Causes of Lower Respiratory Tract Infections and the Use of Diagnostic Biomarkers in Blood Samples from Children in Hohhot, Inner Mongolia, China, Between July 2019 and June 2020

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG 1 **Yanzi Gan**
BC 1 **YuWei Hu**
CD 1 **Hairong Dong**
DE 1 **Lina Wu**
AEF 1,2 **Yan Niu**

1 Neonatal Department, The First Hospital of Hohhot, Hohhot, Inner Mongolia, PR China
2 Medical Experiments Center, Inner Mongolia Medical University, Hohhot, Inner Mongolia, PR China

Background: Lower respiratory tract infection (LRTI) in children is due to various pathogens. Appropriate diagnosis and early treatment are important for reducing the mortality rate of LRTI. Data on the epidemiology profiles of LRTI are scarce in northern China. The aim of this study was to provide data on the pathogen pattern of LRTI in hospitalized children in Hohhot, Inner Mongolia, China.

Material/Methods: From July 2019 to June 2020, nasopharyngeal swabs were collected from 265 children in Hohhot with LRTI, and pathogens were detected with RT-PCR and PCR. The correlations among procalcitonin (PCT), C-reactive protein (CRP), and white blood cells (WBC) with acute respiratory infections were evaluated.

Results: The highest prevalence of LRTI was detected in 2- to 6-year-old children (149, 56.2%) in winter. Eleven respiratory pathogens were evaluated, and respiratory syncytial virus, *Streptococcus pneumoniae* and *Haemophilus influenza* were the most common pathogens in this region. Single viruses, bacteria, mycoplasma, and multiple pathogens were identified in 24.2, 15.8, 5.3, and 54.7% of patients, respectively. The mean blood biomarker values of patients with LRTI were significantly different from those of healthy children. Furthermore, The AUCs were 0.90, 0.74, and 0.84 for bacteria, virus, and mycoplasma PCT values, which were significantly higher than that of WBC and CRP.

Conclusions: This evaluation of the regional pattern of pathogens in children with acute respiratory infections and the correlation with blood biomarkers provides valuable information for the prevention and treatment of LRTI in children.

Keywords: **C-Reactive Protein • China • Epidemiology • Procalcitonin • Respiratory Tract Infections**

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Background

Lower respiratory tract infection (LRTI) remains one of the leading causes of death among children under 5 years of age and is estimated to be responsible for 652 500 deaths globally each year [1]. China, with more than 30 000 deaths annually, is among the countries with a high incidence of acute respiratory tract infection [2-5]. Owing to their young age, children cannot clearly describe the discomfort associated with an ARI, which is not conducive to the accurate diagnosis of the disease, and therefore the detection of pathogens is prolonged. Bacterial and viral agents are the leading causes of pneumonia, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, influenza virus, and respiratory syncytial virus (RSV) [6]. Some patients develop mixed viral and bacterial etiology. In addition, ARIs can be caused by several pathogens simultaneously [7,8]. The prevalence of respiratory pathogens varies from region to region and may be due to virus strain, season, and geographical region. China is a large country with different climatic characteristics in different regions.

Therefore, it is difficult to find rapid and effective biological indicators to assist diagnosis in clinical practice. In recent years, inflammation-related markers in pediatric LRTI have received increased attention. At present, procalcitonin (PCT), C-reactive protein (CRP), and white blood cells (WBC) have been widely used to identify pediatric respiratory tract infections and have specific clinical value. CRP is a blood biomarker for the presence of an inflammatory response [9]. Previous studies in developed countries have shown that hospitals using CRP testing reduces antibiotic use in patients with cough in a way that does not affect the patient's recovery [10]. One study by Esposito et al reported a mean CRP level of 32.2 mg/L in 74 patients with bacterial infection, compared with 9.4 mg/L in 16 patients with viral infection [11]. PCT is a precursor to the calcitonin produced in parafollicular cells [12] and reflects the severity of overall bacterial LRTI, safely and effectively guiding individualized decisions to initiate antimicrobial therapy [13]. In another study by Esposito et al, the mean concentration of PCT was 1.1 ng/mL in patients with viral infection, compared with 6.1 ng/mL in patients with bacterial infection [14]. Thetotal WBC count was unstable in the pediatric population. However, some previous studies have shown that 80% of patients with viral infection had WBC counts less than 10×10^9/L [15]. Prealbumin is synthesized in the liver, and the value of prealbumin decreases with infection [16]. Serum prealbumin levels below 16 mg/dL indicate infection [17].

However, there are still 2 major problems in the current research. First, it is difficult for a single inflammatory marker to simultaneously have the advantages of high sensitivity and high specificity. Therefore, the combination of different biomarkers has become the focus of recent research. Second,

pathogens of LRTI in children vary due to the location of infection. Studies on inflammatory markers focus on identifying bacterial and viral infections, and their role in identifying different pathogens is worthy of further study.

In the present study, we report the epidemiological investigation on respiratory pathogens and epidemiology in children with LRTI in Hohhot, Inner Mongolia, China, from July 2019 to June 2020. We also investigated the best combination of blood biomarkers to improve the diagnostic sensitivity and specificity through the research on children with respiratory tract infection. The primary purpose of this study is to guide utilization of vaccines in this region, dependent on our data. Secondly, we aimed to investigate the relationship between blood biomarkers and the pattern of local pathogens.

Material and Methods

Patient Population and Specimen Collection

The Ethics Committee of the First Hospital of Hohhot approved the study protocol (approval no. IRB2021009-1.0). The parents or guardians of all children were informed of the research goals and methods, which followed the First Hospital of Hohhot, Hohhot, Inner Mongolia Ethics Committee requirements. Demographic information and clinical characteristics were recorded for all children. Nasopharyngeal swabs were collected from patients after their parents or guardians gave consent. From July 2019 to June 2020, nasopharyngeal swabs of 265 pediatric patients with more than 1 respiratory symptom (cough, sore throat, and body temperature above 37.5°C) were obtained by trained personnel within 24 h after admission, following standard operating procedures. In addition, 31 nasopharyngeal swabs were collected from healthy children following standard operating procedures.

Pathogen Detection

According to the manufacturer's instructions, RNA and DNA of pathogens were extracted by a genomic DNA extraction kit and total RNA extraction kit (Tiangen, Beijing, China), respectively. cDNA was synthesized with a reverse transcription kit (TransGen Biotech, Beijing, China). Primers and PCR conditions for *Streptococcus hemolyticus*, *Streptococcus pneumonia*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, influenza virus, RSV, adenovirus, human cytomegalovirus, parainfluenza virus, and Epstein-Barr virus (EBV) were as described previously [18-23]. Primers (**Table 1**) were synthesized by BGI Biological Technology Co, Ltd (Beijing, China), and PCR testing was performed using LA-Taq (Takara Biomedical Technology, China). Respiratory bacteria and viruses were identified by the size of the PCR products following agarose gel electrophoresis, with confirmation by DNA sequencing (BGI Biological Technology Co, Ltd).

Table 1. Primers for PCR amplification of acute respiratory infection pathogens.

Type of pathogens	Amplification steps	Sequence (5'-3')	Gene	Gene position	Amplicon size (bp)	References
Adenoviruses	PCR	F: gccgcagtggtcttacatgcacatc	Hexon	18858-18883	308	Polymerase chain reaction for detection of adenoviruses in stool samples
		R: cagcacgccgcggatgtcaaagt		19158-19136		
Influenza virus type A	RT-PCR &PCR	F: gaactcrtycywwatswcaawgrrgaaat	NP	319-347	721	Simultaneous detection of influenza A, B, and C viruses, respiratory syncytial virus, and adenoviruses in clinical samples by multiplex reverse transcription nested-PCR assay
		R: atkgcgcwyrayamwctyarrtcttcawaigc		1040-1009		
Influenza virus type B	RT-PCR &PCR	F: acagagataaagaagagcgtctacaa	NP	217-242	991	
		R: atkgcgcwyrayamwctyarrtcttcawaigc		1208-1177		
Influenza virus type C	RT-PCR &PCR	F: gaactcrtycywwatswcaawgrrgaaat	NP	346-374	738	
		R: atkgcgcwyrayamwctyarrtcttcawaigc		1084-1053		
Respiratory syncytial viruses type A, B	RT-PCR &PCR	F: atggagytgcyratccwcarrrcaartgcaat	F	1-31	737	
		R: aggtgtwgttacacctgcattracactraattc		737-705		
Human cytomegalovirus	PCR	F: gaattcagtggataacctgcggcga	IE	197424-197448	406	PCR optimization: Improving of HCMV PCR to achieve a highly sensitive detection method
		R: ggatccgcatggcattcacgtatgt		197066-197042		
Epstein-Barr virus	PCR	F: ggaacctggtcatccttgc	p143	2944-2962	74	Development of a real-time quantitative assay for detection of Epstein-Barr virus
		R: acgtgcatggaccggttaat		3017-2998		
Haemophilus influenza	PCR	F: aatgcgtgatgctggttatgac	siaT	945-966	138	Simultaneous identification of *Haemophilus influenzae* and *Haemophilus haemolyticus* using real-time PCR
		R: aagagtttgcgatagattcattgg		1081-1058		
Streptococcus pneumonia	PCR	F: taaacagtttgcctgtagtcg	TIGR4	1926036-1926055	155	Identification of *Streptococcus pneumoniae* by a real-time PCR assay targeting SP2020
		R: cccggatatctctttctgga		1926190-1926172		
St. hemolyticus	PCR	F: gtaaagcgtgtattccagatttc	cfb	1496075-1496097	199	A multiplex polymerase chain reaction coupled with high-performance liquid chromatography assay for simultaneous detection of six foodborne pathogens
		R: atatgggatttgggataactaagc		1496273-1496250		
Mycoplasma pneumoniae	PCR	F: gtaatactttagaggagacg	16S rRNA	77-97	225	Application of PCR for *Mycoplasma pneumoniae* detection in children with community-acquired pneumonia
		R: tacttctcagcatagctacac		301-281		

Table 2. Patient characteristics of 265 children treated for lower respiratory tract infection at the First Hospital of Hohhot from July 2019 to June 2020.

Characteristics	Number	Percentage (%)
Female	109	41.10%
Male	156	58.90%
3 months to 5 years	80	30.20%
2-6 years old	149	56.20%
7 to 12 years	36	13.60%
Cough	244	92.08%
Fever	231	87.17%
Running nose	225	84.91%
Rales	170	64.15%
Headache	154	58.11%
Sore throat	217	81.89%
Total	265	

Blood Data Collection

Blood samples were collected with tubes containing EDTA-K2 anticoagulant from all children after admission. The SYSMEX-800i blood analyzer was used to perform blood testing. WBC, serum PCT, and CRP were assessed within 12 h of hospital admission and measured with the SYSMEX-800i blood analyzer. PCT and CRP were expressed in mg/dL.

Statistical Analysis

Statistical analyses were conducted using SPSS (version 19.0., IBM Corp, Armonk, NY, USA). For comparisons of categorical data, the chi-squared or Fisher's exact test was used. A P value of <0.05 indicated statistically significant differences. Comparison of continuous variables, such as body temperature, was conducted using analysis of variance.

Results

Patient Characteristics

From July 2019 to June 2020, nasopharyngeal swabs were collected from 265 children with LRTI after admission to the First Hospital of Hohhot. As shown in **Table 2**, their ages ranged from 6 months to 12 years, the average age was 3.4 years, and most patients were between 2 and 6 years old (149, 56.2%). The ratio of boys to girls was 1.43: 1. The clinical symptoms of LRTI included cough, fever, runny nose, sore throat, expectoration, rales, and headache. Of these, cough (244, 92.08%),

fever (231, 87.17%), and runny nose (225, 84.91%) were the most common clinical symptoms.

Pathogen Analysis

Patients were divided into 4 groups according to the results of pathogen analysis: viruses (DNA or RNA), bacteria, mycoplasma, and multiple-pathogen infection, and all data were ranked in descending order of the number of cases (**Table 3**). Of the 265 patients, 64 were positive for a single viral infection (24.2%), 42 were positive for a single bacterial infection (15.8%), 14 were positive for a single *M. pneumoniae* infection (5.3%), and 145 were positive for multiple pathogens (54.7%). Respiratory viral infections were detected in 24.2% of patients. RSV (23, 8.7%) was the most commonly detected virus, followed by adenovirus (13, 4.9%), influenza A virus (6, 2.3%), and parainfluenza virus (8, 3%). Single respiratory bacterial infections, including *S. pneumonia* (21, 7.9%), *S. hemolyticus* (9, 3.4%), and *H. influenzae* (11, 4.2%) were detected in 15.8% of total patients. *Mycoplasma pneumoniae* (14, 5.3%) was also detected. Multiple-pathogen infections were detected in 145 (54.7%) patients: 2 pathogens were detected in 105 (39.6%) patients, and more than 2 pathogens were detected in 40 (15.1%) patients. Similar to the results of previous studies in other parts of China, RSV infection rates were highest [24-26]. Interestingly, the most common co-infection groups were RSV/*S. hemolyticus* (21, 7.9%), adenovirus/*H. influenzae* (11, 4.2%), and *S. hemolyticus/H. influenzae* (16, 6.0%). Moreover, in the present study, the co-infection of *S. pneumoniae* (84, 31.7%), *H. influenzae* (72, 27.2%), or RSV (58, 21.9%) with other pathogens are the main type of co-infection, accounting for most ARI cases.

During the study period, LRTI showed a seasonal rhythm, with most pathogens detected from November to April (**Figure 1**). **Figures 2-5** show that the seasonal distribution of respiratory pathogens was not equal. In the present study, bacterial and pneumoniae infections were mainly detected in the winter, with 1 or 2 cases detected at other times. However, single RSV infections were only detected from November to February. Adenovirus and other viral infections showed a similar distribution as RSV, nearly disappearing after winter, and with an infection rate that was lower than that of RSV. Therefore, since the Hohhot LRTIs happen in the winter, a rapid preliminary diagnostic technique is urgently needed.

Correlation Among CRP, PCT, and WBC in the Different Etiological Diagnosis

The WBC, PCT, and CRP results of patients were divided into 4 groups: viruses (DNA or RNA) and bacteria and mycoplasma, which were clustered separately and ranked in descending order of the mean value of the blood biomarkers. The mean WBC, CRP, and PCT levels according to etiological diagnosis are

Table 3. Pathogen etiologies for all patients from July 2019 to June 2020.

Single pathogen infection	Number	Percentage (%)	Multiple pathogen infection	Number	Percentage (%)
Virus	64	24.20%	Mixed infection	145	54.72%
DNA virus	26	9.81%	*Streptococcus pneumoniae* & other	84	31.70%
Adenovirus	8	3.02%	*Haemophilus influenzae* & other	72	27.17%
Cytomegalovirus	7	2.64%	Respiratory syncytial virus & other	58	21.89%
Epstein-Barr virus	6	2.26%	Adenovirus & other	29	10.94%
Adenovirus (AV3.4.7.21)	4	1.51%	*Mycoplasma pneumoniae* & other	27	10.19%
Adenovirus (1.2.5.6)	1	0.38%	Epstein-Barr virus & other	26	9.81%
RNA virus	38	14.34%	Cytomegalovirus & other	13	4.91%
Respiratory syncytial virus type B	14	5.28%	Parainfluenza virus & other	11	4.15%
Respiratory syncytial virus	8	3.02%			
Parainfluenza virus type I	4	1.51%			
Parainfluenza virus type IV	3	1.13%			
Influenza B virus	3	1.13%	*Streptococcus pneumoniae* & respiratory syncytial virus	21	7.90%
Metapneumovirus	1	0.38%	*Haemophilus onfluenzae* & adenovirus	11	4.20%
Influenza A (H1N1) virus	1	0.38%	*Streptococcus pneumoniae* & *Haemophilus influenzae*	16	6.00%
Influenza virus A	1	0.38%	*Streptococcus pneumoniae* & Epstein-Barr virus	6	2.26%
Influenza virus B	1	0.38%	*Mycoplasma pneumoniae* & Epstein-Barr virus	6	2.26%
Respiratory Syncytial Virus Type A	1	0.38%	*Streptococcus pneumoniae* & *Mycoplasma pneumoniae*	5	1.89%
Parainfluenza Virus Type II	1	0.38%	*Streptococcus pneumoniae* & parainfluenza virus	4	1.51%
Bacterial	42	15.85%	*Haemophilus influenzae* & Epstein-Barr virus	4	1.51%
Streptococcus pneumoniae	21	7.92%	*Haemophilus influenzae* & *Mycoplasma pneumoniae*	4	1.51%
Haemophilus influenzae	11	4.15%	Adenovirus & *Streptococcus pneumoniae*	4	1.51%
Group A Hemolytic streptococcus	9	3.40%	Epstein-Barr Virus & adenovirus	3	1.13%
Bacillus pertussis	1	0.38%			
Mycoplasma pneumoniae	14	5.28%			

shown in **Table 4**. The mean blood biomarker values of patients with bacterial infections (WBC, 10.56×10^9 L; CRP, 20.39 mg/L; PCT, 0.25 ng/mL) were different from those of patients with viral infections (WBC, 9.18×10^9 L, P=0.739; CRP, 17.32 mg/L, P=0.88; PCT, 0.084 ng/mL, P=0.208) and mycoplasma infection (WBC, 8.00×10^9 L, P=0.177; CRP, 3.75 mg/L, P=0.044; PCT, 0.054 ng/mL, P=0.035), but the differences between bacteria and viruses were not statistically significant. Interestingly, the mean WBC, CRP, and PCT of EBV (WBC, 8.92×10^9 L; CRP, 28.42 mg/L; PCT, 0.078 ng/mL) and adenovirus (WBC, 13.65×10^9 L; CRP,
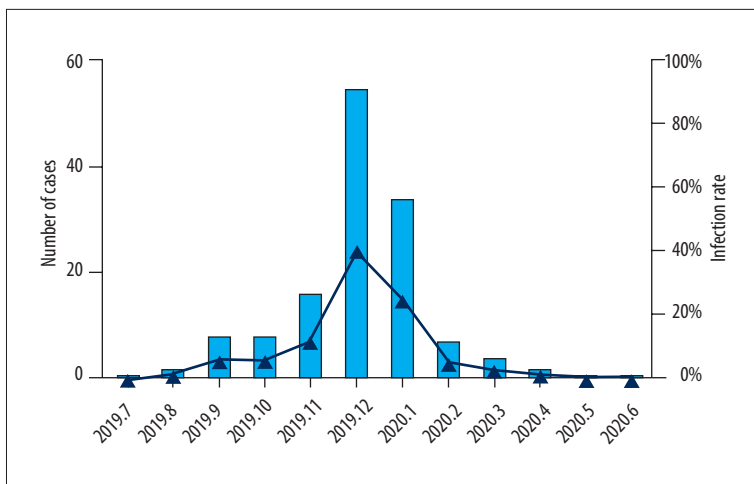
Figure 1. The distribution of all lower respiratory tract infection pathogens from July 2019 to June 2020. The primary y-axis and bars indicate number of cases (left), and the secondary y-axis and lines describe the infection rate (right).
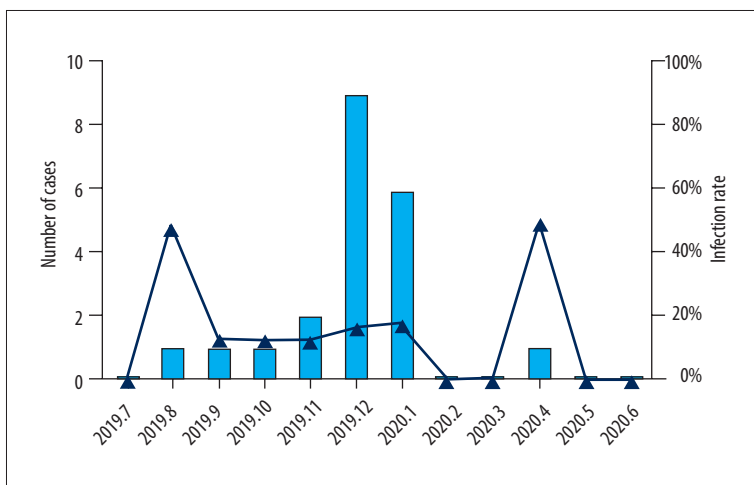

Figure 2. The distribution of streptococcus pneumoniae from July 2019 to June 2020. Primary y-axis and bars describe number of cases (left), while secondary y-axis and lines describes infection rate (right).
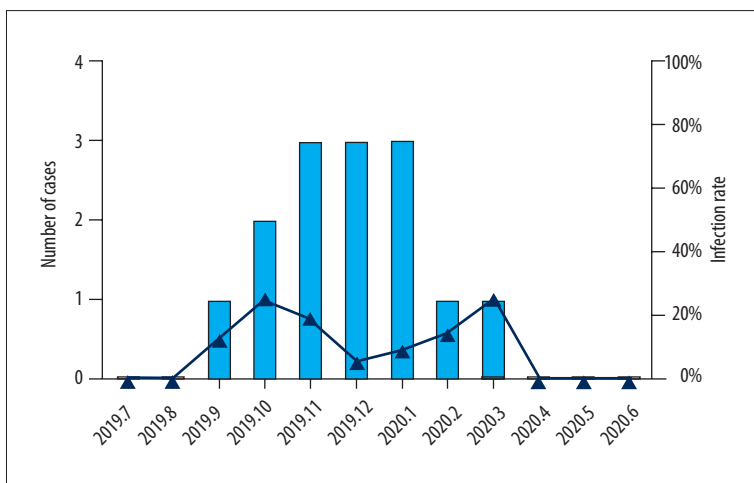

Figure 3. The distribution of *Mycoplasma pneumoniae* from July 2019 to June 2020. The primary Y-axis and bars indicate number of cases (left), and the secondary Y-axis and lines indicate the infection rate (right).

33.60 mg/L; PCT, 0.11 ng/mL) infections were similar to those of patients with bacterial infections. However, the data from this experiment show a rare result that, in patients without EBV and adenovirus infection, the mean values of CRP and PCT of patients with bacterial infections were significantly higher than those of patients with viral infections (*P*<0.05) (**Table 3**). The mean values of WBC, CRP, and PCT of other co-infection groups, such as adenoviruses/*H. influenzae* and *S. hemolyticus/H. influenzae*, were closer to those of bacterial infection groups. For instance, the mean WBC (9.22×10⁹ L), CRP
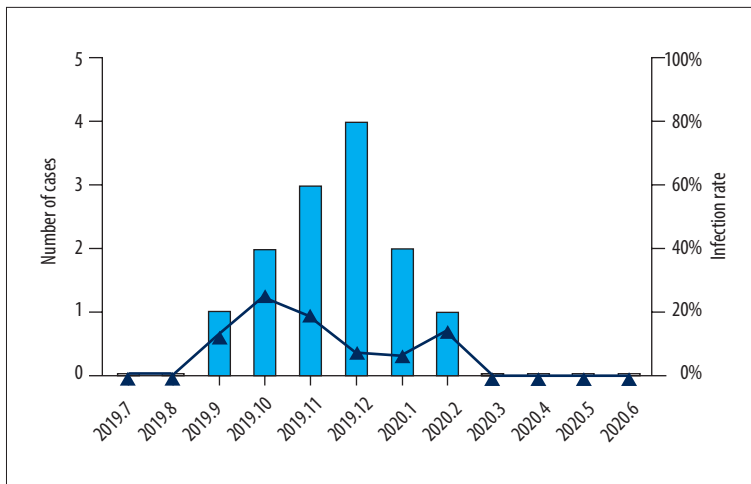
Figure 4. The distribution of adenovirus from July 2019 to June 2020. The primary Y-axis and bars indicate the number of cases (left), and the secondary Y-axis and lines indicate the infection rate (right).
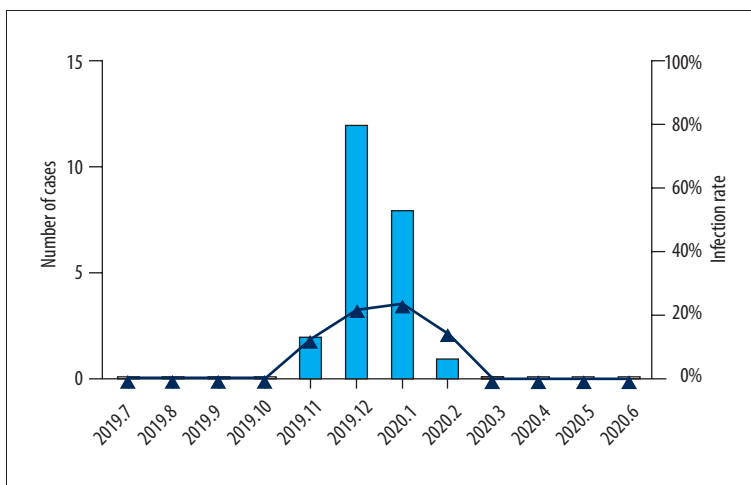

Figure 5. The distribution of respiratory syncytial virus from July 2019 to June 2020. The primary Y-axis and bars indicate number of cases (left), and the secondary Y-axis and lines indicate the infection rate (right).

(10.48 mg/L), and PCT (0.039 ng/mL) levels of RSV/*S. hemolyticus* was closer to that of the *S. hemolyticus* infection group. This suggests that EBV and adenovirus infections induced a similar effect as bacterial infection on the blood biomarkers, and *M. pneumoniae* infection induced a similar effect as other viral infections on the blood biomarkers.

The values of receiver operator characteristic (ROC) curves for WBC, CRP, and PCT for the diagnosis of LRTI are shown in **Table 5**. A positive and highly significant correlation was found among PCT and CRP in bacterial, viral, and mycoplasma vs healthy groups. The area under the curve (AUC) was 0.90, 0.74, and 0.84 for bacteria, viruses, and mycoplasma PCT, respectively; the AUC was 0.67, 0.51, and 0.52 for bacteria, viruses, and mycoplasma CRP, respectively; and the AUC was 0.69, 0.63, and 0.56 for bacteria, virus, and mycoplasma WBC, respectively, indicating a better predictive power for PCT to diagnose different pathogenic infections of LRTI in the study population. When a cut-off value of 0.11 ng/mL was used for PCT, 4.92 mg/dL was used for CRP, and 15.08×10^9 L was used for WBC; the PCT AUC was 0.65, CRP AUC was 0.7, and WBC AUC

was 0.59 in differentiating bacteremia from viral infection, excluding EBV and adenovirus in the infection data (**Figure 6**). Furthermore, when a cut-off value of 0.07 ng/mL was used for PCT, 6.59 mg/dL was used for CRP, and 9.18×10^9 L was used for WBC; the AUCs were 0.7, 0.73, and 0.74, respectively for PCT, CRP, and WBC in differentiating bacteremia from mycoplasma infection (**Figure 7**). It is difficult to distinguish between viral and mycoplasma infection with the AUC (**Figure 8**). The results showed that the combined detection of PCT had higher accuracy in the diagnosis of bacterial infection.

Discussion

The LRTIs in children are usually caused by viral or bacterial infections, which can lead to severe pneumonia and cause death in infants and children worldwide [27]. Several previous studies have been reported on various respiratory pathogens among patients with LRTIs in different parts of China, such as Beijing [24], Shanghai [28], Shenzhen [26], Wuhan [29], Shantou [25], and Lanzhou [30]. However, few studies have reported on

Table 4. Mean values of white blood cells, C-reactive protein, and procalcitonin of indicated pathogenic infections.

Types of pathogens	WBC (10⁹/L)	CRP (mg/L)	PCT (ng/ml)	Number of cases
Virus	9.18	17.32	0.08	64
DNA virus	9.96	24.08	0.07	26
Adenovirus	13.65	33.60	0.11	13
Epstein-Barr virus	8.92	28.42	0.08	6
Cytomegalovirus	7.31	10.23	0.03	7
RNA virus	7.62	33.30	0.06	38
Parainfluenza virus type II	8.96	12.90	–	3
Parainfluenza virus type I	6.49	18.85	0.08	4
Parainfluenza virus type IV	6.96	3.88	0.02	1
Respiratory syncytial virus	8.24	5.90	0.07	23
Influenza virus A	11.00	33.24	0.10	1
Influenza virus B	5.14	10.60	0.06	1
Influenza B virus	5.82	114.06	–	3
Influenza A virus H1N1	4.81	–	–	1
Metapneumovirus	3.08	2.72	0.03	1
Bacterial	10.57	20.39	0.25	42
Group A hemolytic streptococcus	10.80	17.68	0.30	9
Haemophilus influenzae	9.98	20.18	0.33	11
Bacillus pertussis	9.58	0.15	0.03	1
Streptococcus pneumoniae	9.52	11.08	0.10	21
Mycoplasma pneumoniae	8.00	4.34	0.05	14
Haemophilus influenzae & adenovirus	11.21	21.07	0.24	11
Streptococcus pneumoniae & *Haemophilus influenzae*	12.25	33.44	0.27	16
Streptococcus pneumoniae & Respiratory syncytial virus	9.22	10.48	0.04	21
Virus (EB, adenovirus, influenza B virus were removed)	8.29	8.77	0.08	56
Normal chilrden without infection	6.73	5.26	0.03	31

the bacterial and viral etiologies in the northern China; therefore, we conducted a study on data from July 2019 to June 2020 to evaluate the regional common pathogen infection patterns in children in Hohhot, China. Improved clinical diagnostic laboratory tests highlight the significance of viral, bacterial, or mycoplasma etiology and its distribution among children with LRTIs in China. Hence, these data would be equally helpful in similar areas, domestically and internationally. The most common etiological pathogens of ARIs in infants and young children in most regions are RSV, adenovirus, parainfluenza virus, influenza A virus, *S. pneumoniae*, and *H. influenzae*, which have been detected by RT-PCR or PCR methods [31,32]. Similar to previous studies, RSV and *S. pneumoniae* were the primary pathogens in our study, and more than half of the cases were mixed infections. Etiology of 2 or more pathogens was documented in 145 patients (54.7%), and the most detected common co-infection groups were RSV/*S. hemolyticus*, adenovirus/*H. infuenzae*, and *S. hemolyticus*/*H. influenzae*. Our data is consistent with some recent reports on ARIs in Africa, which were validated by RT-PCR multiplex assay [33,34]. Therefore, it is necessary to perform a differential diagnosis between viruses and bacteria. However, the detection based on the PCR or

Table 5. The receiver operating characteristic curve values of white blood cells, procalcitonin, and C-reactive protein for groups with indicated pathogens and the healthy group. Diagonal segments are produced by ties.

Clinical indicators	Pathogens	Cutoff	AUC	Sensitivity	Specificity
WBC	Bacterial	9.73	0.69	48.30%	96.80%
	Virus	7.12	0.63	60.70%	76.20%
	Mycoplasma	7.02	0.51	50.00%	71.00%
	Bacterial infection & virus	15.08	0.59	27.60%	96.40%
	Bacterial infection & mycoplasma	9.18	0.74	51.70%	90.00%
	Virus infection & mycoplasma	7.90	0.70	87.30%	57.10%
CRP	Bacterial	5.02	0.74	69.00%	100.00%
	Virus	4.38	0.51	39.30%	95.20%
	Mycoplasma	4.31	0.66	60.00%	93.50%
	Bacterial infection & virus	4.92	0.7	69.00%	67.90%
	Bacterial infection & mycoplasma	6.59	0.73	65.50%	100.00%
	Virus infection & mycoplasma	–	0.43	–	–
PCT	Bacterial	0.03	0.89	89.70%	93.50%
	Virus	0.03	0.80	78.60%	90.50%
	Mycoplasma	0.03	0.80	80.00%	93.50%
	Bacterial infection & virus	0.11	0.65	41.40%	89.10%
	Bacterial infection & mycoplasma	0.07	0.70	65.50%	70.00%
	Virus infection & mycoplasma	0.15	0.54	17.90%	100.00%

ROC – receiver operating characteristic curve; WBC – white blood cells; PCT – procalcitonin; CRP – C-reactive protein; AUC – area under the curve.

blood culture of pathogens leads to a delayed diagnosis and can lead to the improper use of antibiotics [35]. Therefore, we need to develop a rapid diagnostic method that will be followed by confirmation with molecular diagnostic methods.

Currently, there are no biomarkers in the diagnosis of ARIs for establishing the etiology. Pediatric ARIs have a high incidence. The accurate and effective diagnosis of an ARI is always a significant problem facing clinicians. Traditional primary diagnosis was based on clinical symptoms and laboratory examination, including WBC and ESR, but the single WBC level used in diagnosing bacterial infectious diseases has a specificity of about 40%, which is a low diagnosis coincidence rate [11]. ESR has a particular clinical significance for observing dynamic changes and for the instruction of clinical practice, but the sensitivity is below 50% in upper respiratory tract infection [36]. Therefore, the search for the establishment of the etiology and prediction of the course of pneumonia is essential. To date, there are more than 100 types of biomarkers related to inflammation and infection, but very few of them have proved to have value in

clinical application. CRP and PCT have long been used as biomarkers for the diagnosis of serious infections. CRP is elevated in severe infection, inflammation, and tissue damage, while PCT is elevated only in bacterial infection. PCT is more suitable for the diagnosis of bacteremia [35, 37]. Although PCT and CRP are widely used as biomarkers for discriminating bacterial from other infection, these biomarkers cannot be used for the accurate diagnosis of pneumonia [38]. In the present study, owing to the low prognostic ability and high inter-individual variation in single biomarkers, we sought to use biomarker combinations and disease precursors to diagnose and treat patients.

Our previous studies have found that CRP test and prealbumin test can distinguish different types of ARI pathogens, but the specificity (72.3%) was still not high enough [39]. In the present study, the analysis included a large primary care population with different types of LRTI. Notably, the blood biomarker values of patients with EBV and RSV infection were closer to those of patients with bacterial infection, which was significantly higher than the groups with other viral infections.
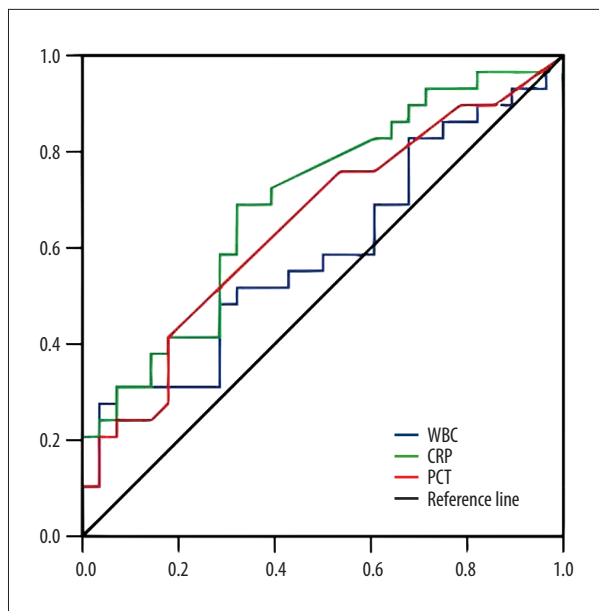
Figure 6. The receiver operating characteristic curve values of white blood cells, procalcitonin, and C-reactive protein for discriminating between bacterial and viral infection. Diagonal segments are produced by ties. Y-axis and bars indicate sensitivity, and Y-axis and bars indicate the value (1-specificity).
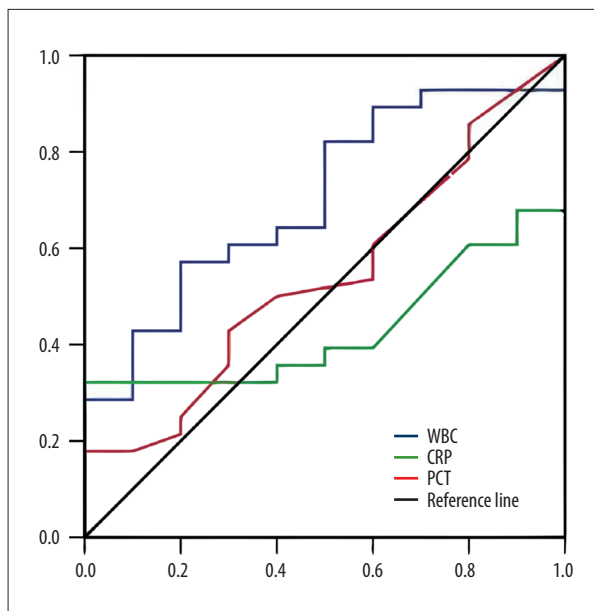

Figure 8. The receiver operating characteristic curve values of white blood cells, procalcitonin, and C-reactive protein for discriminating between virus and mycoplasma infection. Diagonal segments are produced by ties. Y-axis and bars indicate sensitivity, and Y-axis and bars indicate the value (1-specificity).

Figure 7. The receiver operating characteristic curve values of white blood cells, procalcitonin, and C-reactive protein for discriminating between bacterial and mycoplasma infection. Diagonal segments are produced by ties. Y-axis and bars indicate sensitivity, and Y-axis and bars indicate the value (1-specificity).
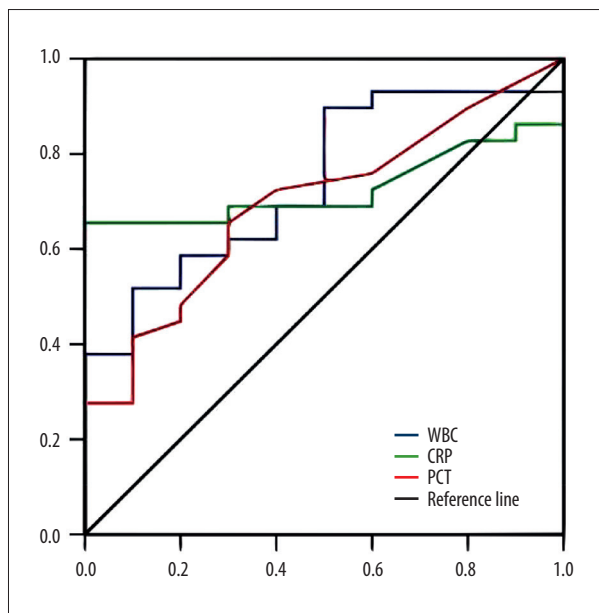
Due to the limitation of detection methods, some co-infection pathogens may not be differentiated upon diagnosis, so the blood biomarker values of patients infected with RSV and EBV were similar to those of patients with bacterial infection. By avoiding the above problems, the coincidence rate of CRP, PCT, and WBC combined in diagnosing children with different infectious pathogens was improved. Also, combined with PCR detection, blood biomarkers can help diagnose the severity of disease progression; however, the sensitivity of detection needs to be improved.

Conclusions

In conclusion, we performed an epidemiological investigation on respiratory pathogens in children with LRTI in Hohhot, Inner Mongolia, China, from July 2019 to June 2020. This study provided information on the profiles of single and multiple lower respiratory tract pathogens and their correlation with blood biomarkers and the seasonal features of LRTI in hospitalized children in northern China. The results of this study may contribute to the use of available vaccines, especially vaccines for RSV, *S. pneumoniae,* and *H. influenzae,* in this region. Although PCR is the most sensitive diagnostic method for bacterial, viral, and mycoplasma infection, blood biomarkers offer valuable information for the modification of therapeutic approaches during the early phase of illness.

CLINICAL RESEARCH

Patient Permission/Consent Declarations

The study was approved by the Research Ethics Committee of the First Hospital of Hohhot, Hohhot, Inner Mongolia, China (no. IRB20211009-1.0). Each patient's participation was voluntary.

Declaration of Figures' Authenticity

All figures and tables submitted have been created by the authors who confirm that the data are original with no duplication and have not been previously published in whole or in part.

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