

Metagenomic next-generation sequencing of BALF for the clinical diagnosis of severe community-acquired pneumonia in immunocompromised patients: A single-center study

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Abstract. The diagnostic methods of conventional microbiological tests (CMTs) for severe community-acquired pneumonia (SCAP) may be too complicated or impossible to use in polymicrobial infections, and it may be difficult to identify unexpected pathogens. CMTs are also limited due to the early application of broad-spectrum or prophylactic antimicrobial drugs and the fastidious or slow-growing pathogenic microorganisms. The present study aimed to investigate the value of mNGS compared with CMTs in the clinical diagnosis of SCAP in immunocompromised individuals. Therefore, 37 patients diagnosed with SCAP in immunocompromised adult patients were enrolled from the Respiratory Intensive Care Unit of the First Affiliated Hospital of Soochow University (Soochow, China) between May 1, 2019, and March 30, 2022. A bronchoalveolar lavage fluid sample from each individual was divided in half. Half was sent to the microbiology laboratory directly for examination, and the other one was sent for DNA extraction and sequencing. In addition, other relevant specimens (such as blood) were sent for CMTs, including culture or smear, T-spot, acid-fast stain, antigen detection, multiplex PCR and direct microscopic examination. Based on a composite reference standard, the diagnostic outcomes were compared between CMTs and mNGS. Among the enrolled patients, 31 patients were diagnosed with microbiologically confirmed pneumonia, with 16

(43.2%) having monomicrobial infections, while 15 (40.5%) had polymicrobial infections. Fungi were the most common etiologic pathogens in immunosuppressive individuals. *Pneumocystis jirovecii* (45.9%) and *Aspergillus* spp. (18.9%) were the most common etiologic pathogens. Initial screening test validity of mNGS [sensitivity=96.8%; specificity=33.3%; positive predictive value (PPV)=88.2%; negative predictive value (NPV)=66.6%; likelihood ratio (LR)+, 1.45; LR-, 0.10] was higher compared with that of CMTs (sensitivity=38.7%; specificity=82.3; PPV=92.3%; NPV=20.8%; LR+, 2.3; LR-, 0.74). The total diagnostic accuracy of mNGS was superior to CMTs and it was statistically significantly different [86.5% (32/37) vs. 45.9% (17/37); $P<0.001$]. In conclusion, the total diagnostic accuracy of mNGS was superior to CMTs for SCAP in immunocompromised patients as an important diagnostic method.

Introduction

Severe community-acquired pneumonia (SCAP) brings serious public health challenges around the world. During recent decades, the number of patients requiring intensive care management due to SCAP has increased globally, especially among the elderly, patients with comorbidities and the immunocompromised. A large population-based surveillance study on hospitalized CAP patients found that 21% of patients required intensive care unit (ICU) admission, with 26% of them needing mechanical ventilation. SCAP hospital mortality is still high, ranging from 25 to >50%. Delays from hospitalization to ICU admission have been related with increased mortality (1). In a multi-center prospective study of SCAP in China, Influenza virus, *S. pneumoniae*, *Enterobacteriaceae*, *Legionella pneumophila* and *Mycoplasma pneumoniae* were the top five most common pathogens (2). The in-hospital mortality of patients diagnosed with SCAP with identified and unidentified pathogens was 21.7% (43/198) and 25.9% (20/77), respectively, in individuals >18 years (2). The incidence rate of SCAP in adults ranged from 1.76 to 7.03 per 1,000 person-years in three cities [General Roca (Argentina), Rivera (Uruguay) and Concepción (Paraguay)] in South America, disclosing the high burden of disease in the region (3). Mixed viral-bacterial co-infections occurred in 15.4% of patients and hospital mortality was as

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high as 13.7% in Singapore between January 2014 and July 2015 (4).

SCAP, characterized by its complexity and lack of predictability, causes an increased number of mortalities each year, especially in immunocompromised patients (5). SCAP is a common disease in hospitalized pneumonia patients with a mortality rate of 30-50%, which is higher in immunocompromised patients (6). Therefore, the ability to accurately detect etiological pathogens is important for guiding optimal antibiotic therapy and improving prognostic results (7). The current tactics of conventional microbiological tests (CMTs) for severe community-acquired pneumonia (SCAP) may be too complicated or impossible to use in polymicrobial infections, and it may be difficult to identify unexpected pathogens. Detectability of pathogens by conventional microbiological tests (CMTs) is also limited due to the early application of broad-spectrum or prophylactic antimicrobial drugs and the fastidious or slow-growing pathogenic microorganisms (8).

Metagenomic next-generation sequencing (mNGS) provides a comprehensive method to identify nearly all potential pathogens—viruses, bacteria, fungi, mycobacteria and parasites in a single assay (8-11). mNGS permits the assessment of etiological pathogens without the need for culture. mNGS is especially applicable to rare, novel and atypical etiologies of complicated infectious diseases (12,13). Due to the high-throughput identification and a relatively rapid turnaround time, mNGS has been widely applied to various clinical diseases in previous years, including diseases of the central nervous (14-16) and respiratory (17) systems, the bloodstream (18), prosthetic joint (19) and urinary tract (20). The present study aimed to evaluate the potential of mNGS compared with CMTs as a first-line diagnostic technology for SCAP in immunocompromised patients.

Materials and methods

Case definition. A total of 66 patients diagnosed with severe pneumonia were admitted to Respiratory Intensive Care Unit of the First Affiliated Hospital of Soochow University (Soochow, China) between May 1, 2019, and March 30, 2022. Among them, 29 patients were excluded from this study, including 20 patients who had an immunocompetent status, and 9 patients who had bronchoalveolar lavage fluid (BALF) samples available for mNGS >72 h after Respiratory ICU (RICU) admission (Fig. 1).

Data collection and participants. The present study is retrospective, and the article does not involve the privacy of patients. Informed consent was obtained from all patients or their legal surrogates. Demographic data and medical records of the 37 study subjects were summarized in Table I. The diagnosis of SCAP was made according to Chinese guidelines, and tuberculosis was required to be excluded from the study (21).

Inclusion and exclusion criteria. For inclusion, patients have to meet the following criteria: i) were immunocompromised; ii) were admitted to the intensive care unit (ICU) due to SCAP; and iii) had BALF samples available for CMTs and mNGS within 3 days after RICU admission (22). Immunocompromised status was defined as having one of

the following conditions: i) Received repeated therapy with glucocorticoids; ii) received chemotherapy during the last 3 months; iii) had hematological malignancies; iv) received an organ transplant during the last 1/2 year; or v) was diagnosed with human immunodeficiency virus infection (22).

Diagnostic tests

BALF collection and sampling processing. Bronchoscopy was carried out under local anesthesia for each patient. Several aliquots of 20 ml of 0.9% normal saline were instilled into the target subsegmental bronchi. The first 20 ml was discarded as recommended (23). BALF samples were separated into 5-ml aliquots. Applying CMTs, one aliquot was used for routine experimental examination with staining by optical microscope [Gram's staining solution: i) Dyed with Gentian violet for 10 sec, washed and dried; ii) dyed with iodized solution for 10 sec, washed and dried; iii) decolorizing solution was added to decolorize for 10-20 sec, washed and dried; iv) Gaze yellow solution was used for re-staining for 10 sec and washed; v) once dry, a light microscopic inspection was conducted (x100 magnification). Lactic acid phenol cotton blue staining solution: On a clean slide, 1 drop of lactic acid phenol cotton blue staining solution and a small amount of culture or sample was added, which was spread out with an inoculation needle. The slide was covered, slightly warmed with an alcohol lamp and the slide was lightly pressed to remove bubbles. Light microscopic examination (x100 magnification) was then conducted], and for cultures of fungi and bacteria [Culture of bacteria: i) After inoculation, all types of agar plates (blood plate, chocolate plate and MacConkey plate) were placed in the incubator for 24 h according to relevant culture requirements, and then the results were observed; ii) the bacteria on various plates were observed for color, transparency, bulge state and edge state, and attention was focused on distinguish between contaminated bacteria and pathogenic bacteria; iii) samples from a suspicious single independent bacterial colony were placed on a clean slide that had been dropped with physiological saline. The samples were then directly smeared thinly until they naturally dried. The manufacturer's instructions were followed. A microscope was used to observe the smear after it had dried. Fungal culture: The collected BALF samples were inoculated on Sabouraud medium and placed in a Heraeus CO₂ constant temperature incubator for 48 h to 1 week, and then the growth and colony morphology were observed using the naked eye], and PCR [i) DNA source: Exfoliated cells and secretions of nasopharynx; ii) method of extraction is based on the full-automatic nucleic acid extraction instrument produced by Bio Perfectus Technologies, performed as elaborated in the reagent specification; iii) Hot Start DNA Polymerase, manufactured by Beijing XABT Biotechnology Co., Ltd., was used; iv) Sequences of the forward and reverse primers: Influenza virus forward, 5'-AGAGACTTGAAGATGTCT TTGC-3' and reverse, 5'-GCTCTGTCCATGTTATTGGA TC-3'; influenza virus forward, 5'-GAAAAATTACACTGT TGGTTCGG-3' and reverse, 5'-AGCGTTCCTAGTTTACT TGCAT-3'; adenovirus forward, 5'-GCCGCAGTGGTCTTA CATGCACATC-3' and reverse, 5'-CAGCACGCCGCGGAT GTCAAAGT-3'; respiratory syncytial virus forward, 5'-AGC ACTTATATGTTAACAATAG-3' and reverse, 5'-TGGGAA GAAAGATACTGATCC-3'; parainfluenza virus (PIV-1)

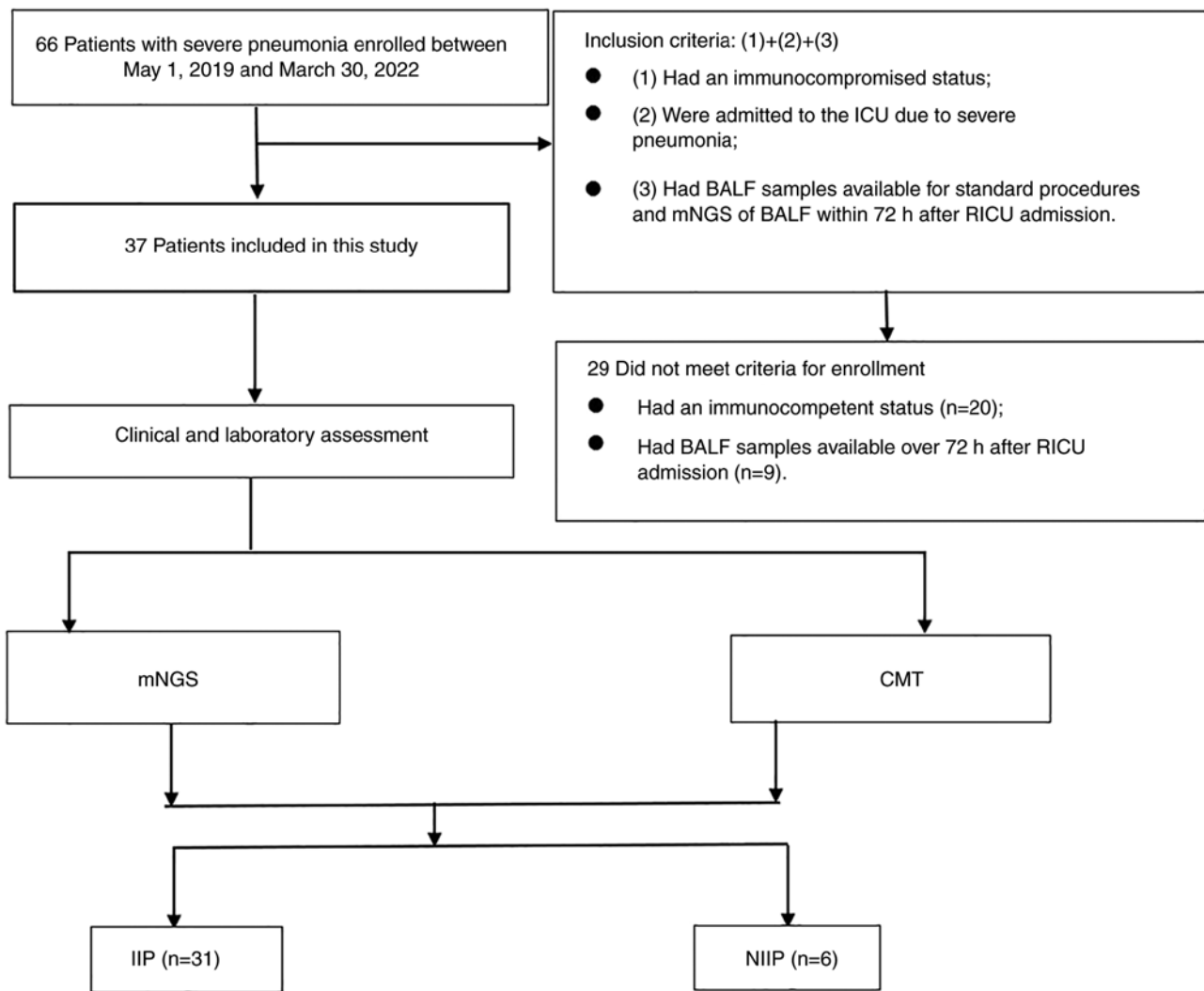


Figure 1. Trial profile. Flow diagram of case inclusion and exclusion. Patients met the inclusion criteria if they: i) Had an immunocompromised status; ii) were admitted to the ICU due to SCAP; and iii) had BALF samples available for CMTs and mNGS of BALF within 3 days after RICU admission. Immunocompromised status was defined as any of the following: i) Repeated therapy with glucocorticoids; or ii) received chemotherapy during the last 3 months; or iii) hematological malignancies; or iv) organ transplant during the last 1/2 year; or v) human immunodeficiency virus infection. Eventually, 37 study subjects were enrolled in the present research. ICU, intensive care unit; SCAP, severe community-acquired pneumonia; BALF, bronchoalveolar lavage fluid; mNGS, metagenomic next-generation sequencing; IIP, identified infectious pathogens; NIIP, non-identified infectious pathogens; RICU, Respiratory Intensive Care Unit.

forward, 5'-ATTTCTGGAGATGTCCCGTAGGAGAAC-3' and reverse, 5'-CACATCCTTGAGTGATTA AGTTTG ATG-3'; PIV-3 forward, 5'-TCGAGGTTGTCAGGATATAG-3' and reverse, 5'-CTTTGGGAGTTGAACACAGTT-3'; v) thermocycling conditions: Initial annealing step at 95°C for 5 min, denaturation step at 95°C for 15 sec, annealing and elongation at 60°C for 45 sec. Fluorescence signals were collected at the last 45th sec. 45 cycles were performed.] for virus detection (including influenza virus) were carried out in every sample. All these routine laboratory methods were known as CMTs. mNGS for the other aliquots were performed parallel with the conventional microbiological testing.

Concerning the serology tests, peripheral blood specimens were used for the detection of immunoglobulin antibodies of influenza A/B, parainfluenza virus, human rhinovirus, adenovirus, Coxsackie virus A/B, *L. pneumophila*, *M. pneumoniae* and *C. pneumoniae* by using commercial ELISA kits (cat. no. YZB/SPA 5210-2009; Figure Bioengineering Co.,

Ltd.) according to the manufacturer's instructions. These diagnostic tests were performed by the First Affiliated Hospital of Soochow University, and they were a part of the clinical treatment.

DNA extraction and sequencing. The collected BALF was tested for the gene of pathogenic microorganisms using mNGS. Bronchoalveolar fluid (600 μ l) samples were mixed with proteinase kinase enzyme (cat. no. DP316; Tiangen Biotech, Co., Ltd.) and glass beads (0.5 mm diameter; zirconia/silica cat. no. 11079105z; Thistle Scientific), before being vortexed at 1509.3 x g for 30 min at 4°C. The TIANamp Micro DNA kit (cat. no. DP316; Tiangen Biotech, Co., Ltd.) was used for extracting the total DNA. The DNA extraction and library construction were performed using an NGS automatic DNA library system (cat. no. MAR002; MatriDx Biotech Corp.) and a total DNA library preparation kit (cat. no. MD001T; MaxtriDx Biotech Corp.). Libraries were then quantified by quantitative PCR using a KAPA Library Quantification

Table I. Summary of the patient population and characteristics.

Characteristics	Values
Age in years, median (IQR)	55.6 (47.5, 67.0)
Male, n (%)	28 (75.7)
Received broad-spectrum antibiotics before BALF (%)	28 (75.7)
Laboratory findings	
C-reactive protein in mg/l, median (IQR)	139.3 (64.5, 186.5)
Procalcitonin in ng/ml, median (IQR)	3.4 (0.3, 1.1)
Lactate dehydrogenase in U/l, median (IQR)	414.1 (213.9, 535.0)
White blood cells count ($10^9/l$), median (IQR)	9.6 (6.3, 11.5)
Lymphocytes count ($10^9/l$), median (IQR)	0.8 (0.4, 1.0)
CD4 ⁺ T cell count ($10^6/l$), median (IQR)	203.7 (116.0, 203.0)
CD8 ⁺ T cell count ($10^6/l$), median (IQR)	259.0 (64.5, 231.0)
Type of immunocompromised status, n (%)	
Prolonged corticosteroid therapy ^a	20 (54.1)
Solid tumor/hematological malignancy receiving chemotherapy	12 (32.4)
Solid organ or Bone marrow transplantation	5 (13.5)
Disease severity	
APACHE II score, median (IQR)	20.7 (18.0, 22.0)
SOFA score, median (IQR)	5.5 (4.0, 6.0)
Invasive mechanical ventilation, n (%)	16 (43.2)
Outcomes	
Death within 30 days, n (%)	12 (32.4)

^aDefined as >0.3 mg/kg/day of prednisone equivalent for ≥ 3 weeks. APACHE II, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; IQR, interquartile range.

75-cycle sequencing kit (cat. no. 20024906; Illumina, Inc.). The library concentration had to pass the quality control cut-off (>50 pmol l⁻¹). A total of 10-20 million 50-bp single-end reads were obtained for each library.

Bioinformation pipeline. In order to generate high-quality data, the raw data were needed to remove adapter, low-quality, low complexity and short reads (<35 bp), with an in-house program. Then, the human sequences were excluded by mapping reads to the human reference genome (hg19) with the application of the Burrows-Wheeler Alignment (<http://bio-bwa.sourceforge.net>). The remaining data were aligned to a microbial genomedatabase (National Center for Biotechnology Information; <ftp://ftp.ncbi.nlm.nih.gov/genomes>). The reference database used for the present study contained 11,910 bacteria, 7,103 viruses, 1,046 fungi and 305 parasites that are all associated with human diseases (8).

Infectious pathogens were defined as that meeting either of the following criteria (20): i) >30% relative abundance at the genus level, regardless of culture or smear result, and there was robust evidence of pathogenicity in the lungs based on the clinical literature; ii) culture and mNGS identified the same microbes, the number of unique reads was ≥ 50 from a single species (24) and there was robust evidence of pathogenicity in the lungs based on the clinical literature. Oral colonization microorganisms were not considered infectious pathogens regardless of their relative abundance unless proven otherwise, or they were deemed significant by

the managing physician. These were based on strict clinical criteria (24), combined with multiple clinician adjudication, to rigorously discriminate infection from colonization and contamination. The 37 patients were categorized into two groups: i) Identified infectious pathogens (IIP) group; ii) and non-identified infectious pathogens (NIIP) group, according to the final diagnosis.

The pathogen for SCAP was defined as follows: i) The final etiology result was assessed by the attending physician teams based on clinical features, microbiological results and response to the treatment; and ii) when disputes arise, the physician group discussed and decided the final results.

Statistical analysis. Continuous variables are reported as the mean and standard deviation when they are normally distributed and as the median and interquartile range (IQR) when they have a skewed distribution, according to the Shapiro-Wilk test. Categorical variables are expressed as frequencies and percentages. Pathogens identified by CMTs or mNGS should be totally identical to those confirmed by the physician group with the reference standards, and the reference standards were combined with clinical composite diagnosis and determination of microbiological etiology. The paired McNemar χ^2 test was used to compare the diagnostic efficiency of mNGS vs. CMTs. $P < 0.05$ was considered to indicate a statistically significant difference. All statistical analyses were utilized using SPSS 26.0 (IBM Corp.).

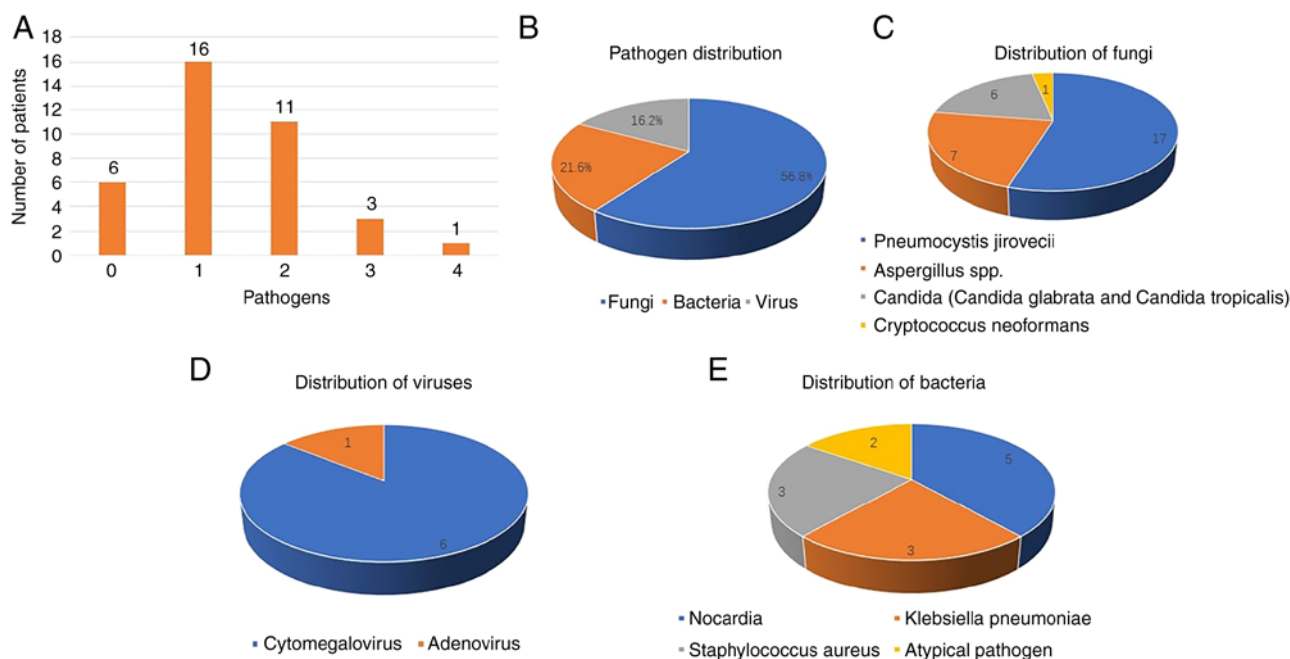


Figure 2. Distribution of pathogens in immunocompromised patients with SCAP. (A) For the enrolled patients, 16 (43.2%) had monomicrobial infections, while 15 (40.5%) had polymicrobial infections (including 11 patients with two pathogens, 3 patients with three pathogens and 1 patient with four pathogens). (B) A total of 21 (56.8%) patients had fungal infection, 8 (21.6%) patients had bacterial infections and 6 (16.2%) patients had viral infections. (C) *Pneumocystis jirovecii* (45.9%) and *Aspergillus* spp. (18.9%) were the most common fungi in immunocompromised patients. (D) Cytomegalovirus (16.2%) were the most common viruses in these patients. (E) *Nocardia* (13.5%), *Klebsiella pneumoniae* (8.1%) and *Staphylococcus aureus* (8.1%) were the common bacteria.

Results

Patient characteristics. During the study period, 66 patients were admitted to RICU due to SCAP, among whom 29 patients were excluded either because mNGS was not performed within 72 h of their admittance or because they did not meet the criteria of immunocompromised status. Thus, 37 patients [median age, 55.6 years; IQR, 47.5, 67.0 years; males, 28 (75.7%)] met the inclusion criteria and were included in the final analysis. Subsequently, 20 patients (54.1%) received prolonged corticosteroid therapy for autoimmune diseases, 12 patients (32.4%) were treated with chemotherapy due to solid tumor or hematological malignancy, whereas 5 patients (13.5%) were treated with solid organs or bone marrow transplantation (Table I). The CD4⁺ T cell count was 203.7x10⁶/l (IQR, 116.0, 203.0), and CD8⁺ T cell count was 259.0x10⁶/l (IQR, 64.5, 231.0). The median APACHE II score was 20.7 (IQR, 18.0, 22.0), and the median SOFA score was 5.5 (IQR 4.0, 6.0). Of the 37 patients, 28 (75.7%) patients received broad-spectrum antibiotics treatment prior to BALF collection. Separately, the average values of the C-reactive protein (≤ 6 mg/l), procalcitonin (0-0.5 ng/ml), lactate dehydrogenase (120-250 U/l), white blood cell counts (3.5×10^9 - 9.5×10^9 cells/l), and lymphocyte counts (1.1×10^9 - 3.2×10^9 cells/l) of the 37 patients were found to be 139.3 mg/l (IQR 64.5, 186.5 mg/l), 3.4 ng/ml (0.3, 1.1 ng/ml), 414.1 U/L (213.9, 535.0 U/L), 9.6×10^9 cells/l (6.3, 11.5×10^9 cells/L), 0.8×10^9 cells/l (0.4, 1.0×10^9 cells/l). Then, 16 (43.2%) patients underwent tracheal intubation and received mechanical ventilation when the BALF was collected. Overall, 12 (32.4%) died within 30 days of being admitted to the ICU (Table I).

Pneumonia pathogens. Based on a retrospective review of clinical manifestations, the diagnosis of SCAP also required judgement by an experienced team of experts. Among the 37 patients enrolled in the present study, pathogenic pathogens have been clearly defined in 31 patients, and not been defined in the other 6 patients. Among these 31 patients, 16 (43.2%) had monomicrobial infections, while 15 (40.5%) had polymicrobial infections (including 11 patients with two pathogens, 3 patients with three pathogens and 1 patient with four pathogens). A total of 21 (56.8%) patients had fungal infections [*Pneumocystis jirovecii* (n=17), *Aspergillus* spp. (n=7), *Candida glabrata* (n=3), *Candida tropicalis* (n=3) and *Cryptococcus neoformans* (n=1)], 8 (21.6%) patients had bacterial infections [*Nocardia* (n=5), *Klebsiella pneumoniae* (n=3), *Staphylococcus aureus* (n=3) and Atypical pathogen (n=2)] and 6 (16.2%) patients had viral infections [cytomegalovirus (CMV; n=6) and adenovirus (n=1)] (Fig. 2).

Diagnostic performance of CMTs and mNGS. Among the 37 patients, an accurate and complete microbiological diagnosis of mNGS was obtained for 31 patients, and 3 out of 6 patients with a non-infectious aetiology had negative mNGS results (data not shown), corresponding to 96.8% sensitivity and 33.3% specificity, positive and negative predictive values were 88.2 and 66.6%, while the positive and negative likelihood ratio were 1.45 and 0.1, respectively. Regarding CMTs, pathogens were detected in 13 patients by CMTs only, among whom 7 (18.9%) patients had fungal pneumonia [*Candida glabrata* (n=3), *Candida tropicalis* (n=2), *Aspergillus* spp. (n=2)], 5 (13.5%) patients had bacterial infections [*Nocardia* (n=3), atypical pathogen (n=1), *Staphylococcus aureus* (n=1)] and 1 (2.7%) patient had a viral infection [CMV (n=1)]. The

Table II. Diagnostic performance comparison of mNGS and CMTs.

Parameter	mNGS	CMTs
Positive detection		
IIP	30	12
NIP	4	1
Negative detection		
IIP	1	19
NIP	2	5
Sensitivity, %	96.80	38.70
Specificity, %	33.30	83.30
Positive predictive value, %	88.20	92.30
Negative predictive value, %	66.60	20.80
LR+	1.45	2.3
LR-	0.1	0.74
Accuracy, %	86.50	45.90 ^a

^aP<0.001 compared with mNGS. IIP, identified infectious pathogens; NIP, non-identified infectious pathogens; LR, likelihood ratio; mNGS, metagenomic next-generation sequencing; CMTs, conventional microbiological tests.

sensitivity and specificity were 38.7 and 83.3%, the positive and negative predictive values were 92.3 and 20.8%, while the positive and negative likelihood ratios were 2.3 and 0.74, respectively. Compared with that of CMTs, the overall diagnostic accuracy of mNGS was higher and it was statistically significantly different [86.5% (32/37) vs. 45.9% (17/37); P<0.001] (Table II).

Discussion

Precise and timely responsible pathogen diagnosis is essential for SCAP in immunocompromised individuals (25). Despite current advanced diagnostic techniques, ~60% infectious diseases fail to identify pathogens (26). CMTs have drawbacks in their abilities of detection and sensitivity, due to the characteristics of being time-consuming, technically intensive and error-prone (27,28). mNGS, which is independent of the etiological hypothesis, can theoretically permit the identification of all known pathogenic microbes (29,30). To the best of our knowledge, the present research is the first study to apply mNGS to the diagnosis of pathogenic microbes in immunocompromised SCAP adult patients. The present study reported our experience in the evaluation of immunocompromised patients, with the majority of the focus on those receiving long-term steroid therapy and chemotherapy with febrile illness or invasive infections by mNGS.

In the present study, of the 37 immunocompromised SCAP patients, 31 patients (83.8%) were identified as having infectious pathogens based on mNGS and CMTs. In total, >40% patients (40.5%) had polymicrobial etiology, with *Pneumocystis jirovecii* (45.9%) and *Aspergillus* spp. (18.9%) as the most common pathogens of mixed infection; however, to the best of our knowledge, none were reported in previous

studies (31,32). The spectrum of pathogens in immunocompromised patients with SCAP is different compared with those in immunocompetent SCAP individuals, with mixed pulmonary infections often reported (31,32). mNGS demonstrated the ability to detect the causative agents of mixed infection, especially in a mixed infection of bacteria, fungi and viruses. The CMTs (such as culture, serology and molecular methods) performed poorly in detecting mixed infections by comparison.

In contrast to previous studies (8,22), broad-spectrum antibiotics were commonly administered in a large proportion of these patients (28/37, 75.7%) before ICU admission, and CMTs showed a relatively poor level of overall diagnostic performance compared with that of mNGS. We hypothesize that the use of broad-spectrum antibiotics had led to a decline in the detection rate of CMTs, while mNGS was not affected by antibiotics. It is noteworthy that the present study confirmed the potential advantages of mNGS in the identification of fungi and viral infection, especially *Pneumocystis jirovecii* and CMV. The current study demonstrated the high sensitivity of mNGS compared with CMTs in immunocompromised patients with SCAP, making it recommended as a front-line test for microbiological diagnosis in suspected infections or as a 'rule-out' method to exclude infection in the immunocompromised patients. mNGS would be most valuable when physicians are unable to find presumed causative pathogens or when a full work-up of CMTs are unavailable in the clinical testing centers. The distinct advantage of mNGS may contribute to more comprehensive evaluation of empiric antibiotics therapy and more effective adjustments for these immunocompromised SCAP patients.

Bacteria were identified in 21.6% patients in which *Nocardia* (13.5% of 37 patients) were the most frequent pathogen in the bacteria etiology. *Klebsiella pneumoniae* and *Staphylococcus aureus* were identified in 8.1% immunocompromised patients with SCAP, respectively. Fungi (56.8% of 37 patients) were the most common pathogenic pathogens in immunosuppressive individuals. *Pneumocystis jirovecii* (45.9% of 37 patients) remained the most frequent pathogen in the fungi etiology. *Aspergillus* spp. (18.9% of 37 patients) and *Candida* (*Candida glabrata* 8.1% and *Candida tropicalis* 8.1%) were identified in immunocompromised patients with SCAP, respectively. A higher number of patients in the present study developed *Pneumocystis jirovecii* compared with other studies (26,27). It may be related to the serious immunosuppression condition of the patients that were included in the present study.

Respiratory viruses play an increasing role in immunocompromised patients with SCAP; for example, influenza virus and covid-19 cause SCAP in immunocompromised patients (33,34). In the present study, cytomegalovirus (16.2% of 37 patients) was the most common viruses in the patients. Therefore, the management strategy of SCAP in immunocompromised patients has to involve the detection of respiratory viruses such as cytomegalovirus (35,36).

Since the outbreak of coronavirus disease-19, mNGS technology has been widely developed in China. The cost of mNGS is ~3,600 RMB, and the cost of CMTs (such as culture technology, antibody detection and PCR) is ~2,000 RMB or more (34). Although mNGS is more expensive when compared with CMTs, the shorter time taken to identify the pathogen can

often bring major adjustments to treatment and, thus, reduce the hospitalization days and expenses (27). mNGS technology is already being performed in numerous hospitals in China. In the Department of Pulmonary and Critical Care Medicine of The First Affiliated Hospital of Soochow University, mNGS testing can be performed independently. For hospitals unable to carry out the technology themselves, it is convenient to entrust a third-party biological company for testing. To the best of our knowledge, there is no relevant research on the clinical benefit to cost ratio. According to our clinical experience, there is no significant difference in the cost of mNGS and CMTs. Therefore, the present study recommends mNGS as a powerful tool.

The current study has several limitations, as follows: i) this study was conducted in one single center, where there was a small sample size in addition to potential selection bias; ii) patients were recruited with highly heterogeneous immunosuppression, including autoimmune diseases with prolonged corticosteroid therapy, solid tumors or hematological malignancies receiving chemotherapy and patients with solid organ or bone marrow transplantation, which may be associated with different pathogen profiles and diagnostic efficiencies of mNGS; iii) due to the lack of standard criteria, the interpretation of mNGS results may influence the diagnostic results. Some bacteria and viruses with relatively low abundance may be regarded as non-pathogens or contamination; and iv) the respiratory tract is an open airway, and therefore there are some confounding variables that may affect results. In fact, the respiratory tract is susceptible to the influence of various colonizing bacteria. Patients with immunosuppressive pneumonia have different basic conditions and complications, and there are indeed many confounding factors. However, the diagnosis of pneumonia pathogens depends not only on the microbiological diagnosis, but also on the clinical characteristics and treatment effects. Ultimately, the comprehensive judgment of clinicians is required. Therefore, the final determination of pneumonia pathogens strictly complies with the comprehensive judgment of the expert group.

In conclusion, mNGS is a revolutionary technology in the microbiological diagnosis of SCAP in immunocompromised patients. mNGS demonstrated its notable advantages in detecting pathogenic microbes, mixed infections and rare pathogens in these patients. The present study indicated that mNGS could quickly offer etiological evidence for SCAP in immunocompromised adult patients. In the future, additional clinical trials need to be carried out to evaluate the clinical usage of mNGS further. In addition, the data analysis strategy could be further improved.

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Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to our team using part of the data for writing another manuscript, but are available from the corresponding author on reasonable request.

Authors' contributions

XZ wrote the manuscript, analyzed and interpreted the patient data. YQ was responsible for acquisition of data, and analyzed and interpreted the patient data. WL and JAH were responsible for designing the study, and the analysis and interpretation of the data. All authors have read and approved the final manuscript. XZ and WL confirm the authenticity of all the raw data.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of the First Affiliated Hospital of Soochow University (approval no. [2023] Ethical Research NO.073). Informed consent was obtained from all patients or their legal surrogates.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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