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ORIGINAL ARTICLE

The Value of Single-Molecule Nanopore DNA Sequencing in the Clinical Diagnosis of Suspected Tuberculosis Patients

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SUMMARY

Background: **Early diagnosis of *Mycobacterium tuberculosis* (MTB) infection is of great significance for the clinical management of tuberculosis (TB). We first explored the efficacy of single-molecule nanopore DNA sequencing in the early diagnosis of suspected TB patients and analyzed the advantages in differentiating and diagnosing MTB and non-*tuberculous Mycobacteria* (NTM).**

Methods: **In this cohort study, we reviewed the clinical data of suspected TB patients admitted from December 1, 2021, through April 15, 2022. All patients underwent 3 - 6 times acid-fast bacilli smear examinations of sputum, all of which were negative. To make a definitive diagnosis, we extracted specimens from the patients and performed specimen culture, Xpert MTB/Rif assay, and single-molecule nanopore DNA sequencing. The efficacy of different diagnostic methods in diagnosing suspected TB patients was compared using "Diagnostic Criteria for Pulmonary Tuberculosis" (WS288-2017) as the gold standard.**

Results: **Among the 25 patients, 15 were infected with MTB, 5 were infected with NTM, 1 had mixed MTB and NTM infection, and 4 were negative. The accuracy of single-molecule nanopore DNA sequencing in diagnosing mycobacterial infection (MTB + NTM) was 92.0%, with a sensitivity of 90.5% and a specificity of 100%; the accuracy of diagnosing MTB infection was also 92.0%, with a sensitivity of 87.5% and a specificity of 100%. Single-molecule nanopore DNA sequencing showed an accuracy of 100% in differentiating MTB and NTM. However, the diagnostic accuracy and sensitivity of specimen culture and Xpert MTB/Rif assay were relatively low (≤ 52%) compared to "specimen culture + Xpert MTB/Rif assay". The diagnostic efficacy of single-molecule nanopore DNA sequencing was not affected by the source of tissue samples, while specimen culture and Xpert MTB/Rif assay could not diagnose mycobacterial infection using extrapulmonary specimens.**

Conclusions: **As a third-generation sequencing technology, single-molecule nanopore DNA sequencing has significant application value in diagnosing suspected TB patients. Compared to traditional diagnostic methods, such as specimen culture and Xpert MTB/Rif assay, single-molecule nanopore DNA sequencing exhibits high diagnostic efficacy, low error rate, and convenient detection.**

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Supplementary Data

Table S1. Clinical data and laboratory test results of 25 patients.

Number	Gender	Age (years)	Specimen	Single-molecule nanopore DNA sequencing			Culture	Xpert MTB/Rif assay	Clinical diagnosis
				Results	Reads	Drug-resistant Gene			
1	Male	55	Ascites	MTB	2	(-)	(-)	(-)	MTB
2	Male	30	BALF	MTB and NTM (*Mycobacterium abscessus, Mycobacterium chelonae*)	51, 28, 10	(-)	(-)	(-)	MTB, NTM
3	Male	23	BALF	MTB	289	Isoniazid	(-)	(+)	MTB
4	Male	70	BALF	(-)	/	(-)	(-)	(-)	MTB
5	Male	71	Sputum	MTB	2	(-)	(-)	(-)	MTB
6	Male	50	Hydrothorax	MTB	4	(-)	(-)	(-)	MTB
7	Male	50	Sputum	(-)	/	(-)	(-)	(-)	(-)
8	Male	46	BALF	NTM (*Mycobacterium abscessus*)	8	(-)	(-)	(-)	NTM
9	Female	69	Urine	NTM (*Mycobacterium abscessus*)	19	(-)	(-)	(-)	NTM
10	Female	43	BALF	MTB	1,728	Streptomycin	(-)	(-)	MTB
11	Male	27	BALF	MTB	5,883	Rifampicin	(+)	(-)	MTB
12	Female	52	BALF	MTB	18,583	Isoniazid, Rifampicin, Ethambutol, Streptomycin, Fluoroquinolones, Pyrazinamide	(+)	(+)	MTB
13	Male	63	BALF	(-)	/	(-)	(-)	(-)	(-)
14	Male	46	BALF	NTM (*Mycobacterium intracellulare*)	318	(-)	(+)	(-)	NTM
15	Male	24	BALF	MTB	8,443	(-)	(-)	(-)	MTB
16	Male	58	BALF	NTM (*Mycobacterium intracellulare*)	76	(-)	(+)	(-)	NTM
17	Male	27	BALF	MTB	352	Lipofen	(+)	(+)	MTB
18	Male	55	BALF	(-)	/	(-)	(-)	(-)	MTB
19	Female	51	BALF	NTM (*Mycobacterium abscessus*)	15	(-)	(-)	(-)	NTM
20	Male	27	BALF	MTB	3,554	Lipofen, Lincomycin	(+)	(+)	MTB
21	Male	64	BALF	(-)	/	(-)	(-)	(-)	(-)
22	Female	52	BALF	(-)	/	(-)	(-)	(-)	(-)
23	Male	37	BALF	MTB	2	(-)	(-)	(-)	MTB
24	Male	60	BALF	MTB	9	(-)	(-)	(-)	MTB
25	Female	48	Hydrothorax	MTB	4	(-)	(-)	(-)	MTB

BALF - bronchoalveolar lavage fluid, MTB - Mycobacterium tuberculosis, NTM - non-tuberculous mycobacteria, (+) – positive, (-) - negative.

Table S2. Comparison of different detection methods for diagnosing mycobacterial infection (MTB + NTM).

Specimen sources	Detection content	Gold standard		Single-molecule nanopore DNA sequencing		Specimen culture		Xpert MTB/Rif assay		Specimen culture + Xpert MTB/Rif	
		Positive	Negative	True-positive	True-negative	True-positive	True-negative	True-positive	True-negative	True-positive	True-negative
Intra-pulmonary (n = 21)	Mycobacterium	17	4	15	4	6	4	4	4	7	4
	MTB	12	9	11	8	4	6	4	8	5	7
Extra-pulmonary (n = 4)	Mycobacterium	4	0	4	0	0	0	0	0	0	0
	MTB	3	1	3	1	0	0	0	0	0	0

MTB - *Mycobacterium tuberculosis*.