Value of metagenomic next-generation sequencing for the clinical diagnosis and prognosis of acute respiratory distress syndrome caused by severe pneumonia

Peng Zhang [1, 2], **Yan Chen** [3], **Shuyun Li** [4], **Chaoliang Li** [2], **Shuang Zhang** [2], **Weihao Zheng** [2], **Yantang Chen** [2], **Jie Ma** [2], **Xin Zhang** [5], **Yanming Huang** [6], **Shengming Liu** [Corresp. 1]

[1] Department of Respiratory Medicine, The First Affiliated Hospital of Jinan University, Guangzhou, Guangdong, China

[2] Department of Critical Care Medicine, Jiangmen Central Hospital, Affiliated Jiangmen Hospital of Sun Yat-sen University, Jiangmen, Guangdong, China

[3] BGI Genomics, BGI-Shenzhen, Shenzhen, Guangdong, China

[4] Department of Neurology, Jiangmen Central Hospital, Affiliated Jiangmen Hospital of Sun Yat-sen University, Jiangmen, Guangdong, China

[5] Clinical Experimental Center, Jiangmen Central Hospital, Affiliated Jiangmen Hospital of Sun Yat-sen University, Jiangmen, Guangdong, China

[6] Department of Respiration Medicine, Jiangmen Central Hospital, Affiliated Jiangmen Hospital of Sun Yat-sen University, Jiangmen, Guangdong, China

Corresponding Author: Shengming Liu
Email address: smliu01@163.com

Background Metagenomic next-generation sequencing (mNGS) is a valuable diagnostic tool used to detect a broad range of pathogens in respiratory infections and severe pneumonia resulting in an earlier diagnosis of these diseases. However, little is known about the value of mNGS for the diagnosis and prognosis of acute respiratory distress syndrome (ARDS) caused by severe pneumonia. Methods We performed a retrospective cohort study of patients with ARDS caused by severe pneumonia. Samples were collected from patients in the intensive care unit (ICU) of Jiangmen Central Hospital from January 2018 to August 2019. The control group (no-NGS group) was composed of patients given conventional microbiological tests to examine sputum, blood, or bronchoalveolar lavage fluid (BALF). The case group (NGS group) was composed of patients tested using mNGS. We evaluated the etiological diagnostic effect and clinical prognostic value of mNGS in patients with ARDS caused by severe pneumonia. Results 42 (44.2%) NGS and 53 (55.8%) no-NGS patients were evaluated. The mortality rate of the NGS group was significantly lower than that of the no-NGS group (21.4% VS 49.1%, $P < 0.05$). The metagenomics NGS positivity rate was higher than that of the serological antibody test plus polymerase chain reaction (PCR) and sputum culture (91.1%, 28.9%, and 62.2% respectively). The pathogens detected by mNGS in the NGS group correlated with those detected by the sputum cultures with a consistency of 31.1%. The majority (62.5%) of the inconsistencies in detecting the pathogen were caused by a negative sputum culture. We compared the clinical data of immunosuppressive patients in the two groups and found that the length of stay in the ICU ($P < 0.01$), the duration of mechanical ventilation ($P < 0.05$), and the cost

of the ICU stay ($P < 0.01$) in the NGS group were significantly lower than those in the no-NGS group. Conclusion mNGS is valuable tool to determine the etiological value of ARDS caused by severe pneumonia to improve the diagnostic accuracy and prognosis for this disease. 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 and guide the use of medications.

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[1] Department of Respiratory Medicine, The First Affiliated Hospital of Jinan University, Guangzhou, Guangdong, China.

[2] Department of Critical Care Medicine, Jiangmen Central Hospital, Affiliated Jiangmen Hospital of Sun Yat-sen University, Jiangmen, Guangdong, China.

[3] BGI Genomics, BGI-Shenzhen, Shenzhen, China.

[4] Department of Neurology, Jiangmen Central Hospital, Affiliated Jiangmen Hospital of Sun Yat-sen University, Jiangmen, Guangdong, China.

[5] Clinical Experimental Center, Jiangmen Central Hospital, Affiliated Jiangmen Hospital of Sun Yat-sen University, Jiangmen, Guangdong, China.

[6] Department of Respiration Medicine, Jiangmen Central Hospital, Affiliated Jiangmen Hospital of Sun Yat-sen University, Jiangmen, Guangdong, China.

*Corresponding Author:

Shengming Liu[1*]

Department of Respiratory Medicine, The First Affiliated Hospital of Jinan University, Guangzhou, Guangdong 510630, China.

E-mail: smliu01@163.com.

Abstract

Background

Metagenomic next-generation sequencing (mNGS) is a valuable diagnostic tool used to detect a broad range of pathogens in respiratory infections and severe pneumonia resulting in an earlier diagnosis of these diseases. However, little is known about the value of mNGS for the diagnosis and prognosis of acute respiratory distress syndrome (ARDS) caused by severe pneumonia.

Methods

We performed a retrospective cohort study of patients with ARDS caused by severe pneumonia. Samples were collected from patients in the intensive care unit (ICU) of Jiangmen Central Hospital from January 2018 to August 2019. The control group (no-NGS group) was composed of patients given conventional microbiological tests to examine sputum, blood, or bronchoalveolar lavage fluid (BALF). The case group (NGS group) was composed of patients tested using mNGS. We evaluated the etiological diagnostic effect and clinical prognostic value of mNGS in patients with ARDS caused by severe pneumonia.

Results

42 (44.2%) NGS and 53 (55.8%) no-NGS patients were evaluated. The mortality rate of the NGS group was significantly lower than that of the no-NGS group (21.4% VS 49.1%, $P < 0.05$). The metagenomics NGS positivity rate was higher than that of the serological antibody test plus polymerase chain reaction (PCR) and sputum culture (91.1%, 28.9%, and 62.2% respectively). The pathogens detected by mNGS in the NGS group correlated with those detected by the sputum cultures with a consistency of 31.1%. The majority (62.5%) of the inconsistencies in detecting the pathogen were caused by a negative sputum culture. We compared the clinical data of immunosuppressive patients in the two groups and found that the length of stay in the ICU ($P < 0.01$), the duration of mechanical ventilation ($P < 0.05$), and the cost of the ICU stay ($P < 0.01$)

50 in the NGS group were significantly lower than those in the no-NGS group.

51 **Conclusion**

52 mNGS is valuable tool to determine the etiological value of ARDS caused by severe
53 pneumonia to improve the diagnostic accuracy and prognosis for this disease. For patients with
54 severe disease, immunosuppression, or cases that cannot be diagnosed by routine methods,
55 mNGS technology can be used to provide more diagnostic evidence and guide the use of
56 medications.
57

Introduction

Acute respiratory distress syndrome (ARDS) is typically caused by an infection, such as pneumonia, and is one of the main causes of death in critically ill patients. Approximately 31%[1] of patients with ARDS were admitted to the intensive care unit (ICU) with a mortality rate of 19.7%-57.7%[2]. 10% of patients in the ICU and 23% of patients on mechanical ventilation had ARDS[3]. The mortality rate of patients with severe ARDS was 46%[3]. ARDS survivors are at greater risk of cognitive decline, depression, post-traumatic stress disorder, and persistent skeletal muscle weakness[4, 5]. Pneumonia brought about by various pathogens[6] may develop into ARDS, leading to multiple organ failure and death. Early pathogen detection is important for the treatment of ARDS caused by severe pneumonia.

The treatment guidelines[7] for ARDS focus on controlling the primary disease, initiating respiratory support therapy, and managing drug therapy. Respiratory support therapy includes sedation and analgesia, protective ventilation, lung reactivation, high positive end-expiratory pressure (PEEP), prone ventilation, high frequency oscillating ventilation, and extracorporeal membrane oxygenation (ECMO). The primary goal of respiratory support therapy is to minimize damage to the lung cells and to avoid the release of additional inflammatory mediators to provide sufficient time for treatment and lung recovery. Early pathogen detection and treatment is critical for patients with ARDS to prevent pathogen-induced pneumonia[6].

Pathogens from bacteria and fungi are typically detected using cultures[8]. This method is not restricted by the pathogen content and can be used to identify and test for drug susceptibility[9], however, there is only a 15-20% detection rate with a long turnover time for the cultures (3-5 days). Nucleic acid hybridization and PCR are highly sensitive, specific tests used to detect pathogenic nucleic acid fragments in viruses, mycoplasma pneumonia, chlamydia, legionella, and other pathogens that are difficult to culture. However, primers should be designed for pathogens and detection types are limited. There is a limited sensitivity in the serological antibody test and there is a specific period during which antibody detection may be successful.

Metagenomics next generation sequencing was first used to diagnose a central nervous system infection of leptospira in 2014[10]. This emerging diagnostic method can quickly detect all nucleic acids in the samples[11, 12] and specimen types from different infection sites including the blood, respiratory tract, central nervous system (CNS), and abscesses. The pathogenic infection characteristics of ARDS caused by severe pneumonia are relatively unknown. The prognostic value of next-generation sequencing in severe pneumonia-induced ARDS has not been well studied. The purpose of this study was to retrospectively evaluate the value of mNGS technology in the diagnosis and clinical prognosis of ARDS caused by severe pneumonia.

Materials & Methods

Ethics approval and consent to participate

The protocol 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 patients or their next of kin.

Study Patients

A retrospective analysis was conducted on all cases of ARDS resulting from severe pneumonia in patients 18 years and older who were admitted to the ICU at Jiangmen Central Hospital from January 2018 to August 2019. For the purposes of our study, ARDS needed to be caused by severe pneumonia and was diagnosed according to the 2012 Berlin definition of the disease[13]. Patients were excluded from the study if their ARDS was not caused by severe pneumonia or they did not follow through with treatment for any reason.

All patients were endotracheally intubated and mechanically ventilated, and fiberoptic bronchoscopy was used to obtain microbial specimens. The mNGS test is typically seen as an optional test with a high cost, despite its use in our study hospital since 2018. Patients were included in the NGS group when relatives signed the informed consent and were willing to test; those who were not tested were grouped in the no-NGS group. Specimens from the two groups

were collected using bronchoscopy and were tested using conventional microbiological tests following a diagnosis of ARDS caused by severe pneumonia. Samples of bronchoalveolar lavage fluid (BALF) were taken from patients in the NGS group for pathogen testing at the BGI Clinical Laboratories (Shenzhen) Co., Ltd.

Clinical treatment

All patients used empirical antimicrobial treatment according to the Chinese Adult Community Acquired Pneumonia Diagnosis and Treatment Guide[14] and the Chinese Adult Hospital Acquired Pneumonia and Ventilator-associated Pneumonia Diagnosis Guide[15], and combined with infection indicators and imaging information. All patients were treated with mechanical ventilation according to the ARDS ventilation guidelines[11]. The no-NGS patients were treated with an antibacterial regimen based on the results of conventional microbiological tests. The NGS patients adjusted the antibacterial regimen according to the NGS results.

Information collection and analysis

Patient data including age, gender, disease status, laboratory test results before treatment, ventilator parameters, conventional microbiological tests, serum biomarkers, ICU special treatment data, APACHE II and SOFA scores. Data were collected and compared between the two groups. The primary outcome was measured by a 28-day all-cause mortality. The secondary outcome was measured as length of stay in the ICU, duration of mechanical ventilation, duration of ECMO, duration of prone position ventilation, and ICU treatment costs. Patients showing signs of immunosuppression were selected from both groups and their prognosis was compared using the same aforementioned outcomes. Cox regression analysis was conducted to analyze the risk factors for the prognosis of ARDS. The mNGS results were compared with the results from conventional microbiological tests in the NGS group.

Statistical Analysis

The t-test was used to determine normal distribution and uniformity of the variance. The Wilcoxon rank test was used to calculate the variance of the measured data that was not normally distributed or had homogeneity of variance. The chi-square test was used to calculate the difference between the two groups. All statistical analyses were conducted using GraphPad 5.0 and R3.4.4 software. $P < 0.05$ was considered statistically significant.

Results

Sample and patient characteristics

105 patients with ARDS caused by severe pneumonia were screened in this study and 10 patients were excluded based on exclusion criteria. 42 patients were placed in the NGS group and 53 patients were placed in the no-NGS group. 3 patients in the NGS group had two mNGS tests and a total of 45 BALF samples were sent for mNGS.

Patients demographics, characteristic baselines, and ICU special treatment in the NGS and no-NGS groups are shown in Tables 1, 2, and 3, respectively. There were no significant differences in age, sex ratio, disease status, laboratory examination, ventilator parameters, APACHE II and SOFA scores before treatment, and incidences of special treatment in the ICU between the two groups ($P > 0.05$).

Comparison outcomes between NGS and no-NGS groups

There was a significant difference in 28-day all-cause mortality between the two groups (P=0.006) (Table 4). The 28-day survival was significantly higher in the NGS group than in the no-NGS group (HR=2.41, 95% CI: 1.21-4.17, P =0.01) (Fig. 1). There was no significant difference in the length of stay in the ICU, the duration of mechanical ventilation, ECMO, prone position ventilation, or the cost of the ICU stay between the two groups ($P > 0.05$) (Table 4).

Prognosis of ARDS patients

Cox univariate analysis was performed on all factors and cox multivariate analysis was performed on the indexes when P<0.2 of the cox univariate analysis. The NGS or no-NGS group, length of stay in ICU, and APACHE II and SOFA scores before treatment were the risk factors in patients with ARDS caused by severe pneumonia. The NGS group had a better prognosis than no-NGS group (P =0.005). Those with a shorter stay in the ICU (P =0.037), and lower APACHE II before treatment (P =0.016) and SOFA scores before treatment (P =0.003) had a better prognosis (Table 5).

Comparison of mNGS results and culture results in the NGS group

Current research shows that mNGS testing is able to detect more pathogens than cultures. We analyzed the consistency of pathogens identified by both techniques. The test results were considered to be consistent when the pathogens identified by mNGS were the same as the pathogens obtained from sputum culture. The test results were also considered to be consistent if mNGS identified more pathogens than the culture method. The result was partially consistent when the pathogens identified by two methods were partially congruent. The result was considered to be inconsistent when the pathogens identified by the two methods varied completely. 31.1% of the identified pathogens in the NGS group were consistent, 15.6% were partially consistent, and 53.3% were completely inconsistent. In the inconsistent samples, 62.5% were negative for sputum culture, while 8.3% were negative for the mNGS results, and 29.2% were mismatched (Figure 2).

Comparison metagenomics of NGS results and conventional microbiological tests

The pathogenic microorganisms that cause severe pneumonia are typically bacteria, fungi, or viruses. Some special pathogens are difficult to obtain through culture, so we defined special pathogens as: Legionella, Tuberculosis, Mycoplasma / Chlamydia, Parasites, K. spores, *etc*. Severe pneumonia is not caused by a single pathogen and is typically accompanied by co-

infections. A co-infection is defined as a non-single pathogenic infection, such as bacteria + fungi / bacteria + virus / fungi + virus / bacteria + fungi + virus.

mNGS was significantly less reliable in detecting viruses than serological antibody tests plus PCR (6.7% *vs.* 26.7%, P =0.021). mNGS in this study only detected DNA viruses from samples, however, the viruses identified by serological antibody tests plus PCR were RNA viruses, such as influenza A and influenza B. mNGS was significantly better at detecting bacteria than serological antibody testing plus PCR (24.4% *vs.* 0%, P =0.001). mNGS was able to detect specific pathogens better than sputum culture (22.2% *vs.* 0%, P =0.001) and serological antibody testing plus PCR (22.2% *vs.* 2.2%, P =0.007). mNGS was significantly better at the identification of co-infections than serological antibody tests plus PCR (26.7% *vs.* 0%, $P < 0.001$). mNGS proved to be significantly better at identifying pathogens than sputum culture (91.1% VS 62.2%, P =0.001) and serological antibody testing plus PCR (91.1% *vs.* 28.9%, $P < 0.001$) (Table 6).

Clinical medication guidance based on mNGS results

In the NGS group, based on mNGS results, thirty patients (71.4%) were treated with empirical antibiotics that did not cover the whole pathogen. These patients adjusted the antibacterial regimen according to the mNGS results (Figure 3).

Immunosuppressive patients

The clinical features of the immunosuppressed patients were complicated. 21 immunosuppressed patients were enrolled in our study, 8 were subjected to metagenomic NGS pathogen detection, and 13 did not undergo mNGS. 3 sputum cultures were positive in the NGS group, consistent with the pathogens identified by mNGS, including 5 *P. jirovecii*, 1 *Rhizopus*, 1 *Cryptococcus,* and 1 human herpesvirus. 5 *P. jirovecii* is an opportunistic pathogen causing pneumonia that leads to death in patients, especially in those with low immune function, such as HIV-infected patients, those with tissue organ transplants, or those undergoing cancer radiotherapy and chemotherapy[16]. Five patients with *P. jirovecii* were found to have nephrotic

205 syndrome, dermatomyositis, multiple myeloma, and lymphoma. In the no-NGS group, 9 cases
206 were positive for sputum culture, and 2 *S. maltophilia*, 2 *A. baumannii*, 1 *S. aureus*, 4 *Candida,*
207 and 1 *Aspergillus* were detected. 4 cases had multi-drug resistant bacteria. There was no
208 significant difference in the 28-day all-cause mortality between the two groups ($P > 0.05$).
209 However, there were significant differences in the length of stay in the ICU ($P = 0.023$), the
210 duration of mechanical ventilation ($P = 0.030$), and the cost of stay in the ICU ($P = 0.004$)
211 between the two groups of immunosuppressed patients (Figure 4).

212 ## Discussion

213 We explored the value of using mNGS for the etiological diagnosis and prognosis of ARDS
214 caused by severe pneumonia and found that there were significant physiological indicators
215 between the NGS and no-NGS groups. The mortality of the NGS group was significantly lower
216 than that of the no-NGS group *(*$P > 0.05$), and the 28-day survival rate was significantly higher
217 than that of the no-NGS group ($P < 0.05$). There were no differences between the two groups in
218 the length of stay in the ICU, duration of mechanical ventilation, duration of ECMO, duration of
219 prolonged ventilation time, and cost in of treatment in the ICU. Our results were consistent with
220 previous studies by Wang[17], who analyzed 178 patients with severe pneumonia and confirmed
221 their diagnosis using mNGS, which increased the 90-day survival rate from 57.7% to 83.3%.
222 This study showed that there was no increase in the ICU cost and that the cost of
223 immunosuppressed patients with mNGS detection in the ICU was lower than that of patients
224 without mNGS detection.

225 Compared with conventional microbiological tests, the mNGS method in this study had no
226 obvious advantages for identifying simple bacteria, fungi and viruses, but was incredibly reliable
227 for detecting special pathogens and patients with co-infections. mNGS quickly detected
228 pathogenic microorganisms and improved treatment accuracy. Immunocompromised patients or
229 those in critical condition were prone to co-infections and the mNGS method had obvious
230 advantages in detecting pathogens in such patients. mNGS detected pathogenic bacteria in

immunosuppressed patients that was difficult to culture, including P.jejuni, Rhizopus, Cryptococcus, and human herpesvirus 5. The NGS group had a lower mortality rate than the no-NGS group, however, this difference was not statistically significant (3/8 *vs.*7/13, P=0.659), which was likely due to the small sample size. The mNGS method could significantly reduce the length of stay in ICU, the duration of ventilation, and the cost of stay in the ICU for immunosuppressed patients (P<0.05).

ARDS caused by severe viral pneumonia is a serious condition with a rapid onset. It is easy to develop from a virus infection to co-infection, and immunosuppressed patients are prone to concurrent viral infections. In the NGS group of patients diagnosed with viral pneumonia, there were 17 patients with bacterial or fungal, or bacterial and fungal infections. It is important to adjust the treatment regimen according to mNGS results and clinical indicators. All 6 cases of severe viral pneumonia in the NGS group were successfully treated with ECMO. mNGS is successful at detecting specific pathogens and a large-scale retrospective study conducted by Hu[18] found that mNGS sensitivity was greater than routine cultures and was better at detecting TB, fungi, viruses, and anaerobic bacteria. The effect of prior antibiotic used on mNGS was smaller than that of routine culture. Parize[19] found that mNGS was valuable for detecting pathogens in immunosuppressed patients. The positive rate virus and bacterial identification by mNGS was 3 times greater than routine methods. mNGS had a greater negative predictive value than routine methods.

There are limitations to the use of mNGS technology, despite its widespread use. There is no authoritative guide to the interpretation of the results of mNGS. The detection of a broad spectrum of pathogens by mNGS has caused problems in the diagnosis of pathogenicity of clinical pathogens with an inability to distinguish between background, colonization and pathogenic bacteria, and pollution. Better technology needs to be developed for mNGS to be used successfully in clinical applications.

mNGS lacks standardized technology, databases, and interpretation of results. mNGS

technology is not a substitute for conventional microbiological tests, however, in patients with severe disease, rapid disease progression, immunosuppression, or cases that cannot be diagnosed by conventional methods, it can be used to provide more evidence for clinical diagnoses and to guide the use of medications. The use of mNGS in clinical applications will: (1) achieve a faster diagnosis of pathogens and obtain information on the drug resistance of related pathogens; (2) identify microbial colonization or infection through monitoring the patient's immune response, which will eventually curb bacterial resistance, achieve a rational application of antibiotics, and ultimately reduce the economic and social burden of infectious diseases; (3) lower the cost of the mNGS test with the development of technology so that more patients benefit.

Our study was limited and the clinical prognosis was affected by many factors. The single-factor and multi-factor analysis of the clinical prognosis of ARDS caused by severe pneumonia found that a long ICU stay, high APACHE II score, and high SOFA score are risk factors for death related to ARDS. The use of mNGS to detect pathogens was protective against death from ARDS. Further studies should include a larger sample size involving a multi-center clinical, prospective, controlled study, which will help us better understand the prognostic value of NGS testing for ARDS caused by severe pneumonia.

Conclusion

mNGS technology is valuable for the treatment and prognosis of ARDS caused by severe pneumonia. mNGS technology is superior to conventional microbiological tests for the detection of special pathogens and co-infections. 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 an accurate diagnosis and to guide proper treatment.

Declarations

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). The data showed 95 patients with ARDS caused by severe pneumonia.

Competing interests

Yan Chen is an employee of BGI Genomics. All authors declare that they have no competing interests.

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Authors' contributions

All authors had accessed 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. All authors have read and approved the final manuscript.

Shengming Liu and Yanming Huang conceived and designed the study. They reviewed and approved the final report.

Peng Zhang, Yan Chen, Shuyun Li, Chaoliang Li, Shuang Zhang, Weihao Zheng, Yantang Chen, Jie Ma, Xin Zhang were involved in the case and sample collection, analysis, interpretation of the data and wrote the first draft of the paper.

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References

[1] Griffiths M J D, McAuley D F, Perkins G D*, et al.* Guidelines on the management of acute respiratory distress syndrome [J]. BMJ Open Respir Res, 2019, 6 (1): e000420.

[2] Bein T, Grasso S, Moerer O*, et al.* The standard of care of patients with ARDS: ventilatory settings and rescue therapies for refractory hypoxemia [J]. Intensive Care Med, 2016, 42 (5): 699-711.

[3] Bellani G, Laffey J G, Pham T*, et al.* Epidemiology, Patterns of Care, and Mortality for Patients With Acute Respiratory Distress Syndrome in Intensive Care Units in 50 Countries [J]. JAMA, 2016, 315 (8): 788-800.

[4] Herridge M S, Moss M, Hough C L*, et al.* Recovery and outcomes after the acute respiratory distress syndrome (ARDS) in patients and their family caregivers [J]. Intensive Care Med, 2016, 42 (5): 725-738.

[5] Herridge M S, Tansey C M, Matte A*, et al.* Functional disability 5 years after acute respiratory distress syndrome [J]. N Engl J Med, 2011, 364 (14): 1293-1304.

[6] Lee K Y. Pneumonia, Acute Respiratory Distress Syndrome, and Early Immune-Modulator Therapy [J]. Int J Mol Sci, 2017, 18 (2).

[7] Fan E, Del Sorbo L, Goligher E C*, et al.* An Official American Thoracic Society/European Society of Intensive Care Medicine/Society of Critical Care Medicine Clinical Practice Guideline: Mechanical Ventilation in Adult Patients with Acute Respiratory Distress Syndrome [J]. Am J Respir Crit Care Med, 2017, 195 (9): 1253-1263.

[8] Rajapaksha P, Elbourne A, Gangadoo S*, et al.* A review of methods for the detection of pathogenic microorganisms [J]. Analyst, 2019, 144 (2): 396-411.

[9] Simner P J, Miller S, Carroll K C. Understanding the Promises and Hurdles of Metagenomic Next-Generation Sequencing as a Diagnostic Tool for Infectious Diseases [J]. Clin Infect Dis, 2018, 66 (5): 778-788.

[10] Wilson M R, Naccache S N, Samayoa E*, et al.* Actionable diagnosis of neuroleptospirosis by next-generation sequencing [J]. N Engl J Med, 2014, 370 (25): 2408-2417.

[11] Ai J W, Zhang H C, Cui P*, et al.* Dynamic and direct pathogen load surveillance to monitor disease progression and therapeutic efficacy in central nervous system infection using a novel semi-quantitive sequencing platform [J]. J Infect, 2018, 76 (3): 307-310.

[12] Guan H, Shen A, Lv X*, et al.* Detection of virus in CSF from the cases with meningoencephalitis by next-generation sequencing [J]. J Neurovirol, 2016, 22 (2): 240-245.

[13] Ards Definition Task Force O, Ranieri V M, Rubenfeld G D*, et al.* Acute respiratory distress syndrome: the Berlin Definition [J]. JAMA, 2012, 307 (23): 2526-2533.

[14] Respiratory Branch of Chinese Medical Association O. Chinese Adult Community Acquired Pneumonia Diagnosis and Treatment Guide (2016 year version) [J]. Chinese

Journal of Tuberculosis and Respiratory Diseases, 2016, 39 (4): 253-279.
[15] Department of Infectious Diseases Chinese Medical Association Respiratory Branch O. Chinese Adult Hospital Acquired Pneumonia and Ventilator-associated Pneumonia Diagnosis and Treatment Guide (2018 Edition) [J]. Chinese Journal of Tuberculosis and Respiratory Diseases, 2018, 41 (4): 255-280.
[16] Sokulska M, Kicia M, Wesolowska M*, et al.* Pneumocystis jirovecii--from a commensal to pathogen: clinical and diagnostic review [J]. Parasitol Res, 2015, 114 (10): 3577-3585.
[17] Xie Y, Du J, Jin W*, et al.* Next generation sequencing for diagnosis of severe pneumonia: China, 2010-2018 [J]. J Infect, 2019, 78 (2): 158-169.
[18] Miao Q, Ma Y, Wang Q*, et al.* Microbiological Diagnostic Performance of Metagenomic Next-generation Sequencing When Applied to Clinical Practice [J]. Clin Infect Dis, 2018, 67 (suppl_2): S231-S240.
[19] Parize P, Muth E, Richaud C*, et al.* Untargeted next-generation sequencing-based first-line diagnosis of infection in immunocompromised adults: a multicentre, blinded, prospective study [J]. Clin Microbiol Infect, 2017, 23 (8): 574 e571-574 e576.

FIGURE LEGENDS

Figure 1. **Analysis of 28-day survival curves of patients in the NGS group and no-NGS group.** The 28-day survival was significantly higher in the NGS group than in the no-NGS group (HR=2.41, 95% CI: 1.21-4.17, P =0.01)

Figure 2. **The consistency of sputum culture and mNGS pathogen detection in NGS group.** 31.1% of the identified pathogens in the NGS group were consistent, 15.6% were partially consistent, and 53.3% were completely inconsistent. In the inconsistent samples, 62.5% were negative for sputum culture, while 8.3% were negative for the mNGS results, and 29.2% were mismatched**.**

Figure 3. **Pathogen coverage of empirical antibiotic therapy in the NGS group.** In the NGS group, based on mNGS results, thirty patients (71.4%) were treated with empirical antibiotics that did not cover the whole pathogen. These patients adjusted the antibacterial regimen according to the mNGS results.

Figure 4. **Clinical data of 21 immunosuppressive patients with NGS and no-NGS were compared.** There were significant differences in the length of stay in the ICU (P =0.023), the

375 duration of mechanical ventilation (P =0.030), and the cost of stay in the ICU (P =0.004)
376 between the two groups of immunosuppressed patients.

Table 1(on next page)

Patient characteristics and baseline of two groups.

There were no any differences in age, sex ratio, basis disease between two groups ($P > 0.05$).

Table 1. Patient characteristics and baseline of two groups.

	NGS (n=42)	no-NGS (n=53)	*P* value
Age (yr)			
≥ 60, n (%)	21 (50.0)	33 (62.3)	0.231
< 60, n (%)	21 (50.0)	20 (37.7)	
Gender			
Male, n (%)	31 (73.8)	38 (71.7)	0.819
Female, n (%)	11 (26.2)	15 (28.3)	
Basis disease			
Hypertension, n (%)	13 (31.0)	17 (32.1)	0.907
Coronary heart disease, n (%)	3 (7.1)	5 (9.4)	0.690
COPD, n (%)	10 (23.8)	17 (32.1)	0.375
Chronic nephrosis, n (%)	7 (16.7)	6 (11.3)	0.452
Diabetes, n (%)	5 (11.9)	9 (17.0)	0.488
Immunosuppression, n (%)	8 (19.0)	13 (24.5)	0.523
Tumor, n (%)	10 (23.8)	11 (20.8)	0.722
Smoking, n (%)	20 (47.6)	17 (32.1)	0.123
Drinking, n (%)	4 (9.5)	5 (9.4)	0.988

Note: chi-square test was utilized to calculate the difference between two groups. $P < 0.05$ was considered statistically significant. Abbreviations: COPD: chronic obstructive pulmonary disease.

Table 2(on next page)

Laboratory examination, Ventilator parameters, APACHE II score and SOFA score before treatment of two groups.

There were no any differences in laboratory examination, ventilator parameters, APACHE II score and SOFA score before treatment between two groups ($P > 0.05$).

Table 2. Laboratory examination before treatment, Ventilator parameters, APACHE II score and SOFA score before treatment of two groups.

	NGS (n=42)	no-NGS (n=53)	*P* value
Laboratory examination before treatment			
PCT (ug/L)	1.3 (0.5, 8.4)	2.5 (0.3, 10.6)	0.516
WBC (10^9/L)	10.5 (6.4, 15.4)	13.1 (7.5, 15.5)	0.189
Hb (g/L)	109 (85, 130)	105 (84, 129)	0.932
PLT (10^9/L)	159 (84, 205)	154 (112, 197)	0.780
Scr (μmol/L)	78 (64, 201)	97 (64, 121)	0.515
T.Bil (mmol/L)	11.8 (5.2, 17.2)	14.4 (7.8, 21.1)	0.071
ALT (IU/L)	28 (20, 47)	27 (20, 45)	0.612
Alb (g/L)	28.0 (23.6, 31.6)	28.2 (24.8, 32.6)	0.880
APTT (sec)	35.6 (31.0, 44.7)	34.7 (26.4, 48.1)	0.614
NT-proBNP (pg/ml)	652 (236, 2747)	656 (311, 2066)	0.482
Lac (mmol/L)	1.6 (1.4, 2.9)	1.7 (1.2, 2.5)	0.763
Ventilator parameters			
FiO_2	0.8 (0.6, 1.0)	0.6 (0.5, 0.8)	0.992
Peep	10 (8, 15)	8 (6, 12)	0.272
OI	124 (76, 177)	156 (108, 194)	0.996
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

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. Abbreviations: PCT: Procalcitonin; WBC: White blood cell; Hb: Hemoglobin; PLT: Platelet count; Scr: Serum creatinine; T.Bil: Total bilirubin; ALT : Alanine aminotransferase; Alb: Albumin; APTT: Activated partial thromboplastin time; NT-proBNP: N-terminal Pro-Brain Natriuretic Peptide;

Lac: Lactate; FiO2: Fraction of inspiration O2; Peep: positive end expiratory pressure; OI: Oxygenation Index; APACHE-II: Acute physiology and chronic health evaluation-II; SOFA: Sequential Organ Failure Assessment.

Table 3(on next page)

ICU special treatment of two groups.

There were no any differences in ICU special treatment between two groups ($P > 0.05$).

Table 3. ICU special treatment of two groups.

	NGS (n=42)	no-NGS (n=53)	*P* value
Use of vasoactive agent, n (%)	24 (57.1)	30 (56.6)	0.958
CRRT, n (%)	9 (21.4)	7 (13.2)	0.288
ECMO, n (%)	6 (14.3)	3 (5.7)	0.177
Prone positioning, n (%)	10 (23.8)	11 (20.8)	0.722

Note: chi-square test was utilized to calculate the difference between two groups. $P < 0.05$ was considered statistically significant. Abbreviations: ICU: Intensive care unit; CRRT: continuous renal replacement therapy; ECMO: Extracorporeal membrane oxygenation.

Table 4(on next page)

Outcome of two groups.

The primary outcome: There was a significant difference in 28-day all-cause mortality between the two groups (P =0.006). The second outcome: There was no significant difference in the length of stay in the ICU, the duration of mechanical ventilation, ECMO, prone position ventilation, or the cost of the ICU stay between the two groups ($P > 0.05$) .

Table 4. Outcome of two groups.

	NGS (n=42)	no-NGS (n=53)	*P* value
Primary outcome			
28-day all-cause mortality	9 (21.4%)	26 (49.1%)	**0.006***
The second outcome			
Length of stay in ICU (d)	12 (7, 20)	11 (8, 15)	0.719
Duration of mechanical ventilation (h)	240 (144, 353)	216 (134, 311)	0.810
Duration of ECMO (d)	15 (11, 18)	10 (10, 23)	0.500
Duration of prone position ventilation (h)	89 (63, 117)	96 (71, 121)	0.345
Cost in ICU (thousand CNY)	82.3 (55.1, 211.1)	98.9 (68.9, 141.1)	0.297

Note: chi-square test was utilized to calculate the difference between two groups. The measured data of patients' outcomes in the above table were shown by: median (interquartile range). $P < 0.05$ was considered statistically significant.

Table 5(on next page)

Cox multivariate analysis of two groups of patients.

The NGS group had a better prognosis than no-NGS group (P =0.005). Those with a shorter stay in the ICU (P =0.037), and lower APACHE II before treatment (P =0.016) and SOFA scores before treatment (P =0.003) had a better prognosis.

Table 5. Cox multivariate analysis of two groups of patients.

	HR	Lower .95	Upper .95	*P* value
mNGS (yes/no)	0.263	0.105	0.663	**0.005***
Age (yr)	1.013	0.988	1.038	0.322
Length of stay in ICU (d)	0.888	0.794	0.993	**0.037***
APACHE Ⅱ score before treatment	1.112	1.020	1.212	**0.016***
SOFA score before treatment	1.339	1.105	1.622	**0.003***
Coronary heart disease (yes/no)	1.660	0.556	4.958	0.364
Bronchiectasis (yes/no)	1.128	0.331	3.843	0.848
Diabetes (yes/no)	0.324	0.088	1.195	0.091
Hb (g/L)	0.993	0.980	1.006	0.284
T.Bil (mmol/L)	0.999	0.987	1.012	0.882
Be	1.063	0.996	1.133	0.064
Use of vasoactive agent (yes/no)	1.443	0.587	3.548	0.424
ECMO (yes/no)	1.212	0.067	21.764	0.896
Cost in ICU (CNY)	1.000	1.000	1.000	0.477

Note: $P < 0.05$ was considered statistically significant.

Table 6(on next page)

Compare the efficiency of three methods for detecting different types of microorganisms

mNGS was significantly less reliable in detecting viruses than serological antibody tests plus PCR (6.7% *vs.* 26.7%, P =0.021). mNGS was significantly better at detecting bacteria than serological antibody testing plus PCR (24.4% *vs.* 0%, P =0.001). mNGS was able to detect specific pathogens better than sputum culture (22.2% *vs.* 0%, P =0.001) and serological antibody testing plus PCR (22.2% *vs.* 2.2%, P =0.007). mNGS was significantly better at the identification of co-infections than serological antibody tests plus PCR (26.7% *vs.* 0%, P < 0.001). mNGS proved to be significantly better at identifying pathogens than sputum culture (91.1% VS 62.2%, P =0.001) and serological antibody testing plus PCR (91.1% *vs.* 28.9%, P < 0.001)

Table 6. Compare the efficiency of three methods for detecting different types of microorganisms

□	Method A (n=45)	Method B (n=45)	Method C. (n=45)	*P* value, A vs. B	*P* value, A vs. C
Only virus, n (%)	3 (6.7)	0 (0.0)	12 (26.7)	0.24	**0.021***
Only bacterial, n (%)	11 (24.4)	15 (33.3)	0 (0.0)	0.486	**0.001***
Only fungus, n (%)	5 (11.1)	5 (50.0)	0 (0.0)	1	0.056
Special pathogen, n (%)	10 (22.2)	0 (0.0)	1 (2.2)	**0.001***	**0.007***
Co-infection, n (%)	12 (26.7)	8 (17.8)	0 (0.0)	0.311	**<0.001***
Overall Positive, n (%)	41 (91.1)	28 (62.2)	13 (28.9)	**0.001***	**<0.001***

Note: Method A: mNGS; Method B: Sputum culture; Method C: Serological antibody test plus PCR. Chi-square test was utilized to calculate the difference between two groups. $P < 0.05$ was considered statistically significant.

Figure 1

Analysis of 28-day survival curves of patients in the NGS group and no-NGS group.

The 28-day survival was significantly higher in the NGS group than in the no-NGS group (HR=2.41, 95% CI: 1.21-4.17, *P* =0.01)

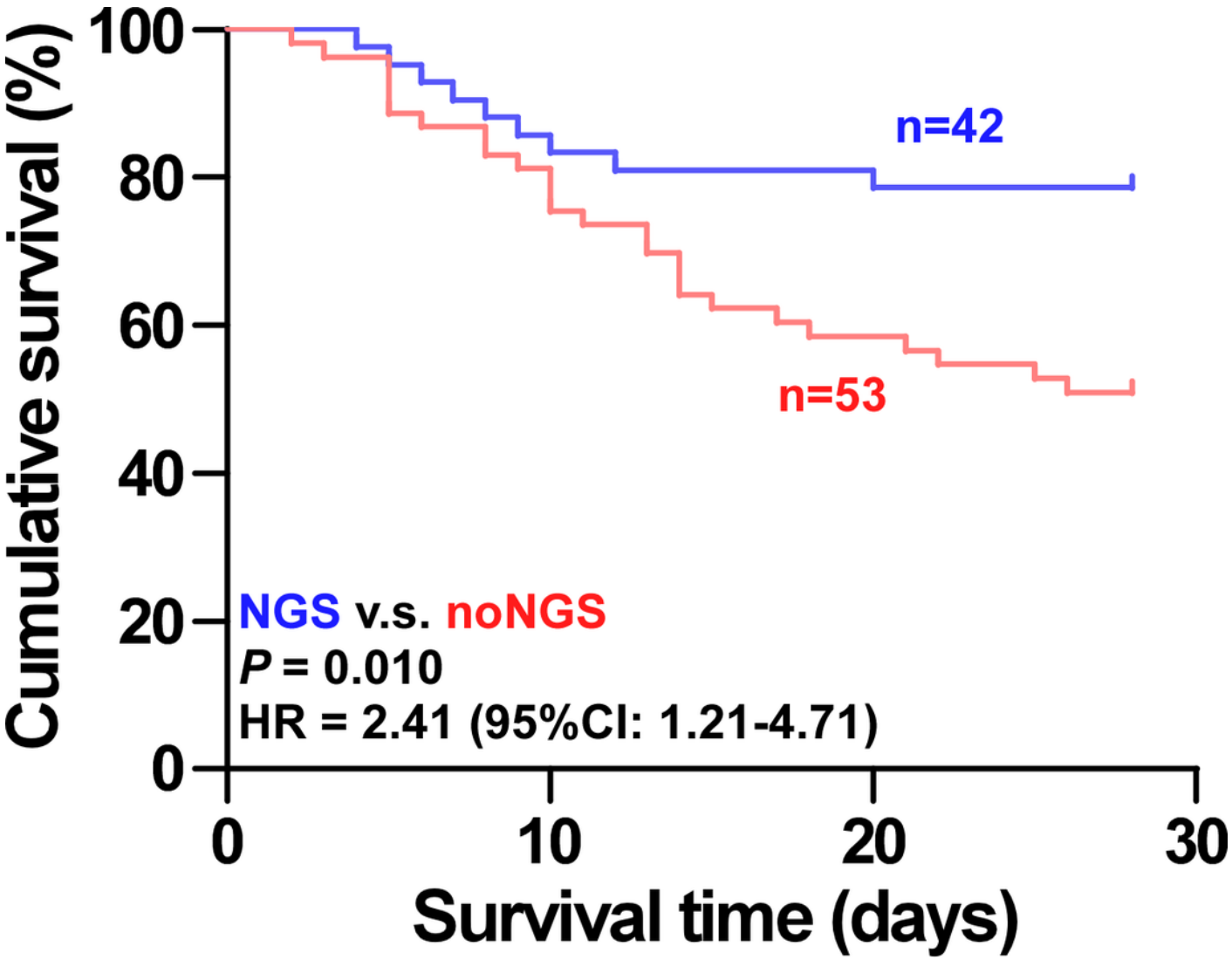

Figure 2

The consistency of sputum culture and mNGS pathogen detection in NGS group.

31.1% of the identified pathogens in the NGS group were consistent, 15.6% were partially consistent, and 53.3% were completely inconsistent. In the inconsistent samples, 62.5% were negative for sputum culture, while 8.3% were negative for the mNGS results, and 29.2% were mismatched

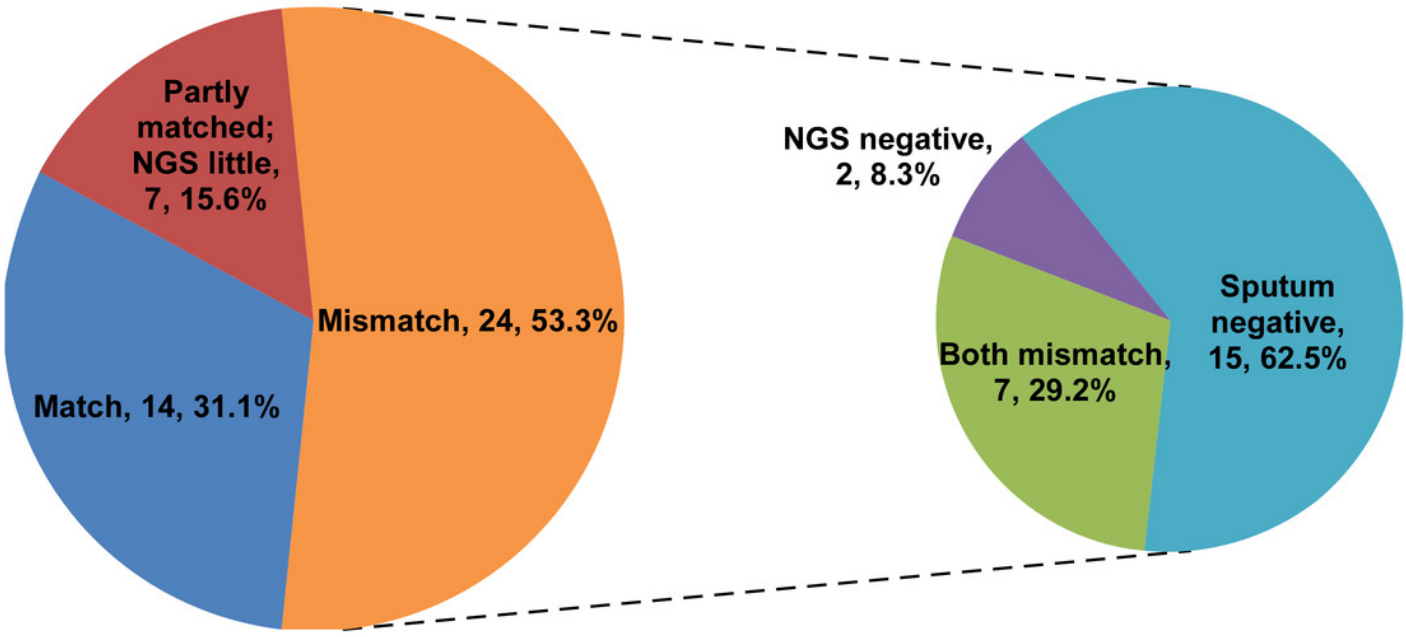

Figure 3

Pathogen coverage of empirical antibiotic therapy in the NGS group.

In the NGS group, based on mNGS results, thirty patients (71.4%) were treated with empirical antibiotics that did not cover the whole pathogen. These patients adjusted the antibacterial regimen according to the mNGS results.

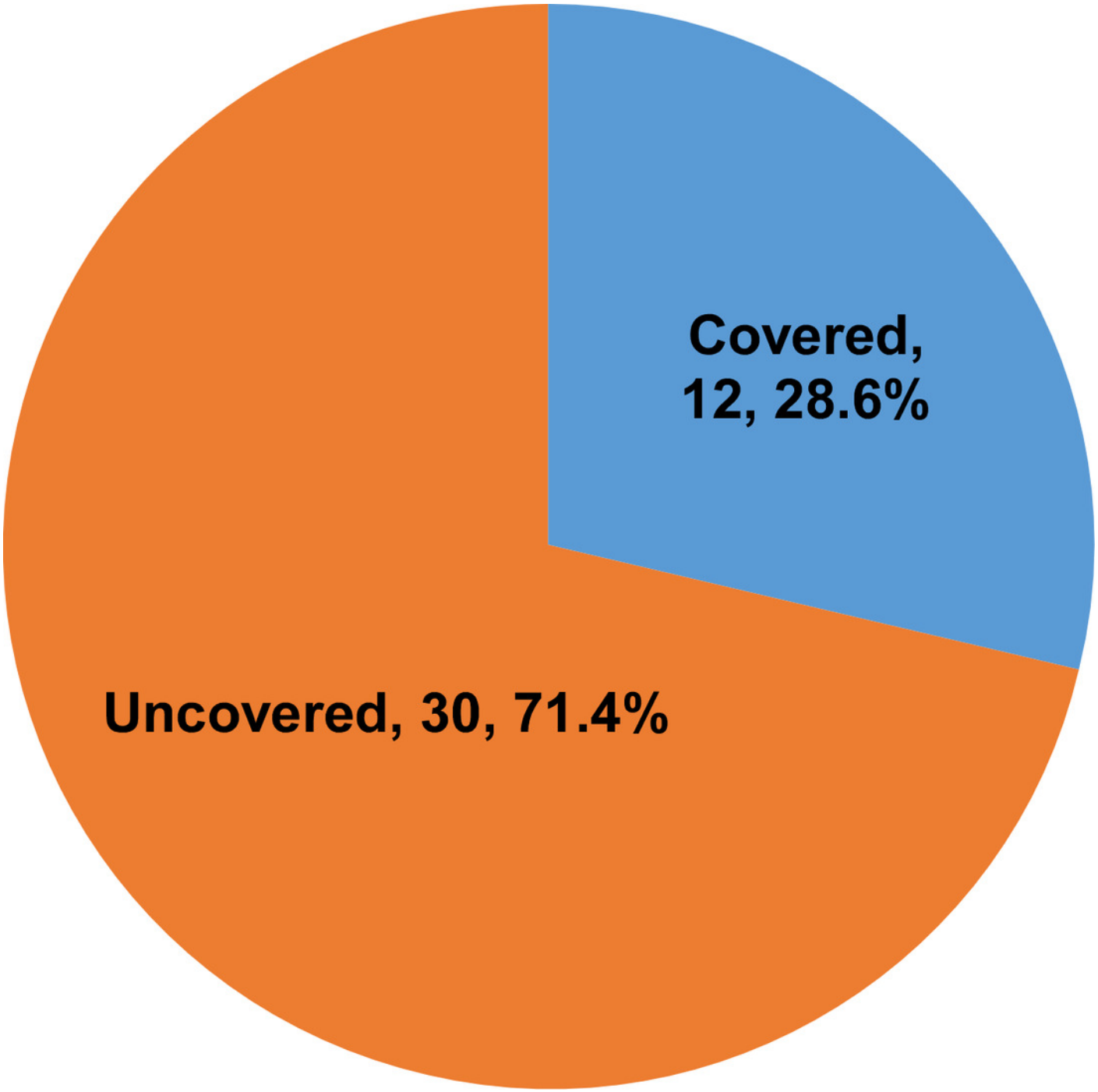
Covered, 12, 28.6%
Uncovered, 30, 71.4%

Figure 4

Clinical data of 21 immunosuppressive patients with NGS and no-NGS were compared.

There were significant differences in the length of stay in the ICU (P =0.023), the duration of mechanical ventilation (P =0.030), and the cost of stay in the ICU (P =0.004) between the two groups of immunosuppressed patients