ORIGINAL RESEARCH

LncRNA RP11-248E9.5 and RP11-456D7.1 are Valuable for the Diagnosis of Childhood Pneumonia

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Background: Pneumonia is a common infection of the lung parenchyma in children, and early and accurate diagnosis of childhood pneumonia (CP) is important for implementing appropriate preventive and treatment strategies. This study aimed to evaluate the diagnostic value of the combination of long non-coding RNA (lncRNA) RP11-248E9.5, RP11-456D7.1, c-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) in CP.

Patients and Methods: A total of 50 healthy children (HC) and 100 CP patients were enrolled. The serum expression of RP11-248e9.5 and RP11-456d7.1 was detected by qRT-PCR. The white blood cell (WBC), hemoglobin (HB), platelet (PLT), neutrophil, and lymphocyte were analyzed by automated hematology analyzer. The serum levels of CRP and procalcitonin (PCT) were analyzed by automatic biochemical analyzer. The receiver operating characteristic (ROC) curves were applied to evaluate the diagnostic value in CP.

Results: The NLR and PLR, expression of RP11-248E9.5 and RP11-456D7.1, and serum levels of CRP and PCT were significantly higher in the CP group than those in the HC group. Both RP11-248E9.5 (AUC, 0.86; sensitivity, 84%; specificity, 78%) and RP11-456D7.1 (AUC, 0.89; sensitivity, 79%; specificity, 92%) exhibited certain diagnostic value in CP. The diagnostic values of PCT, CRP, NLR and PLR in CP were limited by low sensitivity ($\leq$ 71%). The combination of multiple indicators improved the diagnostic value. The combination of RP11-248E9.5, RP11-456D7.1, CRP, NLR, and PLR had the best diagnostic value in CP (AUC, 0.992; Sensitivity, 0.97; Specificity, 0.99).

Conclusion: The combination of RP11-248E9.5, RP11-456D7.1, CRP, NLR, and PLR was a potential diagnostic strategy for CP.

Keywords: childhood pneumonia, long non-coding RNA, C-reactive protein, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio

Introduction

Pneumonia is the most common cause of childhood death around the world.[1] It has been reported that 16% of children under 5 years die of pneumonia every year.[2] The early symptoms of childhood pneumonia (CP) are not obvious, exhibiting mild respiratory symptoms. Patients with CP present cough and difficulty breathing with the disease procession.[3] Although the symptoms of advanced CP are obvious and easy to find,[4] the best period of treatment is missed. Until now, the missed diagnosis is a critical risk factor for death in children with CP complicated by acute respiratory infections.[5] It is vital to explore potential biomarkers for the early diagnosis of CP.

Clinically, the diagnostic criteria for CP mainly includes the symptoms of cough, fever, abnormal breathing, the changes of inflammatory factors and platelet (PLT), and the patchy shadow on chest X-ray.[3] Diverse biomarkers in blood have

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been proved to have potential diagnostic value on CP. For instance, PCT has moderate diagnostic accuracy for bacterial pneumonia in children.[6] C-reactive protein (CRP) has good value in differentiating bacterial and non-bacterial CP.[7] Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are important in the diagnosis and prognostic evaluation of pneumonia.[8,9] However, these indicators are also changed in many other diseases,[10–12] which limited their application alone in the diagnosis of CP.

Long non-coding RNAs (LncRNAs) are a kind of non-coding RNAs that participates in the progression of different diseases.[13,14] Some lncRNAs have been identified as potential diagnostic markers in pneumonia.[15–18] Zhou et al have found that overexpression of lncRNA SNHG16 is related to LPS-induced acute pneumonia.[15] Nong et al have displayed that lncRNA NEAT1 plays an important role in the development of pneumonia.[16] Zhang et al have reported that lncRNA MIAT2 has been determined to be associated with the onset of neonatal pneumonia.[17] Recently, Huang et al have found that lncRNA RP11-248E9.5 and lncRNA RP11-456D7.1 are up-regulated in both the mild and severe pneumonia.[19] However, the diagnostic values of RP11-248E9.5 and RP11-456D7.1, as well as the combination with traditional indicators in CP are still unclear.

In this study, we evaluated the diagnostic values of RP11-248E9.5 and RP11-456D7.1, and the combination with traditional indicators (CRP, NLR, and PLR) in CP. Our findings may reveal a potential diagnostic strategy for CP.

Patients and Methods
Clinical Samples

A total of 100 CP patients (3.12 ± 2.02 years old) were collected at our hospital from April 2018 to March 2019, and 50 healthy children (3.24 ± 1.84 years old) were collected as healthy control (HC). CP was defined according to the World health organization's guidelines for acute respiratory infection.[20] All CP patients met the following criteria: 1) The symptoms of cough, fever, and abnormal breathing, and fixed medium and small alveolar sounds in lung; 2) increased neutrophils (> 8×10^9/L) and PLTs (> 300×10^9/L) increased significantly; 3) patchy shadows observed by Chest X-ray. Patients with chronic renal or hepatic disease, hematological diseases, inflammatory bowel disease, chronic obstructive pulmonary disease, asthma, and congenital diseases were

excluded from this study. This study was approved by the Medical Ethics Committee of our Hospital in accordance with the Declaration of Helsinki. Written informed consent was obtained from the guardians of all patients and HCs.

Detection of Serum Markers

Venous blood samples were collected from patients in the morning with an empty stomach, and the serum was separated by 20 min of centrifugation at 3000 rpm. The white blood cell (WBC), hemoglobin (HB), neutrophil, lymphocyte and PLT were analyzed by BC-6800 Automated Hematology Analyzer (Sun bright, Shanghai, China). NLR and PLR were calculated as the ratio of the neutrophils and PLTs to lymphocytes. The serum levels of C-reactive protein (CRP) and procalcitonin (PCT) were detected by an automatic biochemical analyzer (AU5811, Beckman Coulter, Brea, CA, USA).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The total RNAs were extracted from serum using miRcute Serum/Plasma miRNA Isolation Kit (Tiangen, Beijing, China), and were reverse-transcribed into cDNAs using Prime Script RT reagent kit (Tiangen). qRT-PCR was performed using miRcute PlusmiRNA qPCR Detection Kit (Tiangen). The reaction program was 95°C for 2 min and 30 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 1 min. GAPDH was used as an internal reference. The relative expression level was calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences were enlisted in Table 1.

Statistical Analysis

All statistical analysis was performed using SPSS 23.0 (Version X; IBM, Armonk, NY, USA). Qualitative data were analyzed using the χ^2 test. Quantitative data were presented as the mean ± standard deviation (SD), and comparison between different groups was analyzed by Student's *t*-test (two groups) or one-way ANOVA

Table 1 Primer Sequences Used in qRT-PCR

Name of Primer	Sequences
RP11-248E9.5-F	5′-TTTCCCTTCTCA-ACGACCTG-3′
RP11-248E9.5-R	5′-GAGTAAAGACAAGCT-GAACCCA-3′
RP11-456D7.1-F	5′-GCAA-GGAGAGTAATGCTGGA-3′
RP11-456D7.1-R	5′-CAATGAG-GATGAGAAGAGGTC-3′
GAPDH-F	5′-GACGGCCGCATCTTCTTGT-3′
GAPDH-R	5′-CACACCGACCTTCACCATTTT-3′

followed by Tukey's post hoc test (more than two groups). Receiver operating characteristic (ROC) curves were plotted to evaluate the diagnostic value on CP. P < 0.05 was considered to be statistically significant.

Results
The Characteristics of CP Patients

The clinicopathological characteristics of all cases were shown in Table 2. There were no significantly differences in the age, gender, pneumonia type, and HB between the CP and HC groups. The levels of WBC and PLT in the CP group were markedly higher than those in the HC group (P < 0.0001, Table 2).

The Levels of PCT, CRP, NLR, PLR, RP11-248E9.5, and RP11-456D7.1 in CP Patients

As displayed in Figure 1A and B, the serum levels of PCT and CRP were significantly higher in the CP group than that in the HC group (P < 0.05). The NLR and PLR in the CP group were also remarkably higher than those in the HC group (P < 0.05, Figure 1C and D). In addition, the expression of RP11-248E9.5 and RP11-456D7.1 in the CP group was significantly higher than that in the HC group (P < 0.05, Figure 1E and F). Patients were then classified into three categories based on pathogens. The results displayed that there were no significantly differences in the expression of RP11-248E9.5 and RP11-456D7.1 among children with bacterial, viral, and mycoplasma pneumonia (P > 0.05, Table 3).

The Diagnostic Values of PCT, CRP, NLR, PLR, RP11-248E9.5, and RP11-456D7.1 in CP

The diagnostic values of PCT, CRP, NLR, PLR, RP11-248E9.5, and RP11-456D7.1 in CP were evaluated by ROC curves. As shown in Figure 2A–F and Table 4, RP11-456D7.1 (0.89) exhibited the highest AUC, followed by RP11-248E9.5 (0.86), PCT (0.8093), NLR (0.8063), CRP (0.7827), and PLR (0.7316). The sensitivity of PCT, CRP, NLR, and PLR in the diagnosis of CP were all ≤ 71%. The sensitivity and specificity of RP11-248E9.5 in the diagnosis of CP were 84% and 78%, respectively. The sensitivity and specificity of RP11-456D7.1 in the diagnosis of CP were 79% and 92%, respectively.

### The Diagnostic Values of Indicator Combinations in CP

To improve the diagnostic values of RP11-248E9.5 and RP11-456D7.1 in CP, RP11-248E9.5 and RP11-456D7.1 were combined with CRP, NLR, and PLR. As shown in Figure 3A–E and Table 5, the AUC of the combination of traditional indicators (CRP, NLR, and PLR) was 0.8936. The AUC of the combination of RP11-248E9.5 and RP11-456D7.1 was 0.9608. The combination with traditional indicators increased the diagnostic values of RP11-248E9.5 and RP11-456D7.1. The AUC of the combination of RP11-248E9.5, CRP, NLR, and PLR, and the combination of RP11-456D7.1, CRP, NLR, and PLR were 0.963 and 0.9668, respectively. The combination of the above

Table 2 Comparison of Clinical Cases Between Health Control and Childhood Pneumonia Patients

	Health Control (HC)	**Childhood Pneumonia (CP)**	**P value**
Age (y)	3.12 ± 2.02	3.24 ± 1.84	0.1475
Gender (cases)			0.5272
Male	25	53	
Female	25	47	
Pneumonia types			
Bacterial	–	33	
Viral	–	30	
Mycoplasma	–	37	
WBC (× 10^9/L)	6.80 ± 1.75	10.50 ± 3.40	< 0.0001*
HB (g/L)	119.03 ± 18.26	127.44 ± 21.72	0.0698
PLT (× 10^9/L)	217.71 ± 59.31	385.04 ± 68.58	< 0.0001*

Note: *Significantly different between HC and CP patients.
Abbreviations: WBC, white blood cell; HB, hemoglobin; PLT, platelet.
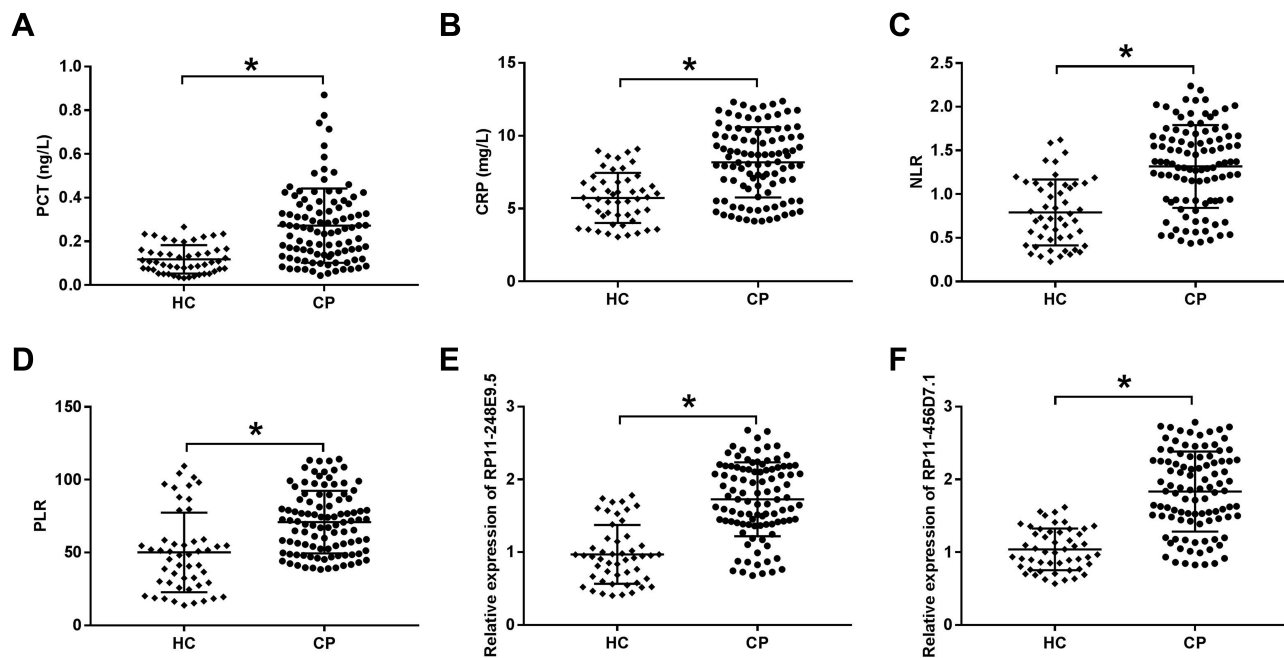
Figure 1 The levels of PCT, CRP, NLR, and PLR, and the expression of RP11-248E9.5 and RP11-456D7.1 in HC and CP. (**A**) PCT. (**B**) CRP. (**C**) NLR. (**D**) PLR. (**E**) RP11-248E9.5. (**F**) RP11-456D7.1. *P < 0.05 vs HC.

five indexes had the best diagnostic value in CP (AUC = 0.992, sensitivity = 0.97, specificity = 0.99).

Discussion

Pneumonia is a serious infection in the lung and remains a major cause of childhood mortality, especially in children under 5 years old.[21] In the present study, the NLR and PLR, serum levels of PCT and CRP, and expression of RP11-248E9.5 and RP11-456D7.1 in CP children were significantly higher than those in HC children. RP11-248E9.5 and RP11-456D7.1 were proved to have a certain value in the diagnosis of CP, and their combination with traditional indicators can further improve the diagnostic value.

LncRNAs are involved in the occurrence and progression of pneumonia.[15–18] Recently, Huang et al have found

that RP11-248E9.5 and RP11-456D7.1 are up-regulated in pneumonia, and there is a difference in the expression of RP11-248E9.5 and RP11-456D7.1 between mild and severe pneumonia.[19] In our study, the expression of RP11-248E9.5 and RP11-456D7.1 was significantly higher in CP patients than those in HC. We speculated that monitoring the expression of RP11-248E9.5 and RP11-456D7.1 may aid in the diagnosis of CP to a certain extent. The diagnostic values of RP11-248E9.5 and RP11-456D7.1 in CP were further analyzed. The ROC curve analysis indicated that both RP11-248E9.5 and RP11-456D7.1 had certain diagnostic values for CP. The AUC of RP11-248E9.5 and RP11-456D7.1 were both higher than 0.8. The sensitivity and specificity values of RP11-248E9.5 were 84% and 78% and the sensitivity and specificity of RP11-456D7.1 were 79% and 92%, respectively. These data indicated that RP11-248E9.5 and

Table 3 Comparison of LncRNA RP11-248E9.5 and RP11-456D7.1 in Children with Different Types of Pneumonia

	Bacterial (n=33)	Viral (n=30)	Mycoplasma (n=37)	P value
Age (y)	3.384 ± 1.829	2.806 ± 1.908	3.471 ± 1.814	0.3004
Gender (cases) Male Female	19 14	18 12	16 21	0.3196
RP11-248E9.5	1.663 ± 0.502	1.797 ± 0.459	1.727 ± 0.552	0.5811
RP11-456D7.1	1.810 ± 0.484	1.867 ± 0.596	1.827 ± 0.579	0.9188
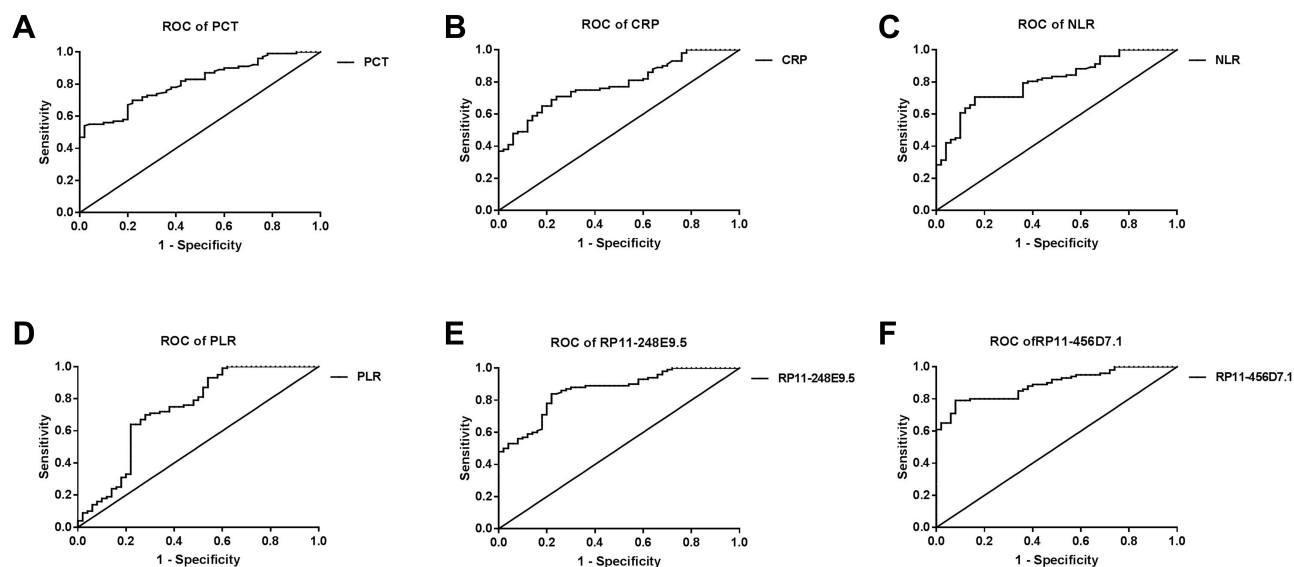
Figure 2 The receiver operating characteristic (ROC) curves of PCT, CRP, NLR, PLR, RP11-248E9.5, and RP11-456D7.1 in the diagnosis of CP. (**A**) PCT. (**B**) CRP. (**C**) NLR. (**D**) PLR. (**E**) RP11-248E9.5. (**F**) RP11-456D7.1.

RP11-456D7.1 may be potential diagnostic markers of CP. Until now, the knowledge on the molecular mechanisms of RP11-248E9.5 and RP11-456D7.1 in pneumonia is still limited. Based on previous studies, we speculated that RP11-248E9.5 may be involved in pneumonia through regulating QRFP/G-protein coupled receptor signaling pathway, and RP11-456D7.1 may be involved in pneumonia via targeting CCL21.[19] The detail molecular mechanisms of these two lncRNAs in CP need to be further explored.

CRP is a non-specific marker of inflammation, and its level is relatively low in the blood of healthy people. CRP increases sharply in a short time when organs or tissues are damaged or infected, which plays a protective role in the process of human natural immunity.[22] Notably, the increased level of CRP is also closely to be associated with co-infections of different pathogens in CP.[23] CRP has been reported to be valuable in distinguishing bacterial from the respiratory syncytial virus-associated pneumonia among children.[24] In our study, the CRP level in CP patients was

markedly higher than that in HC. In addition, ROC curve analysis showed that the AUC of CRP in the diagnosis of CP was 0.7827, and the sensitivity and specificity were only 71% and 76%, respectively. These results indicated that CRP alone is limited in the diagnosis of CP. In inflammatory diseases, the characteristics of NLR are increased neutrophils and decreased lymphocytes.[25] A high level of NLR is usually associated with inflammation in pneumonia.[26] Bekdas et al have found that the combination of NLR and CRP/mean PLT volume ratio can be used for the differential diagnosis of bacterial and viral pneumonia in children.[27] PLR is another marker for disclosing the changes in PLT and lymphocyte counts caused by acute inflammatory states.[28] PLR is positively correlated with CRP in pneumonia, and the activated PLT can trigger inflammation.[29] Kartal et al have concluded that elevated PLR has diagnostic value to identify community-acquired pneumonia.[8] In this study, the levels of NLR and PLR were significantly increased in CP patients. However, NLR and PLR were not

Table 4 The Diagnostic Value of Five Serum Markers in Diagnosis of Childhood Pneumonia (CP)

Markers	Cutoff	Sensitivity (%)	Specificity (%)	AUC	95% CI	P value
PCT	0.1605	70	76	0.8093	0.7423~0.8763	< 0.0001
CRP	6.882	71	76	0.7827	0.7105~0.8549	< 0.0001
NLR	1.145	71	84	0.8063	0.7365~0.8761	< 0.0001
PLR	59.06	64	78	0.7316	0.6372~0.826	< 0.0001
RP11-248E9.5	1.233	84	78	0.86	0.801~0.919	< 0.0001
RP11-456D7.1	1.421	79	92	0.89	0.839~0.9386	< 0.0001

Abbreviations: PCT, procalcitonin; CRP, C-reactive protein; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; RP11-248E9.5, long non-coding RNA RP11-248E9.5; RP11-456D7.1, long non-coding RNA RP11-456D7.1; AUC, area under curve.
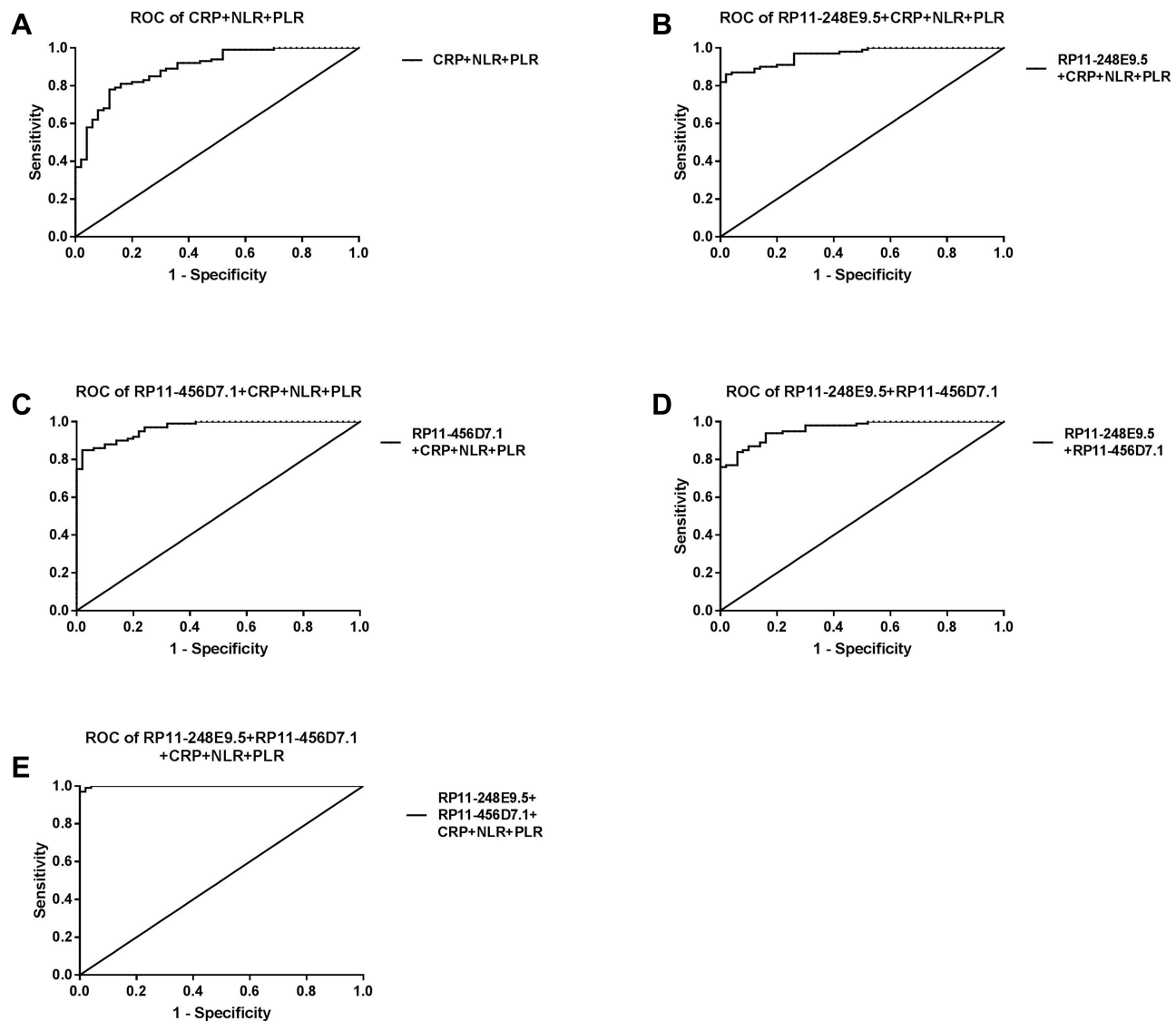
Figure 3 The receiver operating characteristic (ROC) curves of RP11-248E9.5 and RP11-456D7.1 combined with CRP, NLR, and PLR in the diagnosis of CP. (**A**) CRP+NLR +PLR. (**B**) RP11-248E9.5+CRP+NLR+PLR. (**C**) RP11-456D7.1+CRP+NLR+PLR. (**D**) RP11-248E9.5+RP11-456D7.1. (**E**) RP11-248E9.5+ RP11-456D7.1+CRP+NLR+PLR.

sensitive and specific enough in diagnosis of CP (NLR: sensitivity, 71%; specificity, 84%; PLR: sensitivity, 64%; specificity, 84%).

Due to the diversity of pathogenic factors and clinical manifestations of CP, the application value of single traditional indicator in the diagnosis of CP is challenged

Table 5 The Diagnostic Value of RP11-248E9.5 and RP11-456D7.1 Combined with CRP, NLR and PLR in Diagnosis of Childhood Pneumonia

Markers	Sensitivity (%)	Specificity (%)	AUC	95% CI	P value
CRP + NLR + PLR	0.78	0.88	0.8936	0.8421~0.9451	< 0.0001
RP11-248E9.5 + CRP + NLR + PLR	0.86	0.98	0.963	0.9381~0.9879	< 0.0001
RP11-456D7.1 + CRP + NLR + PLR	0.85	0.98	0.9668	0.9439~0.9897	< 0.0001
RP11-248E9.5 + RP11-456D7.1	0.84	0.94	0.9608	0.9348~0.9868	< 0.0001
RP11-248E9.5 + RP11-456D7.1 + CRP + NLR + PLR	0.97	0.99	0.9992	0.9975~1.001	< 0.0001

Abbreviations: CRP, C-reactive protein; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; RP11-248E9.5, long non-coding RNA RP11-248E9.5; RP11-456D7.1, long non-coding RNA RP11-456D7.1; AUC, area under curve.

seriously. The combined application of multiple indicators is imperative which can greatly improve the diagnostic value of individual indicators alone.[30] Liu et al have reported that PCT in combination with hs-CRP facilitates the early diagnosis of pneumonia complicated by sepsis in new-born.[31] In our study, the diagnostic values of the combinations of CRP, NLR, PLR, RP11-248E9.5 and/or RP11-456D7.1 were further evaluated. Compared with the combination of traditional indicators (CRP, NLR, and PLR), the AUC of the combination of RP11-248E9.5 and traditional indicators was increased by 7.77%, and the AUC of the combination of RP11-456D7.1 and traditional indicators was increased by 8.19%. In addition, the combination of five indexes had the best diagnosis value in CP with an AUC of 0.992. These results indicated that the combination of multiple indicators may improve the diagnostic efficacy of a single indicator. To sum up, the combination of RP11-248E9.5, RP11-456D7.1, CRP, NLR, and PLR was recommended in the diagnosis of CP. However, this set of biomarkers may be complicated in clinical practice. More simplified diagnostic strategies still need to be studied in the future.

Conclusion

The NLR and PLR, serum levels of PCT and CRP, and expression of RP11-248E9.5 and RP11-456D7.1 were remarkably increased in CP patients. RP11-248E9.5 and RP11-456D7.1 have a certain value in the diagnosis of CP. The combinations of RP11-248E9.5 and RP11-456D7.1 with conventional indicators (CRP, NLR, and PLR) greatly improved the diagnostic efficiency. The combination of RP11-248E9.5, RP11-456D7.1, CRP, NLR, and PLR is recommended in the diagnosis of CP.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article.

Ethics Approval and Informed Consent

This study was approved by the Medical Ethics Committee of Zaozhuang Maternal and Child Health Care Hospital in accordance with the Declaration of Helsinki. Written informed consent was obtained from the guardians of all patients and HCs.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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