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Mini review

Pediatric Tuberculosis Diagnosis: Evaluating the Xpert Assays

Dachuan Jin[1†]*, Shunqin Jin[2†], Zhongfeng Cui[1]*

[1]*Medical Laboratory, Henan Provincial Infectious Disease Hospital, China*

[2]*Department of Radiology, Hebei Medical University, China*

Corresponding author: Dachuan Jin, Medical Laboratory, Henan Provincial Infectious Disease Hospital, China

Zhongfeng Cui, Medical Laboratory, Henan Provincial Infectious Disease Hospital, China

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Abstract

Tuberculosis (TB) is a significant global cause of mortality, particularly among pediatric patients. The restricted sensitivity and limited accessibility of conventional diagnostic methods have hindered the management of tuberculosis in pediatric patients. In recent years, revolutionary rapid, sensitive, and specific tuberculosis detection techniques based on PCR technology, known as Xpert assays, have emerged. These include Xpert MTB/RIF, Xpert MTB/RIF Ultra, and Xpert MTB/XDR. This mini review provides a concise summary of the characteristics of the Xpert assays, their impact on the clinical outcome of children with tuberculosis, and prospects for future research.

Keywords: Pediatric tuberculosis; diagnosis; Xpert assays

Introduction

Tuberculosis (TB) was identified by the World Health Organization (WHO) as one of the 13 leading causes of global mortality in 2019 [1]. Annually, TB affects approximately 5-7 million individuals worldwide, with approximately 1.1 million cases occurring in pediatric patients [2]. Furthermore, every year, a quarter of children with TB die from the disease [3,4]. However, accurate diagnosis of pediatric TB poses unique challenges. These challenges arise primarily from difficulties in obtaining suitable sputum specimens from pediatric patients, a higher prevalence of extra-pulmonary TB, and low bacterial load, which complicate the isolation of drug-resistant bacteria from pediatric specimens [5,6]. Reports indicate that 96% of TB-related deaths in children occur in individuals who were not promptly diagnosed [7,8]. Considering that the treatment outcomes for tuberculosis in children are generally better than those in adults, early detection and effective treatment of pediatric tuberculosis can lead to a significant cure rate [9]. Therefore, the development of sensitive and rapid tuberculosis diagnostic methods is a core aspect of the WHO's End TB strategy, and Xpert assays are revolutionary tuberculosis diagnostic technologies that have emerged in recent years [10]. The mini review provides a brief evaluation of Xpert assays as diagnostic methods for pediatric tuberculosis, explores the challenges and limitations in implementing Xpert testing in clinical practice, and discusses future research and development directions.

Innovative Tools for Pediatric TB Diagnosis: Exploring Xpert Assays' Features

Despite tuberculosis being a significant and preventable cause of child mortality, the limited sensitivity and restricted accessibility of conventional testing methods, such as the tuberculin skin test (also known as the Mantoux test), chest x-ray, and sputum smear microscopy, pose challenges in managing pediatric TB. As a result, pediatric patients require rapid, reliable, and effective testing methods. The latest Xpert assays, developed by Cepheid in the USA, are rapid and highly specific diagnostic PCR-based tests designed for detecting Mycobacterium tuberculosis (MTB) and resistance to

rifampicin and other drugs. Specifically, Xpert MTB/RIF and Xpert MTB/RIF Ultra have been recommended by the WHO guidelines as the primary diagnostic methods for the initial diagnosis of TB in children. All Xpert assays, including the first generation of Xpert MTB/RIF assay (launched in May 2009), the second generation of Xpert MTB/RIF Ultra assay (launched in March 2017), and the third generation of Xpert MTB/XDR (launched in July 2020), are developed on the same Xpert platform [11-14]. Xpert MTB/RIF and Xpert MTB/RIF Ultra have become extremely widely used molecular diagnostic assays [15,16].

The growth rate of Mycobacterium tuberculosis is slow, usually taking 3-4 weeks of cultivation to obtain results for drug susceptibility testing. This delay hinders the timely diagnosis and initiation of tuberculosis treatment. In contrast, Xpert MTB/RIF assay is a stable and reliable detection method that can provide sputum test results within 100 minutes. This significantly shortens the time needed for tuberculosis diagnosis and the detection of rifampicin drug resistance [17]. This approach reduces the need for operational time and biosafety infrastructure [18]. Due to the ability of the test reagents to kill Mycobacterium tuberculosis, the detection can be conducted in peripheral laboratories. Therefore, it offers advanced TB diagnostic capabilities to physicians working in resource-limited conditions. Moreover, Xpert MTB/RIF assay exhibits much higher sensitivity (with a detection limit of 112.6 CFU/mL) compared to traditional microscopy examination, which requires at least 10,000 CFU/mL for detection [19]. Thus, it is regarded as a groundbreaking laboratory testing method for TB and also the first FDA-approved molecular detection method for identifying drug-resistant tuberculosis.

Previous research has shown that Xpert MTB/RIF is less effective in diagnosing HIV-TB co-infection when compared to standalone TB diagnosis [20]. In response to this issue, Cepheid developed Xpert MTB/RIF Ultra assay as the next generation of rapid molecular detection [21]. The detection limit of Xpert MTB/RIF Ultra is 15.6 CFU/mL, which is ten times more sensitive than Xpert MTB/RIF assay. Therefore, it is particularly suitable for pediatric TB cases with low bacterial load, irrespective of HIV status. It has been reported that Xpert MTB/RIF Ultra exhibits higher sensitivity than Xpert MTB/RIF in children with HIV-TB co-infection, with an increase of 22.2%(88.9% vs. 66.7%) [22].As per the 2022 WHO guidelines, Xpert MTB/RIF Ultra can serve as the primary diagnostic method for lymph node aspirate and biopsy, replacing smear microscopy and culture. If children suspected of having pulmonary tuberculosis receive initial Xpert MTB/RIF Ultra negative results, and the pretest probability is above 5%, it is recommended to repeat Xpert MTB/RIF Ultra test using sputum and nasopharyngeal aspirate specimens. For children under the age of 10 with pulmonary signs and symptoms of tuberculosis, the WHO recommends using Xpert MTB/RIF Ultra method to test gastric aspirate or stool specimens [23]. Regardless of whether it is sputum gastric aspirate, stool, or nasopharyngeal aspirate, Xpert MTB/RIF Ultra is an accurate method for diagnosing pediatric pulmonary tuberculosis and rifampicin resistance.

Early diagnosis, which includes universal drug susceptibility testing for all tuberculosis patients, plays a crucial role for the eradication of TB [24]. Rifampicin resistance is the predominant form of drug resistance in TB. However, due to the growing prevalence of resistance to other drugs such as isoniazid, fluoroquinolones, and injectable second-line anti-TB drugs, as well as the emergence of extensively drug-resistant TB, there is a need for diagnostic assays capable of detecting resistance to all clinically significant drugs. This information is crucial for the timely initiation of effective anti-TB treatment [25]. For instance, in 2009, the World Health Organization estimated that isoniazid-resistant TB affected 1.1 million (11%) TB patients. Additionally, 3.5% of new TB cases and 20.5% or previously diagnosed TB cases were identified as having multidrug-resistant TB [26]. Furthermore, multidrug-resistant TB is rising at a rate exceeding 20% per year. Although Xpert MTB/RIF and Xpert MTB/RIF Ultra are widely used for detecting Mycobacterium tuberculosis and rifampicin resistance, they are incapable of assessing resistance to isoniazid, fluoroquinolones, and second-line drugs. The latest generation of Xpert MTB/XDR integrates resistance testing for isoniazid, fluroquinolones, ethionamide, amikacin, kanamycin and capreomycin with high sensitivity and specificity, especially for isoniazid and fluoroquinolones [27]. It can provide a comprehensive assessment of resistance to isoniazid, fluoroquinolone and second-line anti-tuberculosis drugs in a single test [28-31]. With a similar limit of detection (136CFU/mL) for Mycobacterium tuberculosis as the earlier Xpert MTB/RIF assay, Xpert MTB/XDR is recommended for reflex testing in patients with confirmed tuberculosis infection.

Transforming Paediatric TB Treatment: Significance of Xpert Assays for Prognosis

The development of early and accurate diagnostic methods is crucial for reducing the mortality and morbidity associated with childhood tuberculosis. The introduction of Xpert assays provides an opportunity for children, even in resource-limited areas, to receive early and accurate diagnoses, leading to timely and effective treatment, improved prognosis, and reduced transmission of tuberculosis. So, does the application of Xpert assays improve patients' prognosis? Several studies have found that early drug susceptibility testing using Xpert MTB/RIF significantly improved the accessibility of multidrug-resistant tuberculosis diagnosis, greatly shortened the time to diagnosis, increased the number of cases detected with multidrug-resistant tuberculosis infection by 8 times in adults and children, and doubled the confirmation rate of TB infection [32-36]. The proportion of patients receiving empirical treatment decreased by 35%, and the duration of treatment was also shortened to varying degrees. However, studies have shown no clear evidence that the Xpert assays reduce morbidity or mortality in children with TB.

This may be because physicians applying conventional microscopy applied a higher proportion of empirical treatment and initiate empirical anti-tuberculosis treatment in the absence of bacteriologic confirmation, thus offsetting the benefits of Xpert

in improving diagnostic sensitivity, but the benefits of reducing overtreatment are difficult to detect. It has also been found that the incidence of TB mortality after treatment initiation was significantly lower in the Xpert group than in the conventional microscopy group, but the result was not sufficiently reliable because of the large number of missed cases. In patients with pulmonary tuberculosis and HIV co-infection, although blood based Xpert has lower sensitivity for pulmonary tuberculosis, a positive Xpert result is a highly predictive marker of early mortality, thus demonstrating potential utility as a prognostic indicator. Moreover, nearly all studies on the impact of prognosis are on Xpert MTB/RIF assay, while studies on the impact of Xpert MTB/RIF Ultra assay and Xpert MTB/XDR assay on prognosis are very limited.

Conclusion

In summary, the Xpert assays require only a single-step process and can be implemented at all levels of the healthcare system with basic laboratory skills and equipment. As rapid, stable, feasible, and reliable diagnostic methods, Xpert assays have overcome all the drawbacks of traditional TB detection methods. The high sensitivity and specificity of Xpert assays for TB detection provide unparalleled advantages for the diagnosis of TB in children. In particular, Xpert MTB/RIF Ultra greatly supports case detection in pediatric patients with low mycobacterial loads. The Xpert MTB/XDR can detect resistance to most anti-tuberculosis drugs, but its inability to detect bedaquiline and linezolid is a shortcoming. Future upgrading to be able to detect resistance to the two aforementioned drugs ensures the efficacy of these drugs will be a future research direction [28]. In addition, another limitation of the Xpert assays is that it cannot distinguish between viable and nonviable bacteria, which may result in false-positive results and cannot diagnose reinfection [37]. Further research is also needed to investigate the diagnostic performance of Xpert assays on non-respiratory specimens in pediatric patients.

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