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【 ORIGINAL ARTICLE 】

Frequency and Significance of Coinfection in Patients with COVID-19 at Hospital Admission

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Abstract:

Objective Viral pneumonia is not rare in community-acquired pneumonia (CAP). Mixed or secondary pneumonia (coinfection) can be seen in viral pneumonia; however, its frequency in coronavirus disease 2019 (COVID-19) has only been investigated in a few studies of short duration, and its significance has not been fully elucidated. We investigated the frequency and significance of co-infection in patients with COVID-19 over a 1-year study period.

Methods Coinfection was investigated via multiplex polymerase chain reaction (PCR), culture of respiratory samples, rapid diagnostic tests, and paired sera. We used logistic regression analysis to analyze the effect of coinfection on severity at admission and Cox proportional-hazards model analysis to analyze the effect of coinfection on need for high-flow nasal cannula, invasive mandatory ventilation use, and death, respectively.

Patients We retrospectively investigated 298 patients who suffered CAP due to severe acute respiratory syndrome coronavirus-2 infection diagnosed by PCR and were admitted to our institution from February 2020 to January 2021.

Results Primary viral pneumonia, and mixed viral and bacterial pneumonia, accounted for 90.3% and 9.7%, respectively, of COVID-19-associated CAP, with viral coinfection found in 30.5% of patients with primary viral pneumonia. Influenza virus was the most common (9.4%). Multivariable analysis showed coinfection not to be an independent factor of severity on admission, need for high-flow nasal cannula or invasive mandatory ventilation, and mortality.

Conclusion Viral coinfection was common in COVID-19-associated CAP. Severity on admission, need for high-flow oxygen therapy or invasive mandatory ventilation, and mortality were not affected by coinfection.

Key words: viral pneumonia, coinfection, COVID-19, severe, prognosis

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Introduction

Viral infection is a major component of community-acquired pneumonia (CAP) (1). A recent study investigating the etiology of CAP found that viruses accounted for about 20% of the infections (1). Another study in Japan showed a viral etiology of CAP in 23.1% of cases (2).

In November 2019, severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection became pandemic resulting in a large number of severe cases and deaths, and since then, the importance of viral pneumonia has been recognized. To date, coinfection with not only bacteria but also viruses has been reported in viral pneumonia (1), and some reports have shown coinfection with viruses in coronavirus disease 2019 (COVID-19). However, studies investigating coinfection with COVID-19 have been performed for only a short duration, e.g., for a few weeks. As some coinfecting

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pathogens, typically viruses, show seasonal development, we thus thought it best to investigate coinfection for a complete year. In addition, the significance of coinfection on clinical courses of COVID-19, such as mortality and the requirement for high-grade pulmonary care, also has not been investigated (3, 4). Therefore, the present study aimed to investigate the frequency of coinfection and whether coinfection influences severity, the clinical course during hospitalization, and mortality of patients with COVID-19.

Materials and Methods

We retrospectively analyzed patients who were admitted to Saitama Cardiovascular and Respiratory Center over the 12 months from February 2020 to January 2021 for CAP caused by COVID-19. Data were extracted from medical records. Informed consent was obtained in the form of opt-out on both the hospital web-site and information posted in the hospital. Nursing home residents and patients with non-resected lung cancer were excluded, as were those who declined to participate in the study. SARS-CoV-2 infection was confirmed using polymerase chain reaction (PCR) methods with nasopharyngeal swabs. Swabs were stored at -70℃ and used for the detection of respiratory pathogens on a Rotor-Gene Q instrument (Quiagen, Hilden, Germany) with a multiplex, real-time PCR (RT-PCR) using an FTD Resp 21 Kit (Fast Track Diagnostics, Silema, Malta) (5). The kit detects the following respiratory pathogens: influenza A and B viruses; coronaviruses (NL63, 229E, OC43, and HKU1); human parainfluenza viruses (HPIV) 1, 2, 3, and 4; human metapneumovirus A/B (hMPV); rhinovirus; respiratory syncytial virus (RSV) A/B; adenovirus; enterovirus; human parechovirus; bocavirus; and *Mycoplasma pneumoniae*. An EZ1 Virus Mini Kit v2.0 was used for nucleic acid extraction (Quiagen). Results of RT-PCR were considered positive with a threshold cycle value of <33 as indicated in the instruction manual. Paired sera included antibody titers of *M. pneumoniae*, *Legionella* spp., *Chlamydophila psittaci*, *C. pneumoniae*, influenza virus, RSV, HPIV, and adenovirus. Disease onset was defined as the day on which initial symptoms (e.g., fever, sore throat) developed. Coinfection was surveyed by multiplex PCR, culture, urinary antigen tests, paired sera, and rapid influenza diagnostic tests as reported previously (6). Pneumonia was classified into primary viral pneumonia, mixed viral and bacterial pneumonia, and secondary bacterial pneumonia based on a previous report (7). Severe pneumonia was defined when at least one major criterion or three minor criteria of the Infectious Diseases Society of America/American Thoracic Society guidelines (8) were present. Outcomes used in this study included severity at admission and time to need for high-flow nasal cannula (HFNC), invasive mandatory ventilation (IMV) use and death during the period from admission to final follow-up. The study protocol was approved by the Ethical Committee of Saitama Cardiovascular and Respiratory Center.

Statistical analysis

Risk factors for severity on admission was evaluated by univariate and multivariable logistic regression analysis. Risk factors for need for HFNC or IMV, and mortality from CAP accompanying COVID-19 were evaluated by univariable and multivariable Cox proportional-hazards model. Variables showing significance in the univariable analysis ($p<0.05$) were included in the multivariable regression analysis, considering factors which had been reported to be significant for severity or mortality of COVID-19. The 95% confidence intervals (CIs) were also reported. In all instances, a 2-tailed p value of <0.05 was considered to indicate statistical significance. All statistical analyses were performed with SAS version 9.4 (SAS Institute, Cary, USA).

Results

Patient characteristics

During the study period, 452 patients with laboratory-confirmed COVID-19 were admitted to our institution. A total of 154 patients were nursing home residents, and there were no patients with non-resected lung cancer or patients declined to participate in the study, then, 298 patients were enrolled. All patients admitted in February 2020 were transferred from a cruise ship. Results are presented as frequency and percentage or mean ± standard deviation or median (range) unless otherwise indicated. Patient age was 61.1± 14.6 years old and 205 (68.8%) were men (Table 1). The median number of disease days (range) from onset to admission was 7 (0-19). There were no underlying diseases in 109 (36.6%) of the patients. Chronic obstructive pulmonary diseases were the most common among the underlying pulmonary diseases, and bronchiectasis was found in only 1 (0.3%) patient. Hypertension and diabetes mellitus were common as non-pulmonary underlying diseases. Laboratory tests on admission showed lymphopenia (<500/mm^3) in 21 patients, elevated D-dimer values (≥2 μg/mL) in 40 (13.4%), and elevated serum ferritin value (≥500 ng/mL) in 146 (49.0%).

Pneumonia Subtypes and Microbiological Patterns

Among the pneumonia subtypes, primary viral pneumonia was present in 90.3% of patients, and no patients had secondary bacterial pneumonia. Pathogens coinfected with SARS-CoV-2 and methods used to identify the pathogens are listed in Tables 2 and 3. Bacterial coinfection was found in 10 patients (9.7%), with *M. pneumoniae* being the most common. Viral coinfection was found in 91 (30.5%) patients, with influenza virus being the most common followed by rhinovirus. The numbers of patients with viral infection for each month of the study are shown in Figure. SARS-CoV-2 showed an increase of patients in April, August, and December of 2020. None of patients who were transferred from the cruise ship in February 2020 showed coinfection. Coinfec-

Table 1. Patients' Characteristics, n=298.

Characteristics	Value	Characteristics	Value
Male sex	205 (68.8)	Laboratory data	
Age, years	61.1±14.6	Arterial blood gas analysis	
<65	168 (56.4)	PaCO2, Torr	
65-74	79 (26.5)	Unknown	8 (2.7)
75≤	51 (17.1)	<35	160 (53.7)
Body mass index (BMI), kg/m^2	25.5±4.54	35-45	124 (41.6)
30≤ BMI	44 (14.8)	45≤	6 (2.0)
BMI <18	7 (2.3)	Lactate, mmol/L	
BMI 18≤, <30	233 (78.1)	Unknown	36 (12.1)
BMI, unknown	14 (4.7)	<2	221 (74.2)
Days from onset to admission	7 (0-19)	2≤	41 (13.8)
Antibiotics prior to admission, yes	39 (13.1)	WBC, /mm^3	6,484±3,049
Smoking history, yes	145 (48.7)	Plt, /mm^3	20.9±7.7
Underlying diseases, none	109 (36.6)	Neutrophils, /mm^3	4,971±2,993
Pulmonary diseases		Lymphocytes, /mm^3	1,073±508
COPD	17 (5.7)	Unknown	0
Bronchial asthma	14 (4.7)	<500	21 (7.0)
Bronchiectasis	1 (0.3)	500≤	277 (93.0)
Pulmonary nontuberculous mycobacteriosis	1 (0.3)	D-dimer, μg/mL	1.74±3.36
Old tuberculosis	2 (0.7)	Unknown	3 (1.0)
Interstitial lung diseases	8 (2.7)	<2	255 (85.6)
Post lung cancer operation	4 (1.3)	2≤	40 (13.4)
Pneumoconiosis	1 (0.3)	AST, IU/L	41±38
Chronic pulmonary artery thromboembolism	1 (0.3)	ALT, IU/L	35±33
Non-pulmonary diseases		LDH, IU/L	281±116
None	120 (40.2)	CK, IU/L	150±424
Hypertension	100 (33.6)	BUN, mg/dL	16±9
Congestive heart failure	3 (1.0)	BUN ≥20	
Ischemic heart diseases	19 (6.4)	Cre, mg/dL	0.88±0.34
Diabetes mellitus	92 (30.9)	Na, mmol/L	137±8
Valvular diseases	1 (0.3)	CRP, mg/dL	5.6±5.8
Arrythmias	9 (3.0)	KL-6, U/mL	337±321
Cardiomyopathy	2 (0.7)	Unknown	8 (2.7)
Cerebrovascular diseases	7 (2.3)	<500	251 (84.2)
Dementia	4 (1.3)	500≤	39 (13.1)
Neuromuscular diseases	4 (1.3)	Ferritin, ng/mL	743±703
Post upper digestive system surgery	4 (1.3)	Unknown	8 (2.7)
Chronic liver diseases	5 (1.7)	<500	144 (48.3)
Connective tissue diseases	3 (1.0)	500-1,000	76 (25.5)
Systemic steroids or immunosuppressants	7 (2.3)	1,000≤	70 (23.5)
Psychiatric diseases	2 (0.7)	Procalcitonin, ng/mL	0.235±1.625
Malignancy	9 (3.0)	Unknown	11 (3.7)
Heavy drinker	1 (0.3)	<0.5	275 (92.3)
Chronic kidney disease	6 (20.1)	0.5≤, <1	8 (2.7)
Long-term oxygen therapy	1 (0.3)	1≤	4 (1.3)
Vaccination history, pneumococcus	25 (8.4)	Complications	
Vaccination history, influenza virus	73 (24.5)	Deep vein thrombosis	4 (1.3)
Premorbid performance status		Acute pulmonary thromboembolism	1 (0.3)
0	262 (87.9)	Pneumothorax	1 (0.3)
1-2	29 (9.7)	Pulmonary hemorrhage	1 (0.3)
3-4	6 (2.0)	Acute kidney injury	16 (5.4)
Viral coinfection, yes	91 (30.5)	qSOFA, 2≤	2 (0.7)
Bacterial coinfection, yes	29 (9.7)	Severity, severe	46 (15.4)
		Treatment during hospital stay	
		Antibiotics, yes	114 (38.3)
		Neuraminidase inhibitors, yes	112 (37.6)
		Corticosteroids, yes	100 (33.6)
		Day from onset to start corticosteroid	8 (0-18)
		HFNC	46 (15.4)
		IMV	30 (10.1)
		Continuous renal replacement therapy	1 (0.3)
		ECMO	6 (2.0)
		Days from onset to HFNC	9 (3-15)
		Days from onset to IMV	11 (4-19)
		Days from admission to IMV	2.5 (-1-18)
		Follow-up period, from onset	42 (6-398)
		Mortality	23 (7.7)

qSOFA: quick Sequential Organ Failure Assessment Score, IMV: invasive mandatory ventilation, HFNC: high-flow nasal canula, ECMO: extracorporeal membrane oxygenation

Table 2. Etiology of Mixed Infection.

Pathogens	n (%)
Mycoplasma pneumoniae	23 (7.7)
Streptococcus pneumoniae	3 (1.0)
Legionella spp.	2 (0.7)
Escherichia coli	1 (0.3)
Influenza virus	28 (9.4)
Parainfluenza virus	27 (9.1)
Common cold coronavirus	18 (6.0)
Adenovirus	14 (4.7)
Bocavirus	10 (3.4)
Rhinovirus	9 (3.0)
Parechovirus	7 (2.3)
hMPV	6 (2.0)
RSV	6 (2.0)
Enterovirus	4 (1.3)

hMPV: human metapneumovirus, RSV: respiratory syncytial virus

tion with *M. pneumoniae*, influenza virus, and HPIV increased during the winter season. The number of viruses co-infecting with SARS-CoV-2 included 1 in 68 (22.8%), 2 in 14 (4.7%), 3 in 5 (1.7%), 4 in 2 (0.7%), and 5 in 2 (0.7%) patients, respectively.

Severity on admission, treatment, and clinical courses

Forty-six (15.4%) patients were in severe condition on admission. During the patients' clinical courses including before and after admission to our hospital, antibiotics and neuraminidase inhibitors (favipiravir) were administered in 114 (38.3%) and 112 (37.6%), respectively. Neuraminidase inhibitors were administered >72 h after onset in 108 patients. Corticosteroids were administered in 84 patients (including to 9 patients by local physicians before transfer) when they developed respiratory failure and required oxygen therapy and in 16 patients (all by local physicians before transfer) in

Table 3. Diagnostic Methods.

Methods	Number of positive diagnostic studies	Number of episodes studied
Urinary antigen test		
Legionella spp., positive	2	291
Streptococcus pneumoniae, positive	3	291
Rapid influenza diagnostic test, tested	21	292
Paired sera, tested	2	123
Culture		
Sputum	1	62
Bronchial toilet	1	8
Multiplex PCR		
Nasopharyngeal swabs, sputum	91	298
BALF	2	2

PCR: polymerase chain reaction, BALF: bronchoalveolar lavage fluid

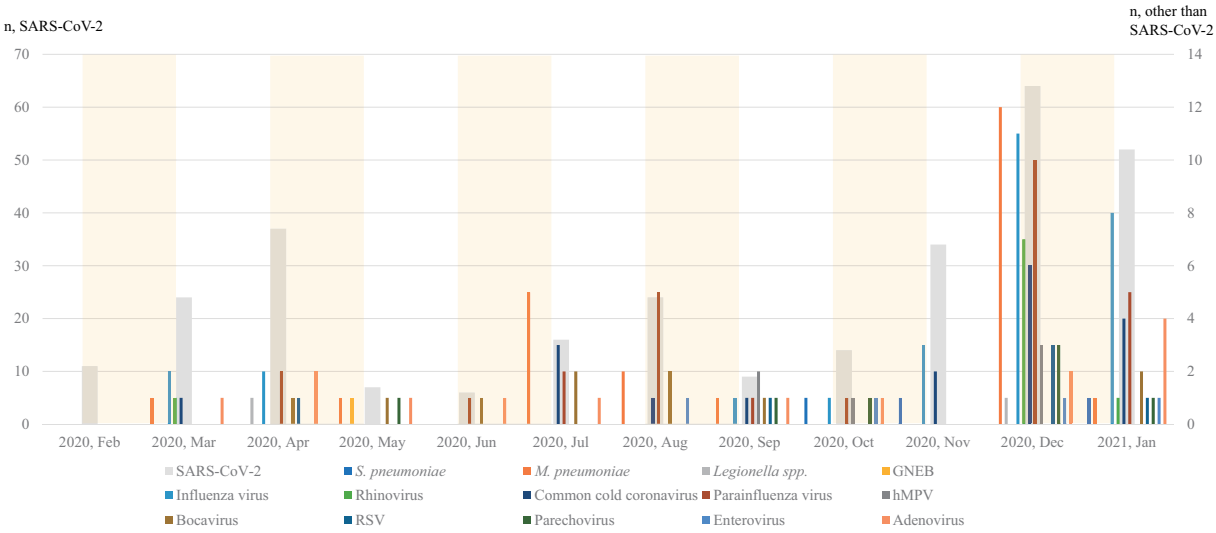

Figure. The numbers of patients with SARS-CoV-2 infection and each co-infecting pathogen by month. The number of patients with COVID-19 increased in April, August, and December of 2020. *Mycoplasma pneumoniae* and influenza virus infections increased in winter.

non-respiratory failure without the requirement for O_2. These 100 (33.6%) patients received corticosteroid therapy with dexamethasone 6 mg/day for 7-10 days. During the disease courses, HFNC and IMV were required in 46 (15.4%) and 30 (10.1%) patients, respectively. One day before their transfer to our hospital, 1 patient had been placed on HFNC and another patient on IMV by local physicians. One patient received continuous renal replacement therapy, 6 received extracorporeal membrane oxygenation, and 23 patients died.

Risk Factors for Severity on Admission

Results of the univariable and multivariable analyses are listed in Table 4. Multivariable analysis showed that the Odds ratio (OR) of age ≥75 years group was 5.61 (95% CI, 2.09 to 15.05) with age <65 years group as the reference, OR of elevated serum ferritin value of 500-1,000 ng/mL and ≥1,000 ng/mL were 2.62 (95% CI, 1.07 to 6.43) and 5.78 (95% CI, 2.33 to 14.33) with serum ferritin value <500 ng/mL as the reference, whereas coinfection with bacteria and viruses were nonsignificant factors.

Risk factors for the need for HFNC or IMV

Risk factors for the need for HFNC or IMV were evaluated except for each one patient who had been placed on HFNC and another patient on IMV by local physicians. Results of the univariable and multivariable analyses are listed in Tables 5 and 6. Multivariable analysis for the need for HFNC showed severe condition on admission [hazard ratio (HR), 4.30; 95% CI (1.52, 12.14) with non-severe condition as the reference], elevated serum KL-6 value of ≥500 U/mL [HR, 3.29 95% CI (1.20, 8.99) with KL-6 value <500 U/mL as the reference], elevated serum ferritin value of 500-1000 ng/mL [HR, 6.01; 95% CI (1.57, 23.04) with serum ferritin value <500 ng/mL as the reference], corticosteroid treatment in non-respiratory failure [HR, 4.31; 95% CI (1.15, 16.17) with non-corticosteroid use as the reference], and corticosteroid treatment in respiratory failure [HR, 2.76; 95% CI (0.81, 9.35), with non-corticosteroid use as the reference] to be the independent factors (Table 5). These were also the independent factors for the need for IMV after admission: severe condition on admission [HR, 3.35; 95% CI (1.06, 10.58)], elevated serum ferritin value of 500-1000 ng/mL [HR, 17.45; 95% CI (2.09, 146.09)], and corticosteroid treatment in respiratory failure [HR, 4.39; 95% CI (1.11, 17.33)] (Table 6). Coinfection with bacteria and viruses were not associated with the need for HFNC or IMV.

Risk factors for mortality

Results of the univariable and multivariable analyses are listed in Table 7. Age ≥75 years old [HR, 8.49; 95% CI (1.79, 40.36) with age <65 years old as the reference], lymphopenia <500/mm^3 [HR, 9.07; 95% CI (1.79, 46.01) with lymphocyte count ≥500/mm^3 as the reference], D-dimer value of ≥2 μg/mL [4.67; 95% CI (1.16, 18.77) with D-dimer value of <2 μg/mL as the reference], serum ferritin value of 500-1000 ng/mL [HR, 15.65; 95% CI (1.70, 144.31) with serum ferritin value <500 ng/mL as the reference], and corticosteroids in non-respiratory failure [HR, 15.62; 95% CI (1.99, 122.68) with non-corticosteroid use as the reference] and in respiratory failure [HR, 10.66; 95% CI (1.57, 72.18) with non-corticosteroid use as the reference] were the factors associated with death. Coinfection with viruses or bacteria was not associated with mortality (Table 7).

Discussion

The present study showed that most of the SARS-CoV-2 pneumonia was primary viral pneumonia, and while bacterial coinfection was not so common, coinfection with other viruses was common. Considering treatment with antivirals and antibiotics, coinfection with *M. pneumoniae* and influenza virus were the most important pathogens. Coinfection did not affect severity on admission, the need for HFNC or IMV, and mortality.

There have been reports investigating the frequency of viral infection in pneumonia, but limited studies have focused on the characteristics of viral pneumonia itself. Crotty et al. investigated patients with viral pneumonia, half of whom were immunocompromised patients. Eighty-four of 284 patients had coinfection (9), with half coinfected with bacteria and the rest coinfected with viruses. Another report showed the rates of single virus infection, virus-virus coinfection, and virus-bacterial coinfection to be 22%, 2%, and 3%, respectively (1). These reports suggested that viral pneumonia without bacterial coinfection is common, which is compatible with our results. No patients in the present study had secondary bacterial pneumonia. Patients can easily consult physician soon after noticing their impaired condition in Japan and can receive diagnostic tests for COVID-19. When diagnosed as having COVID-19, they are immediately transported to hospital and isolated. These practices can lead to early hospitalization and may reduce the incidence of secondary bacterial infection on admission.

Several studies investigated coinfection of SARS-CoV-2. One study showed 23 (19.8%) of 116 patients with COVID-19 had coinfection; rhinovirus and enterovirus were the most common viruses, followed by RSV and common cold coronavirus (10). Another study showed that 18 of 89 patients (20.2%) with COVID-19 showed coinfection, all of which were due to bacteria (11). A multicenter study in the U.S. showed 1,690 of 12,075 (14.0%) patients had coinfection, and the number of pathogens coinfecting with SARS-CoV-2 ranged from 1 to 6 (12). Frequent pathogens included *Staphylococcus aureus*, human herpes virus-4, *M. catarrhalis*, *Klebsiella pneumonia*, hMPV, and adenovirus (12). Another multicenter study of 5,700 COVID-19 patients showed the common coinfecting pathogens to be enterovirus, rhinovirus, of which the common cold coronavirus was the most common, followed by RSV, HPIV, *C. pneumoniae*, hMPV, influenza virus, and *M. pneumoniae* (13). Other studies also showed that coinfection with viruses, including RSV, hMPV, HPIV, and common cold coronavi-

Table 4. Univariable and Multivariable Analysis of Severity on Admission.

	Univariable analysis			Multivariable analysis (final model)		
	OR	95% CI	p value	OR	95% CI	p value
Body mass index (BMI)						
30≤ BMI	1.08	0.45, 2.59	0.8728			
BMI <18	0.92	0.11, 7.87	0.9401			
BMI 18≤, <30	Ref					
Sex, male	1.77	0.84, 3.74	0.1355			
Age, years						
<65	Ref					
65-74	1.83	0.81, 4.11	0.1456	1.86	0.79, 4.36	0.1542
75≤	6.06	2.79, 13.17	<0.0001	5.61	2.09, 15.05	0.0006
Smoking history, yes	0.76	0.41, 1.44	0.402			
Pulmonary diseases						
Chronic obstructive pulmonary disease	0.72	0.16, 3.25	0.6678			
Bronchial asthma	1.53	0.41, 5.71	0.5278			
Bronchiectasis	>999.999	<0.001, >999.999	0.9875			
Pulmonary nontuberculous mycobacteriosis	<0.001	<0.001, >999.999	0.9909			
Old tuberculosis	5.58	0.34, 90.85	0.2271			
Interstitial lung diseases	3.45	0.79, 14.95	0.0984			
Post lung cancer operation	<0.001	<0.001, >999.999	0.9881			
Pneumoconiosis	<0.001	<0.001, >999.999	0.9909			
Chronic pulmonary artery thromboembolism	<0.001	<0.001, >999.999	0.9909			
Non-pulmonary diseases						
Hypertension	1.19	0.62, 2.30	0.5957			
Congestive heart failure	<0.001	<0.001, >999.999	0.9897			
Ischemic heart diseases	2.07	0.71, 6.07	0.1832			
Diabetes mellitus	2.14	1.13, 4.07	0.0201	1.66	0.81, 3.39	0.1692
Valvular diseases	<0.001	<0.001, >999.999	0.9909			
Arrythmias	0.68	0.08, 5.55	0.7175			
Cardiomyopathy	<0.001	<0.001, >999.999	0.9916			
Cerebrovascular diseases	2.25	0.42, 11.94	0.3427			
Dementia	5.69	0.78, 41.42	0.0863			
Neuromuscular diseases	1.84	0.19, 18.13	0.5996			
Post upper digestive system surgery	5.69	0.78, 41.42	0.0863			
Chronic liver diseases	3.77	0.61, 23.23	0.1522			
Connective tissue diseases	11.41	1.01, 128.51	0.0488			
Systemic steroids or immunosuppressants	4.33	0.94, 20.01	0.0609			
Psychiatric diseases	<0.001	<0.001, >999.999	0.9916			
Malignancy	0.68	0.08, 5.55	0.7175			
Heavy drinker	<0.001	<0.001, >999.999	0.9909			
Chronic kidney disease	11.90	2.11, 67.05	0.005			
Long-term oxygen therapy	>999.999	<0.001, >999.999	0.9909			
Vaccination history, pneumococcus	2.21	0.50, 9.71	0.2939			
Vaccination history, influenza	1.98	0.84, 4.64	0.117			
Premorbid performance status						
0	Ref					
1-2	2.48	1.02, 60.35	0.045	0.95	0.31, 2.89	0.9221
3-4	6.52	1.27, 33.58	0.025	3.27	0.41, 26.07	0.2638
Viral coinfection, yes	1.41	0.73, 2.72	0.3053	1.47	0.71, 3.06	0.2995
Bacterial coinfection, yes	1.16	0.42, 3.21	0.7772			
Ferritin, ng/mL						
<500	Ref					
500-1,000	2.91	1.24, 6.82	0.0138	2.62	1.07, 6.43	0.0354
1,000≤	5.74	2.53, 13.05	<0.0001	5.78	2.33, 14.33	0.0002
Procalcitonin, ng/mL						
<0.5	Ref					
0.5≤, <1	5.98	1.44, 24.86	0.0139	2.65	0.49, 14.29	0.2579
1≤	1.99	0.20, 19.62	0.5549	2.65	0.24, 29.13	0.4265

Table 5. Univariable and Multivariable Analysis of the Need for Nasal High-flow Oxygen Therapy during the Hospital Stay.

	Univariable analysis			Multivariable analysis (final model)		
	HR	95% CI	p value	HR	95% CI	p value
Body mass index (BMI), kg/m^2						
30≤ BMI	1.61	0.77, 3.35	0.2073			
BMI <18	0.98	0.13, 7.12	0.9801			
BMI 18≤, <30	Ref					
Sex, male	1.59	0.78, 3.21	0.2005	1.03	0.36, 2.98	0.9501
Age, years						
<65	Ref					
65-74	1.57	0.77, 3.21	0.2132	1.51	0.61, 3.76	0.3747
75≤	2.60	1.27, 5.30	0.0087	0.67	0.20, 2.19	0.506
Smoking history, yes	0.70	0.39, 1.28	0.245			
Pulmonary diseases						
Chronic obstructive pulmonary disease	1.61	0.58, 4.51	0.3623			
Bronchial asthma	0.50	0.07, 3.62	0.4913			
Bronchiectasis	14.05	1.89, 104.43	0.0098			
Interstitial lung diseases	1.76	0.43, 7.29	0.4327			
Non-pulmonary diseases						
Hypertension	1.06	0.57, 1.97	0.8655			
Congestive heart failure	2.59	0.36, 18.82	0.3468			
Ischemic heart diseases	1.56	0.56, 4.36	0.3962			
Diabetes mellitus	2.02	1.12, 3.66	0.0201	0.92	0.37, 2.27	0.8603
Arrythmias	0.74	0.10, 5.36	0.7649			
Cerebrovascular diseases	0.97	0.13, 7.07	0.9795			
Chronic liver diseases	1.42	0.20, 10.32	0.7287			
Systemic steroids or immunosuppressants	1.02	0.14, 7.40	0.9856			
Malignancy	1.43	0.35, 5.90	0.622			
Chronic kidney disease	1.52	0.21, 11.01	0.6813			
Long-term oxygen therapy	-	-	-			
Vaccination history, pneumococcus	0.95	0.34, 2.642	0.9146			
Vaccination history, influenza	2.12	0.90, 5.03	0.0864			
Severity on admission, severe	10.45	5.71, 19.14	<0.0001	4.30	1.52, 12.14	0.0059
Premorbid performance status						
0	Ref					
1-2	1.47	0.62, 3.48	0.3795			
3-4	-	-	-			
Viral coinfection, yes	1.07	0.57, 2.01	0.8399	0.80	0.34, 1.91	0.6149
Bacterial coinfection, yes	0.44	0.11, 1.82	0.2587			
$PaCO_2$, Torr						
<35	1.44	0.77, 2.70	0.2508			
35-45	Ref					
45≤	1.23	0.16, 9.31	0.8414			
Lactate, mmol/L						
<2	Ref					
2≤	1.95	0.96, 3.95	0.0634			
Lymphocytes, /mm^3						
<500	2.38	1.01, 5.64	0.0483	1.37	0.36, 5.15	0.6415
500≤	Ref					
D-dimer, μg/mL						
<2	Ref					
2≤	2.35	1.16, 4.76	0.0176	0.78	0.25, 2.47	0.6757
KL-6, U/mL						
<500	Ref					
500≤	5.34	2.90, 9.84	<0.0001	3.29	1.20, 8.99	0.0205
Ferritin, ng/mL						
<500	Ref					
500-1,000	5.62	2.39, 13.21	<0.0001	6.01	1.57, 23.04	0.0089
1,000≤	5.28	2.17, 12.83	0.0002	2.91	0.76, 11.17	0.1202
Procalcitonin, ng/mL						
<0.5	Ref					
0.5≤, <1	2.22	0.54, 9.17	0.272			
1≤	1.89	0.26, 13.71	0.5311			
qSOFA, 2≤	4.38	0.60, 31.83	0.1447			
Treatment during hospital stay						
Antibiotics, yes	2.226	1.23, 4.04	0.0086	1.29	0.66, 2.52	0.4523
Corticosteroids, no	Ref					
Corticosteroid use in non-respiratory failure	20.86	7.51, 57.96	<0.0001	4.31	1.15, 16.17	0.0304
Corticosteroid use in respiratory failure	9.46	3.72, 24.05	<0.0001	2.76	0.81, 9.35	0.1035
Neuraminidase inhibitors	0.84	0.45, 1.57	0.5855			

KL-6: Krebs von der Lungen-6, qSOFA: quick Sequential Organ Failure Assessment Score

Table 6. Univariable and Multivariable Analysis of the Need for Invasive Mandatory Ventilation during the Hospital Stay.

	Univariable analysis			Multivariable analysis (final model)		
	HR	95% CI	p value	HR	95% CI	p value
Body mass index (BMI)						
30≤ BMI	1.99	0.85, 4.68	0.1156			
BMI <18	-	-	-			
BMI 18≤, <30	Ref					
Sex, male	1.70	0.69, 4.20	0.2489	1.40	0.38, 5.11	0.6123
Age, years						
<65						
65-74	2.19	0.95, 5.05	0.0660	1.50	0.50, 4.51	0.4714
75≤	1.97	0.73, 5.33	0.1817	0.52	0.12, 2.34	0.3917
Smoking history, yes	0.60	0.28, 1.27	0.1801			
Pulmonary diseases						
Chronic obstructive pulmonary disease	1.23	0.29, 5.19	0.7762			
Bronchial asthma	0.80	0.11, 5.85	0.8223			
Bronchiectasis	26.55	3.43, 205.66	0.0017			
Non-pulmonary diseases						
Hypertension	1.12	0.52, 2.43	0.7706			
Congestive heart failure	-	-	-			
Ischemic heart diseases	2.69	0.93, 7.75	0.0671			
Diabetes mellitus	2.07	0.98, 4.35	0.0553	0.84	0.29, 2.48	0.7579
Valvular diseases	-	-	-			
Arrythmias	-	-	-			
Cardiomyopathy	-	-	-			
Cerebrovascular diseases	1.64	0.22, 12.10	0.6252			
Systemic steroids or immunosuppressants	1.66	0.23, 12.22	0.6191			
Malignancy	1.12	0.15, 8.27	0.9088			
Chronic kidney disease	2.55	0.35, 18.75	0.3592			
Vaccination history, pneumococcus	0.79	0.24, 2.60	0.6913			
Vaccination history, influenza	1.53	0.58, 4.04	0.3858			
Severity on admission, severe	8.35	3.97, 17.60	<0.0001	3.35	1.06, 10.58	0.0399
Premorbid performance status						
0	Ref					
1-2	0.33	0.045, 2.46	0.2815			
3-4	-	-	-			
Viral coinfection, yes	1.48	0.69, 3.16	0.3107	1.02	0.39, 2.65	0.9753
Bacterial coinfection, yes	0.71	0.17, 3.01	0.6466			
$PaCO_2$, Torr						
<35	1.37	0.63, 2.97	0.4245			
35-45	Ref					
45≤	-	-	-			
Lactate, mmol/L						
<2	Ref					
2≤	1.05	0.37, 3.03	0.9255			
Lymphocytes, /mm^3						
<500	1.86	0.56, 6.17	0.3086	1.19	0.22, 6.32	0.8416
500≤	Ref					
D-dimer, μg/mL						
<2	Ref					
2≤	3.12	1.37, 7.08	0.0066	0.72	0.21, 2.43	0.5907
KL-6, U/mL						
<500	Ref					
500≤	6.03	2.85, 12.76	<0.0001	3.11	0.90, 10.71	0.0721
Ferritin, ng/mL						
<500	Ref					
500-1,000	13.06	2.97, 57.45	0.0007	17.45	2.09, 146.09	0.0083
1,000≤	13.87	3.10, 61.99	0.0006	5.30	0.63, 44.83	0.1255
Procalcitonin, ng/mL						
<0.5	Ref					
0.5≤, <1	6.45	1.94, 21.46	0.0024			
1≤	3.18	0.43, 23.49	0.2575			
Treatment during hospital stay						
Antibiotics, yes	2.64	1.22, 5.71	0.0139	1.13	0.49, 2.58	0.7807
Corticosteroids, no						
Corticosteroid use in non-respiratory failure	18.32	4.92, 68.28	<0.0001	3.49	0.71, 18.25	0.1254
Corticosteroid use in respiratory failure	13.01	4.14, 40.88	<0.0001	4.39	1.11, 17.33	0.0349
Neuraminidase inhibitors	0.91	0.42, 1.96	0.8012			

KL-6: Krebs von der Lungen-6

Table 7. Univariable and Multivariable Analysis of Mortality.

	Univariable analysis			Multivariable analysis (final model)		
	HR	95% CI	p value	HR	95% CI	p value
Body mass index (BMI)						
45≤	1.57	0.53, 4.65	0.4172			
BMI <18	-	-	-			
BMI 18≤, <30	Ref					
Sex, male	1.25	0.49, 3.18	0.6357	1.07	0.285, 3.983	0.9244
Age, years						
<65	Ref					
65-74	1.65	0.44, 6.16	0.4531	0.48	0.07, 3.17	0.4438
75≤	12.33	4.42, 34.41	<0.0001	8.49	1.79, 40.36	0.0072
Smoking history, yes	0.59	0.25, 1.36	0.2113			
Pulmonary diseases						
Chronic obstructive pulmonary disease	1.39	0.33, 6.93	0.6567			
Bronchial asthma	0.99	0.13, 7.38	0.9952			
Bronchiectasis	40.49	4.98, 329.11	0.0005			
Interstitial lung diseases	3.42	0.80, 14.60	0.0970			
Non-pulmonary diseases						
Hypertension	1.67	0.73, 3.80	0.2262			
Congestive heart failure	6.21	0.83, 46.27	0.0748			
Ischemic heart diseases	2.63	0.78, 8.88	0.1187			
Diabetes mellitus	4.69	1.99, 11.06	0.0004	3.72	0.82, 16.77	0.0878
Arrythmias	1.14	0.15, 8.49	0.8967			
Cerebrovascular diseases	2.02	0.27, 15.02	0.4906			
Neuromuscular diseases	8.89	2.07, 38.28	0.0034			
Post upper digestive system surgery	3.89	0.52, 28.97	0.1852			
Chronic liver diseases	2.76	0.37, 20.52	0.3202			
Systemic steroids or immunosuppressants	2.31	0.31, 17.14	0.4136			
Malignancy	1.23	0.17, 9.09	0.8430			
Chronic kidney disease	3.00	0.40, 22.32	0.2841			
Vaccination history, pneumococcus	0.59	0.18, 1.99	0.3961			
Vaccination history, influenza	1.46	0.50, 4.29	0.4914			
Severity on admission, severe	9.04	3.95, 20.71	<0.0001	0.48	0.10, 2.41	0.3729
Premorbid performance status						
0	Ref					
1-2	3.12	1.15, 8.47	0.0254			
3-4	2.42	0.32, 18.23	0.3903			
Viral coinfection, yes	1.49	0.63, 3.52	0.3672	1.11	0.30, 4.16	0.876
Bacterial coinfection, yes	1.65	0.49, 5.56	0.4225			
$PaCO_2$, Torr						
<35	2.11	0.83, 5.36	0.1151			
35-45	Ref					
45≤	-	-	-			
Lactate, mmol/L						
<2	Ref					
2≤	1.44	0.49, 4.23	0.5094			
Lymphocytes, /mm^3						
<500	8.68	3.51, 21.48	<0.0001	9.07	1.79, 46.01	0.0078
500≤	Ref					
D-dimer, μg/mL						
<2	Ref					
2≤	3.70	1.52, 9.03	0.0040	4.67	1.16, 18.77	0.0300
KL-6, U/mL						
<500	Ref					
500≤	4.03	1.71, 9.51	0.0015	1.76	0.43, 7.21	0.4316
Ferritin, ng/mL						
<500	Ref					
500-1,000	12.74	2.85, 57.01	0.0009	15.65	1.70, 144.31	0.0153
1,000≤	11.65	2.51, 54.03	0.0017	3.67	0.39, 34.77	0.2573
Procalcitonin, ng/mL						
<0.5	Ref					
0.5≤, <1	6.75	1.99, 22.91	0.0022			
1≤	4.95	0.66, 37.23	0.1199			
qSOFA, 2≤	12.38	1.61, 94.85	0.0155			
Treatment during hospital stay						
Antibiotics, yes	4.17	1.64, 10.58	0.0027			
Corticosteroids, no	Ref					
Corticosteroid use in non-respiratory failure	28.03	6.63, 118.50	<0.0001	15.62	1.99, 122.68	0.009
Corticosteroid use in respiratory failure	16.87	4.42, 64.31	<0.0001	10.66	1.57, 72.18	0.0153
Neuraminidase inhibitors, yes	0.86	0.36, 20.26	0.7279			
NSAIDs, yes	0.47	0.06, 3.50	0.4631			
Pneumonia subtypes						
Primary viral pneumonia	Ref					
Mixed viral and bacterial pneumonia	1.65	0.49, 5.56	0.4225			

KL-6: Krebs von der Lungen-6, qSOFA: quick Sequential Organ Failure Assessment Score, NSAIDs: nonsteroidal anti-inflammatory drugs

rus (14, 15), was common.

Previous studies suggested that coinfection is usually connected with the need for a higher level of care, increased length of stay, and development of acute respiratory distress syndrome (16). Because of the serious damage to the immune system caused by the coinfection (17), the condition of patients who are positive for both SARS-CoV-2 and other viruses may be more serious, and their treatment can be more complicated and require a longer treatment cycle (18). However, in the present study, coinfection did not affect severity on admission, the need for HFNC or IMV, and mortality, the results of which were compatible with those of a previous report (19). Another previous study showed mixed viral and bacterial pneumonia to be an independent factor for mortality (20) from influenza-associated pneumonia, and an additional report showed higher mortality from viral pneumonia when coinfected by bacteria, e.g., *Streptococcus pneumoniae* (21, 22). In one study that investigated patients with cystic fibrosis, coinfection of other pathogens in addition to SARS-CoV-2 led to intensive care, antibiotics use, and an increased mortality rate (23). In the present study, the pneumococcal coinfections were minor, and underlying diseases of bronchiectasis and pulmonary non-tuberculous mycobacteriosis, both of which are risk factors of mixed viral and bacterial infection (23), were infrequent. These factors may have affected our results that mixed bacterial coinfection was minor and bacterial coinfection did not affect either severity or mortality. In other words, in COVID-19 patients without such underlying diseases, bacterial coinfection is uncommon, which indicates that the use of routine broad-spectrum antibiotics is not recommended. Prediction models to distinguish bacterial coinfection from primary viral pneumonia are desirable to judge the need for antibiotics therapy. The most frequent bacterial pathogens coinfecting in the present study were *M. pneumoniae* followed by *S. pneumoniae* and *Legionella* spp., and thus, macrolides or quinolones may be recommended in regions with a low rate of infection with macrolide-resistant *S. pneumoniae* for the time being. Future prospective studies are needed to clarify recommendations for routine antibiotics use in COVID-19.

Although the significance of viral coinfection is unknown, the mechanisms of coinfection include virus-induced airway damage, reduced mucociliary clearance, and damage to the immune system (24), which indicates a role of coinfection as a gatekeeper of SARS-CoV-2. Because our study could not clarify this matter, the significance of viral coinfection should be investigated in future studies. Another important issue is the efficacy of antivirals on coinfection. A few studies showed that early use of neuraminidase inhibitors decreased intensive care unit admission and mortality in patients with influenza-associated pneumonia (25). Options for the treatment of viruses other than influenza virus are extremely limited, and the efficacy of antivirals against these viruses coinfecting with COVID-19 remains unknown but should be elucidated in future studies.

Our study has several limitations. First, because this is a non-randomized observational study, the level of confidence was reduced. Second, clinical tests to detect causative microorganisms were not used in all patients. For example, sputum culture was performed in only 62 (20.8%) of 298 patients because of the low frequency at which patients expectorate sputum. This may result in underestimation of the coinfection rate. Third, this study was carried out in a single institution, and the results may not be applicable to other settings. Finally, some viral infections may have been missed in this study because only a limited number of viruses were screened in the assay.

In conclusion, the present study showed that coinfection was frequent in CAP with COVID-19, especially by other viruses, and primary viral pneumonia was dominant. The rate of bacterial coinfection was less than 10%. Coinfection, both of viral and bacterial origin, did not appear to affect severe respiratory conditions or mortality.

The authors state that they have no Conflict of Interest (COI).

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