The Clinical Impact of Metagenomic Next-Generation Sequencing (mNGS) Test in Hospitalized Patients with Suspected Sepsis: A Multicenter Prospective Study.

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Research

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Abstract

Background:

Next Generation Sequencing (NGS) is a newly developed technology and able to detect pathogens rapidly, which may have great importance in early diagnosis and clinical management of infectious diseases. Our study aimed to assess the diagnostic performance and clinical impact of metagenomic NGS (mNGS) in hospitalized patients with suspected sepsis and analyze the suitable population for mNGS test besides culture.

Methods:

A multi-center, prospective cohort study was performed. We enrolled eligible patients with hospitalized infection, collected demographic and clinical characteristics, and record the 30-day survival. Blood samples were collected on the day of enrollment to perform blood culture and mNGS test. Diagnostic efficacy of mNGS test and blood culture were calculated, and clinical impact of antibiotic regimen modification based on pathogenic test were also analyzed with SPSS22.0 (SPSS Inc, Chicago, IL).

Results:

We collected demographic and clinical characteristics of patients, and record the 30-day survival. Blood samples were collected on the day of enrollment to perform blood culture and mNGS test. Diagnostic efficacy of mNGS test and blood culture were calculated, and clinical impact of antibiotic regimen modification based on pathogenic test were also analyzed. A total of 277 patients were enrolled and 162 were diagnosed with sepsis. Among patients with 30-day follow-up data, the mortality was 44.8% (121/270). The mNGS test exhibited shorter turn-out time [27.0(26.0, 29.0) vs 96.0(72.0, 140.3) hours, $P < 0.001$] and higher sensitivity (90.54% vs 36.00%, $P < 0.001$) than blood culture, especially for fungal infections. The mNGS test showed better performance for patients with mild symptoms, prior antibiotics use, and early stage of infection than blood culture. Higher reads of pathogens detected by mNGS was related to 30-day mortality ($P=0.002$). The mNGS test was capable of guiding antibiotic regimen modification and ameliorating prognosis. Negative mNGS results helped with antibiotic de-escalation safely.

Conclusions

mNGS technology may be helpful for patients with possible blood-stream infections, especially in fungal infection and for patients with mild symptoms, prior antibiotics use and early stage of infection. Its role in antibiotic stewardship and ameliorating prognosis warrants further study.

Trial registration

The study was registered on the Chinese Clinical Trial Registry (Number: ChiCTR1800019187) on 01/24/2019 (Retrospectively registered).

1 Introduction

Infections are common causes of hospitalization, which could progress into sepsis. The optimal timing for treatment is often missed when sepsis was clinically diagnosed, making it a serious threat to public health and life [1, 2]. According to the latest definition of sepsis in 2016, sepsis is a disorder of the host inflammatory response to infection, resulting in homeostasis imbalance and life-threatening organ dysfunction syndrome. The clinical diagnostic criteria include systemic inflammatory response syndrome (SIRS), the quick Sequential (Sepsis-related) Organ Failure Assessment (qSOFA) score of ≥ 2 points, and high potentially fatal risk [2].

In recent years, the mortality of sepsis has not improved. The development of novel antibiotics and updating treatments are not enough to deal with drug-resistant pathogens and disease progression [3]. Sepsis is still the primary cause of ICU admission and death.

For one thing, the early diagnosis of sepsis still lacks effective indicators. For another, the rapid identification of pathogens lacks effective methods and block the timely targeted treatment and prognosis improvement of patients with infectious disease [3, 4–6]. Clinical etiological diagnosis technologies of sepsis mainly include smears and cultures of clinical specimens such as blood. As the gold standard for diagnosing bloodstream infections, blood culture has obvious shortcomings including time-consuming and low sensitivity.

Based on the above situation, it is necessary to adopt more sensitive and specific detection methods for patients with infection and sepsis. High-throughput Next Generation Sequencing (NGS) is a newly developed nucleic acid detection technology in recent years. The most prominent advantage of metagenomic NGS (mNGS) is the rapid and accurate detection of pathogens, which dramatically reduces the time required for clinical diagnosis [7–9].

However, there is controversy on the role of mNGS in the management of patients with possible blood-stream infections, including accuracy and the impact on improving clinical management. Therefore, we carried out a prospective multi-center study with the following purposes: (1) Assess the value of mNGS test in pathogenic diagnosis of possible blood-stream infection, and explore whether mNGS test could improve clinical prognosis; (2) Identify the features of patients who might benefit from mNGS.

2 Methods

2.1 Study design and Patients population

A multi-center, prospective cohort study was performed. The participating hospitals and number of cases were listed in Additional file 1. Zhongshan Hospital of Fudan University was the clinical unit responsible for this research. The leading investigator determined the sample size based on experience of clinical research and hospital capacity.

The inclusion criteria were as follows: (1) Age > 18 years and ≤ 90 years old; (2) Patients who met any 1 of the following 3 items: ▯ respiratory rate (per minute) ≥ 22; ▯ Systolic blood pressure ≤100 mmHg; ▯altered mental status. (3) Patients diagnosed with any of the following: ▯ bloodstream infection; ▯ community acquired pneumonia (CAP); ▯ hospital acquired pneumonia (HAP); ▯ peritonitis; ▯ acute purulent bile duct Inflammation; ▯ acute pyelonephritis; ▯ skin and soft tissue infection. (4) Signature of informed consent.

The exclusion criteria were the existence of any of the following: (1) Age < 18 years or > 90 years old; (2) Patients not able to cooperate with observers.

According to the latest definition of sepsis in 2016 [2], eligible patients with qSOFA score of ≥ 2 points were divided into sepsis group, and the remaining patients were classified as non-sepsis group.

2.2 Data collection

Demographic statistics and clinical characteristics were collected after signing the informed consent. Demographic statistics include age, gender, height, and weight. Clinical characteristics including: (1) Previous and present medical history; (2) Assessment of current disease severity, including the Acute Physiologic and Chronic Health Evaluation (APACHE) II score, SOFA and qSOFA score; (3) The number of days from the infection onset to enrollment into the study, the infection onset was defined as the time when the patient had fever and leukocyte elevation; (4) Laboratory examination on the day of enrollment, including blood routine, leukocyte sorting count, PCT; (5) Results of blood culture; (6) Antibiotic regimen records within 30 days after enrollment and modification based on pathogenic test. The modification of antibiotic regimen was defined as any change of antibiotic agents within 2-7 days after the enrollment, cause the results of mNGS test and blood culture were given during this period; (7) 30-day follow-up of survival.

2.3 mNGS Detection

The mNGS detection was conducted according to the manufacturer’s recommendation, which was supplied in Additional file 2.

With reference to previous literature, we have formulated the following criteria for positive mNGS results [10]:

For bacteria (mycobacteria excluded), fungi, virus and parasites: mNGS identified a microbe (genus level) as positive whose reads were 3-fold greater than that of any other microbes. If only one pathogen was detected, it would be regarded as positive.

Mycobacteria: Mycobacterium tuberculosis was considered positive as long as there is more than 1 read mapped to the species or genus level.

Under the condition that the above criteria were met, the patients' clinical characteristics were taken into consideration and results such as parvovirus, parvovirus, human herpes virus 5, etc. were appropriately excluded by clinicians and laboratory staff due to the consideration of laboratory contamination. Other results were treated as positive.

2.4 Blood Culture

The blood culture was carried out in microbiological laboratory of each participated hospital. Blood samples were collected from both sides of the patients on the day of enrollment and cultured following the standard procedures. Samples with positive blood culture on both sides were defined as positive blood culture, and positive results of only one side were considered contaminating bacteria.

The final etiology result was made at the end of the follow-up, and was assessed by the local attending physician group led by principal investigator in each hospital based on microbiological results, clinical features and response to the treatment. When there was a controversy, the leading investigators of all the centers discussed and made the final decision to avoid the bias.

The final etiology results were taken as the responsible pathogens and the reads of responsible pathogens in mNGS reports were applicated in the statistical analysis.

2.5 Determination of Inflammatory Factors

Plasma samples of mNGS-positive patients were taken for inflammatory factor detection, using the Human Cytokine Screening 48-Plex Services Kit (BIORAD, Catalog number: 12007283. California, America) according to the manufacturer's recommendation.

2.6 Statistical Analysis

The analysis was performed with SPSS22.0 (SPSS Inc, Chicago, IL, USA) and GraphPad Prism 8.0.1 (GraphPad Software Inc, San Diego, CA, USA).

Patients were divided into different subgroups according to clinical manifestation. Chi-square analysis was used for categorical variables. One-sample Kolmogorov-Smirnoff test was used to test the normality of continuous variables. The continuous variables with normal distribution were compared by the *t*-test, and the non-normally distributed variables were compared by the Mann-Whitney *U* test. Same statistical method was applied when comparing reads of pathogens in different subgroups. McNemar test was applied to compare the sensitivity and specificity of mNGS test and blood culture. Receiver Operating Characteristic (ROC) curves were analyzed and the value of area under curve (AUC) was calculated. Univariate linear regression analysis was applied to analyze the correlation between reads of pathogens and pro-inflammatory factors. The data with normal distribution were represented as means ± standard deviation, and the others were represented as medians (1^{st} quartile, 3^{rd} quartile).

2.7 Ethics approval

The ethics committee of Zhongshan Hospital approved this study (Number: B2018-182R). All the participants were fully informed of the content of informed consent before enrollment and provided their written informed consent to participate in this study. The study was registered on the Chinese Clinical Trial Registry (Number: ChiCTR1800019187).

3 Results

A total of 285 patients were investigated, and 277 patients were enrolled and included for final analysis after excluding the patients < 18 years old and > 90 years old.

3.1 Demographic And Basic Clinical Information

There were 195 males and 82 females, with an average age of 62 (49, 70) years old. According to the qSOFA score, 162 hospitalized patients with infection were diagnosed with sepsis and 115 patients without sepsis. 270 patients with 30-day follow-up data showed that the mortality was 44.81% (121/270). Table 1 presented the basic information of the patients.

Table 1
Demographic and basic clinical characteristics of hospitalized patients with and without sepsis.

Basic information	Sepsis group (n = 162)	Non-sepsis group (n = 115)	P-value
Gender, male/female	114/48	81/34	0.991
Age, median (q1, q3)	63.0 (49.8, 69.0)	60 (43, 72)	0.451
Comorbidities [n (%)]			
Pulmonary disease [a]	13 (8.02%)	12 (10.43%)	0.490
Congestive heart failure	11 (6.79%)	4 (3.48%)	0.230
Cerebrovascular disease	19 (11.73%)	11 (9.57%)	0.568
Diabetes	34 (20.99%)	14 (12.17%)	0.056
Hepatic cirrhosis	3 (1.85%)	0 (0%)	0.269
Acute/chronic renal failure	23 (14.20%)	15 (13.04%)	0.783
Smoking history [n (%)]	56 (34.57)	32 (27.83%)	0.235
Antibiotic use before enrollment [b] [n (%)]	144 (88.89%)	104 (92.04%)	0.679
Invasive procedures before onset of symptoms [c] [n (%)]	92 (56.79%)	55 (47.83%)	0.141
Corticosteroids/immunosuppressive drug/cytotoxic chemotherapy before onset [n (%)]	38 (23.46%)	48 (41.74%)	**0.001**
Recent surgery/trauma history [d] [n (%)]	54 (33.33%)	39 (33.91%)	0.920
ICU admission [n (%)]	141 (87.04%)	71 (61.74%)	**< 0.001**

a: including chronic obstructive pulmonary disease (COPD), asthma, interstitial lung disease, structural lung disease; b: antibiotics application within 2 weeks before enrollment; c: invasive procedures include punctures and drainages, tracheal intubation, urinary catheterization, arteriovenous catheterization, and superficial vein indwelling needle puncturing; d: surgery/trauma history within 3 months before enrollment.

Abbreviations: ICU, intensive care unit.

3.2 Characteristics Of Infection

The average time from symptom onset to enrollment was 8 (3, 17) days, with leukocyte count of 10.16 (6.70, 15.69) (×10^9/L) and neutrophil count of 9.03 (5.39, 13.38) (×10^9/L). The rest of data was shown in Table 2.

Table 2
The infection characteristics of patients in sepsis and non-sepsis group.

Infection characteristics	Sepsis group (n = 162)	Non-sepsis group (n = 115)	P-value
Time from symptom onset to enrollment (d), median (q1, q3)	5.5 (2, 15)	11 (5, 22)	**0.008**
Site of infection [n (%)]			
Pulmonary infection	114 (80.28%)	70 (60.87%)	0.100
Extrapulmonary infection	18 (11.11%)	8 (6.96%)	0.247
APACHE II (means ± SD)	19.97 ± 8.33	14.87 ± 8.28	**< 0.001**
Shock index (means ± SD)	1.04 ± 0.36	0.81 ± 0.19	**< 0.001**
Fever [n (%)]	117 (72.22%)	82 (71.30%)	0.867
Altered mental status [a] [n (%)]	115 (70.99%)	20 (17.39%)	**< 0.001**
Death within 30 days [n (%)]	82, 51.57%	39, 35.14%	**0.008**
PCT (ug/L) [median (q1, q3)]	1.92(0.36, 11.17)	0.53(0.22, 3.82)	0.214
WBC count (×10^9/L) [median (q1, q3)]	10.83(6.93, 15.65)	9.79(6.10, 15.76)	0.410
Neutrophil count (×10^9/L) [median (q1, q3)]	9.07(5.73, 13.42)	8.84(5.00, 13.03)	0.475
a: including coma, delirium, ambiguity of consciousness, et al.			
Abbreviations: APACHE II, the Acute Physiologic and Chronic Health Evaluation II score; PCT, procalcitonin; WBC, white blood cell.			

Among the 148 patients with confirmed pathogens, bacterial infections were the most common (118 cases). *Klebsiella pneumoniae* (22 cases) counted for the first, followed by *Acinetobacter baumannii* (19 cases) and *Pseudomonas aeruginosa* (11cases). For patients with fungi infections (22 cases), the suspected pathogens were *Pneumocystis* (13cases), *Aspergillus* (7 cases), and *Candida* (2 cases). *Adenovirus* (3 cases) were the most common pathogens in viral infected patients (8 cases). 4 cases were diagnosed with *Mycobacterium tuberculosis* infection while their blood culture results were negative, and only 2 of them had positive mNGS results. Figure 1 listed the etiological examination results of 148 patients.

3.3 Performance Of Mngs Test As Compared To Culture

A total of 217 patients performed blood cultures, of which 50 were positive, and 167 were negative. Among 277 patients' mNGS test results, 140 were positive, 135 were negative, and 2 were missing.

The percentage of patients with identified pathogen increased from 16.25%(45/277)when using only culture to 52.71% (146/277) when combined mNGS with culture. According to the final etiology result, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of blood culture were 36.00% (45/112), 94.57% (87/105), 90.00% (45/50) and 52.10% (87/167), respectively, while the results of mNGS test were 90.54% (134/148), 94.53% (121/128), 95.71% (134/140) and 89.63% (121/135), respectively. The AUC value of blood culture and mNGS test, which were 0.653 and 0.929 (P<0.001), respectively.

It should be noted that among 22 patients diagnosed with fungi infections, only 1 patient had a positive blood culture for *Candida*. Therefore, for patients suspected of fungal infection, mNGS testing should be actively performed to avoid missing diagnosis.

Besides, the average turn-out time required for mNGS tests was 27.0 (26.0, 29.0) (hours), which was significantly shorter than the average time required for blood culture was 96.0 (72.0, 140.3) (hours) (P < 0.001).

3.4 Comparison of mNGS test and blood culture in different subgroups

We divided the patients into different subgroups according to various factors, and analyzed the diagnostic efficacy of mNGS test and blood culture in each subgroup. As results shown in Table 3, the sensitivity of mNGS test significantly preceded than that of blood culture, while the specificity remained not inferior to blood culture.

Table 3
The diagnosis efficacy comparison between mNGS and blood culture in different subgroups.

Subgroups	mNGS test		Blood culture	
	Sensitivity	Specificity	Sensitivity	Specificity
Age				
> 65 years old	90.74%*	90.20%	37.78%	95.00%
≤ 65 years old	90.43%**	98.68%	35.00%	94.44%
Gender				
Male	90.48%**	94.32%	36.05%	95.45%
female	90.70%**	97.44%	35.90%	92.31%
Shock index				
> 1.0	89.09%**	100.00%	34.00%	96.30%
≤ 1.0	91.40%**	93.18%	37.33%	93.85%
Pulmonary infection				
Yes	92%**	92.68%	32.50%	92.59%
No	87.50%**	100%	42.22%	97.36%
Bloodstream infection				
Yes	96.15%*	92.86%	56.00%	90.91%
No	89.34%**	95.58%	31.00%	95.06%
Surgery/trauma history [a]				
Yes	95.83%**	97.78%	34.88%	97.22%
No	88.88%**	93.90%	36.59%	92.86%
Time from infection onset to enrollment				
≤ 5 days	91.23%**	96.30%	26.92%	95.45%
> 5 days	89.77%**	97.18%	41.67%	93.62%

a: surgery/trauma history within 3 months before enrollment; b: invasive procedures include punctures and drainages, tracheal intubation, urinary catheterization, arteriovenous catheterization, and superficial vein indwelling needle puncturing; c: antibiotics application within 2 weeks before enrollment.

Abbreviations: mNGS, metagenomic next-generation sequencing; ICU, Intensive care unit.

*P<0.05, mNGS vs blood culture; **P<0.001, mNGS vs blood culture.

Subgroups	mNGS test		Blood culture	
	Sensitivity	Specificity	Sensitivity	Specificity
Invasive operations before infection onset [b]				
Yes	89.61%**	98.53%	42.19%	91.53%
No	91.54%**	91.53%	29.51%	97.67%
Fever				
Yes	88.68%**	95.65%	40.45%	95.24%
No	95.24%**	94.29%	25.00%	93.10%
Altered mental status				
Yes	86.67%**	95.00%	41.54%	91.89%
No	94.37%**	95.52%	29.31%	96.36%
Antibiotics use before enrollment [c]				
Yes	90.70%**	94.96%	34.58%	94.05%
No	89.47%*	100%	44.44%	100%
ICU administration				
Yes	90.43%**	97.89%	35.58%	94.37%
No	90.91%*	87.50%	38.10%	95.24%
Sepsis				
Yes	89.13%**	98.57%	34.15%	93.88%
No	92.86%**	91.23%	39.53%	95.35%
a: surgery/trauma history within 3 months before enrollment; b: invasive procedures include punctures and drainages, tracheal intubation, urinary catheterization, arteriovenous catheterization, and superficial vein indwelling needle puncturing; c: antibiotics application within 2 weeks before enrollment.				
Abbreviations: mNGS, metagenomic next-generation sequencing; ICU, Intensive care unit.				
*P<0.05, mNGS vs blood culture; **P<0.001, mNGS vs blood culture.				

Although there was no statistical significance, the sensitivity of blood culture exhibited downward trends in subgroups of patients within 5 days of infection onset, patients without prior invasive operation, patients without fever, patients without altered mental status, and patients who applied antibiotics before enrollment. This result indicated that mNGS test might be more suitable for patients with mild symptoms or prior antibiotics application, and more prominent in early etiological diagnosis of infectious diseases.

3.5 Reads of responsible pathogens in mNGS reports related to the prognosis of patients and the level of inflammatory factors.

Among the mNGS-positive patients, we compared the reads of responsible pathogens in mNGS reports in patients with different prognosis (shown in Fig. 2). The results showed that reads in the non-survival group was also higher than that in the survival group, and this trend remained significant between non-survival and survival patients in sepsis group. It suggested that the reads of mNGS reports might be related to the prognosis of infected patients.

We detected the level of inflammatory factors in patients with positive mNGS results and analyzed the correlation between the level of inflammatory factors and the reads of pathogens. The results showed that there was a positive correlation between interleukin 1-β (IL-1β), eotaxin, interferon-α (IFN-α), tumor necrosis factor-α (TNF-α) and the reads of pathogen (shown in Table 4). The positive correlation between eotaxin, IFN-α, TNF-α and the reads of pathogen remained significant in sepsis patients (shown in Additional Table 1).

Table 4
Univariate linear regression of inflammatory factors level and reads of pathogens in mNGS reports.

Inflammatory factor	P-value	B (95% CI)	R-square
IL-1β	0.024	0.349 (0.051, 0.647)	0.182
Eotaxin	0.004	0.465 (0.157, 0.773)	0.247
IFN-α	< 0.001	2.629 (1.450, 3.809)	0.436
HGF	0.761	0.039 (-0.221, 0.299)	0.003
TNF-α	< 0.001	0.905 (0.503, 1.307)	0.432
IL-9	0.149	0.415 (-0.158, 0.988)	0.070

Abbreviations: mNGS, metagenomic next-generation sequencing; B, standardized linear regression coefficient; IL, interleukin; IFN, interferon; HGF, hepatocyte growth factor; TNF, tumor necrosis factor.

3.6 Pathogenic test facilitated antimicrobial prescribing modification ameliorated prognosis of patients

Of all the patients, 151's antibiotic regimen was modified within 2–7 days after the enrollment, while the rest remained the original antibiotic regimen. It was observed that patients who made modification of antibiotic regimen had higher 30-day survival rate (63.3% vs 45.5%, P = 0.005), and the trend was also obtained in sepsis group (58.5% vs 37.7%, P = 0.009).

Among the mNGS-negative patients (135 cases), 25 patients de-escalated antibiotic use with a 30-day mortality of 32.0%, while the other 110 patients did not reduce the administration of antibiotics, and the 30-day mortality was 40.0%. There was no statistically significant difference in mortality. The result

indicated that negative mNGS results may help with de-escalating antibiotics without worsening prognosis.

4 Discussion

The current research results exhibited that mNGS test took less time compared with traditional blood culture, and was able to diagnose infectious diseases with high efficacy, especially in patients suspected with fungi infection, or patients with mild symptoms, prior antibiotic application, or in early stage of infection. Higher reads of pathogen were related to higher 30-day mortality and sepsis state, as well as higher levels of IL-1β, eotaxin, IFN-α, and TNF-α, suggesting that the reads of pathogens reflected the pathogen load and may related to the disease severity and prognosis. In addition, we found that patients who made modification of antibiotic regimen based on pathogenic test had higher 30-day survival rate, while the negative mNGS results may help with the de-escalation of antibiotic administration without worsening the prognosis. The results of our research demonstrated the considerable clinical significance of mNGS test.

Blood culture is the gold standard for pathogen detection in bloodstream infections. However, the sensitivity of blood culture fluctuates with the severity and stage of infection. False negatives often occur in blood culture [7, 9–13]. It was reported that the sensitivity of traditional blood culture was 30–60% [14]. In our study, blood culture possessed high specificity, while the sensitivity only reached 36.00%.

Long Y *et al.* investigated the diagnostic efficiency of mNGS in ICU patients. They reported that the sensitivity of mNGS was 30.77% (24/78), which was significantly higher than 12.82% (10/78) using blood culture [15]. In our study, blood mNGS increased the etiology yield from 16.25–52.71% in hospitalized patients with possible blood-stream infection. Studies also confirmed the applicability of mNGS test in tissues other than blood sample, including resected heart valve [16], cerebrospinal fluid [17], vitreous samples [18], formalin-fixed and paraffin-embedded (FFPE) tissue specimens [19].

Clinically, the time to positivity requires 12–48 hours for blood culture, and pathogen identification often takes 5–7 days [20]. In current study, the average report time of mNGS test was 27.0 (26.0, 29.0) h. Compared with blood culture, mNGS not only exhibited quick detection speed, but also can detect multiple pathogens. The detection of culture-negative pathogens was one of mNGS's most significant advantages [21].

In our study, the performance of mNGS test was high in various conditions, especially those with mild symptoms, prior antibiotic treatment or early stage of disease. This is consistent with Grumaz S, et al.'s study, who compared the blood culture and mNGS test results of patients with septic shock in different periods. With the application of antibiotics and the improvement of the patient's condition, the positive rate of blood culture gradually decreased, while mNGS's test was not affected, and the sensitivity remained higher than that of blood culture [22].

The reads of pathogens in mNGS reports exhibited positive relation with plasma levels of pro-inflammatory factors like IL-1β, which means patients with higher reads of pathogens were suffered from severe inflammation. It indirectly verified that reads of pathogens in mNGS reports could reflect the pathogen load. Besides, patients who died within 30 days possessed higher reads of pathogens, which reminded us that more attention should be paid when handling patients with high reads of pathogens in clinic.

We explored the clinical impact of mNGS test in prognosis. Patients who made modification of antibiotic regimen based on pathogenic test had significant higher 30-day survival rate. However, Grumaz S et al. reported different results. In their study, 24 patients with septic shock changed antibiotic regimen based on mNGS test results, while 17 patients followed the original regimen. The mortality of the former within 28 and 90 days was higher than that of the latter (25% vs 11.8%, P = 0.261; 37.5% vs 23.5%, P = 0.285, respectively) [22]. The difference may be caused by different sample size and patients' population. The overall mortality in our research was pretty high (44.8%), which made the prognosis improvement more evident. Besides, the clinical benefit obtained from pathogenic test should be credited to the combination of mNGS and blood culture instead of mNGS alone. Whether mNGS test can play a positive role in ameliorating the prognosis of infected patients and how needs to be further explored. In addition, we found negative mNGS results could facilitate antibiotic de-escalation without worsening the survival. This might play a role in the appropriate use of antibiotics.

Studies approved that the NGS test was able to detect antimicrobial resistance (AMR) gene [23, 24], and may help clinicians to choose antibiotic therapy. We tried to detect AMR genes using NGS in the study, but the results poorly correlated to culture (data not shown). For the current NGS technology used clinically, challenges remained in detection of AMR genes. Firstly, only through whole-genome sequencing can accurate results of AMR genes be obtained; Secondly, we were unable to determine which microorganism the AMR gene belongs to through plasma detection unless the strain of pathogen was provided. Thirdly, even if an AMR gene was detected, there may not be the corresponding resistance phenotype. Genes can be silenced, inactivated or poor expressed. Susceptibility test of cultured strain and PCR for AMR genes are still standard techniques in evaluating AMR.

Our study had several limitations. Firstly, we carried out blood culture and mNGS test on the day of enrollment but failed to monitor the changes of pathogen detection rate during the entire disease process. Secondly, the changes of cytokines were not monitored, either. Thirdly, there are no statistics on the culture results of other tissue samples except for blood.

5 Conclusions

In conclusion, the mNGS technology was useful in early detecting etiology in hospitalized patients with possible blood-stream infections, especially patients with mild symptoms and history of antibiotics use, or patients in the early stage of infection. It might contribute to the modification of antibiotic regimen and further ameliorating the prognosis, and help with de-escalating antibiotics safely. The reads of pathogens

in mNGS reports could reflect the patient's pathogen load, as well as the severity of sepsis and the prognosis of patients. mNGS is expected to complement traditional detection methods in selected patients.

Declarations

Ethics approval and consent to participate

The ethics committee of Zhongshan Hospital approved this study (Number: B2018-182R). All the participants were fully informed of the content of informed consent before enrollment and provided their written informed consent to participate in this study.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

YHZ participated in the design and implementation of the study, performed the statistical analysis and drafted the manuscript. YXW participated the implementation of the study and helped to draft the manuscript. YC and YPL participated in the design and implementation of the study. ZJS, ZL, MJJ, MHS, SYX, HZ, XL, ZJJ and XDL participated in the implementation of the study. JZ conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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Figures

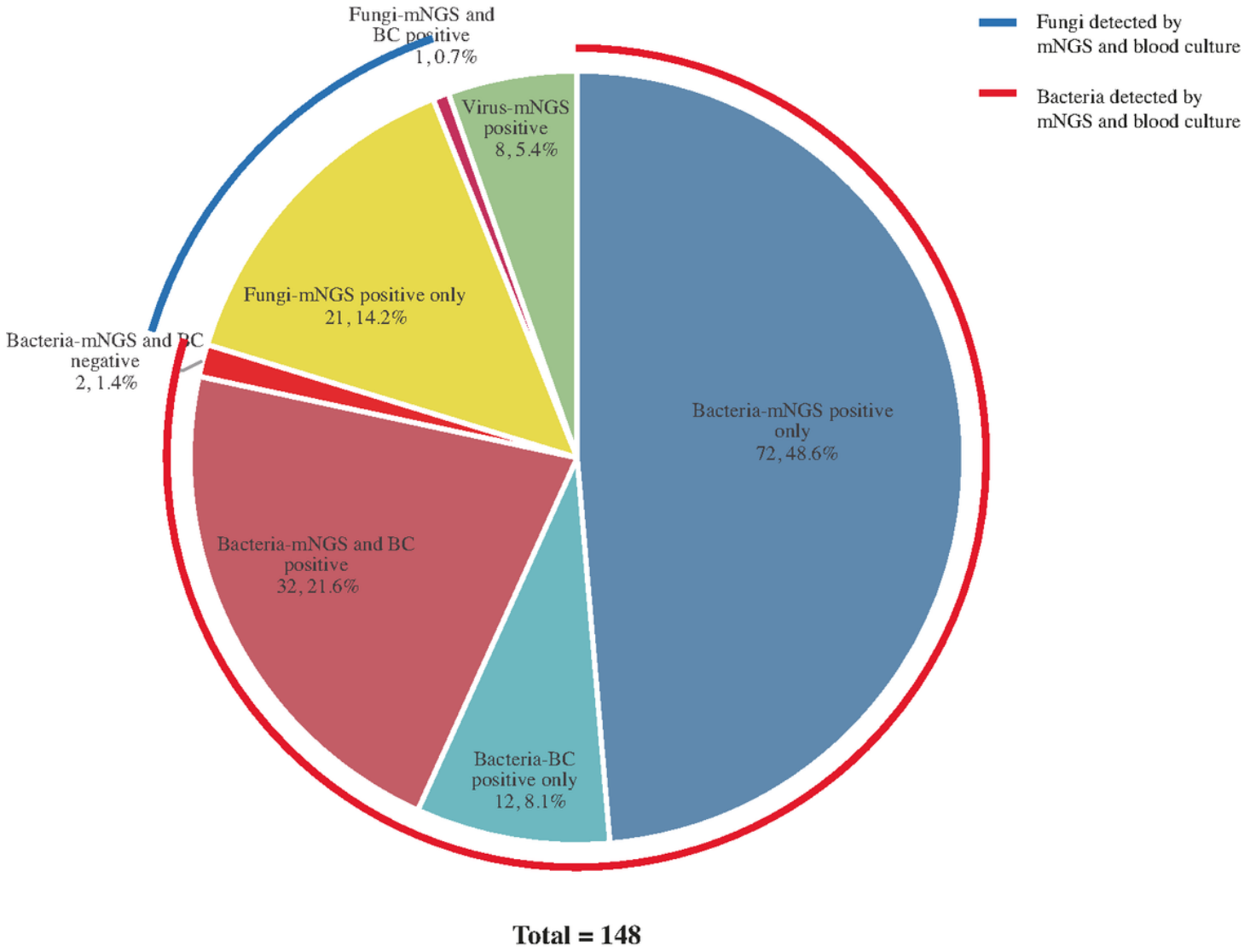

Figure 1. Pathogens detected by mNGS and blood culture (BC)

Figure 1

Pathogens detected by mNGS and blood culture (BC)

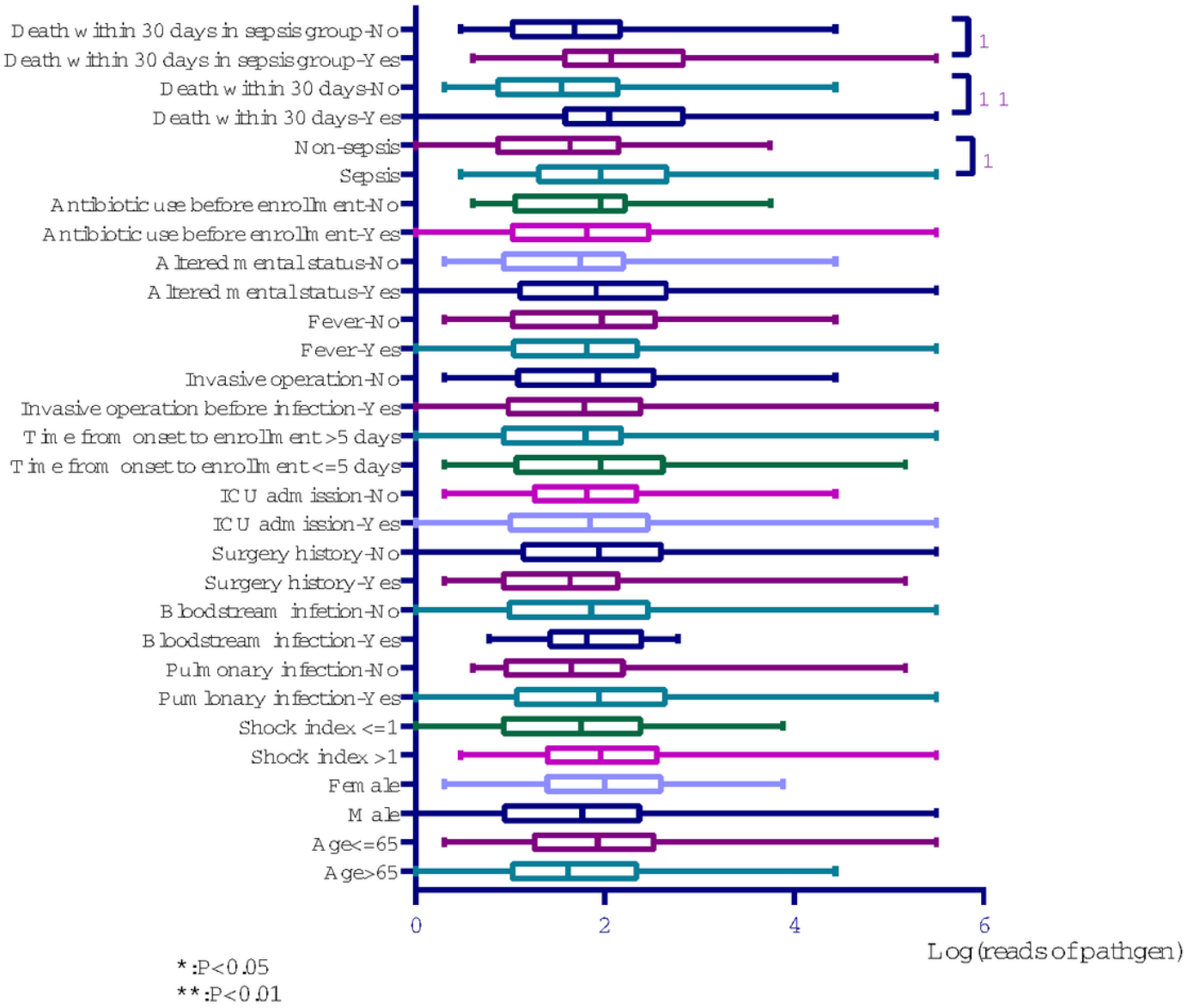

Figure 2

Reads of pathogens in different subgroups

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