Annals of Clinical Microbiology and Antimicrobials

RESEARCH

Open Access

Comparing the diagnostic value of targeted with metagenomic next-generation sequencing in immunocompromised patients with lower respiratory tract infection

Muyun Wei[1,2,3*†], Shaowei Mao[4†], Shuangshuang Li[1], Kangyi Gu[1], Dejian Gu[5], Shengjie Bai[5], Xinhua Lu[1] and Min Li[1,2,3*]

Abstract

Background Accurate identification of the etiology of lower respiratory tract infections (LRTI) is crucial, particularly for immunocompromised patients with more complex etiologies. The advent of next-generation sequencing (NGS) has enhanced the effectiveness of pathogen detection. However, assessments of the clinical diagnostic value of targeted NGS (tNGS) in immunocompromised patients with LRTI are limited.

Methods To evaluate the diagnostic value of tNGS in immunocompromised patients with LRTI, a total of 88 patients, of whom 54 were immunocompromised, were enrolled. These patients underwent tNGS testing of bronchoalveolar lavage fluid (BALF). Results from both metagenomic next-generation sequencing (mNGS) and conventional microbiological tests (CMT) were also available for all participants. The performance of tNGS was assessed by comparing its findings against mNGS, CMT, and the clinical composite diagnosis.

Results In the cohort of 88 patients, tNGS showed comparable diagnostic value to mNGS and was significantly superior to CMT. Compared to CMT and composite reference standard, tNGS showed sensitivity of 94.55% and 90.48%, respectively. In immunocompromised patients, despite a more diverse pathogen variety, tNGS maintained similar sensitivity to mNGS and outperformed CMT. tNGS positively influenced etiologic diagnosis and antibiotic decision-making in 72.72% of cases, leading to a change in antibiotic regimen in 17.05% of cases. We also compared the detection of microbial nucleic acids by tNGS with mNGS and found that tNGS could identify 87.99% of the microbial nucleic acids identified by mNGS.

†Muyun Wei and Shaowei Mao contributed equally to this work and share first authorship.

*Correspondence:
Muyun Wei
wmy2940@sjtu.edu.cn
Min Li
rjlimin@shsmu.edu.cn

Full list of author information is available at the end of the article

Conclusion In summary, our study demonstrated that tNGS offers promising clinical diagnostic accuracy in immunocompromised patients, as evidenced by its favorable comparison with CMT, the composite reference standard, and mNGS.

Keywords Immunocompromised patients, Targeted next-generation sequencing, Pathogen, LRTI, Metagenomic next-generation sequencing

Introduction

With the increased use of immunosuppressive agents, an escalating number of individuals are experiencing immune dysfunction, leading to a heightened risk of life-threatening events, particularly infections [1]. Lower respiratory tract infections (LRTIs) was a leading cause of death globally among immunocompromised individuals [1]. Conventional microbiological testing (CMT) is time-consuming and had low detection rates, falling short in meeting the diagnostic demands for complex pathogen infections in immunocompromised patients [2]. Due to the intricate nature of pathogens infecting such patients, a combination of various detection methods might be needed; however, these may still fail to identify fastidious pathogens, uncommon atypical organisms, or pathogens that have lost viability following antimicrobial therapy [3]. The diagnostic and therapeutic management of LRTIs thus presents formidable challenges [4]. Under these circumstances, the precise identification of pathogens and subsequent targeted therapies are of utmost importance.

NGS is a high-throughput method that directly detects nucleic acids in clinical samples, allowing for the recognition of millions of reads in a single assay. Metagenomic NGS(mNGS) is one of the most widely applied uses of NGS, which had gradually transitioned into clinical practice for the identification of unknown, rare, and atypical pathogens in cases of infections [5, 6]. mNGS serve as a novel tool that can overcome the limitations of traditional diagnostic approaches [7]. Multiple studies have explored the advantages of mNGS in pathogen detection for immunocompromised patients and LRTIs, revealing that mNGS can improve pathogen detection rates by 15–30% and shorten diagnostic timelines [8–12]. However, due to its relatively high cost, mNGS is currently more commonly utilized in Intensive Care Units (ICUs) and for critically ill patients.

Recently, tNGS has emerged as a method that targets a range of pathogens by enriching them through primer amplification or probe capture techniques, followed by sequencing. Several studies have emerged comparing the detection performance of mNGS and tNGS, with tNGS demonstrating similar sensitivity and specificity to mNGS in the identification of LRTI pathogens [13, 14]. However, a dearth of studies has been evident thoroughly investigating the diagnostic performance of tNGS in immunocompromised patients. Our study aims to assess the utility of tNGS in the diagnosis of LRTIs in immunocompromised patients by comparing its performance with mNGS and CMT.

Methods

Patients and sample collection

To assess the diagnostic utility of tNGS in immunocompromised patients, we aimed to include patients diagnosed with LRTI [15] and who have recently undergone mNGS. We retrospectively reviewed 197 suspected cases of LRTI at Renji Hospital, School of Medicine, Shanghai Jiao Tong University between April 2023 and December 2023 (Fig. 1). A total of 88 patients with recent mNGS results available and remaining BALF samples were included in the analysis and divided into two groups: immunocompromised and immunocompetent. The determination of LRTI was based on clinical criteria and etiological results. Clinical criteria included the presence of new or progressive pulmonary infiltration on chest radiographs, and at least two of the following: body temperature > 38 °C or < 36 °C; leukocytosis > 12,000/mm^3 or leukopenia < 4,000/mm^3; or purulent respiratory secretions. The determination of etiological results primarily considered a combination of factors including patient symptoms, various etiological test results (including culture and NGS test) within seven days, and the patient's response to antibiotics that cover the identified pathogen [16]. The final diagnosis of patients serves as the composite reference standard for follow comparison. CMT results encompassed a variety of detection methods including culture for bacteria and fungi, culture and GeneXpert MTB/RIF system (GeneXpert; Cepheid, Inc., Sunnyvale, CA, USA) for *Mycobacterium tuberculosis*, and PCR for *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2) and *influenza* virus. The results of CMT were collected at the time of patient diagnosis, with cultures originating from the same batch of BALF. Other tests might have been derived from different specimens, such as viral PCR. The results of culture for bacteria and fungi and PCR were available for all samples, and other tests were selected based on patient symptoms.

Immunocompromised status is defined by the presence of one or more of the following risk factors [17]: (A) hematologic cancer; (B) chemotherapy wothin the last three months; (C) chronic steroid (>0.3 mg/kg/day of prednisone-equivalent for ≥3 weeks) or biologic drug use for autoimmune diseases or other immunosuppressive therapies; (D) solid organ transplant within the last six

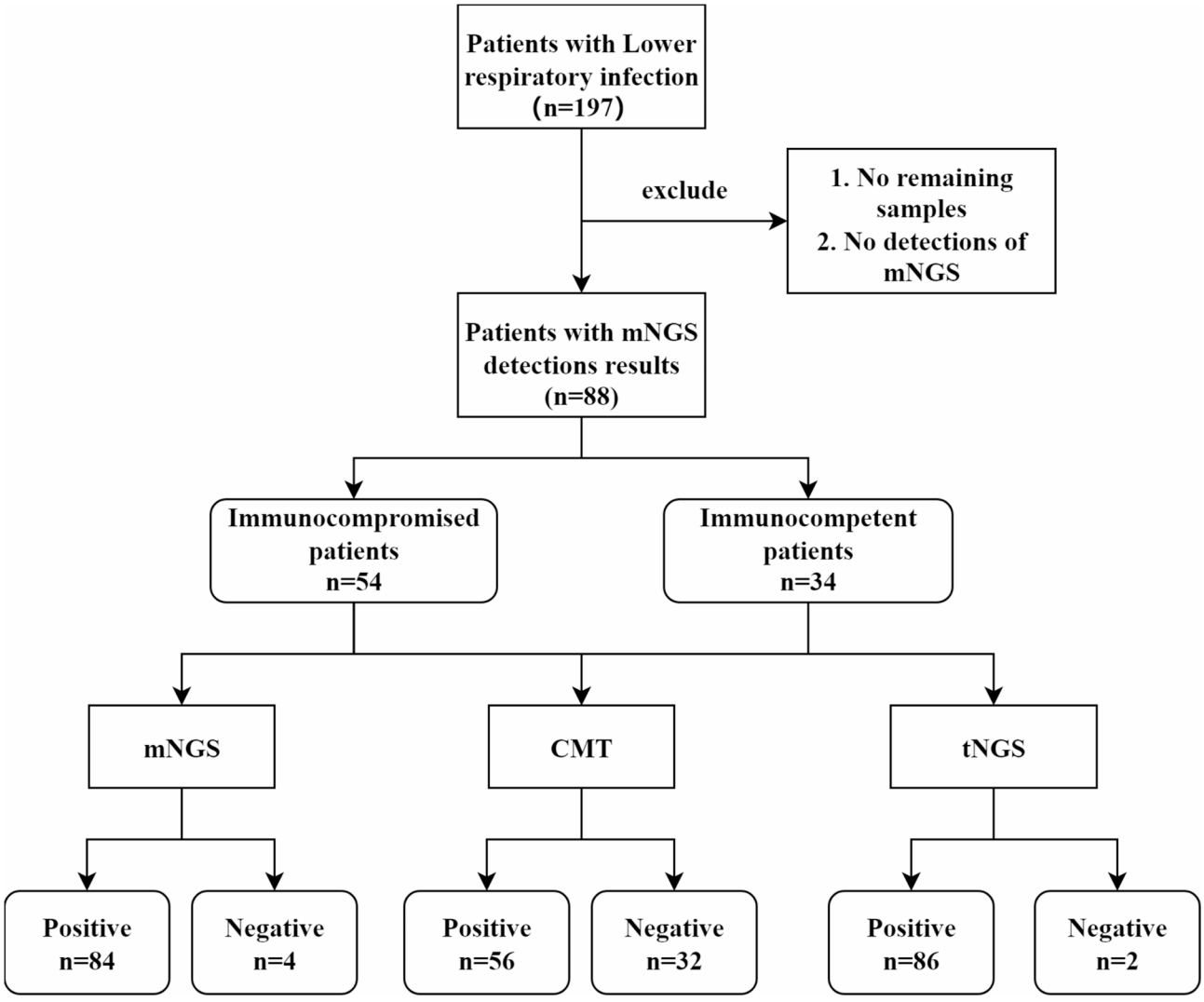

Fig. 1 Flowchart of screening of patients with lower respiratory tract infections and detections results of difference methods

months; (E) neutropenia; (F) acquired or inherited severe immunodeficiency.

The workflow of targeted NGS

A 400 μl volume of BALF samples was taken for extraction. The samples were extracted according to the extraction procedure of previous studies [18]. The samples were then prepared into a library using the HieffNGS®C37P4OnePotcDNA&gDNA Library Prep Kit (Yeasen, Shanghai, China) according to the manufacturer's protocol. Subsequently, the samples were incubated with Geneplus-designed probes for 4 h to complete the probe capture, which were subsequently prepared into DNA nanoballs (DNBs). Sequencing was performed on the Gene+Seq-100 sequencing platform (GenePlus-Beijing) using 100-bp single-end read sequencing, targeting a depth of 5 million reads. The sequenced data was analyzed using the GenePlus's self-built database, which covers 1,872 pathogens identified by tNGS (Supplementary Table 1). Reads aligning to the target capture interval of the probe for corresponding species were defined as target-reads and normalized to the number of reads per million (RPM). The reporting threshold was set at RPM≥6 for common pathogens (excluding *mycobacteria*) and ≥0.5 for fungi and *mycobacteria* [19]. A manual review is conducted. Typically, bacteria (excluding *mycobacteria*), fungi (excluding *aspergillus*), and viruses were reported if they are within the top 30% of RPM. Pathogens with abnormal genomic coverage will be filtered out.

The workflow of metagenomic NGS

The mNGS process was conducted following previous reports [10]. Genomic DNA was extracted from the specimens using the TIANamp Micro DNA Kit after enzymatic treatment and mechanical disruption with beads. Sequencing libraries were prepared through DNA

fragmentation, end-repair, and adaptor ligation. Sequencing was performed on MGISEQ-2000. Short (<35 base pairs), low-quality reads and human sequences were filtered out, and the remaining reads were aligned to an in-house database for identifying microbial species. The reporting of pathogenic microorganisms mainly followed to previous research [10]. Briefly, for common species or genera, an RPM value≥3 was considered reportable. For difficult-to-extract microorganisms, such as *Aspergillus* and *Mycobacterium tuberculosis*, reporting was made when at least one read is detected.

Statistical analysis

The scoring structure for comparing NGS with clinical testing referred to Karius method [20]. For continuous variables, report the results as the median and interquartile range(Q1, Q3). Categorical variables are represented by frequency and percentage. Inter-group comparisons are made using the unpaired t-test or the Mann-Whitney U test. For comparisons between groups of categorical variables, the chi-square test is used. The diagnostic value of difference methods was compared using the Kappa test. All statistical tests were two-sided with a significance level set at 5%. Graphs were generated using GraphPad Prism version 9.5.0 (GraphPad Software LLC., San Diego, CA, USA). Analyses were conducted using Windows SPSS version 22.0(SPSS Inc., Chicago, Illinois, USA). Statistical significance was set at a p-value of <0.05.

Results

Patient characteristics

A cohort comprising 197 patients was enrolled, with 88 of these patients having accessible mNGS results, clinical information, and residual BALF samples, which were included in the ultimate analysis. This cohort comprised 54 immunocompromised individuals and 34 immunocompetent patients. The median age was 64 years, with 47 (53.41%) males enrolled. Twenty-four patients (27.27%) received long-term corticosteroid therapy for solid-organ transplantation or autoimmune diseases, and nine patients (10.2%) were treated with chemotherapy for solid tumors or hematologic malignancies. Demographic features and baselines characteristic are detailed in Table 1. There were no significant differences between the two groups in terms of gender, age, history of antibiotic

Table 1 Characteristics of patients and baseline of two groups

Characteristic	All patients	Immunocompetent patients	Immunocompromised patients	*P*-value
Number, n(%)	88(100.00)	34(38.64)	54(61.36)	
Male gender, n (%)	47(53.41)	19(55.88)	28(51.85)	0.247
Age (year), median (Q1, Q3)	64(55, 69)	61(55, 68)	65(56, 69)	0.833
Laboratory findings				
WBC (10^9/L), median (Q1, Q3)	8.29(5.51, 11.16)	7.46 (5.16,9.79)	8.64(6.10, 13.50)	0.965
Monocyte count(10^9/L), median (Q1, Q3)	0.52(0.35, 0.69)	0.52(0.33, 0.61)	0.52(0.37,0.72)	0.965
Comorbidities, n (%)				0.043
Type 2 diabetes	14(15.91)	5(14.71)	9(16.67)	
cardiovascular and cerebrovascular diseases	24(27.27)	12(35.29)	12(22.22)	
COPD	7(7.95)	5(14.71)	2(3.70)	
Immunocompromised status, n (%)				
Hematologic malignancy	4(4.55)	NA	4(7.41)	
Solid-organ transplantation	31(35.22)	NA	31(57.41)	
Solid tumor receiving chemotherapy	5(5.68)	NA	5(9.26)	
Immunosuppressive therapy	25(28.41)	NA	25(46.30)	
Prolonged corticosteroid therapy[a]	24(27.27)	NA	24(44.44)	
Autoimmune disease	4(4.55)	NA	4(7.41)	
Antibiotics therapy				0.483
No prior antibiotics	15(17.04)	7(20.59)	8(14.81)	
Prior antibiotics	73(82.95)	27(79.41)	46(85.19)	
Disease severity				0.075
Septic shock, n (%)	4(4.54)	0(0)	4(7.41)	
Invasive mechanical ventilation, n (%)	21(23.86)	6(17.65)	15(27.78)	
Outcome				0.965
Death	13(14.77)	5(14.71)	8(14.81)	
survival	74(84.09)	28(82.35)	46(85.19)	
unknown	1(1.13)	1(2.94)	0(0)	

Abbreviations Q1 and Q3, interquartile range; WBC, White blood cell; NA, not available. The difference based on the t-test was calculated. a Defined as >0.3 mg/kg/d of prednisone-equivalent for ≥3 weeks

therapy, or outcome ($p>0.05$). However, immunocompetent patients group had a higher incidence of comorbidities than immunocompromised patients group (64.71% vs. 42.59%, $p=0.04$), and a lower proportion of severe disease than immunocompromised patients (17.65% vs. 35.19%, $p=0.07$).

Comparing the diagnostic value of tNGS, mNGS, and CMT in all patients

To assess the diagnostic value of tNGS, it was compared with culture, CMT (all samples included culture for bacteria and fungi, and PCR for virus), composite reference standard, and mNGS. Among the 88 patients, tNGS showed a similar diagnostic agreement rate to mNGS (88.64% and 87.50%), but higher than CMT and culture (60.23% and 44.32%) (Fig. 2A). In 85.23% of the patients, at least two methods demonstrated diagnostic concordance, while 7.95% of the patients showed concordance only with one NGS method (Fig. 2B). Using culture as a reference, tNGS had a sensitivity of 94.87% and specificity of 10.20%, with an overall concordance rate of 47.73%. When compared to CMT, tNGS demonstrated a sensitivity of 94.55% and a specificity of 15.15%, with an agreement rate of 64.77%. Compared to the composite reference standard, tNGS demonstrated a sensitivity of 90.48%, specificity of 50.00%, and an 88.64% agreement rate (Table 2). Similarly, mNGS demonstrated an 87.50% consistency with the composite reference standard, with

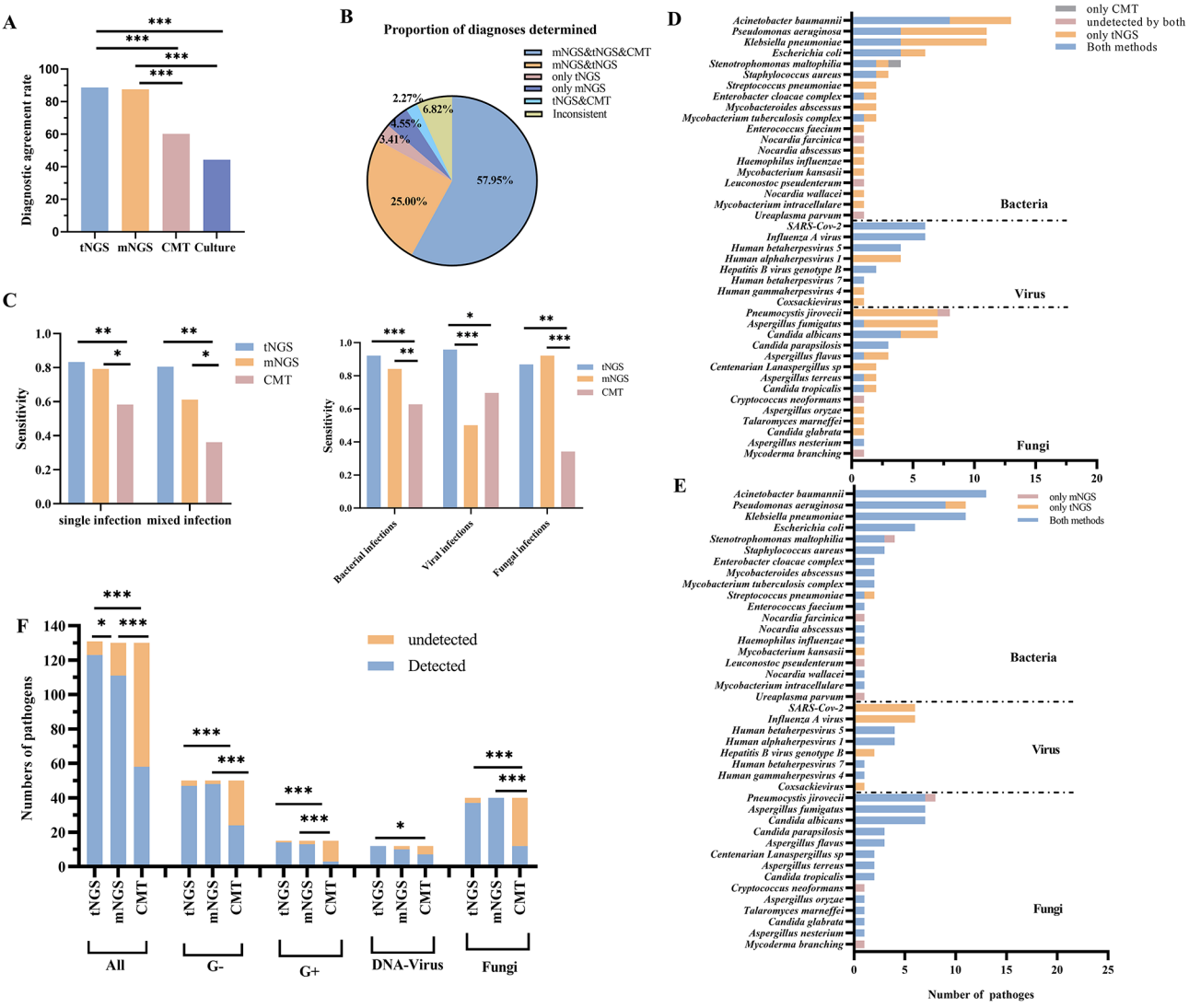

Fig. 2 Diagnostic performance of tNGS in 88 samples. (**A**) Diagnostic agreement rate of tNGS, mNGS, CMT, and culture. (**B**) Pie chart showing the proportion of diagnoses determined by different methods. CMT, conventional microbiological testing. (**C**) Comparison of detection sensitivity of mixed pathogens and various pathogens infections between tNGS, mNGS, and CMT. (**D**) Comparison of pathogen spectrum detected between CMT and tNGS. (**E**) Comparison of pathogen spectrum detected between mNGS and tNGS. (**F**) Comparison of pathogens detected between tNGS, mNGS, and CMT. G+: Gram-positive bacteria; G-: Gram-negative bacteria. *: $p<0.05$, **: $p<0.01$,***: $p<0.001$

Table 2 Performance characteristics of targeted NGS in 88 clinic samples

Patients characteristics ($n=88$)	tNGS positive	tNGS negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Agreement (%)	Kappa value
Positive by culture	37	2	94.87	10.20	45.68	71.43	47.73	0.06
Negative by culture	44	5						
Positive by CMT	52	3	94.55	15.15	65.00	62.50	64.77	0.06
Negative by CMT	28	5						
Positive by composite reference standard	76	8	90.48	50.00	97.43	20.00	88.64	0.21
Negative by composite reference standard	2	2						
Positive by mNGS	81	3	96.43	75.00	98.78	50.00	94.32	0.58
Negative by mNGS	2	2						

Abbreviations CMT: Conventional microbiological test; tNGS: Targeted Next-generation sequencing; mNGS: Metagenomic next-generation sequencing; PPV: Positive predictive value; NPV: Negative predictive value

a sensitivity of 88.09% and specificity of 75.00% (Supplementary Table 2). tNGS detected pathogens in 28/33 samples that were negative in CMT, showing an 78.79% consistency rate with the composite reference standard (Table 2). Then, tNGS compared with mNGS demonstrated a sensitivity of 96.43% and specificity of 75.00%, with an agreement rate of 95.45%. The kappa value between tNGS and mNGS was 0.58. The comparative assessment of mixed infection detection capability across the three methodologies showed that tNGS and mNGS had sensitivities of 80.56% and 61.11%, respectively, significantly surpassing the sensitivity rate of 36.11% demonstrated by CMT (Fig. 2C). This superiority in sensitivity for detecting mixed infections was also observed in cases of single-pathogen infections (tNGS vs. mNGS vs. CMT: 83.33% vs. 79.17% vs. 58.33%). Both tNGS and mNGS showed high sensitivity for detecting bacterial (92.06% and 84.13%) and fungal (86.64% and 92.11%) infection samples, while tNGS exhibited higher sensitivity for detecting viral infections (95.83% vs. 50.00%). Both tNGS and mNGS methods demonstrated higher sensitivity than CMT for bacterial and fungal infection samples (Fig. 2C).

The pathogens detection was compared between tNGS with mNGS and CMT. In total, 130 causative pathogens were confirmed, with tNGS detecting 123 of them, mNGS identifying 111, and CMT finding 58. tNGS and mNGS showed comparable and significantly higher detection rates for pathogens than CMT, regardless of whether the pathogens were fungi, DNA viruses, or bacteria (Fig. 2D-F). The most prevalent bacteria were *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, while the most common fungi were *Pneumocystis jirovecii* and *Aspergillus fumigatus*. SARS-CoV-2 and *Influenza A virus* were common viruses, however, mNGS did not detect them as it only identified DNA. tNGS not only detected SARS-CoV-2 and *Influenza A virus* but also successfully identified the subtypes for all 12 pathogens, with subtypes that are completely consistent with PCR (Supplementary Table 3). While both tNGS and CMT concurrently detected 57 pathogens, tNGS identified an additional 66. Notably, tNGS detected a higher prevalence of DNA viruses than CMT, due to the absence of appropriate testing for these pathogens in CMT. Concurrently, six pathogens, comprising three fungi and three bacteria, remained undetected by both methods but were detected by mNGS. Compared to mNGS, tNGS revealed an additional 19 pathogens. When comparing the fungi and bacteria not detected by tNGS with those detected by both NGS methods, it was found that the Reads Per Million (RPM), an important indicator of the relative abundance of microbial nucleic acids in NGS detection, was lower in mNGS for fungi ($p=0.07$), while there was no significant difference for bacteria ($p=0.54$). Meanwhile, the RPM of bacteria additional detected by tNGS was lower than those detected by both methods (Supplementary Fig. 1).

Comparing diagnostic value of tNGS, CMT and mNGS in immunocompromised patients

We compared the diagnostic agreement rates of difference methods in immunocompromised and immunocompetent patients. The rates are illustrated in Fig. 3A. There were significant differences in the diagnostic agreement rates between NGS methods and CMT ($p<0.001$). However, no difference was found between tNGS and mNGS. Higher mixed pathogens rates and higher proportion of fungi infections was found in immunocompromised patients (44.44% vs. 32.35%, $p>0.05$; 46.30% vs. 32.35%, $p>0.05$, Supplementary Fig. 2). Then, we evaluated the diagnostic performance of tNGS in two groups. Compared to the composite reference standard, tNGS demonstrated sensitivity of 83.87% and 94.34% in both immunocompetent and immunocompromised groups (Table 3), respectively, while mNGS showed 80.65% and 92.45% sensitivity. Furthermore, the pathogen spectrum varied between immunocompetent and immunocompromised individuals (Fig. 3B). The most common pathogens in immunocompetent patients were *Acinetobacter baumannii*, *Influenza A virus*, and *Klebsiella pneumoniae*, whereas immunocompromised patients more frequently exhibited *Pneumocystis jirovecii*, *Aspergillus fumigatus*,

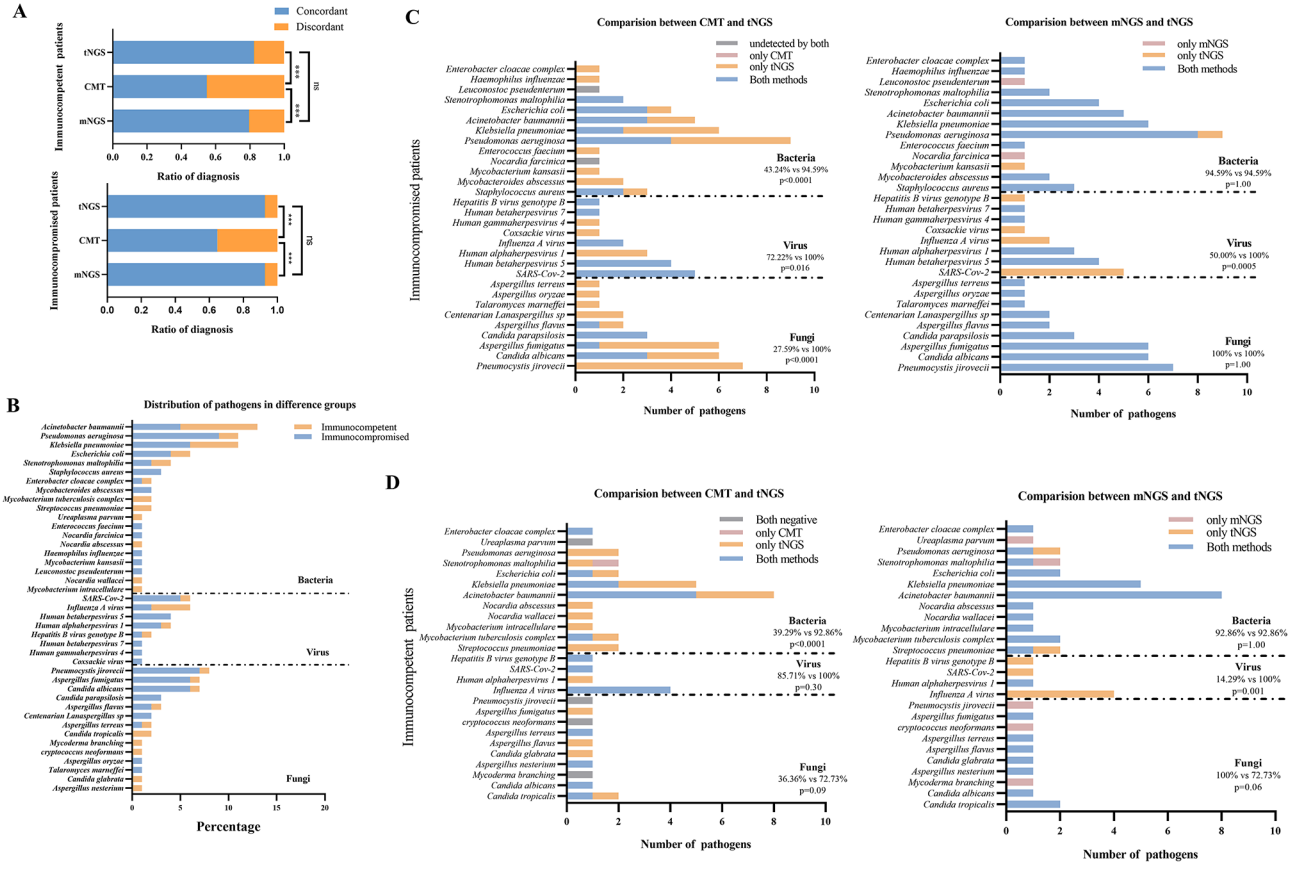

Fig. 3 Comparison diagnosis of tNGS with mNGS and CMT in immunocompromised patients. (**A**) The comparisons of diagnostic accuracy between three methods in immunocompetent patients and immunocompromised patients. (**B**) Pathogen spectrum of immunocompetent and immunocompromised patients. (**C**) Comparison between tNGS with CMT and mNGS in the detection of pathogens in immunocompromised patients. (**D**) Comparison between tNGS with CMT and mNGS in the detection of pathogens in immunocompetent patients. The pathogen detection rate is depicted in the figure. *: $p < 0.05$, **: $p < 0.01$,***: $p < 0.001$

Table 3 Diagnostic performance of NGS in difference groups compared with composite reference standard

Ptients characteristics		tNGS				mNGS			
		Positive	Negative	Sensitivity(%)	Specificity(%)	Positive	Negative	Sensitivity(%)	Specificity(%)
immunocompromised	Positive by composite reference standard	50	3	94.33	0	49	4	92.45	100
	Negative by composite reference standard	1	0			0	1		
immunocompetent	Positive by composite reference standard	26	5	83.87	66.67	25	6	80.65	66.67
	Negative by composite reference standard	1	2			1	2		

Abbreviations tNGS: Targeted Next-generation sequencing; mNGS: Metagenomic next-generation sequencing; PPV: Positive predictive value; NPV: Negative predictive value

Candida albicans, and *non-tuberculous mycobacteria*. In the immunocompromised group, a higher number of fungal species were detected (29 vs. 11), including an increased prevalence of *Aspergillus* spp (12 vs. 5). and *Pneumocystis jirovecii* (7 vs. 1).

We conducted a further comparative analysis of the pathogen detection among immunocompromised and immunocompetent individuals using tNGS, mNGS, and CMT (Fig. 3C&D). Consistent with the aforementioned findings, tNGS demonstrated higher pathogen detection rates compared to CMT across both immunocompromised and immunocompetent patients (97.62% vs. 44.05%, $p<0.0001$; 89.13% vs. 45.65%, $p<0.0001$). Particularly in immunocompromised patients, tNGS showed a higher detection rate for all types of pathogens (Fig. 3C). In immunocompromised patients, tNGS demonstrated a higher pathogen detection rate than mNGS (97.62% vs. 86.90%, $p=0.009$), primarily due to its detected for RNA viruses. tNGS almost identified all pathogens detected by mNGS, with the exception of one *Nocardia farcinica* and one *Leuconostoc pseudenterum* not within the coverage range. In immunocompetent individuals, the pathogen detection rates were comparable (89.13% vs. 82.61%, $p=0.36$), with tNGS detecting more RNA viruses while mNGS had a higher detection rate for fungi.

Clinical impacts of NGS on etiological diagnosis and antibiotic adjustment

Based on the retrospective impact of mNGS testing on etiologic diagnosis and antibiotic decision-making, the effect of NGS was categorized into three groups. As shown in Tables 4, 73.86% (65/88) of mNGS and 72.72% (64/88) of tNGS had a positive impact on etiologic diagnosis and antibiotic decision-making, with 17.05% leading to appropriate antibiotic adjustments. Based on the results of mNGS, 12 patients underwent escalation of therapy, one patient underwent de-escalation of therapy, and two patients had their antibiotic treatment discontinued after infection was ruled out. Compared to mNGS, tNGS could also lead to similar adjustments for these 15 patients. Furthermore, the effectiveness and prognostic outcomes of antibiotic treatments were compared. In patients with positive impact from mNGS, 88.71% (55/62) improved after treatment, and 85.71% in the antibiotic adjustment group showed improvement. The same result was observed in the tNGS assay.

Comparison tNGS with mNGS in the detection of microbial nucleic acids

Given the unbiased nature and ultra-sensitivity of NGS testing, the detection of non-pathogenic microorganisms in BALF samples was inevitable. Among the 88 patients, mNGS detected 283 microorganisms, while tNGS identified 402 microorganisms. In comparison of the RPM between pathogens and other microorganisms of tNGS, no significant differences were observed. The consistency of microbial nucleic acid reporting between mNGS and tNGS was analyzed. Both NGS methods shared the detection of 249 microorganisms, accounting for 87.99% of those identified by mNGS. The positive predictive value for the shared microorganisms was 41.77%, while the positive predictive values for microorganisms exclusively detected by mNGS or tNGS were 20.59% and 12.42%, respectively. Thirty-four microorganisms only

Table 4 Clinical impacts on antibiotic adjustment and prognosis

Groups		mNGS					tNGS				
		N	Rate	N of prognosis	Improved	Deterioration	N	Rate	N of prognosis	Improved	Deterioration
No Impact	Total	23	26.14%	22	77.27%	22.73%	24	27.27%	19	73.68%	26.32%
	Not meet the hypothesis	17	19.32%				18	20.45%			
	Withdrawal from treatment	6	6.82%				6	6.82%			
Meet expected	Total	50	56.82%	48	89.58%	10.42%	49	55.68%	43	88.37%	11.63%
	Meet the hypothesis	50	56.82%				49	55.68%			
Antibiotic therapy adjustment	Total	15	17.05%	14	85.71%	14.29%	15	17.05%	11	90.91%	9.09%
	Escalation of treatment	12	13.64%				12	13.64%			
	De-escalation of treatment	1	1.14%				1	1.14%			
	Ruled out infection	2	2.27%				2	2.27%			

The "No Impact" group refers to cases where the mNGS results did not affect the antibiotic decision-making. The main reasons were that the mNGS results did not match the clinical hypotheses or the patients withdrawal from treatment. The other two groups are the Positive Impact on Antibiotic Decision group, where "Meet expected" refers to cases where the mNGS results were consistent with clinical expectations, maintaining the empirical treatment plan. Patients who underwent antibiotic escalation, de-escalation, or ruled out infection were assigned to the third group

detected by mNGS, and 20.59% (7/34) were not covered in the tNGS assay. Among them, 7/34(20.59%) were identified as causal pathogens, including three of fungi and four of bacteria. Comparing the RPM of these microorganisms with those shared by both NGS, it was found that the RPM for Gram-positive bacteria and fungi in the group detected only by mNGS was lower (Supplementary Fig. 3). One hundred fifty-three microorganisms were detected exclusively by tNGS, including 39.87% bacteria, 9.80% fungi, 33.99% DNA viruses, and 16.34% RNA viruses (Fig. 4C). Among them, 19/153(12.42%) were identified as causal pathogens, including 15 viruses and 4 bacteria. It is worth noting that the majority of these microorganisms are composed of common respiratory tract colonizing microorganisms. (Fig. 4D). Further comparison of the RPM of these additional microorganisms detected by tNGS with those shared by both NGS revealed that the RPM of the shared microorganism group was higher, a trend observed across bacteria, viruses, and fungi.

Discussion

NGS testing offers a rapid, comprehensive pathogen detection in clinical settings [21]. Adoption of various NGS technologies in clinical diagnostics is growing, showing promising sensitivity. However, there is currently a lack of research exploring whether tNGS is a clinically suitable diagnostic aid, particularly for immunocompromised patients with LRTI [3, 8, 22]. We retrospectively analyzed the efficacy of tNGS method based on BALF samples in diagnostic value for immunocompromised patients with LRTI.

Previous studies have reported the application of tNGS in LRTI patients, with sensitivity agreeing with our findings [3, 23]. Chao et al. evaluated t a multiplex PCR amplification-based tNGS method for LRTI diagnosis, achieving 60–90% sensitivity and 60-80% specificity across different pathogens [24]. Lin et al. used the respiratory pathogen ID/AMR enrichment sequencing (RPIP) on BALF samples from children with respiratory infections [25], demonstrating diagnostic performance with sensitivities and specificities of 84.4% and 97.7%, respectively, compared to culture-based standards. In our study, tNGS demonstrated a sensitivity and specificity of 90.48% and 50.00%, respectively. Meanwhile, tNGS and mNGS demonstrated comparable diagnostic performance for patients with LRTI. Prior studies have substantiated that tNGS has diagnostic capabilities comparable to mNGS [3, 22]. In our study, comparing tNGS and mNGS yielded a 95.45% concordance rate and a kappa value of 0.58. In terms of cost, the cost for mNGS services to detect DNA pathogens was around $500, while the tNGS method was approximately $200-$300, roughly half that of the mNGS service.

Although the diagnostic performance of mNGS in immunocompromised patients has been studied [10–12], there is a lack of research on the utility of tNGS in this population. In this study, tNGS demonstrated comparable sensitivity in both immunocompromised and immunocompetent patients, with rates of 94.34% and 83.87%, respectively ($p>0.05$). Additionally, a higher proportion of mixed infections and fungal infections was noted in immunocompromised patients, although this difference was not statistically significant. The spectrum of pathogens in immunocompromised patients was quite different from that in immunocompetent patients, but the diagnostic value of tNGS was similar in two populations [1, 26, 27]. Further, tNGS has demonstrated comparable diagnostic performance with mNGS in both immunocompromised and immunocompetent populations. In a multicenter prospective study reported a higher detection rate (up to 90.3%) and earlier pathogen detection by mNGS of BALF compared to CMTs in 101 immunocompromised patients, [28]. Peng et al. reported similar diagnostic performance of mNGS on BALF and CMTs in 60 immunocompromised patients [10].

The tNGS method offers the advantage of concurrent DNA and RNA detection, providing a convenience over mNGS. In our study of 13 patients, we detected RNA viruses and subtyped 12 of these due to tNGS enrichment. However, this enhancement also had potential drawbacks. In the comparative analysis (Supplementary Figs. 1&3), fungi that were not detected by tNGS were found to exhibit lower RPM, suggesting that tNGS might have missed fungal present at lower concentrations. This could be attributed to the fact that tNGS necessitates the simultaneous extraction of both DNA and RNA from microorganism. To preserve the integrity of nucleic acids of some microorganisms such as virus, this may lead to incomplete cell wall disruption in fungi and other microorganisms present at low concentrations. In the analysis of microorganisms that only detected by mNGS, it was observed that the RPM of Gram-positive bacteria and fungi were lower than those detected by both NGS methods, supporting the notion. Furthermore, this heightened sensitivity may identify colonizing or non-pathogenic microorganisms [3]. In the comparative analysis of tNGS and mNGS, it was found that tNGS can additionally detect microorganisms with low RPM values. However, the positive predictive value of these microorganisms was relatively low, mainly consisting of some respiratory tract commensal microorganisms. Prior research has reported that both mNGS and tNGS typically identify 3–4 microorganisms per sample [3, 8]. This necessitates the gradual optimization of these methods in clinical practice and serves as a reminder to clinical experts to interpret NGS results in conjunction with patients' symptoms for further assessment and diagnosis.

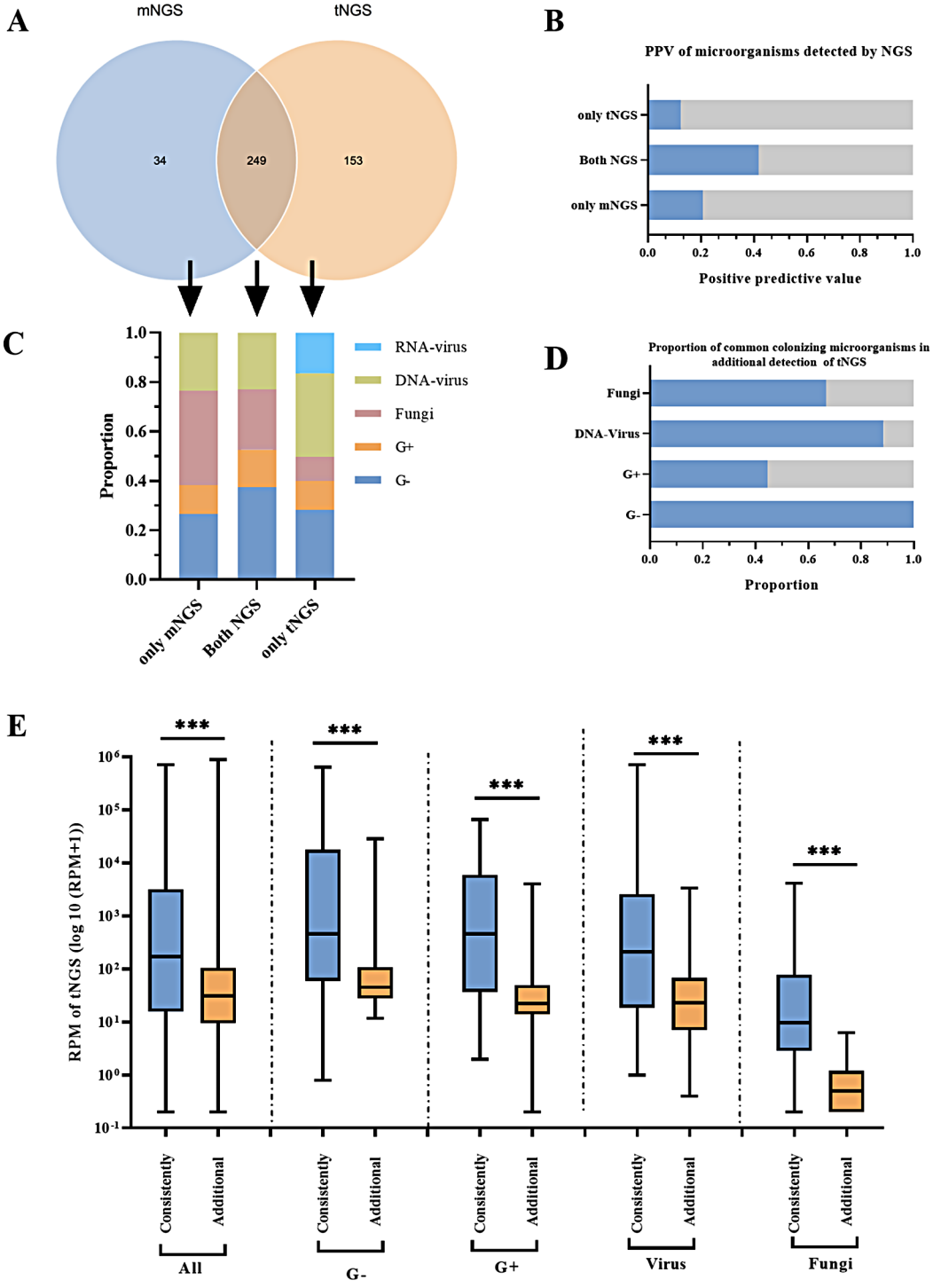

Fig. 4 Comparison between tNGS and mNGS in microorganisms detections. (**A**) Venn diagram showing the results of microorganisms detected in tNGS and mNGS. (**B**) Microorganisms positive predictive values of different groups. (**C**) The proportion of microorganisms types in different groups. G+: Gram-positive bacteria; G-: Gram-negative bacteria. (**D**) The proportion of common microorganisms among the additional detections by tNGS includes *Candida* species as the common fungi, *Human herpesvirus1/4/5* among DNA-viruses, *Streptococcus pneumoniae* among G+, and *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia*, *Burkholderia cepacian*, and *Pseudomonas aeruginosa* among G-. (**E**) Comparisons the RPM of difference microorganisms in tNGS grouped in consistently detection (detected by both NGS) and tNGS additional detection. RPM: read per million. *: $p < 0.05$, **: $p < 0.01$,***: $p < 0.001$

Several studies have discussed the impact of mNGS on etiological diagnosis and antibiotic adjustment. Sun et al. reported that mNGS could guide antibiotic adjustments in 87% of immunocompromised patients with severe pneumonia [29]. Xu et al. showed a positive impact of mNGS on pathogen diagnosis in nearly 45.00% of cases and antibiotic adjustments in 24.31% of cases [11]. In our study, tNGS positively influenced etiological diagnosis and antibiotic decision-making in 72.73% patients and potentially enabled antibiotic adjustments in 17%. Additionally, the improvement rate was higher in the groups where antibiotic decisions were positively influenced by either mNGS or tNGS, as compared to the groups that were not impacted, although these differences were not statistically significant (88.71% vs. 77.27%, $p=0.10$; 88.89% vs. 73.68%, $p=0.22$). Further investigation is warranted to elucidate the effects of NGS methodologies on therapeutic outcomes. Our study had limitations that should be carefully considered. Firstly, its retrospective and single-center design with a small sample size could lead to participant selection bias. Secondly, selection of patients with mNGS test results for analysis may result in compromised patient randomization. Finally, the omission of supplementary mNGS testing for RNA pathogens in our study precludes a direct comparison of the detection capabilities of tNGS and mNGS for RNA viruses. Additionally, this absence may give the impression that tNGS yields more incidental findings.

Conclusion

In summary, our study suggested that tNGS had great potential for pathogen diagnosis in immunocompromised patients. With comparable diagnostic performance to mNGS but cost reduction, tNGS can serve as a valuable tool in addition to CMT.

Abbreviations

tNGS	Targeted Next-generation sequencing
LRTIs	Lower respiratory tract infections
mNGS	Metagenomic next-generation sequencing
BALF	Bronchoalveolar lavage fluid
PPV	Positive predictive value
NPV	Negative predictive value
CMT	Conventional microbiological test
RPM	Reads per million

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12941-024-00749-5.

Supplementary Material 1: Table 1-The list of pathogens in tNGS assay. Table 2-Performance characteristics of mNGS and CMT in 88 clinic samples. Table 3-The subtypes of SARS-Cov-2 and Influenza A virus confirmed by tNGS and PCR.

Supplementary Material 2: Fig. 1 Comparison of RPM values between consistently detected and inconsistently detected pathogens in mNGS and tNGS. RPM: read per million.

Supplementary Material 3: Fig. 2 The proportion of mixed and fungal infections in immunocompetent patients and immunocompromised patients.

Supplementary Material 4: Fig. 3 Comparisons the RPM of microorganisms in mNGS grouped in Both NGS detection (Both detected) and tNGS notdetection(missed).

Acknowledgements

We owe thanks to the patients in the study and their family members. We acknowledge the staffs of all departments for their assistance to this study.

Author contributions

M L, M W, and S M contributed to conception and design of the study. D G, S B, and S L contributed to the operation of the experiments and the collection of information. K G, D G, and X L contributed to confirmation of the authenticity of the data. M W and S M performed data analysis and interpretation. All authors wrote the manuscript and read and approved the final manuscript.

Funding

This work was supported by National Key Research and Development Program (2022YFC2603800), Yangfan Project of Science and Technology Commission of Shanghai Municipality (23YF1431300), and 3-Year Action Plan for Strengthening Public Health System in Shanghai (GWVI-11.1-1).

Data availability

The data that support the findings of this study are available from the corresponding author on request. Sequencing data that support the finding of this study (with human reads removed) have been deposited in the China National Center for Bioinformation - National Genomics Data Center and can be accessed with the BioProject identifier subCRA025931.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the ethical guidelines provided by the Declaration of Helsinki and was approved by the Ethics Committee of Renji Hospital, with the approval number KY2023-109-C. The written informed consent from participants was waived and the data were analyzed anonymously.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Laboratory Medicine, Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine, No.160 Pujian Road, Shanghai, China
[2]Jiading Branch, Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[3]Faculty of Medical Laboratory Science, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[4]School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, China
[5]Geneplus-Beijing Co., Ltd, Beijing, China

Received: 27 April 2024 / Accepted: 20 September 2024
Published online: 30 September 2024

References

1. Azoulay E, Mokart D, Kouatchet A, Demoule A, Lemiale V. Acute respiratory failure in immunocompromised adults. Lancet Respiratory Med. 2019;7(2):173–86.
2. Zhu YG, Tang XD, Lu YT, Zhang J, Qu JM. Contemporary Situation of Community-acquired Pneumonia in China: a systematic review. J Translational Intern Med. 2018;6(1):26–31.
3. Gaston DC, Miller HB, Fissel JA, Jacobs E, Gough E, Wu J, et al. Evaluation of Metagenomic and targeted next-generation sequencing workflows

for detection of respiratory pathogens from Bronchoalveolar Lavage Fluid Specimens. J Clin Microbiol. 2022;60(7):e0052622.
4. Zheng Y, Qiu X, Wang T, Zhang J. The Diagnostic Value of Metagenomic Next-Generation sequencing in Lower respiratory tract infection. Front Cell Infect Microbiol. 2021;11:694756.
5. Simner PJ, Miller S, Carroll KC. Understanding the promises and hurdles of Metagenomic Next-Generation sequencing as a Diagnostic Tool for Infectious diseases. Clin Infect Diseases: Official Publication Infect Dis Soc Am. 2018;66(5):778–88.
6. Filkins LM, Bryson AL, Miller SA, Mitchell SL. Navigating clinical utilization of direct-from-Specimen Metagenomic Pathogen detection: clinical applications, limitations, and Testing recommendations. Clin Chem. 2020;66(11):1381–95.
7. Chiu CY, Miller SA. Clinical metagenomics. Nat Rev Genet. 2019;20(6):341–55.
8. Miao Q, Ma Y, Wang Q, Pan J, Zhang Y, Jin W, et al. Microbiological Diagnostic performance of Metagenomic Next-generation sequencing when Applied to Clinical Practice. Clin Infect Diseases: Official Publication Infect Dis Soc Am. 2018;67(suppl2):S231–40.
9. Liang M, Fan Y, Zhang D, Yang L, Wang X, Wang S, et al. Metagenomic next-generation sequencing for accurate diagnosis and management of lower respiratory tract infections. Int J Infect Diseases: IJID: Official Publication Int Soc Infect Dis. 2022;122:921–9.
10. Peng JM, Du B, Qin HY, Wang Q, Shi Y. Metagenomic next-generation sequencing for the diagnosis of suspected pneumonia in immunocompromised patients. J Infect. 2021;82(4):22–7.
11. Xu CH, Chen X, Zhu GQ, Yi HM, Chen SL, Liu T et al. Diagnostic performance and clinical impacts of metagenomic sequencing after allogeneic hematopoietic stem cell transplantation. Journal of microbiology, immunology, and infection = Wei Mian Yu Gan ran Za Zhi. 2024;57(1):11–9.
12. Ren D, Ren C, Yao R, Zhang L, Liang X, Li G, et al. The microbiological diagnostic performance of metagenomic next-generation sequencing in patients with sepsis. BMC Infect Dis. 2021;21(1):1257.
13. Li N, Cai Q, Miao Q, Song Z, Fang Y, Hu B. High-throughput metagenomics for identification of pathogens in the clinical settings. Small Methods. 2021;5(1):2000792.
14. Xiao M, Liu X, Ji J, Li M, Li J, Yang L, et al. Multiple approaches for massively parallel sequencing of SARS-CoV-2 genomes directly from clinical samples. Genome Med. 2020;12(1):57.
15. Buchan BW, Windham S, Balada-Llasat JM, Leber A, Harrington A, Relich R et al. Practical Comparison of the BioFire FilmArray Pneumonia Panel to Routine Diagnostic Methods and Potential Impact on Antimicrobial Stewardship in Adult Hospitalized Patients with Lower Respiratory Tract Infections. Journal of clinical microbiology. 2020;58(7).
16. Estimates of the. Global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990–2016: a systematic analysis for the global burden of Disease Study 2016. Lancet Infect Dis. 2018;18(11):1191–210.
17. Lin P, Chen Y, Su S, Nan W, Zhou L, Zhou Y, et al. Diagnostic value of metagenomic next-generation sequencing of bronchoalveolar lavage fluid for the diagnosis of suspected pneumonia in immunocompromised patients. BMC Infect Dis. 2022;22(1):416.
18. Li X, Liu Y, Li M, Bian J, Song D, Liu C. Epidemiological investigation of lower respiratory tract infections during influenza A (H1N1) pdm09 virus pandemic based on targeted next-generation sequencing. Front Cell Infect Microbiol. 2023;13:1303456.
19. Zhang P, Liu B, Zhang S, Chang X, Zhang L, Gu D, et al. Clinical application of targeted next-generation sequencing in severe pneumonia: a retrospective review. Crit Care (London England). 2024;28(1):225.
20. Blauwkamp TA, Thair S, Rosen MJ, Blair L, Lindner MS, Vilfan ID, et al. Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease. Nat Microbiol. 2019;4(4):663–74.
21. Guan H, Shen A, Lv X, Yang X, Ren H, Zhao Y, et al. Detection of virus in CSF from the cases with meningoencephalitis by next-generation sequencing. J Neurovirol. 2016;22(2):240–5.
22. Lin Q, Yao Y, Li X, Zhang S, Guo H, Ma X, et al. The application of nanopore targeted sequencing for pathogen diagnosis in bronchoalveolar lavage fluid of patients with pneumonia: a prospective multicenter study. Infect Dis (London England). 2024;56(2):128–37.
23. Li S, Tong J, Liu Y, Shen W, Hu P. Targeted next generation sequencing is comparable with metagenomic next generation sequencing in adults with pneumonia for pathogenic microorganism detection. J Infect. 2022;85(5):e127–9.
24. Chao L, Li J, Zhang Y, Pu H, Yan X. Application of next generation sequencing-based rapid detection platform for microbiological diagnosis and drug resistance prediction in acute lower respiratory infection. Annals Translational Med. 2020;8(24):1644.
25. Lin R, Xing Z, Liu X, Chai Q, Xin Z, Huang M et al. Performance of targeted next-generation sequencing in the detection of respiratory pathogens and antimicrobial resistance genes for children. J Med Microbiol. 2023;72(11).
26. Hardak E, Avivi I, Berkun L, Raz-Pasteur A, Lavi N, Geffen Y, et al. Polymicrobial pulmonary infection in patients with hematological malignancies: prevalence, co-pathogens, course and outcome. Infection. 2016;44(4):491–7.
27. Wang J, Han Y, Feng J. Metagenomic next-generation sequencing for mixed pulmonary infection diagnosis. BMC Pulm Med. 2019;19(1):252.
28. Parize P, Muth E, Richaud C, Gratigny M, Pilmis B, Lamamy A, et al. Untargeted next-generation sequencing-based first-line diagnosis of infection in immunocompromised adults: a multicentre, blinded, prospective study. Clin Microbiol Infection: Official Publication Eur Soc Clin Microbiol Infect Dis. 2017;23(8):574.e1-.e6.
29. Sun T, Wu X, Cai Y, Zhai T, Huang L, Zhang Y, et al. Metagenomic next-generation sequencing for pathogenic diagnosis and antibiotic management of severe community-acquired pneumonia in immunocompromised adults. Front Cell Infect Microbiol. 2021;11:661589.

Publisher's note