Virologica Sinica 40 (2025) 166–175

Contents lists available at ScienceDirect

Virologica Sinica

journal homepage: www.keaipublishing.com/en/journals/virologica-sinica
www.virosin.org

Research Article

Virus profiling of bronchoalveolar lavage fluid in hospitalized non-COVID-19 adult patients with pulmonary infection from November 2020 to November 2021

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ARTICLE INFO

Keywords:
Bronchoalveolar lavage fluid (BLF)
Pulmonary infection
Respiratory virus
Viral load

ABSTRACT

Identifying the cause of respiratory tract infections is important for reducing the burden of diagnosis and treatment. To assess viral etiologies of hospitalized patients with pulmonary infection, bronchoalveolar lavage fluid (BALF) specimens were collected from non-COVID-19 adult patients (n = 333, including patients with lower respiratory tract infection, tuberculosis, lung cancer, and pulmonary nodules) between November 2020 and November 2021. Multiple common respiratory pathogens were detected using multiplex reverse-transcription polymerase chain reaction. The result showed that at least one virus was identified in 35.44% (118/333) of the cases. Among these, influenza virus was the most commonly identified, followed by the parainfluenza virus, coronavirus, human rhinoviruses, and human respiratory syncytial viruses. The tuberculosis group demonstrated the highest viral detection rate, yet paradoxically exhibited the lowest co-infection rate. In contrast, the highest co-infection frequency was observed in the pulmonary nodules group. Patients with viral infections exhibited more severe clinical symptoms compared to those without detected viral infections. However, this observation was only noted in the lower respiratory tract infection group among the different disease groups. Notably, among patients infected with a specific virus, there were no significant differences in viral load between single and co-infections. Our study identified the major causative agents in hospitalized adult patients with pulmonary infection, offering insights for precise disease diagnosis and the prevention of unnecessary use of antimicrobial drugs.

INTRODUCTION

Lower respiratory tract infections (LRTIs) represent the leading cause of mortality within the category of communicable diseases, ranking as the fourth most common cause of death globally, thereby posing a significant threat to public health (World Health Organization, 2022). Among various etiological agents, respiratory viruses are identified as the primary contributors to respiratory tract infections (Jain et al., 2015). Viral infections can create a favorable environment for subsequent bacterial colonization, resulting in increased morbidity and mortality associated with LRTIs (Iuliano et al., 2018; To et al., 2013; Sarna et al., 2018; Qu et al., 2015; Chan et al., 2015). Retrospective studies have established a link between respiratory viral infections and extrapulmonary complications (Kwong et al., 2018; To et al., 2019; Warren-Gash et al., 2018; Blackburn et al., 2018). Consequently, precisely identifying the viral etiology of LRTIs is crucial for reducing the overall disease burden, facilitating diagnosis, and guiding effective treatment strategies.

Understanding and quantifying trends related to the burden of LRTIs is essential for ensuring timely and appropriate investments in interventions aimed at alleviating this burden. Estimates of LRTI incidence

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https://doi.org/10.1016/j.virs.2025.03.005
Received 31 January 2025; Accepted 17 March 2025
Available online 20 March 2025

and their etiological agents are generated annually as part of the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD). While numerous studies have reported viral detection in bronchoalveolar lavage fluid (BALF) among patients with LRTIs, there remains a notable scarcity of research addressing viral infections in populations affected by tuberculosis, lung cancer, or pulmonary nodules. Furthermore, previous investigations predominantly focused on pediatric populations (Sarna et al., 2018; Hammitt et al., 2012), with limited studies examining viral infections in adults, often concentrating on only one or two common viral pathogens (Drori et al., 2020; Howard et al., 2021).

A more definitive understanding of the role of viral load in pulmonary disorders can be achieved through the analysis of viral load in lung specimens, such as lung aspirates or BALF. For instance, Mallia et al. demonstrated a causal relationship between human rhinovirus serotype 16 infection and exacerbations of chronic obstructive pulmonary disease by assessing inflammatory mediators and viral load in blood, sputum, and BALF, both at baseline and following infection (Mallia et al., 2011). Similarly, Soccal et al. conducted a study involving 343 BALF and biopsy specimens from 77 patients, along with 283 nasopharyngeal and BALF specimens. revealing that the presence of the virus coinciding with acute rejection negatively impacted transplant function recovery (Soccal et al., 2010).

In this study, we collected BALF specimens from adult inpatients presenting with pulmonary infections, including patients with lower respiratory tract infection, tuberculosis, lung cancer, pulmonary nodules. We characterized the demographic and clinical profiles of the enrolled patients, explored the viral spectrum present in the lower respiratory tract, and evaluated the implications of virological analyses of BALF for the diagnosis and management of pulmonary diseases.

RESULTS

Virus detection and clinical characteristics

Overall, 333 inpatients were included in the analysis (Fig. 1). The mean age and IQR were 54.21 years (IQR 45–65 years) and 56.76% of participants were male. The cases were predominantly from respiratory medicine (n = 180, 54.05% of total cases) and thoracic surgery (115, 34.53%). The average length of hospital stay was 15.52 days (IQR: 8–19 days). Cough (157, 47.15%) and sputum (115, 34.53%) were the main symptoms. At least 152 patients (45.65%) received antimicrobial agents more than 24 h before virus identification test (Table 1).

Patients were categorized into the following groups based on their clinical diagnosis: LRTI (151, 45.35%), tuberculosis (TB, 44, 13.21%), lung cancer (LCA, 119, 35.74%), and pulmonary nodules (LN, 19, 5.71%). Based on the PCR results, patients were further categorized into either the viral (118, 35.44%) or non-viral (215, 64.56%) group. The clinical characteristics between virus and non-viral infection patients from four groups are analyzed. In LRTI group, pateints with viral infections were younger than non-viral infection patients. Additionally, the viral group had shorter durations of sampling and fewer sick days, as well as a lower percentage of basophils (Fig. 2).

To investigate viral profiles, we first performed a statistical analysis of viral detection rates among the enrolled patients. The results revealed that, of the 333 participants included in the study, the most commonly detected viruses were influenza viruses (Flu, 111, 33.33%), parainfluenza viruses (PIV 1–4, 11, 3.30%), human coronaviruses (CoVs, 10, 3.00%), human rhinovirus (HRV, 7, 2.10%), and respiratory syncytial virus (RSV, 5, 1.50%). Influenza virus A (Flu-A), PIV-3, and CoV-229E were most prevalent among the Flu viruses (94/111), PIVs (7/11), and CoVs (6/10), respectively (Fig. 3). The viral positivity rate was highest in the TB (24/44) group, followed by the LN (8/19), LRTI (52/151), and LCA (34/119) groups. Flu was detected in all four groups, but hMPV and Flu-A3 were not detected in any group. In single infection patients, Flu-A was more prevalent in the TB group than Flu-B. However, co-infection was more common in the other disease groups (Table 2).

No significant differences were observed in personal characteristics or clinical presentation between the various viral subgroups (Supplementary Fig. S1). Together, among adult hospitalized patients with respiratory tract infections, the most commonly detected viruses were Flu and PIV viruses. The TB group exhibited the highest viral detection rate, and

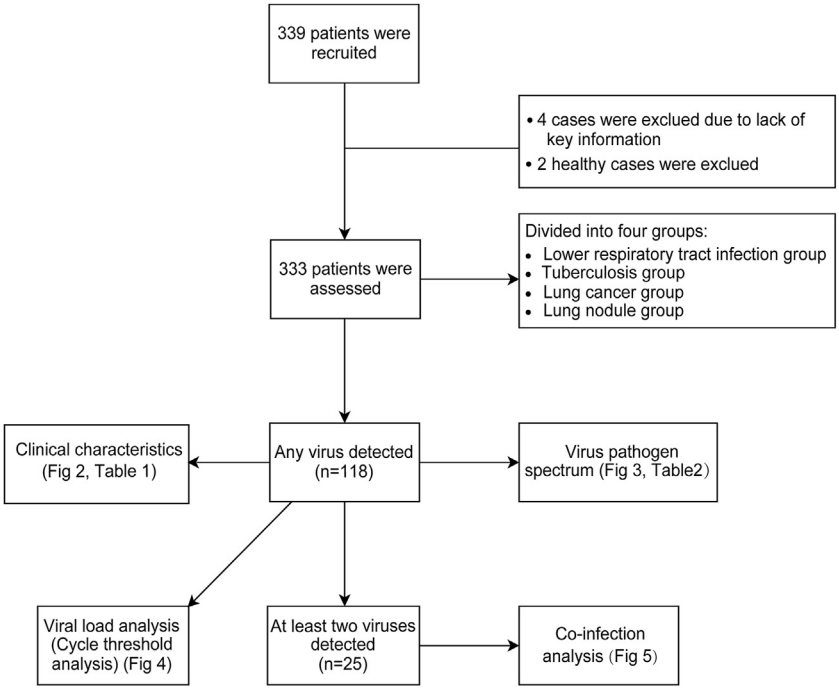

Fig. 1. The process flow diagram for gathering and sorting data.

Table 1
Demographic and clinical characteristics of the study participants.

Variable	LRTI (n = 151)	TB (n = 44)	LCA (n = 119)	LN (n = 19)	*P*-value	Virus (n = 118)	Non-virus (n = 215)	*P*-value
General condition								
Age, years	52.18 (42,63.75)	50.02 (29.5,65)	59.25 (54,67)	51.63 (39,62.5)	0.000293	52.92 (40.5, 65)	55.19 (48.75, 64.25)	0.190
Sampling day	4.19 (2,5)	3.13 (1.75,4)	4.09 (2,5)	4.27 (2,4.5)	0.492	3.62 (2, 4.25)	4.28 (2, 5)	0.137
Hospital length of stay	14.53 (8,16)	7.26 (5,9)	20.03 (14,23)	13.84 (9,15.5)	2.81E-07	15.4 (7.25, 18)	15.59 (9, 19)	0.899
Sick day	75.76 (5,30)	49.5 (7,37.5)	59.45 (10,60)	43.43 (9,17.5)	0.884	64.7 (7, 30)	68.28 (7, 30)	0.897
Body temperature (°C)	36.56 (36.3,36.6)	36.47 (36.3,36.5)	36.43 (36.3,36.6)	36.39 (36.3,36.5)	0.0346	36.5 (36.3, 36.6)	36.49 (36.3, 36.6)	0.807
Pulse (per min)	85.11 (75,94)	86.6 (77.5,96.5)	84.14 (77,90)	78.79 (76.5,85)	0.279	83.66 (75, 90)	85.11 (77, 94)	0.415
Breath (per min)	19.47 (19,20)	19.65 (19,20)	20.09 (18,20)	21.53 (18,19)	0.475	19.81 (18, 20)	19.85 (19, 20)	0.957
Systolic pressure (mmHg)	129.36 (115,140)	121.84 (110,126.5)	128.52 (119,141)	134.16 (124,143.5)	0.101	130.13 (118, 141)	127.35 (114, 140)	0.244
Diastolic pressure (mmHg)	76.51 (69,84)	74.91 (68.5,79)	79.5 (74,86)	77.95 (73.5,84.5)	0.0447	77.93 (72, 84.25)	77.2 (70,85)	0.416
Laboratory values								
WBC 10^9 cells/L	6.88 (4.72,8.22)	6.26 (4.95,7.04)	6.62 (5,7.55)	6.27 (5.14,6.82)	0.616	6.73 (4.85, 7.79)	6.65 (4.88, 7.63)	0.816
Neutrophils %	61.68 (51.58,73.35)	60.62 (54.35,69.75)	62.15 (52.9,68.8)	60.23 (57.2,64.65)	0.91	63.25 (53.2,72.58)	60.81 (51.95,69.55)	0.126
Lymphocyte %	25.88 (17.38,35.53)	24.94 (17.95,31.75)	26.37 (18.78,34.55)	28.73 (24.45,33)	0.703	25.59 (17.55,33.35)	26.35 (18.73,34.93)	0.562
Monocyte %	8.01 (6.5,9.7)	9.19 (6.8,11)	8.52 (7.08,9.53)	8.59 (7.23,8.3)	0.129	8.23 (6.7,9.7)	8.47 (6.9,9.9)	0.354
Eosinophil %	2.87 (0.9,3.2)	2.08 (1.03,2.58)	2.53 (1.3,3.2)	2.06 (1.4,2.98)	0.692	2.44 (1.28,2.98)	2.68 (1,3.2)	0.583
Basophil %	0.66 (0.4,0.9)	0.77 (0.5,0.9)	0.69 (0.5,0.9)	0.66 (0.5,0.8)	0.478	0.66 (0.4,0.9)	0.7 (0.5,0.9)	0.283
Neutrophils ($\times 10^9$ cells/L)	4.42 (2.45,5.79)	5.39 (3.08,4.87)	4.23 (2.65,5.03)	3.82 (2.85,4.22)	0.422	4.36 (2.59,5.65)	4.47 (2.65,5.03)	0.823
Lymphocyte ($\times 10^9$ cells/L)	2.17 (1.09,2.03)	2.41 (1.01,2.02)	1.59 (1.2,1.91)	1.75 (1.3,2.14)	0.754	1.58 (1.06,2.03)	2.16 (1.2,1.96)	0.336
Monocyte ($\times 10^9$ cells/L)	0.52 (0.37,0.63)	0.61 (0.47,0.69)	0.56 (0.37,0.64)	0.54 (0.4,0.58)	0.335	0.55 (0.37,0.65)	0.55 (0.39,0.64)	0.978
Eosinophil ($\times 10^9$ cells/L)	0.35 (0.04,0.18)	0.14 (0.1,0.18)	0.17 (0.08,0.19)	0.12 (0.06,0.18)	0.747	0.16 (0.07,0.18)	0.27 (0.06,0.18)	0.551
Basophil ($\times 10^9$ cells/L)	0.05 (0.02,0.05)	0.05 (0.03,0.07)	0.04 (0.03,0.06)	0.04 (0.03,0.06)	0.861	0.04 (0.02,0.06) 55	0.05 (0.03,0.06)	0.311
Department					4.37E-16			0.017
Respiratory medicine	108	36	30	6		76	104	
Thoracic surgery	20	5	79	11		33	82	
Others	23	3	10	2		9	29	
Gender					0.325			0.648
Male	86	22	65	8		65	124	
Female	59	21	50	11		52	89	
Antibiotic					1.75E-15			0.02
Used	104	21	21	6		64	88	
Unclear	47	23	98	13		54	127	
Virus detection	52	24	34	8				
Clinical feature						59	98	
Cough	89	27	38	3	1.11E-06	45	70	0.44
Sputum	69	21	24	1	1.61E-06	17	27	0.306
Fever	33	6	4	1	0.0001107	7	10	0.61
Pharynx discomfort	11	0	4	2	0.1255	13	18	0.60
Chest pain	11	3	15	2	0.4552	15	34	0.41
Chest tightness	24	6	15	4	0.7447	36	77	0.47
Nodular shadow	21	13	71	8	1.41E-13	12	28	0.36
Wheezing	22	9	7	2	0.04306	14	25	0.444
Hemoptysis	25	3	9	2	0.09232	26	54	0.93
Lung auscultation	48	12	20	0	0.002191	12	25	0.56
Smoking (past or current)	22	5	8	2	0.2461	9	14	0.71
Drinking (past or current)	15	1	5	2	0.1481	25	53	0.68
Hypertension	36	5	33	4	0.1825	9	20	0.50
Diabetes	13	3	11	2	0.9561	7	12	0.62
Hyperlipidemia	6	1	9	3	0.1049	10	18	0.88
Chronic heart disease	11	3	12	2	0.8173	14	32	0.96
Chronic lung disease	28	3	11	4	0.05638	13	21	0.46
Chronic liver disease	13	6	13	2	0.789	6	8	0.69
Chronic kidney disease	9	0	5	0	0.2714	4	12	0.57
Cerebrovascular accident	6	2	8	0	0.543	15	29	0.38
Digestive Disease	22	0	17	3	0.06211	6	8	0.87
Neurological disease	6	1	7	0	0.5545			0.57

Notes: Data are presented as means (Interquartile Range) or number. Abbreviations: LRTI, lower respiratory tract infection; TB, tuberculosis; LCA, lung cancer; LN, lung nodule.

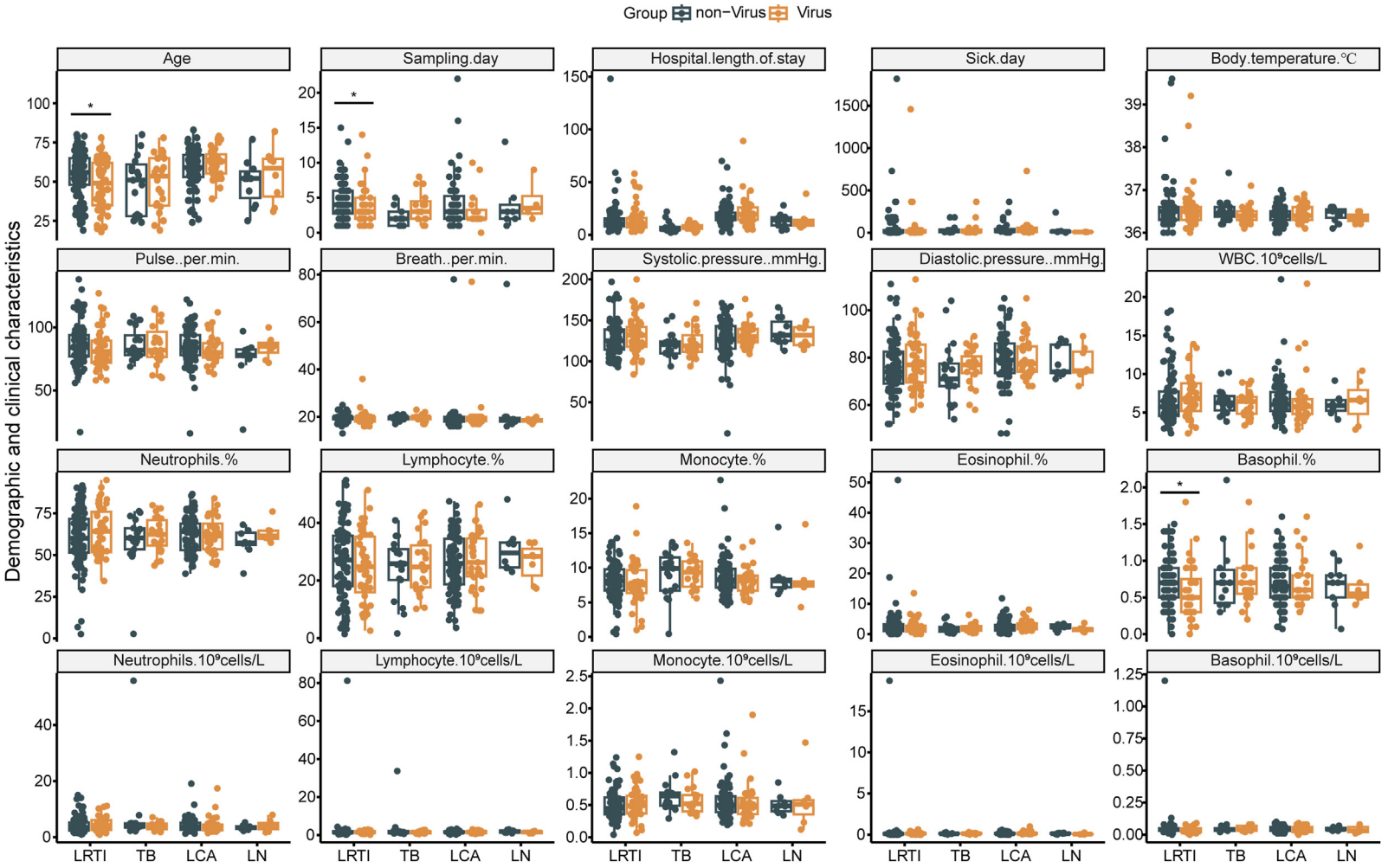

Fig. 2. Clinical characteristics of virus and non-viral infections in different disease groups. Abbreviations: LRTI, lower respiratory tract infection; TB, tuberculosis; LCA, lung cancer; LN, lung nodule.

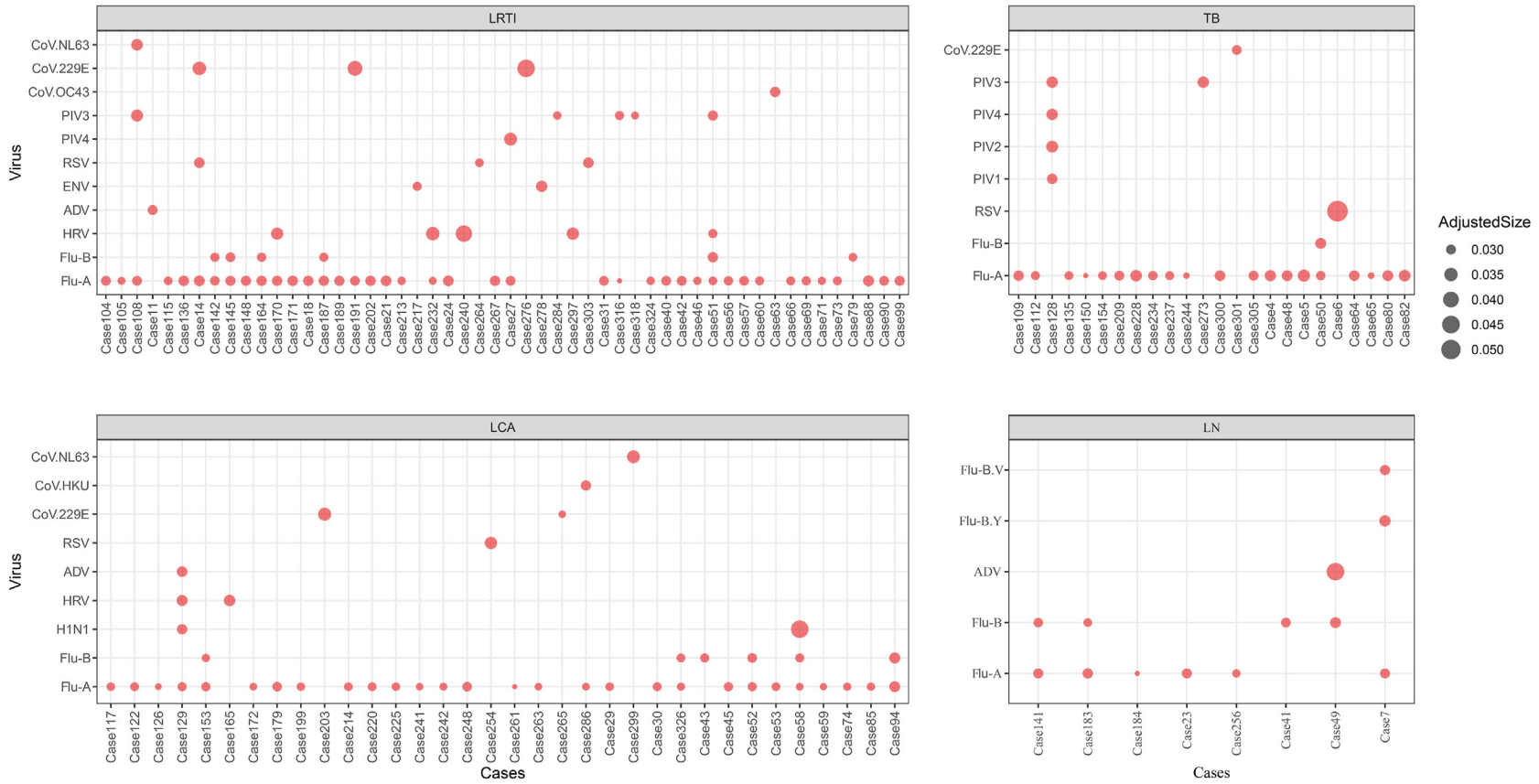

Fig. 3. The distribution of viruses detected in patients withdifferent disease groups. The size of the dots represents the value of 1/Ct. Abbreviations: LRTI, lower respiratory tract infection; TB, tuberculosis; LCA, lung cancer; LN, lung nodule; Flu-A, influenza virus A; Flu-B, influenza virus B; H1N1, influenza A (H1N1) pdm09; HRV, human rhinoviruses; ADV, adenoviruses; ENV, enterovirus; RSV, respiratory syncytial virus; PIV, parainfluenza viruses; CoV, human coronavirus; Flu-B Y, influenza virus B Yamagata lineage; Flu-B V, influenza virus B Victoria lineage; Ct, cycle threshold.

Table 2
Virus distribution in the different disease groups.

Virus	LRTI (52/151, 34.43%)		T_{LRTI}	TB (24/44, 54.55%)		T_{TB}	LCA (34/119, 38.57%)		T_{LCA}	LN (8/19, 42.10%)		T_{IN}	Total (118/333, 35.44%)
	Single (40)	Co (12)		Single (22)	Co (2)		Single (27)	Co (7)		Single (4)	Co (4)		
Flu-A	29	11	40	19	1	20	21	7	28	3	3	6	94
Flu-B	1	5	6		1	1	1	5	6	1	3	4	17
H1N1								2	2				2
HRV	3	2	5				1	1	2				7
ADV	1		1					1	1		1	1	3
ENV	2		2										2
RSV	2	1	3	1		1	1		1				5
PIV1					1	1							1
PIV2					1	1							1
PIV4		1	1		1	1							2
PIV3	2	3	5	1	1	2							7
CoV OC43	1		1										1
CoV 229E	1	2	3	1		1	2		2				6
CoV HKU								1	1				1
CoV NL63		1	1				1		1				2
Flu-B Y											1	1	1
Flu-B V											1	1	1

Notes: Case number or case number with proportion are presented. Abbreviations: Flu-A, influenza virus A; Flu-B, influenza virus B; H1N1, influenza A (H1N1) pdm09; HRV, human rhinoviruses; ADV, adenoviruses; ENV, enterovirus; RSV, respiratory syncytial virus; PIV, parainfluenza viruses; CoV, human coronavirus; Flu-B Y, influenza virus B Yamagata lineage; Flu-B V, influenza virus B Victoria lineage; LRTI, lower respiratory tract infection; TB, tuberculosis; LCA, lung cancer; LN, lung nodule; Single, single infection; Co, co-infection.

clinical differences were observed in relation to the presence or absence of viral infections.

Viral load among patients

To assess the differences in viral load across different groups, we conducted a statistical analysis of the Ct values for the detected viruses. Only Flu-A and Flu-B were detectable in all four groups, and Flu-B had a lower Ct value in the LCA group (Fig. 4, Supplementary Table S1). CoV-229E (n = 3, 20.5) had the lowest Ct in the LRTI group than in the TB (n = 1, 33.54) and LCA (n = 2, 28.16) groups (Supplementary Table S1). The viral group was further subdivided into single infection (93, 79.66%) and co-infection (25, 21.19%) subgroups, or by specific virus types, depending on the viruses detected. In both single and co-infections, Flu-A has the highest viral load in the LRTI and LCA groups, respectively (Fig. 4A and B). In the context of the same virus, a comparison between single and co-infections revealed that patients co-infected with Flu-A, Flu-B, ADV, PIV3, and CoV-229E had lower Ct values (Fig. 4C). In contrast, among single infections, patients infected with HRV and RSV also exhibited lower Ct values (Fig. 4, Supplementary Table S1). However, the limited number of positive cases identified for each individual virus precluded more detailed statistical analysis (Table 2). Together, there were no significant differences in viral load between single infections and co-infections for the same virus.

Viral co-infection among patients

To assess the co-infection status of different viruses, we analyzed the co-infection patterns of the detected viruses. At least two viruses were co-detected in 25 patients, and three viruses were co-detected in seven patients (Fig. 5A). Neither ENV nor CoV-OC43 were detected in co-infection patients. H1N1, PIV1, PIV2, PIV4, CoV, and HKU were detected exclusively in patients with co-infections. Notably, Flu viruses were the most common viruses detected in patients with co-infections (Fig. 5B). Flu-B infections were rare in the TB group, while co-infections were more common in the other disease groups (Table 2). The TB group exhibited the lowest co-infection rate (2/24), whereas the LN group had the highest rate (4/8). Together, Flu virus was the most commonly associated virus in co-infection events, although it rarely co-infected with tuberculosis.

Due to the low detection rate of viruses, only Flu-A was detected in all four disease groups. Therefore, the clinical characteristics of patients with Flu-A infection were analyzed across different disease groups and infection statuses (Supplementary Fig. S2). Among patients infected with Flu-A, individuals with co-infection had shorter sampling durations and lower percentages and absolute counts of basophils compared to those with single influenza infections. In specific disease groups, the co-infection rate was lowest in the TB group, making direct comparisons challenging. However, the clinical characteristics trend of co-infection across the other groups was consistent.

DISCUSSION

In this study, we investigated the prevalence of common viral pathogens in adult patients with LRTI, LCA, TB, and LN, revealing a distinct pattern of viral pathogen distribution in the central region of China. We employed real-time PCR to ensure the sensitivity of viral detection, and the results indicated a positive detection rate of 35.44%. This finding is consistent with studies based on surveillance data from the China Center for Disaese Control Prevention (China CDC) regarding the epidemiological characteristics of acute respiratory infections from 2009 to 2019 (Li et al., 2021). Our results indicate that there is no difference in viral load between the co-infection group and the single infection group for the same virus, which contrasts with findings from other studies (Burstein et al., 2022). This discrepancy can primarily be attributed to the fact that in our study, co-infections predominantly occurred alongside Flu, where Flu virus infection may competitively inhibit the infection or replication of other viruses (Kaaijk et al., 2022). Thus, influenza co-infection could reduce the viral load of co-infecting viruses (Burstein et al., 2022). In adult individuals, viral infections alone may not significantly alter symptoms or length of hospitalization; however, their subsequent impacts, such as secondary bacterial infections, can be substantial. Therefore, it is crucial to continue monitoring respiratory viral infections and their characteristics while minimizing antibiotic use to prevent the negative consequences associated with overuse.

Our analysis demonstrated that among the common viral pathogens studied, the detection rate of Flu was the highest at 33.33%, which aligns with findings from CDC research (Li et al., 2021). From May 2016 to December 2017, Shi et al. collected BALF samples from 408 pneumonia-associated respiratory patients in Wuhan and performed unbiased total RNA sequencing. Their results showed that Flu (mainly Flu-A) and HRV were identified as the leading viral pathogen in both adults and older people, which is partly consistent with our findings (Shi et al., 2022). Additionally, they detected all four common cold-associated

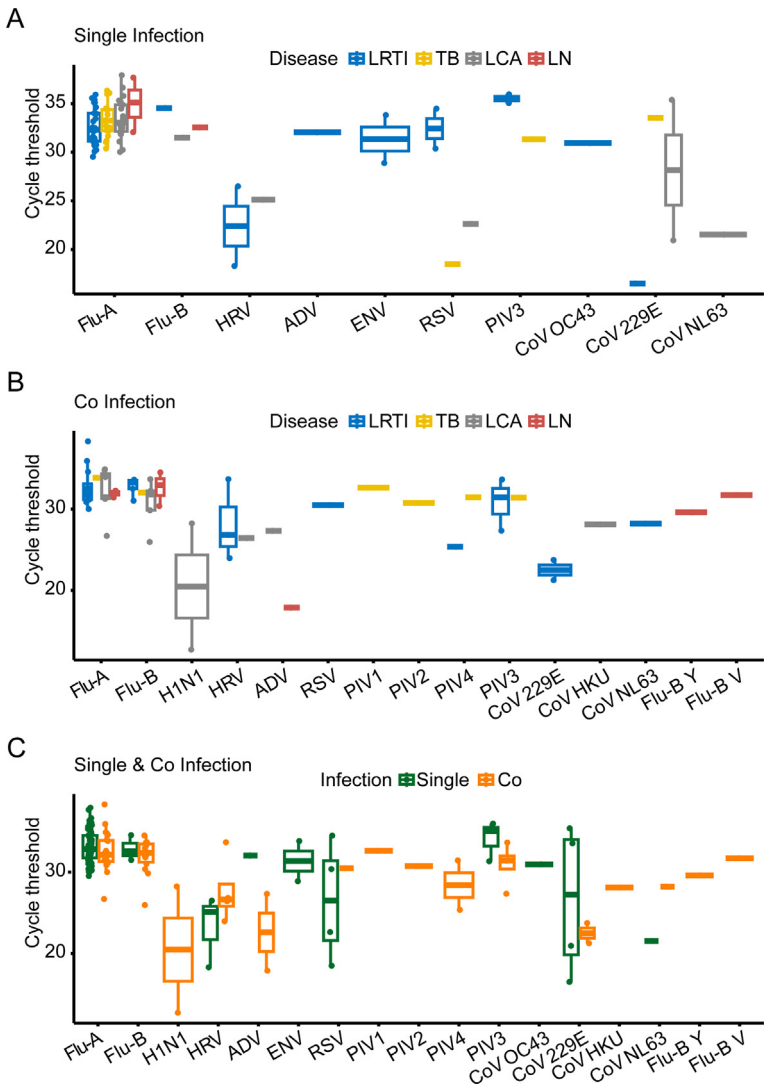

Fig. 4. The Ct values of different disease groups among various viruses. **A** Single infection; **B** Co-infection; **C** Single and co-infection. Abbreviations: Flu-A, influenza virus A; Flu-B, influenza virus B; H1N1, influenza A (H1N1) pdm09; HRV, human rhinoviruses; ADV, adenoviruses; ENV, enterovirus; RSV, respiratory syncytial virus; PIV, parainfluenza viruses; CoV, human coronavirus; Flu-B Y, influenza virus B Yamagata lineage; Flu-B V, influenza virus B Victoria lineage.

CoVs, OC43 (n = 4), HKU1 (n = 4), 229E (n = 6), and NL63 (n = 1), all of which were also detected in our cohort.

In the study by Choi et al., a total of 198 adult hospitalized patients with severe pneumonia admitted to the ICU were included. Viruses were identified in 72 (36.4%) patients. 49 patients (68.1%) underwent fiber-optic bronchoscopy BAL. Viruses were detected from BALF specimens of 40 patients (55.6%) and from nasopharyngeal aspirates or swabs of 47 patients (65.3%). In 15 patients (20.8%), viruses were detected in BALF and nasopharyngeal samples. Among the 23 patients who underwent simultaneous respiratory virus PCR testing of BAL and nasopharyngeal samples, 5 patients were BAL positive but nasopharyngeal negative and 3 patients were BAL negative but nasopharyngeal positive (Choi et al., 2012). There are differences in the viruses detected from different samples. BALF is more representative of the true state of pulmonary infection; however, LRT samples do not preclude URT contamination. Nevertheless, our findings regarding viral profiling differed from those observed in URT sample studies (Qu et al., 2015; Shaman et al., 2018; Mallia et al., 2011) and were more similar to those from LRT sample experiment (Choi et al., 2012). In contrast to previous studies (Howard et al., 2021; Li et al., 2012), we did not detect hMPV infections. Here, the HRV detection rate was relatively high and mainly distributed in the LRTI and LCA groups. Civljak et al. reported that aside from Flu virus, pneumoviruses, HRVs, and PIVs were also important in the etiology of acute respiratory infections in adults (Civljak et al., 2019). Therefore, we presume that this is representative of LRT viral infections. Further studies are required comparing these patients' upper and LRT viral infections.

Flu infection causes serious, complex illnesses, particularly LCA. In addition, it modifies the tumor microenvironment, which might accelerate the spread of lung cancer and impede the efficacy of anticancer therapies (Angrini et al., 2021). For the other viruses, including HRV, ADV, PIV, and CoV, the Ct values fared poorly in separating asymptomatic children from those with community-acquired pneumonia (Self et al., 2016). Similar viral loads were observed in respiratory tract infections and non-respiratory tract infection controls for most viruses, except for RSV (Feikin et al., 2017). In our study, we found that in cases of infections with the same type of virus, the severity of symptoms was greater in the infected and co-infected groups compared to the non-viral and single-viral infection group. However, we observed no statistical difference in the viral load between the disease groups. Feikin et al. also noted that for most viruses, there was no correlation between a larger viral load and worsening pneumonia (Feikin et al., 2017). Furthermore, most of these studies focused exclusively on the viral load's central tendency. However, they failed to show a distinct dichotomy in the viral load distribution based on case status or severity category.

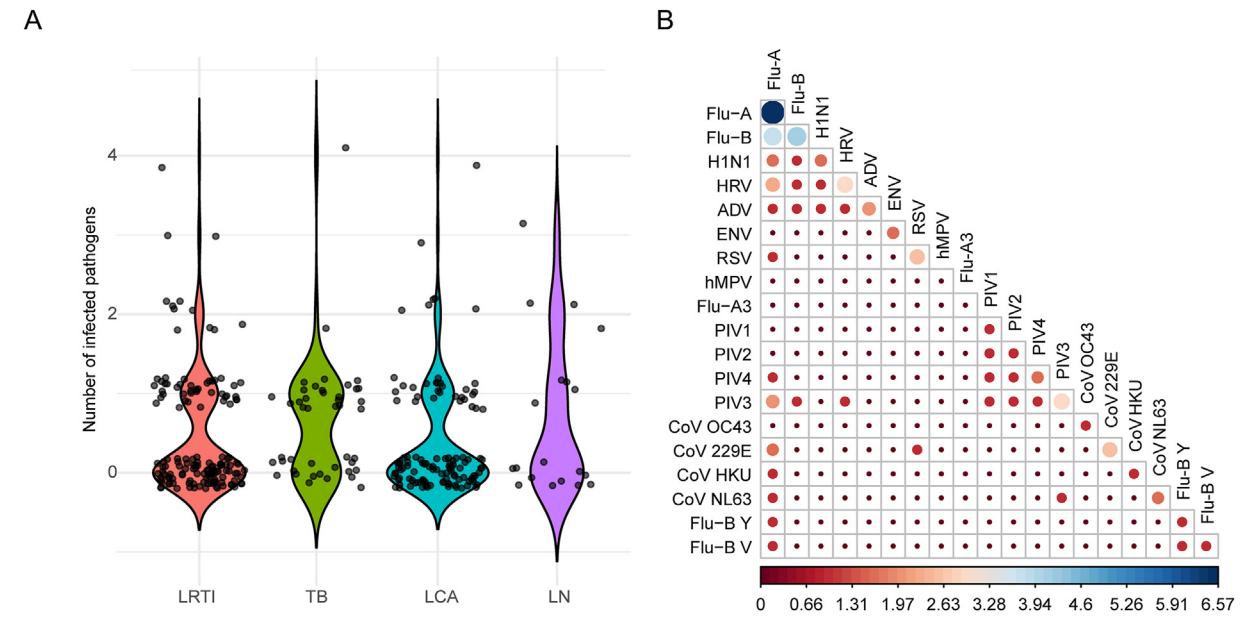

Fig. 5. Analysis of co-infection situations. **A** Distribution of the number of pathogens infected by patients in different disease groups. A dot represents a case. **B** Co-infection pattern of pathogens in included cases. The horizontal axis represents $\log_2$(Patient+1); the darker the blue and the larger the dot, the greater the number of cases; the darker the red, the smaller the number of cases. Abbreviations: Flu-A, influenza virus A; Flu-B, influenza virus B; H1N1, influenza A (H1N1) pdm09; HRV, human rhinoviruses; ADV, adenoviruses; ENV, enterovirus; RSV, respiratory syncytial virus; hMPV, human metapneumovirus; Flu-A H3, influenza virus A H3; PIV, parainfluenza viruses; CoV, human coronavirus; Flu-B Y, influenza virus B Yamagata lineage; Flu-B V, influenza virus B Victoria lineage.

In our study, the detection rate of viruses was highest in the tuberculosis group. Viral respiratory co-infections have been linked to TB disease's rapid progression or worsening, according to earlier investigations (Walaza et al., 2020). Interferon (IFN)-γ+IL-17+CD4$^+$ and IFN-γ+IL-17-CD8$^+$ cells were substantially greater in patients with TB/IFN co-infected than in those TB single-infected (Mendy et al., 2018). According to Kang et al., an enhanced and prolonged type I IFN response to viral co-infection before the pulmonary localization of *Mycobacterium tuberculosis* (Mtb)-specific Th1 cells worsens TB immunopathogenesis by preventing the inflow of Mtb-specific Th1 cells (Kang et al., 2022). In addition, Mulenga et al. reported that respiratory viral infections might accelerate the course of TB, or immune failure might make people more vulnerable to viral and Mtb infections. Participants with respiratory viral organisms had a five-fold higher chance of developing TB than those without any viral organisms (Mulenga et al., 2021). However, Flu infection is not a significant risk factor for developing clinically evident TB in an endemic nation, such as Indonesia (de Paus et al., 2013). The possibility that viral co-infection increases the risk of TB is an intriguing discovery that should be further investigated in follow-up research.

This study had some limitations. First, we recruited inpatients who underwent electronic bronchoscopy at a single hospital in Wuhan and included inpatients older than 18 years only. The retrospective nature of the study biased data collection. Second, the positivity rate for each virus was too low to allow for further analysis. However, this restriction appears to be prevalent in surveillance studies with related research designs (Li et al., 2021). Antiviral medications used before sampling might be the primary reason for the poor detection rates. In addition, the appearance and prognosis of severe community-acquired pneumonia are affected by viral-bacterial co-infection (Voiriot et al., 2016). Third, this study utilized a multiplex PCR approach to detect common respiratory viral pathogens rather than providing a comprehensive profile of microbial infections. It focused solely on routine pathogens using PCR and did not utilize high-throughput sequencing for broader detection, which excludes the potential identification of bacterial, fungal, and uncommon viral infections. However, subsequent experiments were conducted to address these gaps, and the corresponding findings have been published (Li et al., 2024). Fourth, we did not measure the viral load directly; instead, we used Ct values as surrogate indicators. Although direct viral load measures are more acurate in respiratory virus diagnosis, RT-PCR tests are more accessible than direct viral load measurements in clinical laboratories. Ct data could be considered as an auxiliary parameter to be incorporated into patient treatment.

CONCLUSIONS

In this study, we investigeted viral infections among patients with LRTI, as well as those diagnosed with tuberculosis, lung cancer, and lung nodules. Approximately 35.44% of the cases were found to have a viral infection, with Flu and PIV showingshowing thethe highest detection rates. These finding suggest that antiviral therapy can be considered when other infections are excluded and the current therapeutic effect is poor. Larger-scale studies are still needed to further delineate the contribution of respiratory viruses to different underlying diseases.

MATERIALS AND METHODS

Design and study population

This cohort study was conducted at Renmin Hospital of Wuhan University between November 2020 and November 2021. The inclusion criteria were referenced from the Chinese Thoracic Society (CTS) guidelines (Cao et al., 2018), with minor modifications: (1) Aged >18 years; (2) Suspected pulmonary infection: Chest radiograph showing new patchy infiltrates, lobar or segmental consolidation, ground-glass opacities, or interstitial changes, with or without pleural effusion; new onset of cough or expectoration, or aggravation of existing symptoms of respiratory tract diseases, with or without purulent sputum, chest pain, dyspnea, or hemoptysis; (3) The patient was able to tolerate bronchoscopy and enough bronchoalveolar lavage fluid was collected for further analysis. The bronchoscopy procedure was performed as previously described by Tufvesson et al. (Tufvesson et al., 2011). Patients with coronavirus disease 2019, acute bleeding, or uncooperative were excluded from the study. Informed consent was obtained from participants. Patients included in the study typically received standard antimicrobial therapy

due to the presence of infection indicators. Furthermore, all included patients required bronchoscopy as an integral part of their diagnostic and therapeutic process, rather than as an additional procedure. The Ethics Committee of Wuhan University Renmin Hospital approved this study. Here, the procedures were conducted following the principles of the Declaration of Helsinki (1964, amended most recently in 2008) of the World Medical Association.

Virus detection/microbiological measurements

Each BALF sample was aspirated to 200 μL, and total DNA and RNA were extracted using the QIAamp MinElute Virus Spin Kit (Qiagen, USA). Quadruple real-time quantitative PCR was conducted on 3 μL nucleic acid using the Multiplex Combined Real-time PCR Detection Kit for Respiratory Pathogens (Version 3.0 A) (Jiangsu Bio-uninovo, China). The kit is amplified by TaqMan fluorescent probe method. PCR reactions were performed and analyzed on a Bio-Rad CFX96 PCR instrument according to the manufacturer's instructions. PCR was conducted to detect influenza virus A (Flu-A), influenza virus B (Flu-B), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (*ORF1ab* gene), SARS-CoV-2 (*N* gene), RNase P (internal controls), influenza A (H1N1) pdm09, human rhinoviruses (HRV), adenoviruses (ADV), enterovirus (ENV), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), influenza A H3 (Flu-A H3) viruses, parainfluenza viruses 1–4 (PIV 1–4), human coronavirus (CoV): OC43, 229E, HKU, and NL63; influenza B virus Yamagata lineage (Flu-B Y) and Victoria lineage (Flu-B V). A cycle threshold (Ct) value below 40 was selected as the cutoff for a positive result. One sample with a SARS-CoV-2 (N) result of 38.19 was retested using the Novel Coronavirus 2019-nCoV Nucleic Acid Test Kit (Fluorescent PCR Method) (DaAn Gene, Guangzhou, China), and all results for SARS-CoV-2 were negative.

Statistical analysis

Clinical and personal characteristics were recorded, including patient age, department, sampling day, sick days, hospital length of stay, symptoms, signs, blood count, and chest computed tomography. Descriptive statistics included the frequency statistics of categorical variables and the mean and interquartile range (IQR) of continuous variables. In addition, categorical variables between the different groups were compared using Pearson's chi-squared or Fisher's exact tests. Analyses of all statistics were conducted using R version 4.2.1 and the statistical package for the social sciences version 26. A P-value <0.05 for two-sided was considered statistically significant. Further details of the statistical analyses are provided in the Supplementary Information.

DATA AVAILABILITY

All data relevant to the study are included in the article or uploaded as supplementary information.

ETHICS STATEMENT

Informed consent was obtained from participants. All included patients required bronchoscopy as part of their diagnostic and therapeutic process, rather than as an additional procedure. This study was approved by the Ethics Committee of Wuhan University Renmin Hospital (WDRY2024-K190). Here, the procedures were conducted following the principles of the Declaration of Helsinki (1964, amended most recently in 2008) of the World Medical Association. Informed consent was obtained from participants.

AUTHOR CONTRIBUTIONS

Liangyu Li: investigation, formal analysis, visualization, writing-original draft. Haiyue Zhang: investigation, data curation, writing-original draft, writing-review & editing. Pei Xiong, Chan Liu, Lu Wan, Mengling Liu, Jieyu Mao, Ruiyun Li, Min Shang, Hailing Liu, Yuchuan Luo, Jing Yin: data curation. Xiaojun Wu: data curation, funding acquisition, project administration, resources, supervision, writing-review & editing. Jianjun Chen: conceptualization, funding acquisition, Investigation, methodology, project administration, resources, supervision, writing-review & editing.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by The National Natural Science Foundation of China (82370008, 32470169), National Key R&D Program of China (2023YFC260550), and Major Project of Guangzhou National Laboratory (GZNL2023A01001). The authors acknowledge Yi Yan Ph.D., Mengchan Hao Ph.D., Meng Xu M.M., Sanling Fan Ph.D., Zhiyong Wu M.M., and others for their help in our work.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2025.03.005.

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