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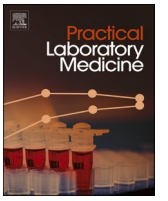

Detection of respiratory pathogenic bacterial nucleic acid detection by Loop-mediated Isothermal Amplification in patients with bacterial pulmonary infections

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ABSTRACT

Objective: Nucleic acid testing can accurately and rapidly identify the presence of pathogenic bacteria. In this study, we analyzed respiratory pathogenic bacteria nucleic acids by LAMP (Loop-mediated isothermal amplification) to clarify the clinical application in patients with bacterial pulmonary infections.

Methods: Clinical data and specimens were collected from 99 patients with bacterial pulmonary infections from June 2021 to April 2023. We compared the differences between nucleic acid detection of LAMP and sputum culture. The correlation between inflammation manifestations of pulmonary imaging and the nucleic acid detection of LAMP was compared and analyzed. And the relationship between LAMP and blood inflammatory markers were analyzed.

Results: The positive rate of LAMP using sputum specimens was significantly higher than that of sputum culture ($P < 0.05$). Pathogenic bacteria in sputum samples are more likely to be detected by LAMP in patients with inflammatory on lung imaging examination. The coincidence rate of elevated PCT and CRP expression with positive LAMP results were 83.87 % and 88.71 %, respectively. Moreover, PCT, CRP and WBC were significantly higher in LAMP positive group than those in negative group ($P < 0.05$).

Conclusion: Nucleic acid testing of sputum specimens for pathogenic bacteria by LAMP on the basis of imaging examination can provide a rapid and accurate experimental basis for clinical diagnosis of bacterial pulmonary infections.

1. Introduction

Respiratory infections are the most common infectious diseases among clinically serious patients, with pulmonary infections remaining the leading cause of death [1]. And lower respiratory infections are still one of the leading causes of death worldwide [2]. Due to the infectious and pathogenic nature of Covid-19 in recent years, pneumonia caused by Covid-19 accompanying bacterial infection has become the leading cause of death from infectious diseases [3,4]. Furthermore, lower respiratory infections are associated with more than 1.5 million deaths from bacterial resistance, which makes it the most high-burden infectious disease syndrome [5]. Therefore, early and accurate detection of pathogenic bacteria is particularly important for the timely and correct treatment of

Abbreviations: LAMP, Loop-mediated isothermal amplification; PCT, Procalcitonin; WBC, white blood cell count; CRP, C-reactive protein.

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patients with respiratory bacterial infections.

Currently, the main pathogens of respiratory tract infections include bacteria, viruses, mycoplasma and chlamydia, for example, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, novel coronavirus, respiratory syncytial virus, and *Mycoplasma pneumoniae* [4,6–8]. Due to genetic variation of the pathogen, one pathogen may lead to different clinical symptoms, and different pathogens may lead to similar clinical manifestations [9,10]. Therefore, empirical administration of drugs based only on the clinical manifestations of patients may lead to clinical treatment failure and can cause an increase in pathogen resistance in the clinic. For the detection of respiratory pathogenic bacteria, traditional bacterial culture is an important tool commonly used in clinical testing. However, the interference of the presence of miscellaneous bacteria in the specimen, the long time of bacterial culture, and the need for special culture conditions for different bacteria lead to difficulties in culturing some pathogenic bacteria, e.g., the culture medium for *Haemophilus influenzae* needs to contain coagulation factors V and X as well as iron ions, and certain antibiotics need to be added as selective culture medium [11]. *Methicillin-resistant staphylococci* require drug sensitivity testing to determine the presence of methicillin and other β-lactams. All these reasons lead to the low sensitivity and poor specificity of traditional bacterial culture tests.

Among the detection techniques of pathogens, PCR is a rapid, highly sensitive and specific molecular diagnostic technique that can be widely used for the detection and differential diagnosis of various pathogens [12–14]. Loop-mediated isothermal amplification (LAMP) technology can simultaneously amplify DNA fragments of multiple common respiratory pathogens and their associated drug resistance genes at once [15,16]. However, it is still not very clear about the clinical value of respiratory pathogenic bacterial nucleic acid testing for clinical use in patients with pulmonary infections. In this study, we will analyze the compliance of the nucleic acid test for respiratory bacterial pathogens with sputum culture results, and correlate the nucleic acid test results with pulmonary imaging manifestations of inflammation, as well as conventional inflammatory markers (procalcitonin (PCT), white blood cell count (WBC), C-reactive protein (CRP)) in order to gain a comprehensive understanding of the value of the clinical application of the nucleic acid test for pathogenic respiratory bacteria.

2. Materials and methods

2.1. Study subjects

Ninety-nine patients suffering from pulmonary infections admitted to the Second People's Hospital of Anhui Province from June 2021 to April 2023 were selected for retrospective analysis and studied, and all patients had informed consent for laboratory tests. Moreover, this study is only a retrospective analysis of clinical laboratory test data and does not reveal any patient privacy. The clinical data of the patients were collected mainly including: gender, age, combined underlying diseases, hospitalization department, specimen type, sputum culture results, etc.

2.2. Specimen collection and pathogenic bacteria nucleic acid detection

The sputum specimen should meet the following criteria: the number of leukocytes under the microscope is more than 25/low magnification field, and the number of squamous epithelial cells is less than 10/low magnification field, and the volume of sputum specimen is $\geq$ 0.6 mL. Nucleic acid detection of eight pathogenic bacteria (*Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Methicillin-resistant Staphylococcus*, *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa*) can be simultaneously performed at one time by LAMP technique with the following simple detection steps. Firstly, the nucleic acid extraction reagent is used to break the bacteria and release the nucleic acid components into the nucleic acid extraction solution, and the extracted nucleic acid sample is clear and not turbid. Secondly, add 34.5 μL of nucleic acid sample to 20 μL of thermostatic amplification reagent and mix thoroughly. 50 μL of the sample was added to the amplification chip and the chip was centrifuged at 6000 rpm for 30 s. After that, the microarray was put into the nucleic acid amplification instrument for DNA amplification detection. All of the above steps were performed according to the kit instructions, and the assay instrument (RTisochip™-A) and its reagents were obtained from Boao Jingdian Biotechnology Co (Bejing,China). The relevant operation flow is shown in Fig. 1.

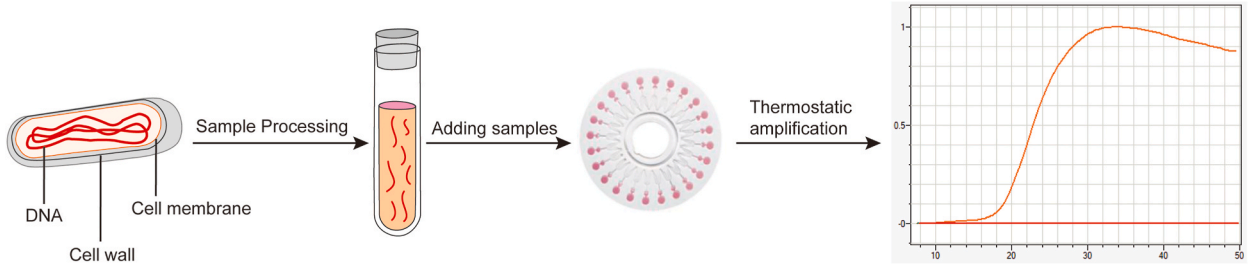

Fig. 1. Respiratory pathogens nucleic acid test operation flow.

2.3. Imaging of pulmonary inflammation

All patients underwent lung examination by CT (Siemens, Germany) or chest radiograph, and the imaging results were interpreted by a highly qualified imaging physician.

2.4. Detection of PCT, WBC, CRP inflammatory markers

WBC was obtained by routine blood analysis, and the normal reference range of WBC was 3.5–9.5 $\times$ 10^9/L. PCT and CRP were detected by chemiluminescence and immunoscatter turbidimetry, respectively. And the reference ranges of PCT and CRP were 0.00–0.05 ng/mL and 0.00–6.00 mg/L, respectively. The differences of the expression of inflammatory markers were analyzed in the group of pathogenic bacteria nucleic acid detection positive and the negative group.

2.5. Statistical analysis

SPSS 20.0 statistical software (IBM, USA) and Graphpad Prism 7.0 statistical software (GraphPad Software Inc., La Jolla,CA, USA) were used for statistical analysis and plotting in this study. Normally distributed data were presented as mean $\pm$ standard deviation (Mean $\pm$ SD). Non-normally distributed data are represented by the median (P25, P75), and the difference between two groups was tested by Mann-Whitney *U* test. Differences in composition ratios within each group were tested by χ^2 test or Fisher's exact test. $P < 0.05$ indicates that the difference is statistically significant.

3. Results

3.1. Clinical characteristics of the patients

As shown in Table 1, the 99 patients with pulmonary infections included 61 males and 38 females, and their ages ranged from 26 to 93 years, with a mean age of 64.95 $\pm$ 15.83 years. Among them, there were 18 patients aged $\leq$ 50 years, 23 patients aged $>$ 50 years to $\leq$ 60 years, 16 patients aged $>$ 60 years to $\leq$ 70 years, 26 patients aged $>$ 70 years to $\leq$ 80 years, and 16 patients aged $>$ 80 years. The main inpatient departments were: 38 in respiratory and critical care department, 42 in intensive care unit, 9 in cardiothoracic surgery, 6 in department of infectious diseases, and 4 in other departments. There were 89 patients with signs of inflammation on the imaging examination and 10 patients without signs of inflammation. Among the patients with underlying medical conditions, 36 had cerebrovascular accidents (including cerebral infarction, cerebral hemorrhage, etc.), 30 had hypertension, 16 had renal damage (including renal failure, renal insufficiency, etc.), 14 had cardiovascular disease (mainly including coronary heart disease, cardiac insufficiency, etc.), 12 had diabetes mellitus, 9 had a history of tumor, 6 had chronic obstructive pulmonary disease (COPD), and 27 had other conditions. In some cases, one patient had several underlying medical conditions. The types of specimens collected were 23 cases of lavage fluid and 76 cases of sputum, respectively.

3.2. Comparison of the results of nucleic acid testing by LAMP and sputum culture

Since the results of LAMP are greatly influenced by the type of specimen, the differences between the two methods were analyzed according to the different specimen types. Using sputum specimens for LAMP, 28 cases were positive for both testing methods, 17 cases were negative, 30 cases were positive for LAMP but negative for sputum culture, and only 1 cases were negative for LAMP but positive for sputum culture. Therefore, the positive rate of LAMP (58/76 = 76.31 %) was significantly higher than the positive rate of sputum

Table 1
Basic clinical characteristics of patients with pulmonary infections.

Characteristics	Number (%)	Characteristics	Number (%)
Gender		Imaging inflammatory signs	
Male	61 (61.62)	Yes	89 (89.90)
Female	38 (38.38)	No	10 (10.10)
Age (years)		Specimen type	
≤50	18 (18.18)	Sputum	76 (76.77)
>50 ~ ≤ 60	23 (23.23)	Lavage fluid	23 (23.23)
>60 ~ ≤ 70	16 (16.16)	Underlying medical conditions	
>70 ~ ≤ 80	26 (26.26)	Cerebrovascular accidents	36 (36.36)
>80	16 (16.16)	Hypertension	30 (30.30)
Inpatient departments		Renal damage	16 (16.16)
Respiratory and critical care department	38 (39.39)	Cardiovascular disease	14 (14.14)
Intensive care unit	42 (42.42)	Diabetes mellitus	12 (12.12)
Cardiothoracic surgery	9 (9.09)	Tumor	9 (9.09)
Department of infectious diseases	6 (6.06)	COPD	6 (6.06)
Others	4 (4.04)	Others	27 (27.27)

COPD: chronic obstructive pulmonary disease.

culture (29/76 = 38.16 %) ($P = 0.0008$) (Table 2). Using lavage fluid specimens for LAMP, the positive rate of LAMP(4/23 = 17.39 %) was slightly higher than the positive rate of sputum culture (1/20 = 5.00 %), but the difference in the positive rate between the two test methods was not statistically significant ($P = 0.173$) (Table 2).

Conformity analysis of the results of two testing methods for detecting eight respiratory pathogenic bacteria

Due to certain differences between the two detection methods and the fact that sputum culture can detect bacteria other than the eight respiratory pathogenic bacteria, inconsistent results existed between bacterial culture and nucleic acid detection. To further analyze this inconsistency, we analyzed whether the nucleic acid detection results of the eight respiratory pathogens were consistent with the culture results. There were 17 cases of *Acinetobacter baumannii* detected by LAMP and only 6 cases by sputum culture, with a compliance rate of 35.29 % (6/17); 14 cases of *Klebsiella pneumoniae* detected by LAMP and only 1 case by sputum culture, with a positive compliance rate of 7.14 % (1/14); 7 cases of *Streptococcus pneumoniae* detected by LAMP and 0 cases by sputum culture, with a positive compliance rate of 0.00 % (0/7); 9 cases of *Staphylococcus aureus* detected by LAMP and 2 cases by sputum culture, with a positive compliance rate of 22.22 %(2/9); LAMP detected *Haemophilus influenzae* in 6 cases and sputum culture in 1 case, with a compliance rate of 16.67 %(1/6); LAMP detected *methicillin-resistant Staphylococcus* in 45 cases and sputum culture in 3 cases, with a compliance rate of 6.67 % (3/45); LAMP detected *Stenotrophomonas maltophilia* in 5 cases and sputum culture in 1 case, with a compliance rate of 20.00 %(1/5); LAMP detected *Pseudomonas aeruginosa* was detected in 9 cases, while sputum culture in 2 cases, with a compliance rate of 22.22 % (2/9). The overall compliance rate between the results of the nucleic acid test and the results obtained by culture was only 16.29 % (16/112), and the overall negative compliance rate was 91.89 % (34/37). All results are shown in Table 3.

Comparison of analysis of pulmonary imaging inflammatory manifestations with pathogenic bacterial nucleic acid detection

Since inflammatory manifestations of lung infection can be detected by imaging examination (CT or chest X-ray), nucleic acid detection was analyzed separately according to the different specimen types (sputum and lavage fluid). Among the 76 sputum specimens tested for nucleic acid by LAMP, imaging lung examination showed inflammatory manifestations in 73 cases and no inflammatory manifestations in 3 cases. Among 73 cases with inflammatory manifestations of imaging, 55 (55/73 = 75.34 %) were positive and 18 (18/73 = 24.66 %) were negative using the LAMP assay; among the 3 cases without inflammatory manifestations of imaging, 3 (3/3 = 100 %) were positive (Table 4). Nucleic acid was detected in 23 lavage fluid specimens, and imaging lung examination was performed in 23 cases (16 cases with inflammatory manifestations and 7 cases without inflammatory manifestations). Among the 16 cases with pulmonary imaging inflammatory manifestations, 3 cases (3/16 = 18.75 %) were positive and 13 cases (13/16 = 81.25 %) were negative using LAMP; among the 7 cases without inflammatory manifestations, 1 case (1/7 = 14.28 %) was positive and 6 cases (6/7 = 85.71 %) were negative using LAMP (Table 4). There was no statistically significant difference between LAMP and imaging findings ($P > 0.9999$).

3.3. Analysis and comparison of imaging inflammatory manifestations with sputum culture results

Because of the large difference between sputum culture and pathogenic bacteria nucleic acid detection results, it is necessary to analyze and compare the imaging examination results with sputum culture results. Among the 89 cases with inflammatory manifestations on imaging lung examination, only 30 cases (30/89 = 33.71 %) were positive for sputum culture and 59 cases (59/89 = 66.29 %) were negative; among the 10 cases without inflammatory manifestations, 0 cases (0.00 %) were positive for sputum culture and 10 cases (10/10 = 100 %) were negative, and there was a difference between the imaging results and sputum culture results ($P = 0.030$) (Table 5).

Correlation analysis of the number of respiratory pathogenic bacterial infections and the expression of inflammatory indicators in patients with nucleic acid testing

Since LAMP can detect multiple bacteria in the same sample at the same time, then multiple infections of bacteria may exist in one patient. In this study, there were 30 patients with one bacterial infection, 22 patients (73.33 %) with PCT over 0.05 ng/mL, 26 patients (94.12 %) with CRP over 6.0 mg/L, and only 12 patients (40.00 %) with WBC over the reference range. The number of patients with

Table 2
Comparison of nucleic acid test results of respiratory pathogenic bacteria and sputum culture results.

Specimen Type	LAMP	Sputum bacterial culture		Total number	Fisher's exact test (*P* value)
		Positive	Negative		
Sputum	Positive	28	30	58	0.0008
	Negative	1	17	18	
	Total number	29	47	76	
Lavage fluid	Positive	1	3	4	0.173
	Negative	0	19	19	
	Total number	1	22	23	

Table 3
Compliance between LAMP and sputum culture results of 8 common respiratory pathogenic bacteria.

Bacteria	LAMP (n)	Sputum culture (n)	Positive conformity rate (%)	Negative conformity rate (%)
Acinetobacter baumannii	17	6	35.29	
Klebsiella pneumoniae	14	1	7.14	
Streptococcus pneumoniae	7	0	0.00	
Staphylococcus aureus	9	2	22.22	
Haemophilus influenzae	6	1	16.67	
Methicillin-resistant Staphylococcus	45	3	6.67	
Stenotrophomonas maltophilia	5	1	20.00	
Pseudomonas aeruginosa	9	2	22.22	
Total number of positive	112	16	16.29	
Total number of negative	37	34		91.89

Table 4
Comparison of LAMP detection and analysis of imaging inflammatory manifestations in different specimen types.

Imaging pulmonary inflammatory manifestations	n	LAMP (sputum)		Fisher's exact test (*P* value)
		Positive	Negative	
Yes	73	55 (75.34 %)	18 (24.66 %)	> 0.9999
No	3	3 (100 %)	0 (0.00 %)	
Total	76	58	18	
Imaging pulmonary inflammatory manifestations	n	**LAMP (lavage fluid)**		
		Positive	**Negative**	
Yes	16	3 (18.75 %)	13 (81.25 %)	> 0.9999
No	7	1 (14.28 %)	6 (85.71 %)	
Total	23	4	19	

Table 5
Comparative analysis of the relationship between sputum culture and imaging signs of inflammation.

Imaging pulmonary inflammatory manifestations	n	Sputum culture		Fisher's exact test (*P* value)
		Positive	Negative	
Yes	89	30 (33.71 %)	59 (66.29 %)	0.030
No	10	0 (0.00 %)	10 (100 %)	
Total	99	30	69	

two bacterial infections was 18, the number of patients with PCT over 0.05 ng/mL was 17 (94.44 %), the number of patients with CRP over 6.0 mg/L was 17 (94.44 %), and the number of patients with WBC over the reference range was 11 (61.11 %). The number of patients with three or more bacterial infections was 14, the number of patients with PCT over 0.05 ng/mL was 13 (92.86 %), the number of patients with CRP over 6.0 mg/L was 12 (85.71 %), and the number of patients with WBC over the reference range was 7 (50.00 %). Thus, the overall concordance rate between increased PCT and positive respiratory nucleic acid test was 83.87 %, the overall concordance rate between elevated WBC and positive respiratory nucleic acid test was 48.39 %, and the overall concordance rate between increased CRP and positive respiratory nucleic acid test was 88.71 %. All results are shown in Table 6.

Differences in inflammatory markers expression between the negative and positive respiratory pathogenic bacteria nucleic acid test groups

To further analyze the relationship between respiratory pathogenic bacteria nucleic acid detection and PCT, WBC, and CRP expression, the differences in PCT, WBC, and CRP expression between the two groups of positive and negative LAMP detection were analyzed. The results showed that the expression level of PCT in the nucleic acid positive group (0.487 (0.113, 2.998) ng/mL) was significantly higher than that in the negative group (0.081 (0.039, 0.277) ng/mL) ($P < 0.0001$); the expression level of WBC in the

Table 6
Comparative analysis between the status of multiple bacterial infections tested by LAMP and inflammatory markers.

Patient bacterial infection status	n	PCT > 0.05 ng/mL	CRP > 6.0 mg/L	WBC > 9.5×10^9/L
One bacteria	30	22 (73.33 %)	26 (94.12 %)	12 (40.00 %)
Two bacteria	18	17 (94.44 %)	17 (94.44 %)	11 (61.11 %)
Three or more bacteria	14	13 (92.86 %)	12 (85.71 %)	7 (50.00 %)
Total	62	52 (83.87 %)	55 (88.71 %)	30 (48.39 %)

nucleic acid positive group (9.015 (6.150, 14.49) $\times 10^9$) was significantly higher than that in the negative group (6.585 (4.725, (8.658) $\times 10^9$) ($P = 0.006$); CRP expression level in the nucleic acid positive group (56.45 (28.58, 128.0) mg/L) was significantly higher than that in the negative group (18.42 (4.510, 56.05) mg/L) ($P = 0.0003$) (Fig. 2).

4. Discussion

Currently, infectious diseases, especially lower respiratory tract infections, remain the most deadly infectious diseases worldwide. The main pathogens causing respiratory tract infections include: bacteria, viruses, chlamydia and mycoplasma, etc. And the most important pathogens are bacteria, such as *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus haemolyticus*, etc [1]. Traditional sputum bacterial culture is the most classical method to identify respiratory bacteria and drug sensitivity test. However, the difficulty in collecting deep sputum specimens from the respiratory tract, the long incubation time, and the tedious experimental operations have resulted in poor sensitivity and specificity of sputum culture in identifing bacteria, leading to difficulties in the identification of pathogenic bacteria of respiratory tract infections, and delaying the clinical treatment with medication [17,18]. Therefore, there is a need to find a more sensitive and accurate rapid test to identify respiratory pathogenic bacteria.

In recent years, the amplification of bacterial-specific DNA fragments by PCR technique can accurately identify the presence of bacteria, which is characterized by easy operation, short detection cycle, high sensitivity and specificity [19]. Therefore, PCR technology used to identify bacteria will likely replace the traditional sputum culture technique in the future. However, the differences between PCR and sputum culture techniques in the identification of bacteria and the value of PCR techniques for clinical applications in respiratory tract infections require further analysis. In this study, we detected the gene expression of *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *methicillin-resistant Staphylococcus*, *Haemophilus influenzae*, and *Stenotrophomonas maltophilia* in sputum and lavage specimens simultaneously by a PCR technique (LAMP). We found significant differences between the results of nucleic acid testing of sputum specimens for respiratory pathogens and sputum culture identification, and the positive rate of nucleic acid testing (76.31 %) was significantly higher than the positive rate of sputum culture (38.16 %). However, There was no statistically significant difference in the positivity rate of detecting pathogenic bacteria by nucleic acid testing in lavage fluid versus sputum culture, and the positivity rate for both methods was low. Thus, the two techniques were compared separately for different bacteria and the results showed a low positive compliance rate for both techniques, for example, 6.67 % for *methicillin-resistant staphylococci*, while the overall positive compliance rate for both techniques was only 16.29 % but the negative compliance rate reached 91.89 %. The reasons for this may exist in the following three aspects: firstly, specimens from patients with deep lung infections are difficult to obtain. Secondly, sputum specimens may be contaminated with normal flora from oral secretions leading to the presence of a large number of miscellaneous bacteria in sputum specimens, and even the presence of fungi may be present in some critically ill patients, all of which seriously interfere with the results of sputum culture. Finally, as the collection of lavage fluid may avoid the interference of miscellaneous bacteria, but it tends to cause an increase in negative results of nucleic acid testing. Therefore, sputum specimens should be used for pathogenic bacterial nucleic acid testing in clinical practice whenever possible.

Imaging examination is the primary diagnostic tool for pulmonary infections and is an important indicator of the severity of pulmonary infections. Chest CT or chest X-ray can provide a comprehensive observation of the inflammatory status, exudation, and infiltration of the lung [20,21]. In this study, the imaging results were compared with the pathogenic bacterial nucleic acid tests of sputum and lavage specimens, respectively. And there was good concordance between imaging and nucleic acid test results. The percentage of patients with inflammatory lung manifestations on imaging and positive nucleic acid tests on sputum specimens was 75.34 % of the total. However, the percentage of patients with inflammatory lung manifestations on imaging and positive nucleic acid tests on lavage fluid specimens was only 18.75 % overall. In addition, we analyzed and compared imaging examinations with sputum culture results, which showed significant differences between the two methods. Therefore, once the presence of inflammatory manifestations in the lungs is detected by imaging, further pathogenic bacteria nucleic acid testing of sputum specimens could greatly increase the detection rate of pathogenic bacteria in respiratory tract infections and thus effectively reflect the real situation of respiratory tract infections. Relying on sputum culture alone may lead to missed pathogens and misdiagnosis of patients.

Procalcitonin (PCT), white blood cell count (WBC), and C-reactive protein (CRP) are all commonly used inflammatory response markers in clinical practice. PCT is a non-hormonally active precalcitonin peptide substance consisting of 116 amino acids. When the body is in a severe systemic inflammatory response due to bacterial and fungal infections, etc., the PCT level in the blood increases significantly [22,23]. WBC is also a common indicator of bacterial infection and tends to increase significantly in the presence of bacterial infection in the lung [24,25]. As an acute temporal response protein and a commonly used non-specific clinical marker of inflammation, CRP can reflect the degree of infection in the body. The interaction between CRP and complement exhibits a number of biological activities, such as the body's defense response to infection and regulation of inflammation, etc [26]. Since multiple infections of bacteria may exist in the same patient, the results of this study showed that the percentage of patients with elevated PCT and CRP were higher than 80 % when they were infected with two or more bacteria at the same time, and the overall compliance rate between elevated PCT and CRP and positive respiratory nucleic acid test was 83.87 % and 88.71 %, respectively. The concordance between elevated WBC and positive respiratory nucleic acid test was only 48.39 %, which may be related to the condition of different underlying diseases, medication and other factors that limit the variation of WBC. In addition, we compared PCT, CRP and WBC between the pathogenic bacteria nucleic acid detection positive and negative groups, respectively, and the results showed that PCT, CRP and WBC were significantly higher in the nucleic acid positive group than that in the nucleic acid negative group, which further suggest that pathogenic bacteria detected by nucleic acid have a good correlation with inflammatory markers.

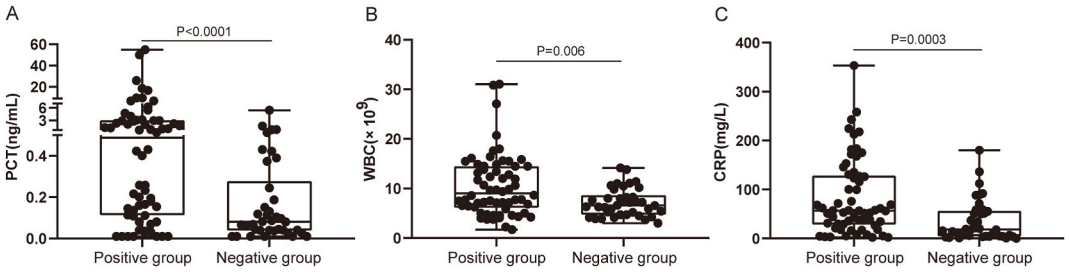

Fig. 2. Expression levels of PCT, WBC and CRP in positive and negative groups of respiratory pathogenic bacteria nucleic acid by LAMP. (A) The expression levels of PCT in the two groups. (B) The expression levels of WBC in the two groups. (C) The expression levels of CRP in the two groups.

In summary, the pathogenic bacteria nucleic acid by LAMP and imaging examination, PCT, CRP, WBC are in good concordance, which avoids missed and misdiagnosed cases of routine sputum culture pathogen identification, and it can be a good alternative to sputum culture identification of lung infections and provide a good help for timely and rapid clinical drug treatment. However, there are still some shortcomings in this study. Firstly, although LAMP can identify eight bacteria at once and rapidly, and can identify different bacteria in one patient, there are still other lung infection bacteria that cannot be detected, which may be the main reason for the negative results of LAMP despite the inflammatory manifestations on imaging tests. Second, whether tracheal intubation resulted in bacterial infection of the lungs and whether the specimen originated from the tracheal intubation are unknown and therefore requires further detailed investigation. Finally, the sample size used in this study was small and still needs to be expanded for further analysis.

Author contribution statement

CW and ZZ collected and organized the data; BZ and CW analyzed the data and wrote the manuscript. XW developed the experiments. BZ supervised this manuscript; Final approval of manuscript: All authors.

Declaration of competing interest

All authors declare that there are no conflicts of interest.

Data availability

Data will be made available on request.

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