopore sequencing with serous effusions might need to be treated differently from pulmonary TB specimens, and a positive result with a low reads count might still be relevant for tuberculous serous effusions. Among the different types of serous effusions, the diagnostic accuracy of the tests associated with pericardial effusions was the highest, suggesting that the level of MTB in pericardial effusions may be higher than that in pleural and abdominal effusions; however, due to the extremely small sample size of pericardial effusions, this result may not be credible and requires further confirmation. The accuracy of nanopore sequencing was similar in different types of serous effusions, suggesting that nanopore sequencing can be effectively applied to a wide range of serous effusions.

The assay turnaround time for nanopore sequencing is 48 hours. While this exceeds that of commercial molecular assays like Xpert MTB/RIF, it remains significantly shorter than conventional mycobacterial culture. Consequently, nanopore sequencing still qualifies as a rapid diagnostic tool and is unlikely to impact patient management timelines. However, current requirements for nanopore sequencing—including specialized laboratory infrastructure, instrumentation, and operator expertise—remain relatively high. Additionally, the per-test cost exceeds that of Xpert MTB/RIF. These factors may limit accessibility, particularly in resource-limited settings. The potential of nanopore sequencing to detect drug-resistant mutations or co-infections may be substantial, but this study targeted paucibacillary tuberculous serous effusions and failed to further evaluate these aspects.

Some limitations inevitably arose in our study; First, due to the retrospective design of this study with a fixed sample size (105 cases in the TB group and 27 cases in the non-TB group), prospective sample size estimation was not possible. The post hoc power analysis was based on the observed AUC value (0.89) versus the specified comparative value (Xpert MTB/RIF, AUC=0.63) at α=0.05 (one-sided). Calculations showed that the statistical validity of detecting an AUC significantly greater than 0.63 was greater than 99.99% at the current sample size. This indicated that the sample size was fully adequate for detecting the observed significant advantage (ΔAUC=0.26). Nevertheless, we fully recognized that an important limitation of this study is the relatively small sample size of the non-TB group (n=27). This is reflected in the wide 95% CI for specificity: 85.2% (95% CI: 66.3%–95.3%). This suggested that there may be considerable uncertainty in the true specificity. However, the present study provided valuable information in the early diagnosis of tuberculous serous effusions by nanopore sequencing. The observed sensitivity (93.3%), AUC (0.89) and comparison with Xpert MTB/RIF showed the potential of this method to have significant advantages. Second, tuberculous serous effusions contain very low levels of MTB, and with limited specimen volume, preference was given to tests with potentially higher sensitivities, so that comparisons of relevant tests were not paired. Third, the sample size for each type of serous effusions was limited, and evaluation of these specific sites still requires prospective large sample size studies for confirmation. Fourth, nanopore sequencing to detect drug resistance mutations or co-infections was not included in this study. Finally, this study did not include a cost-effectiveness analysis, which would require a prospective, large-sample study to further confirm.

Conclusion

Nanopore sequencing was efficient for the rapid diagnosis of tuberculous serous effusions and had a very positive effect. For paucibacillary tuberculous serous effusions, nanopore sequencing might become an effective method for detecting pathogenic bacteria. These findings warrant further validation through larger, prospective studies and may have implications for TB diagnostics in high-burden settings.

Data Sharing Statement

Data is available from the corresponding author on reasonable request via email: dabaitwo@163.com.

Ethics Approval and Consent to Participate

The study protocol was approved by the Ethics Committee of Hangzhou Red Cross Hospital (2024-YS-015). The informed consent of patients or guardians were waived by the Ethics Committee of Hangzhou Red Cross Hospital because this is a retrospective study conducted on already available data and will not have any impact on patients. This study is in accordance with the Declaration of Helsinki.

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Disclosure

The authors declare that they have no conflict of interest.

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