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Diagnostic value of tNGS vs Xpert MTB/RIF in childhood TB

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ABSTRACT

Objectives: To evaluate the diagnostic value of targeted next generation sequencing (tNGS) in childhood tuberculosis (TB) and compare the accuracy with Xpert MTB/RIF method.

Methods: Children aged ≤18 years with symptoms suggestive of TB during July 2021 to December 2022 at Beijing Children's Hospital were included, and the performances of tNGS and Xpert were evaluated.

Results: A total of 103 children with suspected TB were recruited, including 72 discharge diagnosis of TB and 31 non-TB cases. The mean age was 7.37 ± 4.77 years, and 62.1 % were male. The most common type of specimens was gastric aspirate (GA) (59, 57.3 %). Among all the 72 TB patients, tNGS showed higher sensitivity than Xpert, but the difference was not significant (34.7 %, 25/72 vs 20.8 %, 15/72; $P = 0.063$). The specificities of tNGS and Xpert were 87.1 % (27/31) and 96.8 % (30/31), respectively ($P = 0.162$). Among different types of specimen, the highest sensitivity of tNGS on sputum and pus was observed (80.0 %, 4/5), followed by pleural effusion (50.0 %, 2/4). One rifampin resistance and one protionamide resistance were detected in bacteriologically confirmed TB by tNGS.

Conclusion: tNGS had a higher sensitivity but lower specificity compared to Xpert in diagnosis of children TB. tNGS yielded higher sensitivity than Xpert on gastric aspirate and sputum and pus.

1. Introduction

Tuberculosis (TB) in children is a major public health threat worldwide, with an approximately 1.2 million TB cases in 2021 [1]. However, it has been estimated that almost two-thirds of childhood TB have not been reported [2]. And the diagnosis of TB in children is challenging due to the paucibacillary nature, nonspecific symptoms and radiological features, resulted in the underestimation of TB burden in children [3–5]. Furthermore, the bacteriological confirmation by acid-fast bacilli (AFB) smear microscopy and mycobacterial culture has low sensitivity in children [6–8]. Though the molecular diagnostic tests Xpert MTB/RIF and Xpert MTB/RIF Ultra can rapidly identify *Mycobacterium tuberculosis* (MTB) and rifampicin (RIF) resistance, the cost, sophisticated instruments and limited diagnostic sensitivity in children constrained its implementation in resource-limited areas with high burden of TB [9,10].

Targeted next generation sequencing (tNGS) of MTB, a feasible option for comprehensive, fast, and clinically relevant sequencing,

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can provide deep data from uncultured samples, used for diagnosis of TB and drug-resistant TB (DR-TB) [11,12]. The tNGS developed by Hugobiotech. Co., Ltd., can simultaneously detect the presence of MTB and all first line drugs (rifampicin [RIF], isoniazid [INH], ethambutol [EMB] and pyrazinamide [PZA]) resistance. So we aimed to evaluate the diagnostic value of tNGS in childhood TB and compare the accuracy with Xpert MTB/RIF method.

2. Material and methods

2.1. Study design

The samples were collected at Beijing Children's Hospital (Beijing, China) during July 2021 to December 2022. The diagnostic performance of tNGS and Xpert MTB/RIF was compared against the gold standard of discharge diagnosis and composite reference standard (CRS), respectively. The CRS was composed of clinical, histopathological, laboratory, and radiological features. The written informed consent was obtained from a parent or legal guardian, and from children older than 7 years.

2.2. Patient recruitment

Participants with eligibility criteria of suspected TB were: 1) age ≤18 years old; 2) clinical symptoms suggestive of TB including night sweats, fever or cough for more than two weeks, weight loss, and abnormal chest radiograph; and (3) able to collect adequate biological specimens.

Based on the discharge diagnosis and anatomic site, the children were categorized into (1) pulmonary tuberculosis (PTB): any bacteriologically confirmed or clinically diagnosed case of TB involving the lung parenchyma or the tracheobronchial tree, including tuberculous intrathoracic lymphadenopathy (mediastinal and/or hilar), without radiographic abnormalities in the lungs; (2) extrapulmonary tuberculosis (EPTB): any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs (e.g. pleura, peripheral lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges); (3) non-TB: cases with other diagnosis.

According to the CRS, the children with TB were categorized into (1) bacteriologically confirmed TB: positive results of smear microscopy or culture; (2) clinically diagnosed TB: at least 1 symptom and sign, X-ray abnormalities suggestive of tuberculosis, and at least 1 of the following: clinical and radiologic improvement after *anti*-TB treatment, exposure history of active TB, positive results of tuberculin skin test, or interferon-γ release assay (IGRA).

2.3. Procedures

The samples, such as gastric aspirate, bronchoalveolar lavage fluid, sputum and pus, cerebrospinal fluid, and pleural effusion, collected from children were subjected to microscopy testing, solid culture, mycobacteria growth indicator tube (MGIT) 960 culture, Xpert, and tNGS. The blood samples were used for IGRA. Sterile specimens were directly processed. Non-sterile specimens were decontaminated with *N*-acetyl-L-cysteine 2 % sodium hydroxide, then neutralized with sterile saline phosphate buffer (PBS) to a final volume of 45 ml, followed by centrifuged at 3000×*g* for 15 min at 4 °C. The sediments were resuspended in 2 ml PBS buffer with 0.5 ml inoculated into the MGIT 960 system and Lowenstein-Jensen solid medium, the remaining 1.5 ml was used for Xpert and tNGS. The pellet was smeared on a slide for Ziehl-Neelsen acid fast staining.

2.4. Interferon-γ release assay

According to the manufacturer's instructions (QIAGEN, Australia), 1 ml of whole blood was collected into each of the three separate test tubes, including a nil control tube, a positive control tube with mitogen, and a TB antigen tube (containing ESAT-6, CFP-10 and TB7.7), followed by incubated for 16–24 h at 37 °C. Then the tubes were centrifuged and the supernatant were collected to assess the concentration of IFN-γ (IU/mL) via ELISA. The result was interpreted in Supplementary Table 1.

2.5. Xpert assay

As the manufacturer's instructions, 2 ml sample processing reagent was added to 1 ml specimen, then vortexed for 30 s. Followed by incubated at room temperature for 10 min, the mixture was transferred into Xpert cartridges, and loaded into the GeneXpert instrument. The results of the detection of MTB and rifampin resistance were generated automatically by the instrument.

2.6. tNGS

The DNA was extracted and purified according to the instruction of Quick-DNA™ Miniprep Plus Kit (ZYMO, CA, USA). Extracted genomic DNA was quantified by Qubit Fluorometer 4.0 (Thermo Fisher Scientific, MA, USA). Genomic DNA libraries were constructed using the Illumina Nextera kit following the manufacturer's protocol and sequenced on Nextseq 550 platform (Illumina, San Diego, USA). All sequencing procedures were performed by Hugobiotech. Co., Ltd. (Beijing, China).

2.7. Statistical analysis

Categorical variables were presented as percentages, while continuous variables were presented as means and standard deviations. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated at the 95 % confidence interval (CI), while concordance between Xpert and tNGS was performed with Kappa test. $P < 0.05$ was considered as statistically significant. Data analyses were conducted using SPSS version 20.0.

3. Results

3.1. Participants

A total of 103 children with suspected TB were recruited, including 72 discharge diagnosis of TB and 31 non-TB cases. Of the 72 TB cases, 44 PTB and 28 EPTB were confirmed according to disease sites. And there were 23 bacteriologically confirmed TB, 49 clinically confirmed tuberculosis based on CRS (Fig. 1). The mean age was 7.37 ± 4.77 years, and 62.1 % were male. Nine patients (8.7 %) were smear positive, 15 (14.6 %) were solid culture positive, and 19 (18.4 %) had a positive liquid culture. While 59 (57.3 %) patients had a positive IGRA results (Table 1). The types of specimens included gastric aspirate (GA) (59, 57.3 %), bronchoalveolar lavage fluid (23, 22.3 %), sputum and pus (11, 10.7 %), cerebrospinal fluid (6, 5.8 %), and pleural effusion (4, 3.9 %) (Supplementary Fig. 1).

3.2. Comparision of Xpert and tNGS for the detection of pediatric tuberculosis

Among all the 72 TB patients, tNGS showed higher sensitivity than Xpert, but the difference was not significant (34.7 %, 25/72 vs 20.8 %, 15/72; $P = 0.063$). The higher sensitivity of tNGS than Xpert for MTB detection was observed among PTB (34.1 %, 15/44 vs 22.7 %, 10/44; $P = 0.237$) and EPTB patients (35.7 %, 10/28 vs 17.9 %, 5/28; $P = 0.131$), although the difference was not statistically significant. Of the 23 TB patients with bacteriological evidence, tNGS had 69.6 % (16/23) sensitivity, slightly higher than that of Xpert (52.2 %, 12/23; $P = 0.227$). Among the 49 clinically confirmed TB, tNGS yielded slightly higher sensitivity than Xpert, 18.4 % (9/49) and 6.1 % (3/49), respectively ($P = 0.064$). The specificities of tNGS and Xpert were 87.1 % (27/31) and 96.8 % (30/31), respectively ($P = 0.162$) (Table 2). Compared to Xpert, NPV of all enrolled children were higher in tNGS, indicating the low false positivity for diagnosing TB. Two severe combined immunodeficiency diseases (SCID) were diagnosed as mycobacterium bovis infection by tNGS. One central nervous system infection and 1 sepsis cases were diagnosed as TB by tNGS, while 1 severe combined immunodeficiency disease was diagnosed as TB by Xpert.

3.3. Comparision of detection rates among various types of specimens

Due to the small sample size of cerebrospinal fluid and pleural effusion, we exclude them for further analysis. Considering discharge diagnosis as the reference, among different types of specimen, the highest sensitivity of tNGS on sputum and pus was observed (80.0 %, 4/5). Besides, the specificity of tNGS on gastric aspirate was the highest (90.0 %, 18/20). And tNGS yielded higher sensitivity than Xpert on gastric aspirate (30.8 %, 12/39 vs 15.4 %, 6/39; $P = 0.107$) and sputum and pus (80.0 %, 4/5 vs 40.0 %, 2/5; $P = 0.197$), although the difference was not statistically significant (Table 3). And identical sensitivity on bronchoalveolar lavage fluid (35.0 %, 7/20) was observed between tNGS and Xpert. Furthermore, similar specificities were observed in tNGS and Xpert assays on different types of specimen.

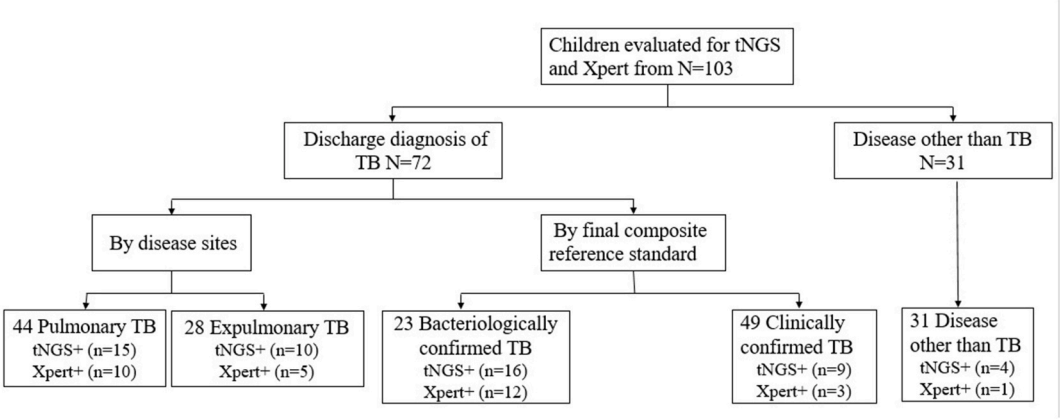

Fig. 1. Flow chart of the study population.

Table 1
Basic characteristics of the patients.

Characteristic	Total (n = 103), n (%)	PTB (n = 44), n (%)	EPTB (n = 28), n (%)	Bacteriologically confirmed TB (n = 23), n (%)	Clinically confirmed tuberculosis (n = 49), n (%)	Non-TB (n = 31), n (%)
Gender						
Male	64 (62.1 %)	27 (61.4 %)	19 (96.4 %)	17 (73.9 %)	29 (59.2 %)	18 (58.1 %)
Female	39 (37.9 %)	17 (38.6 %)	9 (3.6 %)	6 (26.1 %)	20 (40.8 %)	13 (41.9 %)
Age group, y	7.37 ± 4.77	7.62 ± 4.72	6.81 ± 4.83	4.63 ± 5.08	8.56 ± 4.06	7.54 ± 4.90
Interferon-γ release assay						
Positive	59 (57.3 %)	30 (68.2 %)	17 (60.7 %)	18 (78.3 %)	29 (59.2 %)	12 (38.7 %)
Negative	31 (30.1 %)	7 (15.9 %)	9 (32.1 %)	3 (13.0 %)	13 (26.5 %)	15 (48.4 %)
Indeterminate	3 (2.9 %)	1 (2.3 %)	0 (0.0 %)	0 (0.0 %)	1 (2.0 %)	2 (6.5 %)
No data	10 (9.7 %)	6 (13.6 %)	2 (7.1 %)	2 (8.7 %)	6 (12.2 %)	2 (6.5 %)
Acid-fast bacilli smear						
Positive	9 (8.7 %)	6 (13.6 %)	3 (10.7 %)	9 (39.1 %)	0 (0.0 %)	0 (0.0 %)
Negative	91 (88.3 %)	36 (81.8 %)	24 (85.7 %)	14 (60.9 %)	46 (93.9 %)	31 (100.0 %)
No data	3 (2.9 %)	2 (4.5 %)	1 (3.6 %)	0 (0.0 %)	3 (6.1 %)	0 (0.0 %)
LJ Medium						
Positive	15 (14.6 %)	8 (18.2 %)	7 (25.0 %)	15 (65.2 %)	0 (0.0 %)	0 (0.0 %)
Negative	79 (76.7 %)	34 (77.3 %)	19 (67.9 %)	7 (30.4 %)	46 (93.9 %)	26 (83.9 %)
No data	9 (8.7 %)	2 (4.5 %)	2 (7.1 %)	1 (4.3 %)	3 (6.1 %)	5 (16.1 %)
Liquid culture						
Positive	19 (18.4 %)	9 (20.5 %)	9 (3.2 %)	18 (78.3 %)	0 (0.0 %)	1 (3.2 %)
Negative	75 (72.8 %)	33 (75.0 %)	17 (60.7 %)	4 (17.4 %)	46 (93.9 %)	25 (80.6 %)
No data	9 (8.7 %)	2 (4.5 %)	2 (7.1 %)	1 (4.3 %)	3 (6.1 %)	5 (16.1 %)

Table 2
The accuracy of Xpert and tNGS for the diagnosis of TB in children.

Group	Sensitivity, % (n/N)		Specificity, % (n/N)		PPV		NPV	
	Xpert	tNGS	Xpert	tNGS	Xpert	tNGS	Xpert	tNGS
All enrolled children	20.8 % (15/72)	34.7 % (25/72)	96.8 % (30/31)	87.1 % (27/31)	93.8 % (15/16)	86.2 % (25/29)	34.5 % (30/87)	36.5 % (27/74)
PTB	22.7 % (10/44)	34.1 % (15/44)			90.9 % (10/11)	78.9 % (15/19)	46.9 % (30/64)	48.2 % (27/56)
EPTB	17.9 % (5/28)	35.7 % (10/28)			83.3 % (5/6)	71.4 % (10/14)	56.6 % (30/53)	60.0 % (27/45)
Bacteriologically confirmed TB	52.2 % (12/23)	69.6 % (16/23)			92.3 % (12/13)	80.0 % (16/20)	73.2 % (30/41)	79.4 % (27/34)
Clinically confirmed tuberculosis	6.1 % (3/49)	18.4 % (9/49)			75.0 % (3/4)	69.2 % (9/13)	39.5 % (30/76)	40.3 % (27/67)

Table 3
The accuracy of Xpert and tNGS for the discharge diagnosis of TB among various specimens of TB in children.

Specimens	Sensitivity, % (n/N)		Specificity, % (n/N)		PPV		NPV	
	Xpert	tNGS	Xpert	tNGS	Xpert	tNGS	Xpert	tNGS
Gastric aspirate	15.4 % (6/39)	30.8 % (12/39)	100.0 % (20/20)	90.0 % (18/20)	100.0 % (6/6)	85.7 % (12/14)	37.7 % (20/53)	40.0 % (18/45)
Bronchoalveolar lavage fluid	35.0 % (7/20)	35.0 % (7/20)	66.7 % (2/3)	66.7 % (2/3)	87.5 % (7/8)	87.5 % (7/8)	13.3 % (2/15)	13.3 % (2/15)
Sputum and pus	40.0 % (2/5)	80.0 % (4/5)	100.0 % (6/6)	83.3 % (5/6)	100.0 % (2/2)	80.0 % (4/5)	66.7 % (6/9)	83.3 % (5/6)

3.4. Agreement between Xpert and tNGS

Among 103 children, the concordant results were obtained for 80 children with 11 tNGS + Xpert + and 69 tNGS − Xpert −, and the results were weakly consistent (κ = 0.361). However, agreement between tNGS and Xpert was moderate among PTB patients (κ = 0.505), bronchoalveolar lavage fluid (κ = 0.617) and sputum and pus specimen (κ = 0.421) (Supplementary Table 2).

3.5. Resistance detection

One indeterminate result for rifampin resistance was observed in gastric aspirate of bacteriologically confirmed TB by Xpert. Besides, one rifampin resistance in bronchoalveolar lavage fluid of bacteriologically confirmed TB, one protionamide (PTO) resistance

in sputum of bacteriologically confirmed TB, and one pyrazinamide (PZA) resistance in gastric aspirate of non-TB cases were observed by tNGS (Table 4).

4. Discussion

Rapid diagnosis of MTB from clinical specimen is especially important in young children, which is crucial to prevent disease transmission and mortality. Besides, timely initiation of therapy and effective treatment regimen relies on accurate diagnosis and drug-susceptibility testing (DRS) [13]. However, DRS results are often lacking in children, and molecular detection of drug resistance was recommended by WHO as clinical reference [8]. The tNGS has a lower limit of detection (LOD) (5 copies/mL) and can identify targets in multiple drug resistance-associated genes in MTB [14]. However, no data regarding the diagnostic value of tNGS was explored in childhood TB.

A higher sensitivity of tNGS and lower specificity compared to Xpert was observed in this study, irrespective of the reference standard used. And compared to Xpert, greater added value of tNGS were observed among EPTB cases (17.8 %, from 17.9 % to 35.7 %) than among PTB cases (11.4 %, from 22.7 % to 34.1 %) for MTB detection, suggesting that tNGS was highly beneficial for paucibacillary EPTB patients. For children clinically confirmed TB with bacteriological negative, 15 % were tNGS positive. As all these children were symptomatic and improved with TB therapy, but without previously treated for TB, indicating the true positive tNGS results. So tNGS had an additional role in detecting cases of childhood TB with culture negative. However, a lower, although not statistically significant, specificity, was observed in tNGS as compared to Xpert, which probably due to the detection of dead bacilli in patients with a history of treated TB [15]. It's worth noting that two SCID were diagnosed as mycobacterium bovis by tNGS, and Bacille Calmette-Guerin (BCG) derived disease has been described in infants with SCID [16,17]. Besides, 1 central nervous system infection and 1 sepsis cases were also diagnosed as TB by tNGS, and it is possible that the positive result may have been caused by nonspecific amplification, which should be confirmed by another molecular assay.

Varied detection rates of MTB from different types of specimens were observed by tNGS. The sensitivity of tNGS in sputum and pus and gastric aspirate samples were much higher than that of Xpert, indicating superiority for tNGS in diagnosis of these specimens from children with suspected tuberculosis. Gastric aspirate is a stable technique for collecting qualified samples from young children [18–20], and it is the most common types of specimens tested in this study. According to a previous meta-analysis, the pooled sensitivity of GA samples with Xpert was higher than that of sputum samples in children with pulmonary TB [9]. However, in the current study, both the sensitivity of tNGS and Xpert on sputum and pus was higher than that of GA, respectively. Various sensitivities among different specimens may be attributed to the heterogeneity in study populations and settings. In this study, the agreement between tNGS and Xpert among different specimens was moderate and poor, which was mainly attributed to the low bacterial loads in children with Xpert-/tNGS + results, illustrating that tNGS was a more sensitive method.

In this study, one children was detected as RIF-indeterminate by Xpert but RIF-sensitive by tNGS. This phenomenon may be due to the low bacterial load, as the detection of MTB was extremely low on the Xpert. In addition, one case was RIF- sensitive tested by Xpert but RIF-resistant by tNGS. As no phenotypic drug susceptibility testing was available, whether this result was from laboratory induced contamination or mixed infection need further verification [5]. Except for RIF, tNGS can simultaneously detect the resistance of other first line drugs due to its high sequencing depth. We found 1 PTO resistance in bacteriologically confirmed TB and 1 PZA resistance in non-TB cases, suggesting that more children with drug resistant isolates will be detected with the highly sensitive molecular tests of tNGS, which will rapid guidance of clinical treatment.

5. Conclusion

In conclusion, tNGS had a higher sensitivity but lower specificity compared to Xpert in diagnosis of children TB. tNGS yielded higher sensitivity than Xpert on gastric aspirate and sputum and pus.

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Ethical approval

The study was approved by the Ethics Committee of Beijing Children's Hospital, Capital Medical University ([2022]-E−130-Y). Written informed consent was obtained from all the patients.

Data availability statement

Data will be made available on request from the corresponding author.

CRediT authorship contribution statement

Huiwen Zheng: Writing – original draft. **Haiming Yang:** Supervision, Methodology. **Yonghong Wang:** Validation, Methodology.

Table 4
The resistance results detected by Xpert and tNGS.

Group	Specimens	Xpert	tNGS
Bacteriologically confirmed TB	gastric aspirate	RIF- indeterminate	RIF-sensitive
	bronchoalveolar lavage fluid	RIF-sensitive	RIF- resistance
	sputum	RIF-sensitive	PTO-resistance
Non-TB	gastric aspirate	–	PZA-resistance

Feina Li: Methodology, Formal analysis. **Jing Xiao:** Validation, Formal analysis, Data curation. **Yajie Guo:** Resources, Formal analysis, Data curation. **Hao Chen:** Validation, Methodology. **Xiaotong Wang:** Validation, Methodology, Formal analysis. **Huimin Li:** Writing – review & editing, Visualization, Supervision. **Chen Shen:** Writing – review & editing, Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23217.

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