The Value of Metagenomic Next -Generation Sequence In Acquired Immunodeficiency Syndrome With Opportunistic Infections

Li Liu (✉ billowheart@sina.cn)

Hunan Provincial People's Hospital <https://orcid.org/0000-0003-2730-1762>

Mingjuan Yuan

the first peoples hospital of yueyang

Siqing Sun

the second hospital of nanjing

Jinrong Wang

Hengshui peoples hospital

Yi Shi

Nanjing Jinling Hospital: East Region Military Command General Hospital

Xin Su

Nanjing Jinling Hospital: East Region Military Command General Hospital

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Abstract

Background: Metagenomic next-generation sequence (mNGS) is an emerging powerful pan-pathogen test for the diagnosis of infectious diseases. However, the application of mNGS in acquired immunodeficiency syndrome with opportunistic infections is limited to clinical cases reports and central nervous system infection. In this study, we evaluated the diagnostic value of mNGS in acquired immunodeficiency syndrome (AIDS) with opportunistic infections (OIs).

Methods: From January 2018 to February 2021, 86 cases were enrolled in this retrospective analysis. All patients underwent mNGS. Clinical data were recorded.

Result: In the present study, mNGS identified 76 of 86 infection cases (88.37%). Human betaherpesvirus 5 (CMV) (40.70%), Human gammaherpesvirus 4 (EBV) (40.70%), pneumocystis (31.40%) were the most common pathogens detected. The sensitivity of mNGS (88.37%, 76/86) was higher than that of culture (22.10%, 19/86), smear (7%, 6/86) and PCR (46.51%, 40/86). In the detection of viruses such as (CMV and EBV), the consistency between PCR and mNGS of CMV and EBV was 100%, 73.33% respectively. All PCP cases were detected by mNGS. The consistency in detection of *talaromyces* between culture and mNGS was 75%. mNGS is superior to the common methods such as culture and smear in the detection of mycobacterium tuberculosis and non-mycobacterium tuberculosis. The mNGS findings led to changes in treatment strategies in 47/86 (54.65%) cases. Compared with the patients' treatment before mNGS, patients had lower rate of broad-spectrum antibiotic drugs use during clinical treatment after mNGS 78/86 (90.70%) vs 34/86 (39.53%) ($P < 0.0001$).

Conclusion: mNGS showed a satisfying diagnostic performance in acquired immunodeficiency syndrome with opportunistic infections. mNGS may lead to a more precise antimicrobial treatment and reduced the use of antibiotic medicine.

Introduction

Human Immunodeficiency Virus (HIV) infection is still a great public health threat around the world. Since the epidemic of the acquired immunodeficiency syndrome, approximately 78 million people have infected HIV, and 35 million people have died because of acquired immunodeficiency syndrome (AIDS)-related diseases. The increasing use of antiretroviral treatment can effectively suppress the HIV viral load to undetectable level, so the morbidity and mortality of AIDS is sharply decreased [1–3]. However, opportunistic infections often occur in AIDS patients even under the antiretroviral therapy (ART). It has a significant influence on patients' well-being, life quality, health care costs and their survival [4–6]. What is more, opportunistic infections is the major cause of morbidity and mortality among AIDS patients [7, 8].

The difficulty of diagnosis of opportunistic infections in AIDS patients is a great challenge to clinicians because of its broad spectrum of potential aetiologies. The diagnosis of opportunistic infections with AIDS relies on a detailed history, clinical examination, radiological findings and conventional tests before

mNGS availability. However, conventional tests often result in delayed and missed diagnosis because current microbiological tests such as culture-based methods are time consuming and have low yields rates of positive detection. At same time, the overlap and co-infection of etiologies are often missed which may increase AIDS-related death, and prompt identification of causative agents is critical for the timely application of effective treatment.

With the fast development of molecular biology, metagenomic next-generation sequence (mNGS), a novel and promising approach, has emerged as pan-pathogen assays for infectious diseases diagnosis, especially for the detection of atypical, rare, or slow-growing microbes[9–11]. To date, many studies have addressed the value of mNGS in detecting the causative pathogens and guided targeted antimicrobial therapy more quickly[12–17].

However, mNGS in AIDS with opportunistic infections is limited to clinical cases reports and central nervous system infection[18–21]. Its utility in AIDS patients with opportunistic infections is still not well established. Therefore, we conducted a retrospective observational study in mainland China to analyze the diagnostic value of mNGS for diagnosis of opportunistic infections in adult AIDS patients.

Methods

2.1 Study design and participants

The retrospective cohort was enrolled at The First Yueyang People's hospital (Hunan, China), Second Hospital of Nanjing (Nanjing, China) and Hengshui People's hospital (Hebei, China) between January 2018 and February 2021. Patients, who admitted to our department due to AIDS with opportunistic infections and did mNGS, were included. For each case, clinical information and the results of other conventional tests were collected from electronic patient records. The final diagnosis was made by two independent clinicians based on combination of both mNGS results and its accordance with clinical manifestation. This retrospective study was approved by the Ethics Committee of the The First Yueyang People's hospital, Second Hospital of Nanjing and Hengshui People's hospital.

In our real-life clinical practice, patients suspected to have opportunistic infections underwent three times blood culture, epstein-barr virus DNA, cytomegalovirus DNA, chest computer tomography after informed consent. Sputum samples were also sent for conventional testing, including bacterial and fungal smear; acid-fast stain; and culture of bacteria, fungal organisms and Mycobacterium species in all patients. When patients were suspected to have central nervous system infection, cerebrospinal fluid (CSF) samples were sent for bacterial and fungal smear; acid-fast stain, and culture of bacteria, fungal organisms and Mycobacterium species.

2.2 Procedure

Samples were collected, and then sent to BGI-Huada Genomics Institute (Wuhan, China) for pathogens detection as described previously[17]. Briefly, the mNGS procedure for samples includes nucleic acid

extraction,library construction,sequencing,information analysis and quality-controlled.Low-quality and short (length < 35bp) reads were removed.Then the remaining sequence were aligned to the current virus, bacterial, fungal, and protozoan databases (NCBI; ftp://ftp.ncbi. nlm.nih.gov/genomes),which is comprised of whole genome sequences of 4061 viral taxa,2473 bacterial genomes or scaffolds,and genomic sequences for 199 fungi related to human infection and 135 parasites associated with human diseases.

2.3 Statistical analysis

Continuous variables were expressed as the medians.Categorical variables were summarized as the counts and percentages in each category.Statistical analyses were performed using SPSS version 20.0 (IBM Corporation, Armonk, NY, USA).Figures were conducted using GraphPad Prism 5 software.A p value less than 0.05 was considered statistically significant.

Results

3.1 Population characteristics

There were 86 patients,who presenting with acquired immunodeficiency syndrome with opportunistic infections underwent mNGS between January 2018 and February 2021.The baseline characteristics of patients are shown in Table 1.

Table 1 Clinical characteristics of AIDS with opportunistic infections patients

N=86	
Demographics	
Age ,median(rang,years)	42.83(range27-77)
Female/male	17/69
Clinical feature	
Increased WBC(×109 /L)	18/86(20.93%)
IncreasedCRP(mg/L)	26/86(30.23%)
Increased PCT (ng/mL)	21/86(24.42%)
Median CD4 count, cells/mm3	56.86 cells/mm3
Anti-retroviral therapy	54/86 62.80%

Abbreviations: WBC:white blood cells ;CRP:C-reactive protein; PCT: procalcitonin.

There are 17 women and 69 men were included.Their median age was 42.83years(range 27–77).All(100%) patients had advanced clinical symptoms (WHO clinical stage III or stage IV) and

80/86(93.02%) advanced disease (CD4 count of below 200 cells/mm³).The median CD4 T cell count was 56.86 cells/mm³ (range from 7 to 283) .

3.2 Summary of pathogens detected by mNGS

The main sample types of mNGS in our patients were bronchoalveolar lavage fluid(BALF) (n = 31), while other sample types included blood(n = 30), bone marrow(n = 5),cerebrospinal fluid(n = 18),pleural(n = 2). The identified infection cases of mNGS in BALF,the sensitivity of blood, bone marrow,cerebrospinal fluid,pleural were 30/31,28/30,4/5,12/18,2/2,respectively.

In our cohort,mNGS identified 76 of 86 (88.37%) infection cases within 72 hours.Among these patients, human betaherpesvirus 5 (CMV) (n = 35,40.70%), human gammaherpesvirus 4 (EBV) (n = 35,40.70%),pneumocystis (n = 27,31.40%),were the most common pathogens detected,followed by talaromyces (n = 16,18.60%),torque tenovirus (n = 16,18.60%) and mycobacterium tuberculosis (n = 11,12.79%).

Among pathogens detected by mNGS,it showed that a total of 68 patients were virus-positive (68/86 = 79.07%) and the most frequently detected viruses were Human betaherpesvirus 5 (CMV) (n = 35) human gammaherpesvirus 4 (EBV) (n = 35),and torque tenovirus (n = 16).In addition,the mNGS detected another 6 viruses, including torque tenovirus16 (n = 10),torque tenovirus19 (n = 8),torque tenovirus15(n = 7),herpes simplex virus (HSV, n = 6), human polyomavirus type 5(n = 4),varicella zoster virus(n = 2) (Fig. 1).

According to the results of mNGS,the most commonly fungus detected by mNGS were pneumocystis jirovecii (n = 27),talaromyces (n = 16),and aspergillus fumigatus (n = 6) (Fig. 2).

Some patients (n = 32) were detected bacteria,the most commonly bacteria detected by mNGS was mycobacterium tuberculosis(n = 11),followed by streptococcus pneumoniae (n = 6),enterococcus(n = 5), haemophilus influenzae(n = 3) and non-mycobacterium tuberculosis(n = 3)(Fig. 3).

Mixed infection was defined when two or more infectious pathogens were identified by mNGS.Multiple pathogens were detected in 34 patients.The most frequent pattern of mixed infection was virus and fungi mixed infection(17patients,17/86 = 9.77%),followed by bacteria,virus and fungi mixed infection (7 patients,7/86 = 8.14%).Six patients were diagnosed as bacteria and fungi mixed infection (6/86 = 7%);two patients were diagnosed as bacteria and virus mixed infection (2/86 = 1.16%);two patients were diagnosed as bacteria,pneumocystis jirovecii and mycoplasma mixed infection(2/86 = 1.16%) (Fig. 4).

However,mNGS missed detecting pathogens in 16.28% (14/86) patients whose diagnosis based on symptoms, other laboratory tests and response to empirical therapy.The most common pathogens missed by mNGS were nontuberculosis mycobacteria,mycobacterium tuberculosis,followed by syphilis,toxoplasma gondii.

3.3 Test performance of mNGS, culture, smear and PCR

We evaluated the performance of mNGS, culture, smear and PCR respectively. The sensitivity of mNGS (88.37%, 76/86) was higher than that of culture (22.10%, 19/86), smear (7%, 6/86) and PCR (46.51%, 40/86) in total (Fig. 5). Then we compared the consistency between the mNGS and conventional methods in pathogens detection. In the detection of viruses such as CMV and EBV, the consistency between PCR and mNGS of CMV and EBV was 100%, 73.33% respectively. All PCP cases were detected by mNGS. The consistency in detection of *talaromyces* between culture and mNGS was 75%. mNGS is superior to the common methods such as culture and smear in the detection of *mycobacterium tuberculosis* and non-*mycobacterium tuberculosis* (Fig. 6).

3.4 Impact on treatment decisions by mNGS

Empiric antimicrobial of 86 cases were prescribed early in their course for initial consideration of possible infections. On the admission, before performing mNGS, 78/86 (90.70%) patients received broad-spectrum antimicrobial (more than two different types antimicrobial often cover Gram-negative, Gram-positive bacteria, atypical pathogens, virus and fungi) therapy compared with 34/86 (39.53%) after mNGS ($P < 0.0001$) (Fig. 7).

Meanwhile mNGS provided positive identification of the pathogens leading to prompt changes in the anti-infection treatment. Antiviral treatment (ganciclovir or aciclovir) was added as VZV infection in two cases was established based on mNGS results. Anti-*Toxoplasma gondii* treatment was also initiated in one patient based on mNGS results. For patients mNGS provided positive identification of *mycobacterium avium* infection, leading to precise changes in the anti-infection treatment. For patients with PJP confirmed by mNGS, sulphanilamide dose increased from prophylactic medication to therapeutic medication. For patients with *talaromyces* infection, we added amphotericin B, discontinued to use antimicrobial treatment.

3.5 Outcomes

In the present study, 15 patients died, the overall death rate was 17.44% (15/86). 12 patients died of respiratory failure caused by severe pneumonia, two patients died of sepsis shock, one patient died of Secondary Hemophylic syndrome. The remaining patients continued to improve greatly after targeted treatment according with mNGS (see image 1 and 2).

Discussion

We retrospectively enrolled patients diagnosed with acquired immunodeficiency syndrome with opportunistic infections during the period from January 2018 to February 2021, and attempted to identify the causal pathogens. We accordingly found that CMV, EBV, pneumocystis were the most common pathogens detected. It is different from previous studies. Louie found that TB, cryptococcosis and pneumocystis pneumonia (PCP) are among the major causes of hospitalisation and mortality among adults living with HIV [22]. In Bin Luo's study, bacterial pneumonia was the most prevalent opportunistic infections in HIV-infected patients, followed by candida infection, PCP and TB [23]. We assumed possible

explanations for this divergence are due to the different enrolled cohorts, different sample types, as well as the different type of infectious diseases.

Mixed infection and pneumocystis jirovecii pneumonia (PJP) are common in immunosuppressed patients. mNGS has its potential to detect multiple pathogens simultaneously in one test. So immunosuppressed patients are more likely to be the largest beneficiaries. In this study, mNGS detected 34 patients who had mixed infection of multiple pathogens but conventional methods only detected 14 cases ($P < 0.0001$). Our data indicated that mNGS was valuable for the early detection of mixed infection in AIDS patients. It has similar findings in other studies. More mixed pathogens were found in immunocompromised than immunocompetent patients [24]. mNGS exhibited apparent advantages in detecting viruses and identifying mixed infections [25]. Yi-Yi Qian recommends timely use of mNGS when infection of mixed or rare pathogens is suspected, especially in immunocompromised individuals and individuals with severe conditions that require urgent treatment [26].

Meanwhile we found all PJP cases were detected by mNGS, no matter BALF or blood. It indicated mNGS has excellent performance in detecting PJP. There were 6 cases were detected in blood. Jie Chen has reported a case of pneumocystis jirovecii pneumonia in a kidney transplant recipient, which was diagnosed by mNGS of blood [14]. Accordingly, if PJP is clinically suspected and bronchoscopy is not appropriate, blood is a good choice.

The undiagnosed cases usually force physicians to prescribe empirical broad-spectrum antibiotic treatments, which will greatly increase the risk of emergence of multidrug-resistant bacteria. So early aetiological diagnosis is necessary and critical for acquired immunodeficiency syndrome with opportunistic infections which always present severe manifestations and high mortality. In this study, mNGS provided with definite pathogens of 76/86 (88.37%) patients within 72 hours for us. It also supports the clinical decision including prompt changes and discontinuation of unnecessary antimicrobial therapy. In the present study, antibiotic treatment decreased sharply after mNGS. Meanwhile there were adjustments of treatment in 47 patients after mNGS, they had improved greatly after precise treatment according with mNGS. These findings strongly indicate that mNGS may assist in clinical decision making, especially for reducing unnecessary antibiotic usage and improving prognosis in the treatment of acquired immunodeficiency syndrome with opportunistic infections. Ting Sun had the same conclusion. Their study showed mNGS may offer the probability for targeted therapy and improved clinical antibiotic overuse in immunocompromised patients [24]. Zhang also found that the detection of mNGS can significantly shorten the ICU stay, the ventilation time and reduce the ICU cost in immunosuppressed patients with acute respiratory distress syndrome (ARDS) caused by severe pneumonia [27].

As we know, due to the lower sensitivity of conventional methods, mNGS is appealing. Many studies reported that mNGS is sensitive, fast and superior to the common methods [28, 29]. In our study, mNGS detected pathogens in (88.37%, 76/86) patients but culture, smear and PCR sensitivity were 22.10%, 7%, 46.51% respectively. mNGS has excellent advantages compared with conventional methods in total. It is superior to the common methods such as smear and culture in detection of PJP, mycobacterium

tuberculosis and non-mycobacterium tuberculosis. Meanwhile mNGS can identify VZV, human herpesvirus that were not detected using conventional PCR. However, mNGS diagnostic performance has limitations. When we compared the consistency and the full turnaround time between mNGS and PCR in detecting the viruses such as CMV and EBV, the full turnaround time of PCR was significantly shorter than mNGS. No superiority was noted between mNGS and culture when detecting *talaromyces*. Other studies have had similar findings [30, 31].

Our study has some limitations. First, this was a retrospective study. It included small cases. It needs more further observation to investigate the diagnostic value of mNGS in AIDS with opportunistic infections. Second, we used preliminary data for analysis but still lack of control group simultaneously in our cohort. Further, the mNGS results and identification may be easily influenced by many factors. Besides, lack of a gold standard comparator for diagnostics, and classification bias were also the limitations of this study.

In our study, we diagnosed acquired immunodeficiency syndrome with opportunistic infections by mNGS. It demonstrated that in AIDS with opportunistic infections patients, mNGS had a superior advantage in detecting potential pathogens than conventional methods and mNGS can enable earlier initiation of targeted antimicrobial therapy.

Declarations

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None.

Authors' contributions: LL, MY, SS and JW collected the clinical data and processed statistical data, and LL and MY drafted and edited the manuscript. YS and XS participated in the design and revised the manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in the published article.

Ethics approval and consent to participate

All procedures performed in this study involving human participants were conducted in accordance with the ethical standards of the Ethics Committee of The First People's Hospital of Yueyang, the 1964 Helsinki declaration and its later amendments, and with comparable ethical standards.

Consent for publication

Informed consent was obtained from all participants included in the study.

Competing interests

All authors declare no conflict of interest.

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Figures

Figure 1

Viruses detected by mNGS

Figure 2

Fungis detected by mNGS

Figure 3

Bacterias detected by mNGS

Figure 4

mNGS detected mixed infection n=34

Figure 5

Sensitivity of mNGS and conventional methods

Figure 6

consistency between mNGS and conventional methods

Figure 7

The use proportion of broad-spectrum antibiotic