The Value of Alveolar Lavage Fluid mNGS in The Peripheral Pulmonary Among Children

Kun-yin Qiu

Sun Yat-sen Memorial Hospital

Xiong-yu Liao

Sun Yat-sen Memorial Hospital

Jian-pei Fang

Sun Yat-sen Memorial Hospital

Dun-hua Zhou (✉ zdunhua@163.com)

Sun Yat-sen Memorial Hospital

Research Article

Keywords: NGS, children, pulmonary

Posted Date: January 27th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-150661/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Objectives The aim of this study was to evaluate the value of alveolar lavage fluid mNGS in the diagnosis of peripheral pulmonary infectious lesions among children.

Methods Twenty-eight consecutive pediatric patients suspected of pulmonary infections were retrospectively.

Result The detection rate of traditional pathogen method was 17.9%, while the detection rate of mNGS was 71.4% ($P < 0.01$). The smoothing curve also showed that, there is a negative linear correlation between WBC and microbial reads ($P < 0.05$). Further we found when CRP is less than 60mg/dl, CRP increases with the increase of microbial sequencing reads. With the ROC curves, we demonstrated the different detection methods of alveolar lavage fluid in the diagnosis of peripheral pulmonary infectious lesions. The results showed that mNGS was more sensitive (100%) and specific (90.9%) compared to traditional method (83.3%, 18.2%). The PPV and NPV of mNGS were 100% and 75%. The AUC of mNGS was 95.45%, while the AUC of traditional methods was 50.76%, which was with a significant difference ($P < 0.001$). The PPV and NPV of traditional pathogen detection were 80% and 21.7%.

Conclusions Metagenomic NGS analysis can provided fast and precise pathogen detection and identification among children, and WBC and CRP could be used biomarkers of anti-infective efficacy.

1. Introduction

Pneumonia is a common infectious disease in children, with an annual increase of 120 million children with pneumonia worldwide, severe pneumonia accounts for 7%-13%, and 1 million people die^[1]. Children with severe pneumonia have many sequelae and die of illness. The rate is high, which brings burden to the family and society^[2]. Pathogen detection in children with severe pneumonia is very important to guide clinical accurate drug use, shorten hospital stay, reduce sequelae and reduce mortality^[3]. At present, the methods of diagnosing pneumonia pathogens mainly include pharynx test, sputum culture, serum pathogenic antibody detection, blood culture and so on, but they can not fully meet the needs of clinical rapid and accurate treatment. In recent years, the second generation base. The development of metagenomic next-generation sequencing(mNGS) not only enriches the traditional pathogen detection methods, but also improves the detection rate of clinical respiratory tract infection pathogens. However, reports on mNGS application in diagnosis respiratory tract pulmonary infections, particularly by using bronchoscopy samples among children, remains rare. The aim of this study was to evaluate the value of alveolar lavage fluid mNGS in the diagnosis of peripheral pulmonary infectious lesions among children.

2. Patients And Methods

2.1 Patients

Twenty-eight consecutive pediatric patients suspected of pulmonary infections were retrospectively at Sun Yat-sen Memorial Hospital, Sun Yat-sen University between February 2019 to May 2020. Among the patients enrolled, 19 patients had underlying disease, including 16 cases of hematological malignancies and 3 cases of autoimmune disease. This retrospective study was approved by the hospital institutional review committee and informed consent was obtained from all patients' guardian. (Ethics Committee of the Sun Yat-sen Memorial Hospital, China, file number 20200156). We confirm that all methods were performed in accordance with the relevant guidelines and regulations.

2.2 Definition

Although bronchus alveolus lavage fluid (BALF) culture, and smear microscopy were used as traditional pathogen detection methods, the final clinical diagnosis was confirmed by comprehensive evaluation of traditional pathogen detection, mNGS results and other clinical examination results. A mNGS or traditional test result was considered positive only if the pathogen(s) detected was in consistence with the final clinical diagnosis. If the patient's final clinical diagnosis was non-pulmonary infectious disease, the tests with positive results were considered as false positives. If the patient's final clinical diagnosis was pulmonary infectious disease, the tests with positive results were considered as true positives, while the tests with negative results were considered as false negatives^[4].

2.3 Detection method

Samples were collected from 28 children before bronchoscopy, including blood routine, c-reaction protein (CRP), procalcitonin (PCT) and pharynx swab collection for virus detection. All the patients enrolled in the study were perfected with routine examination before bronchoscopy, and bronchoscopy was performed after the patients and their families signed. After fasting for 6 hours and water deprivation for 2 hours before operation, Olympus V160 electronic bronchoscope was used for suction and debridement of airway secretions. Normal saline was given to each segment or lobe of the diseased lung for 10–20 ml each time, and each part was repeatedly lavaged for 2 to 3 times. Double-tube bronchoalveolar lavage fluid was collected which was not less than 5 ml. The bronchoalveolar lavage fluid of the patient was collected and divided into two parts, one was sent for general bacteria, fungi, bacterial smears and culture, and the other was sent for mNGS, and the sample transport met the requirements of the specification. The bronchoalveolar lavage fluid was sent to the BGISEQ-100 platform (Shenzhen Huada Gene Co, Ltd.). The nucleic acid was extracted and sequenced with high throughput. The results were analyzed by bioinformatics and compared with the pathogen database to get the final result. Standard references for reporting pathogenic bacteria.

2.4 Statistical Analyses

With the final clinical diagnosis as the gold standard, the patients were divided into pulmonary infection group and non-pulmonary infection group, and the pulmonary infection group was the positive reference group. The student t-test and χ^2 test were used to calculate differences in continuous variables between groups. P-values < 0.05 were considered to be statistically significant. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy (ACC) were calculated, and

sensitivity and specificity were compared between mNGS and traditional pathogen detection methods using the χ^2 test. All statistics were reported as absolute values with their 95% confidence interval (95% CI) and all statistics were calculated by SPSS 22.0 software.

3. Results

3.1 Baseline characteristics of patients

A total of 28 eligible pediatric patients were enrolled in this study. The baseline characteristics of the children are showed in Table 1. Of them, 14 (50%) are male and 14(50%) are female with a median age of 4 years old. In our study, the most prevalent hematological malignancies were hematopoietic stem cell transplantation (n = 5, 17.9%), acute lymphoblastic leukemia (n = 4, 14.3%), acute myelocytic leukemia (n = 2, 7.1%), hemophagocytic syndrome (n = 2, 7.1%), chronic myelogenous leukemia(n = 1, 3.6%), lymphoma (n = 1, 3.6%) and aplastic anemia (n = 1,3.6%). Idiopathic pulmonary hemosiderosis (n = 2, 7.1%) accounted for the majority of autoimmune diseases. The immune function was normal in nine children, while the other 19 had immune deficiency. For the final clinical diagnosis, twenty-two patients were diagnosed with pulmonary infection, and eight were diagnosed with non-pulmonary infection. Among the patients with pulmonary infections, the percentage with mNGS-positive result was 20/28 (90.9%), which was significantly higher than that in non-pulmonary infection group ($P < 0.01$).

Table 1
Characteristics of participants

Characteristic	Total	Pulmonary infection		<i>P</i> value
		Yes(n = 22)	No(n = 6)	
Age,median(range), <i>years</i>	4.0 (1.0–15.0)	4.0 (1.0–15.0)	3.0 (1.0–6.0)	0.153
Gender, <i>n</i> (%)				
Male	14 (50%)	9 (40.9%)	5 (83.3%)	0.065
Female	14 (50%)	13 (59.1%)	1 (16.7%)	
Immune function, <i>n</i> (%)				
Immune function deficiency	19 (67.9%)	14 (63.6%)	5 (83.3%)	0.360
Immune function normal	9 (32.1%)	8 (36.4%)	1 (16.7%)	
Temperature, median(range), °C	37.8 (35.4–39.6)	37.9 (35.4–39.6)	37.5 (36.6–39.0)	0.997
Inflammatory index, median(range)				
WBC(×10 ⁹ /L)	3.2 (0.5–18.9)	3.1 (0.5–18.9)	6.0 (2.2–17.2)	0.364
ANC(×10 ⁹ /L)	2.1 (0.0-14.6)	1.9 (0.0-14.6)	3.3 (0.6–11.0)	0.615
CRP(mg/dl)	10.7 (0.0-133.4)	12.6 (0.0-133.4)	7.0 (3.0–65.0)	0.463
PCT(ng/ml)	0.1 (0.0-8.4)	0.2 (0.0-8.4)	0.1 (0.0-0.8)	0.447
NGS, <i>n</i> (%)				
Positive	20 (71.4%)	20 (90.9%)	0	< 0.001
Negative	8 (28.6%)	2 (9.1%)	6 (100.0%)	
Traditional pathogen detection, <i>n</i> (%)				
Positive	5 (17.9%)	4 (18.2%)	1 (16.7%)	0.932
Negative	23 (82.1%)	18 (81.8%)	5 (83.3%)	
Detection time, median(range), <i>hours</i>				
NGS	48.5 (24.0–56.0)	48.0 (24.0–56.0)	50.5 (35.1–55.0)	0.813
Blood culture	72(72–168)	75(72–150)	80(75–168)	

Characteristic	Total	Pulmonary infection		<i>P</i> value
		Yes(n = 22)	No(n = 6)	
Sequencing reads,median(range), <i>reads</i>	184.0 (0.0-1469565.0)	670.5 (0.0-1469565.0)	0.0 (0.0–0.0)	< 0.001

3.2 Pathogen physical examination

A total of 28 bronchoalveolar lavage fluid samples were sent for traditional pathogen detection, 5 strains were detected, the detection rate was 17.9%, and the median reported recovery time was 72 (72–168) hours. The other half of bronchoalveolar lavage fluid samples were sent for mNGS, and 20 strains were detected with the detection rate of 71.4%, while the median detection time was 48.5 (24.0–56.0) hours. The detection rate in mNGS group was significantly higher than that in traditional pathogen detection group ($P < 0.01$, Table 2).

Table 2
Comparison of the results of mNGS and traditional method

NGS	Traditional pathogen detection		Total	<i>P</i> value
	Positive	Negative		
Positive	2	18	20	< 0.001
Negative	3	5	8	
Total	5	23	28	

3.3 Etiological analysis between traditional pathogen detection and mNGS

Five cases were positive by bacterial culture, which were 3 cases of bacteria and 2 cases of fungi (Table 3). Twenty-five cases were detected by mNGS. Among them, the physical examination rate of *Mycoplasma pneumoniae* was the highest ($n = 5$, 20%), all of which are found in pediatric patients with normal immune function. Whereas, Virus ($n = 9$, 36%) were the most common pathogen in immunodeficient children, and the others were *Pneumocystis carinii* ($n = 2$, 8%), *Pseudomonas aeruginosa* ($n = 2$, 8%), *Acinetobacter baumannii* ($n = 2$, 8%), virus ($n = 9$, 36%) and *Aspergillus* ($n = 2$, 8%), respectively.

Table 3
Detection results of Organism of Pulmonary Infection in mNGS
Compared with Traditional Detection Method

Pathogen	Traditional detection	NGS
Mycoplasma		
Mycoplasma pneumoniae		5(20%)
Bacteria		
Pneumocystis Yersini		2(8%)
Pseudomonas aeruginosa	1(20%)	
Streptococcus parasanguis		2(7.1%)
Acinetobacter baumannii		2(7.1%)
Lactococcus acidophilus		1(3.6%)
Klebsiella pneumoniae		1(3.6%)
Streptococcus in children		1(3.6%)
Oral streptococcus	1(20%)	
Human staphylococci	1(20%)	
Virus		
Human adenovirus type 7		2(7.1%)
Human herpesvirus 4		1(3.6%)
Human parvovirus B19		1(3.6%)
CMV virus		3(10.7%)
EB virus		1(3.6%)
Human polyomavirus type 3		1(3.6%)
Fungus		
Candida tropicalis	1(20%)	
Aspergillus		2(7.1%)
Candida albicans	1(20%)	
Total	5(17.9%)	25(89.3%)

3.4 The association between WBC, CRP and microbial reads

WBC and ANC were compared between patients with and without pulmonary infection, but we don't find any difference (Table 1). Otherwise, samples with the highest microbial reads (> 10,000 reads) had significantly lower WBC than samples with less microbial reads found, the smoothing curve also showed that, there is a negative linear correlation between WBC and microbial reads ($P < 0.05$, Fig. 1). Microbial sequencing reads was significantly higher in patients when pulmonary infection was identified ($P < 0.001$, Table 1). Then we explored the relationship between CRP and microbial reads by the smoothing plot, with an adjustment for potential confounders (Fig. 2). Further we found when the threshold level of CRP was 60mg/dl, the relationship between microbial reads and CRP level began to change and became notable was determined using a trial method. When CRP is less than 60mg/dl, CRP increases with the increase of microbial sequencing reads.

3.5 Performance of mNGS and Compare with Traditional Pathogen Detection

With the ROC curves, we demonstrated the different detection methods of alveolar lavage fluid in the diagnosis of peripheral pulmonary infectious lesions. The results showed that mNGS was more sensitive (100%) and specific (90.9%) compared to traditional method (83.3%, 18.2%). The PPV and NPV of mNGS were 100% and 75%, respectively. The AUC of mNGS was 95.45%, while the AUC of traditional methods was 50.76%, which was with a significant difference ($P < 0.001$). The PPV and NPV of traditional pathogen detection were 80% and 21.7%, respectively (Fig. 3, Table 4).

Table 4

Comparison of Sensitivity and Specificity Between mNGS and Traditional Detection Method

Method	Cases	PPV(%)	NPV(%)	Sensitivity(%)	Specificity(%)	AUC	<i>P</i> value
NGS	28	100	75	90.9	100	95.45	< 0.001
Traditional Detection	28	80	21.7	18.2	83.3	50.76	
Positive predictive value (PPV) ; negative predictive value (NPV); Area under the Curve of ROC(AUC)							

4. Discussion

In this study, through the detection of mNGS and traditional pathogens in the specimens obtained by bronchoscopy, the application value of mNGS in peripheral pulmonary infection was evaluated retrospectively. Most of the patients enrolled in this study had hematological malignancies or autoimmune disorders. These patients have chronic immune dysfunction due to long-term use of large doses of antineoplastic drugs, cytotoxic drugs or glucocorticoids, and are prone to diseases, especially pulmonary complications [5-6].

When there are pathological changes in the lungs, the pathological changes should be judged quickly and accurately and the correct follow-up treatment should be guided. Because the clinical manifestations of

some infectious and non-infectious diseases are very similar, it is difficult to distinguish this point by routine laboratory examination and imaging analysis, and there is often overlap between these diseases. This present study revealed that mNGS has obvious differential significance between pulmonary infection and non-pulmonary infection, and the detection rate of pathogens in pulmonary infection is significantly higher than that in non-pulmonary infection, and accompanied by a higher microbial sequencing reads. Moreover, mNGS was able to identify nearly 71.4% of patients with pulmonary infection, which was higher than traditional pathogen detection, at 17.9% ($P < 0.001$), and the median detection time is shorter (48.5 hours vs 72 hours). We speculated that the reason may be the effect of the use of antimicrobials on bacterial culture among the bacteria with negative bacterial culture but positive by mNGS. Because mNGS was to directly extract all nucleic acid fragments from samples and detect them. The use of antibiotics had no obvious effect on the detection results, which was one of the advantages of mNGS.

At present, the most common pathogen detection methods in specimens are microbial culture, histopathology and smear microscopic examination. The microbial culture in tracheoscopes included tissue culture and BALF culture^[7-8], but the sensitivity was low, and the positive rate was related to many factors, histopathology and smear microscopic examination could only identify a limited number of fungal species or *Mycobacterium tuberculosis*. For virus detection, the above test methods are not applicable^[9-12]. In our study, interventional specimens were obtained from patients with suspected pulmonary infection by bronchoscopy and sent for mNGS analysis. In addition, mNGS was more specific and sensitive, with a specificity of 100% and a sensitivity of 90.9%, while the specificity of traditional methods was only 83.3% and 18.2% ($P < 0.001$), which may be due to the traditional methods were unable to detect more microorganisms, resulting in a higher true negative rate of non-pulmonary infections^[13-18]. Above of all, the AUC of mNGS was 95.45%, while the AUC of traditional pathogen detection was 50.76%, which indicated that mNGS may play an important role in the diagnosis of peripheral pulmonary infectious lesions among children.

Interestingly, we found that decreased WBC counts were associated with the presence of microbial DNA, and was inversely proportional to the number of sequencing reads, which was consistent with previous reports^[19-20]. It's known to all, neutropenia often occurs in pediatric patients with malignant hematology after chemotherapy, resulting in severe infection. This result also suggested that we should increase the counts of WBC in time during the interval of chemotherapy, which plays a significant role in reducing the infection rate. On the other hand, we also discovered that when CRP is less than 60mg/dl, CRP increases with the increase of microbial sequencing reads, which suggested that CRP can be used as an index to detect the curative effect of anti-infection.

The study has several limitations. Most importantly, it is a single centre retrospective study, so bias in this study was inevitably. Secondly, the sample size of this research was still small. In spite of these limitations, this study does provide a new perspective for studying the value of alveolar lavage fluid metagenomic next-generation sequencing in the diagnosis of peripheral pulmonary infectious lesions among children.

Declarations

Acknowledgements

Authors gratefully acknowledge help of all parents and children. I would like to express my special thanks to Chun-yan Wu for her strong support for this article.

Funding

This work was supported by the Guangzhou Science and Technology Program key projects (No.201803010032).

Ethics approval and consent to participate

The study has been approved by Sun Yat-sen Memorial Hospital Ethics Committee (China).

Data availability statements

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

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bhuiyan MU. Combination of clinical symptoms and blood biomarkers can improve discrimination between bacterial or viral community-acquired pneumonia in children [J]. BMC Pulm Med, 2019,19(1): 71.
2. Shanshan Z,Sammon P,King I,et al. Cost of management of severe pneumonia in young children: systematic analysis [J]. Global Health,2016,6(1):010408.
3. Yun X,Jiang D ,Wei J ,et al. Comparison the pathogen diagnosis of severe pneumonia by using next generation sequencing and traditional detection methods,China,2010–2018 [J]. J Infect,2019,78(2): 158–169.
4. Huang Jie,Jiang Erjie,Yang Donglin et al. Metagenomic Next-Generation Sequencing versus Traditional Pathogen Detection in the Diagnosis of Peripheral Pulmonary Infectious Lesions.[J].Infect Drug Resist, 2020, 13: 567–576.

5. Nisar MK, Ostor AJ. Pulmonary complications of biological therapies in children and adults with rheumatic diseases. *Paediatr Respir Rev*. 2013;14(4):236–241. doi:10.1016/j.prrv.2012.12.007.
6. Wingard JR, Hiemenz JW, Jantz MA. How I manage pulmonary nodular lesions and nodular infiltrates in patients with hematologic malignancies or undergoing hematopoietic cell transplantation. *Blood*. 2012;120(9):1791–1800. doi:10.1182/blood-2012-02-378976.
7. Menon LR, Divate S, Acharya VN, Mahashur AA, Natrajan G, Almeida AF. Utility of bronchoalveolar lavage in the diagnosis of pulmonary infections in immunosuppressed patients. *J Assoc Physicians India*. 2002;50:1110–1114.
8. Miller SA, van Zante A, Schwartz BS. Cytologic evaluation can predict microbial culture results for infectious causes of pulmonary nodules in patients undergoing fine needle aspiration biopsy. *Diagn Microbiol Infect Dis*. 2010;68(3):330–333. doi:10.1016/j.diagmicrobio.2010.08.005.
9. Al-Nakeeb Z, Gupta V, Bell C, Woodhead M. Are we missing opportunities to confirm the diagnosis of tuberculosis by microbial culture? *Respir Med*. 2013;107(12):2022–2028. doi:10.1016/j.rmed.2013.09.016.
10. Shenai S, Ronacher K, Malherbe S, et al. Bacterial loads measured by the xpert MTB/RIF assay as markers of culture conversion and bacteriological cure in pulmonary TB. *PLoS One*. 2016;11(8):e0160062. doi:10.1371/journal.pone.0160062
11. Samuel CM, Whitelaw A, Corcoran C, et al. Improved detection of *Pneumocystis jirovecii* in upper and lower respiratory tract specimens from children with suspected pneumocystis pneumonia using real-time PCR: a prospective study. *BMC Infect Dis*. 2011;11:329. doi:10.1186/1471-2334-11-329
12. Becker MJ, de Marie S, Fens MH, Verbrugh HA, BakkerWoudenberg IA. Effect of amphotericin B treatment on kinetics of cytokines and parameters of fungal load in neutropenic rats with invasive pulmonary aspergillosis. *J Antimicrob Chemother*. 2003;52 (3):428–434. doi:10.1093/jac/dkg367.
13. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kauffman CA, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis*. 2008;46(12):1813–21.
14. Guan H, Shen A, Lv X, Yang X, Ren H, Zhao Y, Zhang Y, Gong Y, Ni P, Wu H, et al. Detection of virus in CSF from the cases with meningoencephalitis by next-generation sequencing. *J Neurovirol*. 2016;22(2):240–5.
15. Patterson TF, Thompson GR 3rd, Denning DW, Fishman JA, Hadley S, Herbrecht R, Kontoyiannis DP, Marr KA, Morrison VA, Nguyen MH, et al. Executive Summary: Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2016;63(4):433–42.
16. Toma I, Siegel MO, Keiser J, Yakovleva A, Kim A, Davenport L, Devaney J, Hoffman EP, Alsubail R, Crandall KA, et al. Single-molecule long-read 16S sequencing to characterize the lung microbiome

- from mechanically ventilated patients with suspected pneumonia. *J Clin Microbiol.* 2014;52(11):3913–21.
17. Chiche L, Forel JM, Roch A, Guervilly C, Pauly V, Allardet-Servent J, Gainnier M, Zandotti C, Papazian L. Active cytomegalovirus infection is common in mechanically ventilated medical intensive care unit patients. *Crit Care Med.* 2009;37(6):1850–7.
 18. Grahame-Clarke C, Chan NN, Andrew D, Ridgway GL, Betteridge DJ, Emery V, Colhoun HM, Vallance P. Human cytomegalovirus seropositivity is associated with impaired vascular function. *Circ.* 2003;108(6):678–83.
 19. Gyarmati P, Kjellander C, Aust C et al. Metagenomic analysis of bloodstream infections in patients with acute leukemia and therapy-induced neutropenia. [J]. *Sci Rep*, 2016, 6: 23532.
 20. Potgieter, M., Bester, J., Kell, D. B. & Pretorius, E. The dormant blood microbiome in chronic, inflammatory diseases. *FEMS Microbiol. Rev.* 39, 567–91 (2015).

Figures

Figure 1

The relationship between WBC and the microbial Sequencing reads. The smoothing curve also showed that, there is a negative linear correlation between WBC and microbial reads.

Figure 2

The relationship between CRP and the microbial Sequencing reads. When CRP is less than 60mg/dl, CRP increases with the increase of microbial sequencing reads.

Compare 2 models

Figure 3

The receiver operating characteristics curves of mNGS and traditional method compared in diagnosis of peripheral pulmonary infectious lesions. Model 1 was traditional method, and model 2 was mNGS.