

# Value of mNGS in guiding clinical prognosis of ARDS caused by severe pneumonia

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## Research article

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# Abstract

Background ARDS caused by severe pneumonia develops rapidly, causes complex pathogens, and has high morbidity and mortality. Convenient and efficient methods are needed in clinical practice for the detection of pathogen of ARDS caused by severe pneumonia.

Methods This study retrospectively assessed the data of 105 patients with ARDS caused by severe pneumonia. Patients were divided into NGS group and no-NGS group. Clinical characteristics, laboratory examination, ICU cost and prognosis in the two groups were compared. Meanwhile, compared patients in NGS group, mNGS and routine detection methods had different detection efficiency for bacteria, fungi, viruses, unusual pathogens and co-infections.

Results The mortality rate of NGS group was significantly lower than that of no-NGS group (21.43% VS 49.06%,  $P < 0.05$ ). In NGS group, the pathogen detected by Metagenomics NGS (mNGS) method was consistent with sputum culture by 31.1%. More than half (62.5%) of the inconsistencies were caused by negative sputum culture. The efficiency of mNGS method for unusual pathogens, only bacteria or co-infection is better than that of serological antibody test plus PCR. The positive rate of mNGS detection of unusual pathogens was significantly higher than that of sputum culture (22.22% vs 0,  $P < 0.01$ ). The overall positive rate of mNGS detection of ARDS caused by severe pneumonia (91.11%) was greater than that of serological antibody test plus PCR (31.11%) and sputum culture (62.22%). The clinical data of immunosuppressive patients in the two groups were compared, and it was found that the ICU time ( $P < 0.01$ ), ventilator time ( $P < 0.05$ ) and ICU cost ( $P < 0.01$ ) in NGS group were significantly lower than those in no-NGS group.

Conclusion mNGS technology has special significance for the treatment and prognosis of ARDS caused by severe pneumonia. MNGS technology is superior to routine pathogen detection methods for the detection of unusual pathogens and co-infection. For patients with severe disease, immunosuppression, or cases that cannot be diagnosed by routine methods, mNGS technology can be used to provide more diagnostic evidence for clinical diagnosis and to guide clinical medication.

## Background

Acute respiratory distress syndrome (ARDS) is mostly caused by infection, such as pneumonia with numerous pathogens. There are also various other non-infectious events, such as drowning, blunt chest contusion, multiple injuries, aspiration burns, pancreatitis, and multiple blood transfusions[1]. ARDS is one of the main causes of death in critically ill patients. The incidence of ARDS in ICU is about 5–20%[2] [3] [4][5], and the mortality rate is 35–60%[6][7]. It was found that 10% of ICU patients and 23% of patients on mechanical ventilation had ARDS[8]. The mortality rate of patients with severe ARDS was 46%[8]. ARDS survivors were at higher risk of cognitive decline, depression, post-traumatic stress disorder and persistent skeletal muscle weakness[9][10]. Pneumonia brought about by various pathogens [1] may

develop into ARDS, leading to multiple organ failure and death. Early diagnosis of pathogens was of particular important for the treatment of ARDS caused by severe pneumonia.

There was not any specific treatment for ARDS. According to the guidelines[11], the treatment focuses on: control of the primary disease, respiratory support therapy and drug therapy. Respiratory support therapy includes sedation and analgesia, ventilator lung protective ventilation, lung reactivation, high peep, prone ventilation, high frequency oscillating ventilation and ECMO. The primary goal of respiratory support therapy was to minimize lung cell damage and avoid further release of inflammatory mediators, thus buying valuable time for etiological treatment and lung recovery. For patients with ARDS caused by severe pneumonia, early antimicrobial treatment for pathogen-induced pneumonia, such as the use of appropriate anti-infective treatment, was critical to reduce pathogens and pathogens of origin[1]. Therefore, early and rapid pathogen detection was critical in the treatment of ARDS.

At present, common pathogenic detection in clinical practice[12] common bacteria and fungi is mainly detected by culture. This method is not restricted by the pathogen content, and can be used for identification and drug susceptibility test[13]. The detection rate is about 15–20%, and the turnover time is long (3–5 days). For viruses, mycoplasma pneumonia, chlamydia, legionella and other difficult to culture pathogens, nucleic acid hybridization and PCR were used to detect the pathogenic nucleic acid fragments. The method has high sensitivity and specificity. However, primers should be designed for pathogens, and detection types are limited. Serological antibody test, the sensitivity of some pathogens is limited, and there is a window period of antibody detection. Metagenomics next generation sequencing (mNGS) was first reported being used to diagnose central nervous system infection of leptospira in 2014[14]. This method can quickly detect all nucleic acids in the samples[15][16]. As an emerging diagnostic method, it has been reported to detect specimen types of different infection sites such as blood flow, respiratory tract, CNS, and abscess. There is the absence of research on the pathogenic infection characteristics of ARDS caused by severe pneumonia. The next-generation sequencing has not already been discussed in terms of its prognostic value after diagnosis of severe pneumonia-induced ARDS. The purpose of this study was to retrospectively evaluate the value of mNGS technology in diagnosis and clinical prognosis of ARDS caused by severe pneumonia.

## Methods

### Case recruitment and management

A retrospective analysis of all cases of ARDS patients caused by severe pneumonia aged 18 years and older who were admitted to the ICU of Jiangmen Central Hospital from January 2018 to August 2019. ARDS diagnostic criteria: it conformed to the Berlin definition of ARDS proposed in 2012[17], and ARDS was caused by severe pneumonia. Exclusion criteria: The patient was discharged automatically, the patient died not due to ARDS caused by severe pneumonia and the patient or relatives quit treatment. Before the use of antibiotics, the sputum were taken from the airway, and the microbial chamber was sent for aerobic and anaerobic culture and sputum smear examination. After the bronchoscopy specimens

were taken, the patients who agreed to the mNGS technology were taken bronchoalveolar lavage fluid (BALF) or sputum to the BGI Clinical Laboratories (Shenzhen) Co., Ltd. for pathogens detection. According to the guidelines [12], the ARDS patients were initially treated with empirical broad-spectrum antibiotics, and then combined with the mNGS and routine examination results to adjust the anti-infective program to observe the clinical efficacy.

## Information collection

Routine microbiological indicators: bacterial, anaerobic, fungal culture, drug sensitivity, fungal d-glucan detection, bacterial, cryptococcus, acid-fast bacilli smear, mycoplasma, virus antibody detection, bacterial and virus nucleic acid hybridization PCR. Other infection indicators: blood routine, procalcitonin (PCT), fungal G test, c-reactive protein, serum endotoxin and other related infection indicators. And collect liver, kidney, heart, coagulation function, chest film, CT and other related examination results. APACHE II score and SOFA score were performed when the patient was admitted to the ICU and transferred out of the ICU or died.

## Sample group comparison

Patients were divided into NGS group and no-NGS group according to whether the patient performed mNGS detection. Clinical characteristics, laboratory tests, 28-day mortality, ICU time, ventilator time, ECMO time, prone position ventilation time, and ICU treatment costs were compared between the two groups. In NGS group, compared mNGS and routine pathogens detection methods for the detection of bacteria, fungi, viruses and co-infection.

## Statistical Analysis

T-test was used for the measurement data that conforms to the normal distribution and the variance was uniform. Wilcoxon rank test was used for variance calculation of the measured data that did not conform to a normal distribution or with homogeneity of variance. For counting data, chi-square test was utilized to calculate the difference between two groups. All statistical analysis was conducted using R3.4.4 software.  $P < 0.05$  was considered statistically significant. Survival analysis was performed utilizing Mantel-Cox test, and the multivariate influencing factors of ARDS patients were analyzed by cox model.

## Results

### Sample and patient characteristics

A total of 105 ARDS patients caused by severe pneumonia were enrolled in this study. There were 49 patients in NGS group and 56 patients in no-NGS group. In the NGS group, 3 patients were discharged automatically, 1 patient died of internal organ bleeding, and 3 relatives gave up treatment. In the no-NGS group, 3 cases were automatically discharged from the hospital. In the NGS group, 3 patients had twice mNGS tests, and a total of 45 samples were sent for mNGS, including 40 BALF and 5 sputum. Characteristics of patients in NGS and no-NGS groups are shown in Table 1. There was not any difference in age, sex ratio, infection index, APACHEII score, SOFA score at admission, ICU time, ventilation time, ECMO time on severe ARDS patients, prolonged ventilation time on moderate or severe ARDS patients, and ICU cost between two groups. There was a difference in outcome between the two groups ( $P \leq 0.05$ ) (Table 1).

## Comparison between NGS and no-NGS groups

In the NGS group, 45 cases of sputum specimens, 28 cases were culture positive, 53 cases of no-NGS group sputum specimens, 46 cultures were positive. There was a significant difference in the positive rate of sputum culture between the two groups (Figure 1,  $P \leq 0.01$ ). The distribution of pathogens positively detected in the two groups was shown in Figure 2. Compared with the NGS group, the no-NGS group detected more opportunistic pathogens such as *A.baumannii*, *Paeruginosa*, *E.coli*, *S.aureus* and *C. albicans*. (Figure 2) It was found that there was a difference in 28-days survival rate between the two groups ( $P \leq 0.05$ ). (Figure 3).

## Comparison mNGS results and routine pathogens detection results in the NGS group

Based on current research, metagenomic NGS detects more pathogens than culture. The researcher analyzed the concordance of pathogens identified by two techniques. Judging criteria: Test results were consistent when the pathogens identified by mNGS were completely consistent with the pathogens obtained from sputum culture (including mNGS identified more pathogens than culture method). The result was partially consistent when the pathogens identified by two methods were partially consistent. The result was inconsistent when the pathogens identified by two methods are completely inconsistent. In the NGS group, 31.1% of the identified pathogens were consistent, 15.5% were partially consistent, and 53.3% were completely inconsistent. Of the inconsistent samples, 62.5% were negative for sputum culture. There were 2 cases (8.3%) of the samples because the mNGS results were negative (Figure 4).

In the NGS group, mNGS method identified 3 only virus infection, 11 only bacterial infection, 5 only fungal infection, 10 cases of unusual pathogens (*P.japonicum*, parasites, tuberculosis or mycoplasma/chlamydia) infection, 12 co-infection (bacterial + fungal / bacterial + virus / fungal + virus / bacterial + fungal + virus). The sputum cultured and identified 15 only bacterial infection, 5 only fungal infection, 8 bacterial and fungal co-infection, and 17 negative. Serological antibody test plus PCR, 9

influenza A virus, 2 Influenza B virus, 1 CMV IgG positive, 1 influenza A virus and CMV IgG positive, 1 *M.pneumoniae* and 31 negative.

For merely virus, serological antibody test plus PCR efficiency was significantly better than mNGS detection efficiency (13/45 VS 3/45,  $P=0.05$ ). For merely bacterial, mNGS detection efficiency was significantly better than serological antibody test plus PCR (11/45 VS 0/45,  $P=0.01$ ). There was no significant difference between the mNGS method and sputum culture for merely bacterial detection. For merely fungal, there were no significant differences among the three methods. For specific pathogen detection, the mNGS method was significantly superior to the sputum culture and serological antibody test plus PCR (10/45 VS 0/45,  $P=0.01$  and 10/45 VS 1/45,  $P=0.05$ ). For co-infection detection, mNGS method was significantly better than serological antibody test plus PCR (12/45 VS 0/45,  $P=0.01$ ), and there was no significant difference between mNGS method and sputum culture. The positive rate of pathogen detection by mNGS was significantly higher than that of sputum culture and serological antibody test plus PCR (41/45 VS 28/45,  $P=0.05$  and 41/45 VS 14/45,  $P=0.01$ ). (Table 2).

### *Prognosis of ARDS patients*

Cox univariate analysis was performed on all factors, and then cox multivariate analysis was performed on the indexes whose P value of cox univariate analysis was less than 0.2. NGS (yes/no), ICU time, APACHE II score before treatment and SOFA score before treatment four factors was the risk factors in patients with ARDS caused by severe pneumonia. NGS group patients had a better prognosis than no-NGS group patient ( $P=0.01$ ). The shorter stay in ICU, the better the prognosis. Patients with lower APACHEII and SOFA scores before treatment had better prognosis ( $P=0.05$ ). (Table 3 and Table 4).

## **Immunosuppressive patient**

The clinical features of immunosuppressed patients are complicated. In this study, 21 immunosuppressed patients were enrolled, 8 were subjected to metagenomic NGS pathogen detection, and 13 were rejected to metagenomic NGS. In NGS group, 3 sputum culture were positive, consistent with the pathogens identified by mNGS. In the NGS group, 5 *P.jirovecii*, 1 Rhizopus, 1 Cryptococcus and 1 human herpesvirus 5 were detected. *P.jirovecii* is an opportunistic pathogen causing pneumonia, which causes severe death in patients. People with low immune function, such as HIV-infected patients, tissue organ transplanters, or cancer radiotherapy and chemotherapy, are susceptible populations of *P.jirovecii* [18]. Five patients with *P.jirovecii* were found to have nephrotic syndrome, dermatomyositis, multiple myeloma, and lymphoma. In the no-NGS group, 9 cases were positive for sputum culture, and 2 *S.maltophilia*, 2 *A.baumannii*, 1 *S.aureus*, 4 Candida and 1 Aspergillus were detected. 4 cases were multi-drug resistant bacterial. There was a significant differences in ICU time ( $P=0.05$ ), ventilator time ( $P=0.05$ ) and ICU cost ( $P=0.05$ ) between the two groups of immunosuppressed patients. (Figure5)

## **Discussion**

ARDS caused by severe pneumonia is one of the main causes of death in critically ill patients. Recently, with the emergence of new pathogenic microorganisms, the increase of drug-resistant pathogenic microorganisms and the increase of immunosuppressive hosts, the incidence and mortality of infections remain high, the mortality rate of ARDS patients was 35–60%[6][7]. Severe pneumonia infection is acute, rapid, and complex. It is essential to identify pathogenic microorganisms in a short time. Routine pathogens detection methods include morphological detection, culture, biochemical detection, immunology and nucleic acid detection. Because of simple, rapid, and less technically demanding, it is still widely used in clinical practice. However, it has limitations in sensitivity, specificity, timeliness, and amount of information, and cannot be quickly identified for unknown or rare pathogenic microorganisms. Metagenomics NGS (mNGS) does not rely on routine microbial culture and directly perform high-throughput sequencing of nucleic acids from clinical samples. Then, compared to the microbial database and identified the pathogenic microorganism contained in the sample. It can quickly and objectively detect more pathogenic microorganisms (including viruses, bacteria, fungi, *Mycobacterium tuberculosis*, parasites) in clinical samples and does not require specific amplification. It has been widely used in the diagnosis of critically ill and difficult infections.

This study took the lead in exploring the guiding value of mNGS for clinical prognosis of ARDS caused by severe pneumonia. We found that the physiological indicators of two groups patients were at a considerable level. The mortality of the NGS group was significantly lower than that of the no-NGS group ( $P < 0.05$ ), and the 28-day survival rate was significantly higher than that of the no-NGS group ( $P < 0.05$ ). There were no differences between the two groups in ICU cost, ventilation time, ECMO time for severe ARDS patients, prolonged ventilation time for moderate or severe ARDS patients. This conclusion was consistent with previous studies. Ruilan Wang[19] analyzed 178 patients with severe pneumonia and confirmed the diagnosis through mNGS. Adjusted therapeutic regimen based on comprehensive clinical diagnosis, the patient's 28-day or 90-day survival rates were improved. The 90-day survival rate increased from 57.7% to 83.3%. This study showed that there was no increase in the ICU cost, but the ICU cost of immunosuppressed patients with mNGS detection was lower than that of patients without mNGS detection.

In this study, the positive rate of sputum culture in patients with no-NGS was significantly higher than that in patients with NGS. It should be the logic of the grouping caused this difference. Sputum cultures can identify pathogens, patients tend not to use mNGS.

Compared with routine pathogens detection methods, the mNGS method in this study have no obvious advantages for simple bacteria, fungi and virus detection, but have special significance for special pathogens and co-infection patients. MNGS quickly detect the pathogenic microorganisms of patients and achieve the accuracy treatment of pathogens. Especially for patients with difficult and immunocompromised conditions, such low immunity patients are prone to get co-infections. The mNGS method has obvious advantages in detecting pathogens in such patients. In the study, mNGS detected immunosuppressive patients infected with pathogenic bacteria that are difficult to culture, such as *P. jejeuni*, *Rhizopus*, *Cryptococcus*, and human herpesvirus 5. For effective anti-infective treatment of

pathogens, we found that the NGS group had a lower mortality rate than the no-NGS group, but it was not statistically significant (3/8 Vs 7/13), probably due to small sample size. MNGS method can significantly reduce ICU time, ventilation time and hospitalization cost in immunosuppressed patients ( $P < 0.05$ ).

ARDS caused by severe viral pneumonia often has a serious condition and develops rapidly. It is easy to develop from a simple virus infection to co-infection. Immunosuppressed patients are also prone to concurrent viral infections. In the NGS group of patients diagnosed with viral pneumonia in this study, there were 17 patients with bacterial or fungal or bacterial and fungal infections. It is important to adjust the anti-infective regimen in combination with mNGS results and clinical indicators. P32 patient with severe viral pneumonia infection, mNGS detected adenovirus, sputum culture negative, serological antibody test negative. The combination of piperacillin sodium tazobactam + ribavirin was used for antibacterial and antiviral treatment, and VV-ECMO was also treated. Frequent fever occurred during the treatment period. After 9 days, the mNGS results were reviewed for *G.Phloem*, *G.glabrata*, and *A.fumigatus*, and the sputum was cultured as *A.baumannii*. The anti-infective regimen was adjusted again to cefoperazone sulbactam + tigecycline + caspofungin. The patients gradually improved, and finally ECMO treatment was discontinued for 16 days and successfully treated. All 6 cases of severe viral pneumonia in the NGS group of this study were successfully treated with ECMO. It has been reported that mNGS has important significance in detecting specific pathogens. A large-scale retrospective study conducted by Hu Bijie[20] found that mNGS sensitivity is higher than routine culture, and the advantages of TB/fungal/virus and anaerobic diagnosis are more obvious. The effect of antibiotic use on mNGS is smaller than that of routine culture. Parize[21] found that mNGS has important clinical value in the pathogen diagnosis of immunosuppressive patient infection. The positive rate of mNGS in virus and bacteria diagnosis is 3 times more than that of routine methods. The mNGS has higher negative predictive value than routine methods.

Although mNGS technology is widely used, there are still some limitations and challenges. There is no authoritative guide to the interpretation of the report on mNGS for clinically infected cases. The mNGS detection of a broad spectrum of pathogens has caused problems in the diagnosis of pathogenicity of clinical pathogens - inability to distinguish between background, colonization and pathogenic bacteria and pollution. For the application of clinical metagenomics, there are many criteria for judging the detection of pathogens. Somasekar S[22] uses mNGS to detect acute liver failure-related viruses, it is considered that the number of virus-detected reads is greater than 25, and the coverage  $> 2\%$  can be judged as positive for mNGS detection. Patricia J. Simner[23] analyzed the mNGS of cerebrospinal fluid. There is a corresponding control sample for each sample. It is considered that the mNGS result must be satisfied that the rpm (read per million) of case should  $\geq 10$  times rpm of the corresponding control sample. In this study, the judgment of positive detection of mNGS is based on the previous study, that is, the strictly map reads number (SMRN) normalized to 20M (SDSMRN). Excluding microorganisms present in the background database, an in-house database, which contains microorganisms appearing in more than 50% samples in the laboratory in past three months. Compared to the negative control, species with significant differences in SDSMRN can be considered candidate suspected pathogens. The candidate

pathogens are then determined for the candidate microorganisms in combination with the patient's clinical status, laboratory findings, imaging findings, therapeutic effects, and microbial characteristics.

The mNGS technology has a low detection rate for intracellular and thick-walled microorganisms, even that the reads number of certain intracellular/grown bacteria is not high, it is considered as a pathogenic pathogen.

Although mNGS technology can be utilized for drug resistance, it has not been able to replace the sputum culture plus drug susceptibility test. Drug resistance gene testing requires knowledge of the genome-wide information of microorganisms in the sample, which requires sequencing more data and matching more costs. In addition, there is no way to establish the correlation between microbial resistance genotypes and clinical drug resistance phenotypes. Therefore, clinical mNGS has certain limitations in accurately predicting microbial antibiotic resistance. Moreover, although the clinician has obtained the drug resistance gene data, there is still no drug susceptibility test so intuitively to determine which drug is resistant and use antibiotics.

The mNGS assay can be performed separately for DNA and RNA. Since RNA has higher abundance and complexity than DNA, and RNA is easily degraded, and has high requirements for transportation and storage, there are still some difficulties in clinical detection of RNA. In this study, there were 12 cases of influenza A or B virus infection in the NGS group, which belonged to RNA virus and have been detected by serological antibody test. Considering the economic cost in clinical testing, most patients only perform DNA testing. P1 patients simultaneously performed DNA and RNA detection procedures and found consistent pathogen, but the RNA detection process failed to find influenza A virus. For the RNA detection process, comprehensive considerations such as specimen transport and laboratory conditions are required. Regardless of the cost, the DNA and RNA detection processes can be carried out together to detect pathogens more comprehensively.

Finally, as an emerging technology, mNGS lacks standard solutions for technologies and databases and interpretation of results. We need to objectively treat the application of mNGS to pathogen infections without abuse. mNGS technology is not a substitute for conventional routine pathogen detection methods. For patients with severe disease, rapid disease progression, immunosuppression, or cases that cannot be diagnosed by conventional methods, mNGS technology can be used to provide more diagnostic evidence for clinical diagnosis and to guide clinical medication. We expect that clinical mNGS will achieve important breakthroughs in the following aspects: (1) to achieve faster diagnosis of pathogens and to obtain information on drug resistance of related pathogens; (2) to identify microbial colonization or infection through monitoring of patient immune response. This will eventually curb bacterial resistance, achieve rational application of antibiotics, and ultimately reduce the economic and social burden of infectious diseases; (3) with the development of technology, the cost of macrogene testing is lower, so that more patients benefit.

In addition, the study still has certain limitations. First, there is a certain shift in retrospective analysis. There was a significant difference in the positive rate of sputum culture between the NGS group and the

no-NGS group. Whether or not to perform mNGS is influenced by the subjective will of the physician or relatives. When the doctor evaluates the condition and found positive for sputum culture. It was not possible to objectively recommend the patient to perform mNGS. The relatives are subjectively unable to accept mNGS new technology or are worried that the cost is too expensive and refuses mNGS. Secondly, the clinical prognosis is affected by many clinical factors. The single-factor and multi-factor analysis of clinical prognosis of ARDS caused by severe pneumonia found that long ICU stay, high APACHE II score and high SOFA score are risk factors for clinical death of ARDS. The mNGS detection is a protective factor for the clinical death of ARDS. We expect a larger sample size involving a multi-center clinical prospective controlled study to better understand the prognostic value of NGS testing for ARDS caused by severe pneumonia.

## Conclusion

mNGS technology has special significance for the treatment and prognosis of ARDS caused by severe pneumonia patients. MNGS technology is superior to routine pathogen detection methods for the detection of unusual pathogens and co-infection. For patients with severe disease, immunosuppression, or cases that cannot be diagnosed by routine methods, mNGS technology can be used to provide more diagnostic evidence for clinical diagnosis and to guide clinical medication.

## Abbreviations

ARDS: Acute Respiratory Distress Syndrome; mNGS: metagenomic next-generation sequencing; PCR: Polymerase Chain Reaction; BALF: bronchoalveolar lavage fluid; ICU: Intensive care unit; APACHE-II: Acute physiology and chronic health evaluation-II; SOFA: Sequential Organ Failure Assessment; SDSMRN: number of reads stringently mapped to species.

## Declarations

## Ethics approval and consent to participate

The protocols used in this retrospective study was reviewed and approved by the Ethical Review Committee of Jiangmen Central Hospital (No: 2019–15). Formal consent was obtained from the patient or the next of kin.

## Consent to publication

Not applicable.

## Availability of data and material

The datasets generated and/or analyzed during the current study are available in the (Figshare) repository([https://figshare.com/articles/data\\_xlsx/10308617](https://figshare.com/articles/data_xlsx/10308617)). The data showed 98 cases with ARDS caused by severe pneumonia.

## Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

All the authors had access to the full dataset (including the statistical reports and tables) and take responsibility for the integrity of the data and the accuracy of the data analysis. SL and YH conceived and designed the study. PZ, YanC, SL, CL, SZ, WZ, YantangC, JM, XZ were involved in the case and sample collection, analysis, interpretation of the data and wrote the first draft of the paper. SL and YH reviewed and approved the final report. All authors have read and approved the final manuscript.

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## Tables

Table1 Patient characteristics of the two groups

	NGS [n=42]	no-NGS [n=53]	P
<b>Characteristics of patients</b>			
	60 (47, 70)	64 (45, 71)	0.122
Male/female)	31/11	38/15	1
Survival (good/death)	33/9	27/26	<b>0.011*</b>
ICU (d)	12 (7, 20)	11 (8, 15)	0.719
ICU time (h)	240 (144, 353)	216 (134, 311)	0.810
APACHE II score before treatment	22 (18, 26)	21 (17, 26)	0.500
SOFA score before treatment	7 (5, 8)	7 (4, 8)	0.875
<b>Comorbid disease</b>			
Coronary artery disease (yes/no)	13/29	17/36	1
Myocardial infarction (yes/no)	3/39	5/48	0.978
Diabetes (yes/no)	10/32	17/36	0.511
Hypertension (yes/no)	1/41	6/47	0.207
Chronic kidney disease (yes/no)	7/35	6/47	0.651
Stroke (yes/no)	5/37	9/44	0.688
Immunosuppression (yes/no)	8/34	13/40	0.696
Aspirin (yes/no)	10/32	11/42	0.914
Statins (yes/no)	20/22	17/36	0.183
Anticoagulants (yes/no)	4/38	5/48	1
<b>Physical examination</b>			
HR (b/min)	1.3 (0.5, 8.4)	2.5 (0.3, 10.6)	0.516
SpO <sub>2</sub> (%)	10.5 (6.4, 15.4)	13.1 (7.5, 15.5)	0.189
MAP (mmHg)	109 (85, 130)	105 (84, 129)	0.932
HRV (ms)	159 (84, 205)	154 (112, 197)	0.780
HRV (ms)	78 (64, 201)	97 (64, 121)	0.515
HRV (ms)	11.8 (5.2, 17.2)	14.4 (7.8, 21.1)	0.071
HRV (ms)	28 (20, 47)	27 (20, 45)	0.612
HRV (ms)	28.0 (23.6, 31.6)	28.2 (24.8, 32.6)	0.880
HRV (ms)	35.6 (31.0, 44.7)	34.7 (26.4, 48.1)	0.614

ml	652 (236, 2747)	656 (311, 2066)	0.482
pl/L	1.6 (1.4, 2.9)	1.7 (1.2, 2.5)	0.763
<b>or parameters</b>			
	124 (76, 177)	156 (108, 194)	0.996
	0.8 (0.6, 1.0)	0.6 (0.5, 0.8)	0.992
	10 (8, 15)	8 (6, 12)	0.272
lock (yes/no)	24/18	30/23	1
<b>reatment</b>			
es/no)	9/33	7/46	0.431
yes/no)	6/36	3/50	0.283
me (d)	15 (11, 18)	10 (10, 23)	0.500
sitioning (yes/no)	10/32	11/42	0.914
sitioning time (h)	89 (63, 117)	96 (71, 121)	0.345
: (CNY)	82344 (55098, 211061)	98912 (68912, 141089)	0.297

Note: the measured data of patients' physiological indicators in the above table were shown by:median (interquartile range). P<0.05 was considered statistically significant.

	mNGS (n=45)		Routine clinical pathogen detection (n=45)			
			Sputum culture	P	Serological antibody test plus PCR	P
Only virus	3	0	0.240		13	0.013*
Only bacterial	11	15	0.485		0	0.001*
Only fungus	5	5	1		0	0.066
Special pathogen	10	0	0.003*		1	0.010*
Co-infection	12	8	0.447		0	0.001*
Overall Positive	41	28	0.011*		14	0.001*

Table2 Comparison of mNGS with routine clinical pathogen detection methods in NGS group

Table3 Cox univariate analysis of two groups of patients

	coef	exp(coef)	se(coef)	z	P
NGS (yes/no)	-0.9585	0.3835	0.3872-2.475	0.013*	
Age (yr)	0.02023	1.02044	0.01063	1.904	0.057*
Sex (male/female)	0.09157	1.0959	0.3742	0.245	0.807
Time in ICU (d)	-0.06728	0.93493	0.03017	-2.23	0.026*
Ventilator time (h)	-0.0006620	0.99933850	0.0010875	-0.608	0.543
APACHE II score before treatment	0.10713	1.11308	0.03087	3.471	0.001*
SOFA score before treatment	0.16765	1.18252	0.05409	3.099	0.002*
Hypertension (yes/no)	-0.1145	0.8918	0.3743	-0.306	0.760
Coronary heart disease (yes/no)	0.8483	2.3357	0.4834	1.755	0.079*
COPD (yes/no)	-0.1003	0.9045	0.3868	-0.259	0.795
Bronchiectasis (yes/no)	0.7603	2.1389	0.5321	1.429	0.153*
Chronic nephrosis (yes/no)	-0.2128	0.8083	0.5315	-0.4	0.689
Diabetes (yes/no)	-0.3358	0.7148	0.5313	-0.632	0.527
Immunosuppression (yes/no)	0.3641	1.4392	0.3742	0.973	0.331
Tumor (yes/no)	0.3259	1.3853	0.3744	0.871	0.384
Smoking (yes/no)	0.06306	1.06509	0.34507	0.183	0.855
Drinking (yes/no)	0.3113	1.3652	0.5314	0.586	0.558
PCT ug/L	-0.006918	0.993106	0.008162	-0.848	0.397
WBC 10 <sup>9</sup> /L	0.02517	1.02549	0.02384	1.056	0.291
Hb g/L	-0.014806	0.985303	0.006503	-2.277	0.023*
PLT 10 <sup>9</sup> /L	-0.002613	0.997391	0.002214	-1.18	0.238
Cr umol/L	-4.68E-05	10.0007256	-0.065	0.949	
T.Bil mmol/L	0.007238	1.007264	0.004939	1.465	0.143*
ALT IU/L	-0.0013	0.998701	0.001818	-0.715	0.475
Alb g/L	0.02717	1.02754	0.0305	0.891	0.373
APTT sec	0.001949	1.001951	0.010613	0.184	0.854
BNP pg/ml	-1.06E-05	1	4.105E-05	-0.259	0.796
Lac mmol/L	-0.1029	0.9022	0.127	-0.81	0.418
PH	1.28	3.598	1.995	0.642	0.521
PaO2	0.00017711	1.00017710	0.0057154	0.031	0.975
PaCO2	0.009267	1.009311	0.009976	0.929	0.353
Be	0.03927	1.04005	0.02537	1.548	0.122*
OI	-0.001195	0.998805	0.002628	-0.455	0.649
FiO2	0.6049	1.831	0.8365	0.723	0.470
Peep	0.02499	1.02531	0.04861	0.514	0.607
Septic shock (yes/no)	0.4582	1.5812	0.3562	1.286	0.198*
CRRT (yes/no)	0.4549	1.5761	0.4026	1.13	0.259

ECMO (yes/no)	-1.4414	0.2366	1.0147	-1.42	0.155*
Prone positioning (yes/no)	0.3034	1.3545	0.3744	0.81	0.418
ICU cost (CNY)	-3.69E-06	1	2.228E-06	-1.655	0.098*

Table 4 Cox multivariate analysis of two groups of patients.

	$\beta$	HR	SE	Wald	P	lower .95	upper .95
NGS (yes/no)	-1.3340	0.2634	0.4712	2.8310	0.005*	0.10462	0.6633
Age (yr)	0.01262	1.013	0.01273	0.991	0.322	0.98774	1.0383
Time in ICU (d)	-0.11870	0.8881	0.05702	2.0820	0.037*	0.79416	0.9931
APACHE II score before treatment	0.106	1.112	0.04399	2.4090	0.016*	1.01996	1.2119
SOFA score before treatment	0.2916	1.339	0.09782	2.9810	0.003*	1.10508	1.6215
Coronary heart disease (yes/no)	0.5068	1.66	0.5583	0.908	0.364	0.55573	4.9579
Bronchiectasis (yes/no)	0.1202	1.128	0.6256	0.192	0.848	0.33093	3.8432
Diabetes (yes/no)	-1.1280	0.3238	0.6664	1.692	0.091	0.0877	1.1953
Hb (g/L)	-0.007002	0.9930	0.006539	1.071	0.284	0.98038	1.0058
T.Bil (mmol/L)	-0.0009512	0.9990	0.006422	0.148	0.882	0.98655	1.0117
Be	0.06065	1.063	0.03277	1.85	0.064	0.99641	1.133
Septic shock (yes/no)	0.3668	1.443	0.4589	0.799	0.424	0.587	3.5476
ECMO (yes/no)	0.1921	1.212	1.474	0.13	0.896	0.06747	21.7642
ICU cost (CNY)	-4.454E-06	1	6.27E-06	-0.71	0.477	0.99998	1

Cox univariate analysis was performed on all factors, and then cox multivariate analysis was performed on the indexes whose P value of cox univariate analysis was less than 0.2.

Note: NGS (yes/no), ICU time, APACHE II score before treatment and SOFA score before treatment four factors was the risk factors in patients with ARDS caused by severe pneumonia. NGS group patients had a better prognosis than no-NGS group patient ( $P=0.01$ ). The shorter stay in ICU, the better the prognosis. Patients with lower APACHE II and SOFA scores before treatment had better prognosis ( $P=0.05$ ).

## Figures

# Figure 1

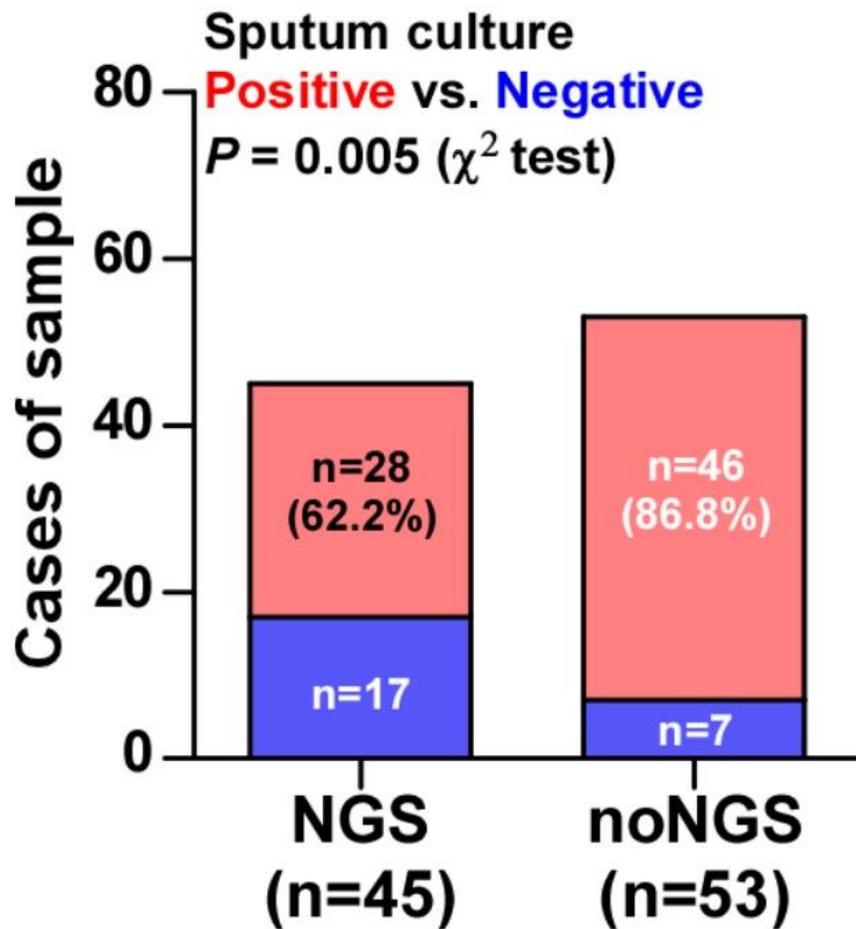
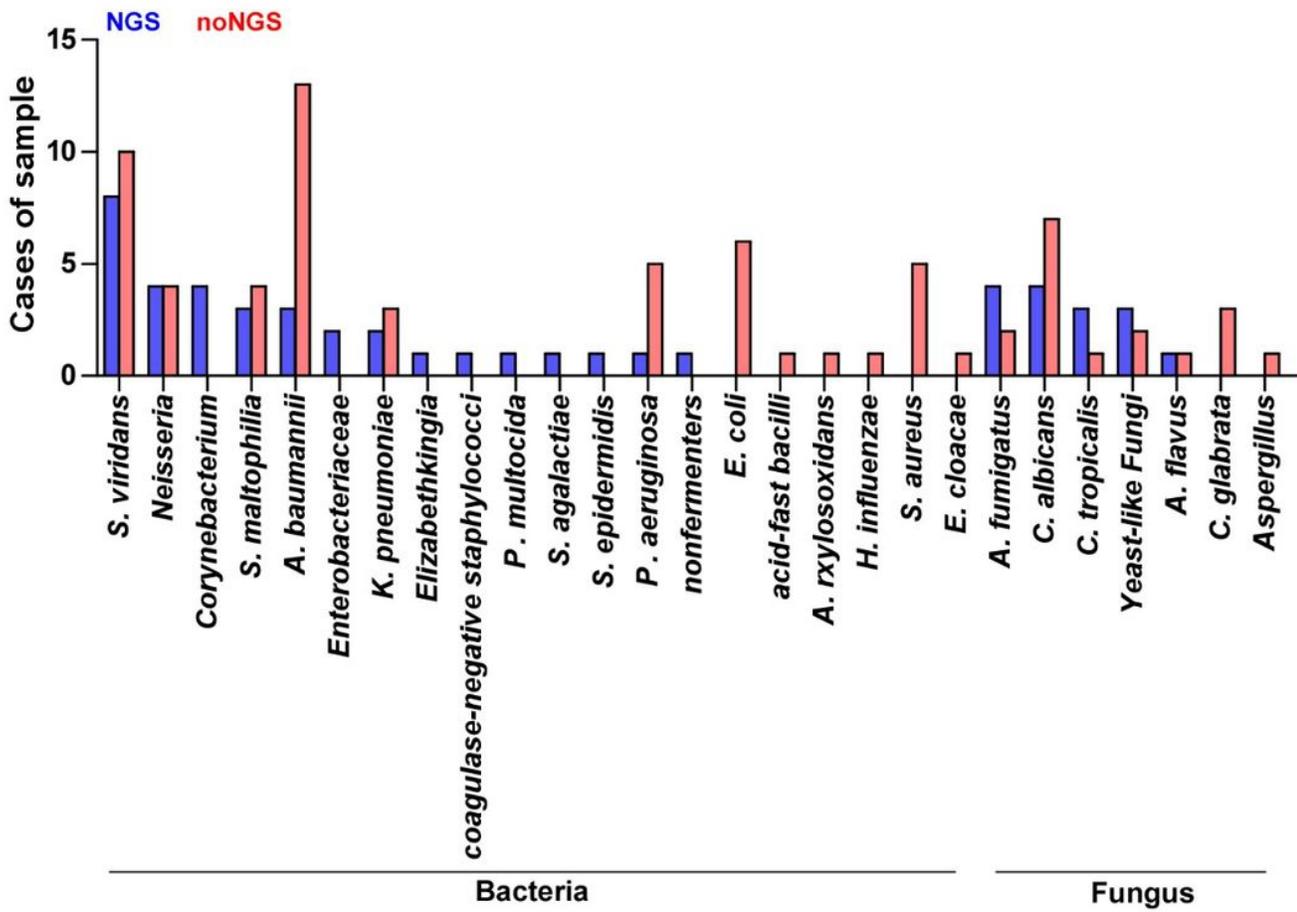


Figure 1

Sputum culture positive rate in NGS group and no-NGS group. In the NGS group, 45 cases of sputum specimens, 28 cases were culture positive, 53 cases of no-NGS group sputum specimens, 46 cultures were positive. There was a significant difference in the positive rate of sputum culture between the two groups ( $P < 0.01$ ).

**Figure 2**



**Figure 2**

Distribution of pathogens from sputum culture in the NGS group and no-NGS group. Compared with the NGS group, the no-NGS group detected more opportunistic pathogens such as *A. baumannii*, *Paeruginosa*, *E. coli*, *S. aureus* and *C. albicans*.

# Figure 3

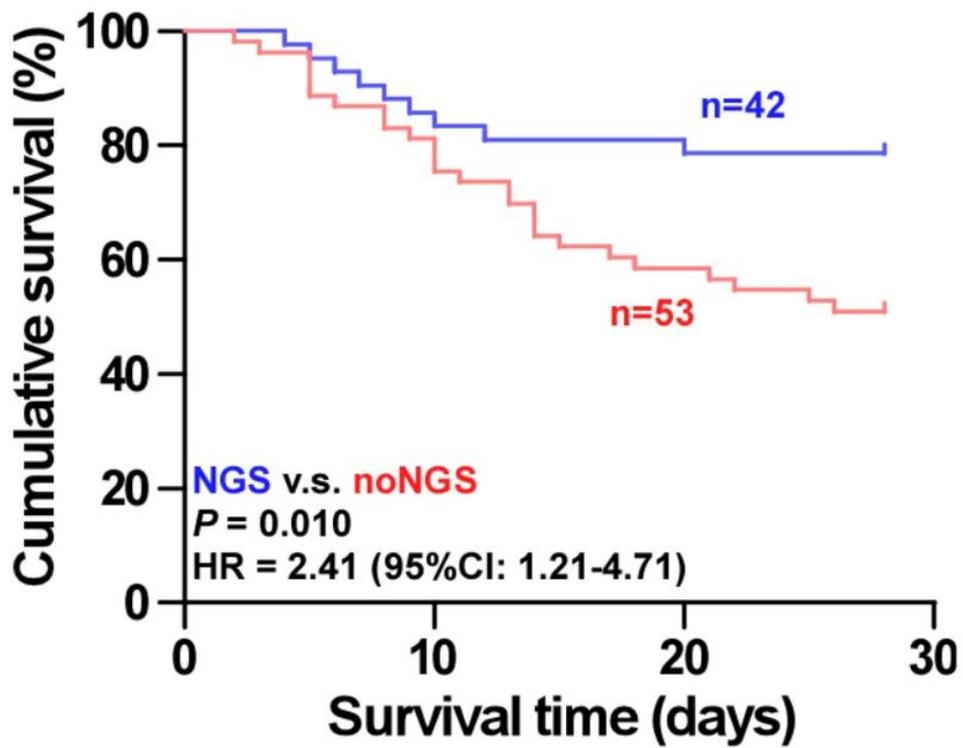
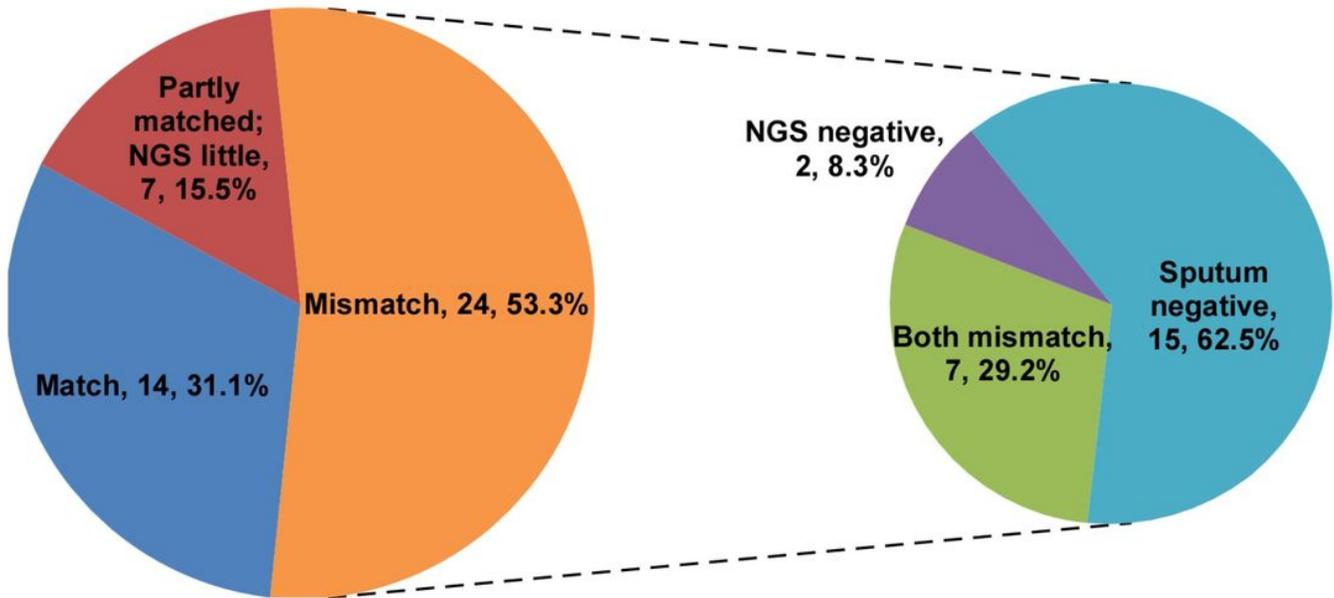


Figure 3

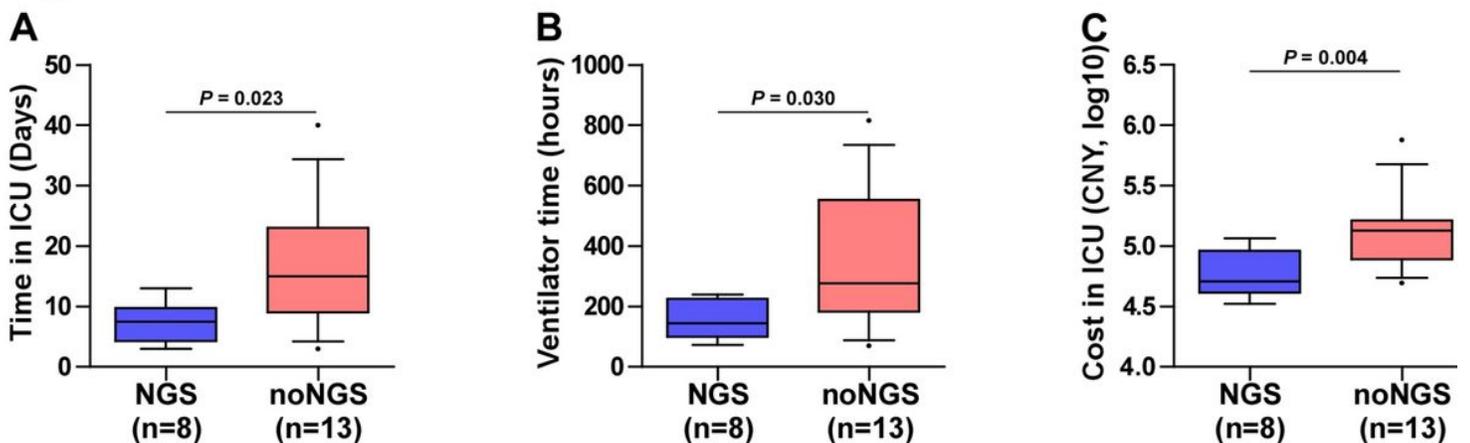
Analysis of 28-day survival curves of patients in the NGS group and no-NGS group. HR=2.41, 95%CI: 1.21-4.17,  $P < 0.05$ , was considered to have a difference in 28-day survival analysis between the two groups.



**Figure 4**

The consistency of sputum culture and mNGS pathogen detection in NGS group. In the NGS group, 31.1% of the identified pathogens were consistent, 15.5% were partially consistent, and 53.3% were completely inconsistent. Of the inconsistent samples, 62.5% were negative for sputum culture. There were 2 cases (8.3%) of the samples because the mNGS result was negative.

**Figure 5**



**Figure 5**

Clinical data of 21 immunosuppressive patients with NGS and no-NGS were compared. There was a significant differences in ICU time ( $P \leq 0.05$ ), ventilator time ( $P \leq 0.05$ ) and ICU cost ( $P \leq 0.05$ ) between the

two groups of immunosuppressed patients.