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The role of metagenomic next-generation sequencing to diagnose HIV-TB coinfection: a promising technology

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Abstract

The human immunodeficiency virus (HIV) pandemic has caused a resurgence of tuberculosis (TB), resulting in increased morbidity and mortality. Meanwhile, HIV-TB coinfection lead to diagnostic difficulty. Sputum smear microscopy, mycobacterial culture, and the GeneXpert MTB/RIF assay (Xpert) are generally endorsed to detect *M. tuberculosis* in HIV-TB coinfection. However, these methods cannot diagnose TB in an accurate and timely manner, which increases the rates of HIV-related morbidity and mortality in TB patients. Hence, there is a considerable need for a better diagnostic tool for HIV-TB coinfected individuals. Metagenomic next-generation sequencing (mNGS) is a novel detecting platform which is widely used in infectious disease, antimicrobial resistance and the microbiome and human host gene expression. Herein, we first summarized its diagnostic advantages in infectious disease. Then we assessed the efficiency of mNGS in the detection of *Mycobacterium tuberculosis (M. tuberculosis)* in different specimens and a few cases of in HIV-TB coinfection. We concluded that mNGS is an acceptable diagnostic method in HIV-TB coinfection although limited research is available.

Key words: Metagenomic next-generation sequencing (mNGS), HIV-TB

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coinfection, diagnosis

1. Introduction

People living with HIV (PLHIV) have a high risk (15–21 folds higher than people without HIV) of developing active tuberculosis (TB), and TB is the leading cause of death in PLHIV. In 2020, about 214 000 people died of HIV-associated TB (1). The diagnosis of HIV-TB coinfection poses particular challenges due to the high incidence of smear-negative TB and extrapulmonary TB (2, 3). Smear microscopy, mycobacterial culture, and the GeneXpert MTB/RIF assay (Xpert) are commonly used to diagnose TB. Theron, et al. reported that the sensitivity of smear microscopy was only 20–50%. Although mycobacterial culture is the gold standard for detecting *Mycobacterium tuberculosis (M. tuberculosis)*, it takes high cost and spends long time about 2–8 weeks (4). In 2010, Xpert was initially approved for the diagnosis of HIV-TB coinfection by the WHO. A retrospective study was conducted in high HIV prevalence setting established that the overall sensitivity of Xpert in smear-positive culture-positive TB cases is significantly higher than in smear-negative culture-positive TB cases (94.7% vs 46.8%) (4). Therefore, an accurate diagnostic approach is highly important for HIV-TB coinfected patients.

Metagenomic next-generation sequencing (mNGS) is a novel technique for

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infectious diseases. In 2008, mNGS was first applied in human infectious diseases and was used to successfully identify a then-new virus called arenavirus (5). This emerging approach can detect all potential pathogens—bacteria, viruses, fungi, and parasites. It also offers unbiased sequencing and identification of microbial genetic material. The use of mNGS in infectious diseases was reviewed by Chiu, et al. They discovered that mNGS has irreplaceable diagnostic value in rare, novel, atypical, and complicated infectious diseases (6). It has been reported that the sensitivity of mNGS in smear-negative TB is significantly higher than that of MGIT 960 culture and Xpert (7). In 2022, The authors provided a comprehensive overview of neurological infections and coinfections in HIV-infected Ugandan adults with subacute meningitis using unbiased cerebrospinal fluid mNGS (8). However, mNGS has been little used in HIV-TB coinfection, and only a few studies are available. Our perspective shows that mNGS may have value in the diagnosis of HIV-TB coinfection. Therefore, in the following pages, we provide evidence showing that this technology is equally applicable to HIV-TB coinfection.

2. Detection advantages of this promising technology

With the advent of next-generation sequencing technology in 2005, metagenomics emerged in the field of pathogenic microorganism detection. mNGS is a promising tool which has many advantages in the diagnosis of infectious diseases. Firstly, high-throughput sequencing allows simultaneous independent detection of thousands to billions of DNA fragments. Secondly, mNGS can sequence full-length

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genome of pathogens. Therefore, it can not only accurately detect pathogenic microorganisms in cases of suspected complications or immunosuppression but also can screen drug resistance pathogens and virulence genes. Thirdly, mNGS can be applied directly to original specimens without microorganism culture. mNGS has been successfully applied in various types of samples, such as cerebrospinal fluid (CSF), respiratory secretions, feces, urine, blood, and other types of tissue (6). Finally, mNGS, which is an unbiased and hypothesis-free approach that can detect all types of pathogens, has considerable potential for discovery of novel or unexpected pathogens.

These attractive advantages have been verified in clinical settings. In 2014, mNGS was initially and successfully used to diagnose a rare case of *Leptospira* infection, when traditional methods such as culture, serologic tests, and PCR had failed to identify the causative pathogen (9). mNGS also plays a vital role in diagnosing various newly emerging infectious diseases. For example, it rapidly identified a novel coronavirus named 2019-nCoV (10). The widespread application and development of mNGS has changed the diagnostic situation of infectious diseases considerably. As we know, detecting *M. tuberculosis* in HIV-TB coinfection is an intractable, mNGS is expected to overcome the problem by virtue of these detection advantages.

3. Application of mNGS in detecting *M. tuberculosis*

Early researches evaluated the potential capacity of mNGS to detect *M. tuberculosis* (11). With the development of sequencing technology, the role of mNGS in TB came to be recognized in many clinical studies. Chen et al. reported that the

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sensitivity of mNGS to all clinical TB specimens was far superior to that of *M. tuberculosis* culture (66.7% vs 36.1%) and Xpert (76.9% vs 61.5%) (12). A retrospective study showed that the diagnostic accuracy of mNGS to active TB was higher than those of cultures on liquid medium (the MGIT 960 system) (49.6% vs 35.2%), and the turnaround time was markedly shortened from 2-6 weeks to 32–36 hours. (13). Another prospective study discovered that the sensitivity of mNGS (44%) was similar to Xpert (42%) but much higher than conventional methods including culture and biopsy (29%) (14).

mNGS, as a promising method to diagnose TB, can detect *M. tuberculosis* from different specimen or extrapulmonary tuberculosis types (Table 1). Zhu et al. reported that mNGS provides sensitive detection of *M. tuberculosis* in bronchoalveolar lavage fluid (BALF) or lung tissue biopsy samples in smear-negative cases with suspected PTB (15). Another large cohort study reported the use of mNGS to detect 208 extrapulmonary specimens, and the final result showed that mNGS had the highest sensitivity (56.11%) than MTB/RIF Xpert (36.11%) and MGIT960 culture (13.89%) (7). Meanwhile, a massive number of case reports and retrospective studies on its clinical application have shown its superiority in diagnosing EPTB, including hepatic tuberculosis and tuberculous meningitis (TBM). Ai et al. was the first to discovery a faster diagnosis of local hepatic TB using the mNGS assay (16). In another study, mNGS was used to detect *M. tuberculosis* from cerebrospinal fluid (CSF), which had higher sensitivity (66.67%) than AFB, PCR, and *M. tuberculosis* culture (33.33, 25, and 8.33%), the specificity of mNGS was excellent (100%) (17).

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4. Applicability of mNGS in HIV-TB coinfection

HIV-TB is a specific type of TB, and its diagnosis remains difficult due to the paucibacillary nature of the disease. Existing methods such as smear microscopy, mycobacterial culture, and Xpert MTB/RIF cannot achieve accurate and rapid diagnosis, especially cases of smear-negative TB and extrapulmonary tuberculosis. Based on the diagnostic ability and advantages of mNGS, we hypothesized that this technology could overcome the diagnostic difficulties of HIV-TB coinfection. In HIV-TB coinfection, mNGS has been reported to diagnose tuberculosis meningitis in HIV-infected individuals. It was able to do this early, rapidly, and accurately, which improves the diagnosis of aseptic meningitis and the treatment of acute HIV infections (18). In another prospective study with 368 HIV-infected Ugandan adults, Ramachandran et al. first developed a combined diagnostic approach to achieve high sensitivity (88.9%) and specificity (86.7%) of tuberculosis meningitis and its many mimics by exploiting the specificity of mNGS and the sensitivity of an MLC created from cerebrospinal fluid (CSF) host. Additionally, they achieved comparable combinatorial method performance at sequencing depths, which was more suitable for diagnostic mNGS in low-income countries (8). Overall, although a few studies are available, the ability of mNGS in diagnosing HIV-TB coinfection cannot be overlooked.

5. Discussion

mNGS is a promising technology, which was used widely in infectious diseases. Despite its great accomplishments, there are still some limitations: i) lack of quality

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control standards for sample preparation and the sequencing process; ii) insufficient bioinformatics analysis for mNGS data; iii) relatively high detection costs, which limit the widespread application of this promising technology, especially in resource-poor areas (19,20); v) although the use of mNGS in diagnosing TB and HIV-TB coinfection has shown good performance in some cases and samples, large-cohort, muti-center clinical studies of HIV-TB coinfection patients are required to establish it in this area. With the improvement of existing technology, particularly the optimization of the sequencing process, the construction of the complete database, and the progress of bioinformatics, these challenges can be overcome. In the future, mNGS will have potential to become a more accurate, economical, and universal diagnostic technology, which can address the diagnostic dilemmas associated with complicated infectious diseases such as HIV-TB coinfection.

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