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 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 coinfection in patients with COVID-19 over a 1-year study period.

Methods Coinfection was investigated via multiplex polymerase chain reaction, 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 SARS-CoV-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.

Conclusions 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

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 (COVID-19) 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 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 coinfected 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.

Material 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°C and used for the detection of respiratory pathogens on aRotor-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; enteroovirus; 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 IDSA/ATS 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, Inc., Cary, NC).

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/mm³) 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. Coinfection 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.
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 | 8 (2.7) |
| 65-74           | 79 (26.5) | Unknown | 160 (53.7) |
| 75+             | 51 (17.1) | <35 | 124 (41.6) |
| Body mass index (BMI), kg/m² | 25±4.54 | 35-45 | 6 (2.0) |
| 30≤BMI          | 44 (14.8) | 45± |  |
| BMI<18          | 7 (2.3) | Lactate, mmol/L | 36 (12.1) |
| BMI 18≤<30      | 233 (78.1) | Unknown | 221 (74.2) |
| BMI, unknown    | 14 (4.7) | <2 |  |
| Days from onset to admission | 7 (0-19) | ≥2 | 41 (13.8) |
| Antibiotics prior to admission, yes | 39 (13.1) | WBC, /mm³ | 6,484±3,049 |
| Smoking history, yes | 145 (48.7) | Plt, /mm³ | 20±7,7 |
| Underlying diseases, none | 109 (36.6) | Neutrophils, /mm³ | 4,971±2,993 |
| Pulmonary diseases | 17 (5.7) | Lymphocytes, /mm³ | 1,073±508 |
| COPD            | 14 (4.7) | Unknown |  |
| Bronchial asthma | 1 (0.3) | <500 | 21 (7.0) |
| Bronchiectasis  | 100 (33.6) | BUN, mg/dL | 16±9 |
| Pulmonary nontuberculous mycobacteriosis | 1 (0.3) | D-dimer, µg/mL | 1.7±3.36 |
| Old tuberculosis | 92 (30.9) | CL-6, U/mL | 137±8 |
| Interstitial lung diseases | 8 (2.7) | Unknown | 3 (1.0) |
| Post lung cancer operation | 4 (1.3) | <2 | 255 (85.6) |
| Pneumococcal    | 1 (0.3) | AST, IU/L | 41±38 |
| Chronic pulmonary artery thromboembolism | 1 (0.3) | ALT, IU/L | 35±33 |
| Non-pulmonary diseases | 120 (40.2) | LDH, IU/L | 281±116 |
| Hypertension    | 3 (1.0) | CK, IU/L | 150±424 |
| Congestive heart failure | 3 (1.0) | BUN<20 |  |
| Diabetes mellitus | 19 (6.4) | Cre, mg/dL | 0.88±0.34 |
| Valvular diseases | 93 (30.9) | Na, mmol/L | 36±12 |
| Arrhythmias     | 1 (0.3) | CRP, mg/dL | 5.6±5.8 |
| Cardiomyopathy  | 9 (3.0) | KL-6, U/mL | 337±321 |
| Cerebrovascular diseases | 7 (2.3) | <500 | 251 (84.2) |
| Dementia        | 4 (1.3) | 500± | 39 (13.1) |
| Muscular diseases | 4 (1.3) | Ferritin, ng/mL | 74±3703 |
| 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 | 0.23±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 | 262 (87.9) | Acute pulmonary thromboembolism | 1 (0.3) |
| 0               | 112 (37.6) | Pneumothorax | 1 (0.3) |
| 1               | 100 (33.6) | Pulmonary hemorrhage | 1 (0.3) |
| 3               | 100 (33.6) | Acute kidney injury | 16 (5.4) |
| Viral co-infection, yes | 91 (30.5) | qSOFA, ≥2 | 2 (0.7) |

qSOFA: quick Sequential Organ Failure Assessment Score, IMV: invasive mandatory ventilation, HFNC: high-flow nasal cannula, 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

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 non-respiratory failure without the requirement for O2. 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 trans-

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.
fer 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 Mortality**

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 (12). 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 coinfected with SARS-CoV-2 ranged from 1 to 6 (12). Frequent pathogens included *Staphylococcus aureus*, *HHV4*, *M. catarrhalis*, *Klebsiella pneumonia*, hMPV, and adenovirus (12). Another multicenter study of 5,700 COVID-19 patients showed the common coinfesting pathogens to be enterovirus, rhinovirus, of which the common cold coronavirus was the most common, 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 coinfected with SARS-CoV-2 ranged from 1 to 6 (12). Frequent pathogens included *Staphylococcus aureus*, *HHV4*, *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 coronavirus (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 were the factors associated with death. Coinfection with viruses or bacteria was not associated with mortality (Table 7).
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 | <.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 mycobacteriosi | <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                             |          |          |                                      |
| Pneumococci             | <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                             |          |          |                                      |
| Atrioventricular disease | 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                               |          |          |                                      |
| Neumuscular 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 co-infection, yes  | 1.41     | 0.73, 2.72 | 0.3053                               | 1.47     | 0.71, 3.06 | 0.2995                               |
| Bacterial co-infection, 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 | <.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)** |
|-------------------------|--------------------------------------|
| **Body mass index (BMI), kg/m²** | |
| 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 |
| **Age, years** | |
| <65 | 1.57 | 0.77 3.21 | 0.2132 |
| 75- | 2.60 | 1.27, 5.30 | 0.0087 |
| **Smoking history, yes** | 0.70 | 0.39, 1.28 | 0.4245 |
| **Pulmonary diseases** | |
| Chronic obstructive pulmonary disease | 1.61 | 0.58, 4.51 | 0.3623 |
| Bronchial asthma | 0.50 | 0.07, 7.12 | 0.4913 |
| Bronchiectasis | 14.05 | 1.89, 104.45 | 0.0098 |
| **Non-pulmonary diseases** | 1.76 | 0.43, 7.29 | 0.4272 |
| 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 |
| 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 | 0.2287 |
| 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, 2.64 | 0.9146 |
| Vaccination history, influenza | 2.12 | 0.90, 5.03 | 0.0964 |
| Severity on admission, severe | 10.45 | 5.71, 19.14 | <0.001 |
| Premorbid performance status | 4.30 | 1.52, 12.14 | 0.0059 |
| 0 | Ref | |
| 1-2 | 1.47 | 0.62, 3.48 | 0.3795 |
| 3-4 | - | - | - |
| Viral co-infection, yes | 1.07 | 0.57, 2.01 | 0.8399 |
| Bacterial co-infection, yes | 0.44 | 0.11, 1.82 | 0.2587 |
| PaCO₂, Torr | |
| <35 | 1.44 | 0.77, 2.70 | 0.2508 |
| 35≤ | Ref | |
| Lactate, mmol/L | 1.23 | 0.16, 9.31 | 0.8414 |
| <2 | Ref | |
| 2% | 1.95 | 0.96, 3.95 | 0.0634 |
| Lymphocytes, /mm³ | 2.38 | 1.01, 5.64 | 0.0483 |
| <500 | Ref | |
| 500≤ | 1.37 | 0.36, 5.15 | 0.6415 |
| D-dimer, μg/mL | 0.78 | 0.25, 2.47 | 0.6757 |
| <2 | Ref | |
| 2% | 2.35 | 1.16, 4.76 | 0.0176 |
| KL-6, U/mL | 0.78 | 0.25, 2.47 | 0.6757 |
| <500 | Ref | |
| 500≤ | 5.34 | 2.90, 9.84 | <0.001 |
| Ferritin, ng/mL | 3.29 | 1.20, 8.99 | 0.0205 |
| <500 | Ref | |
| 500≤ | 5.62 | 2.39, 13.21 | <0.001 |
| 1,000≤ | 6.01 | 1.57, 23.04 | 0.0089 |
| Procalcitonin, ng/mL | 2.91 | 0.76, 11.17 | 0.1202 |
| <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 |
| Corticosteroids, no | 2.29 | 0.66, 2.52 | 0.4523 |
| Corticosteroid use in non-respiratory failure | 20.86 | 7.51, 57.96 | <0.001 |
| Corticosteroid use in respiratory failure | 9.46 | 3.72, 24.05 | <0.001 |
| 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.

| Variable                                           | 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      | 1.40 0.38, 5.11 0.6123             |
| BMI<18                                             | -                    | -                                   |
| BMI 18≤,<30                                       | Ref                  |                                     |
| Sex, male                                         | 1.70 0.69, 4.20      | 1.50 0.50, 4.51 0.4714             |
| Age, years                                        |                      |                                     |
| <65                                                | 2.19 0.95, 5.05      | 0.52 0.12, 2.34 0.3917             |
| 65-74                                              | 2.17 0.73, 5.33      |                                     |
| Smoking history, yes                              | 0.60 0.28, 1.27      |                                     |
| Pulmonary diseases                                |                      |                                     |
| Chronic obstructive pulmonary disease             | 1.23 0.29, 5.19      |                                     |
| Bronchial asthma                                  | 0.80 0.11, 5.85      |                                     |
| Bronchiectasis                                    | 26.55 3.43, 205.66   | 0.0017                              |
| Non-pulmonary diseases                            |                      |                                     |
| Hypertension                                      | 1.12 0.52, 2.43      | 0.84 0.29, 2.48 0.7579             |
| Congestive heart failure                          | -                    | -                                   |
| Ischemic heart diseases                           | 2.69 0.93, 7.75      | 0.0671                              |
| Diabetes mellitus                                 | 2.07 0.98, 4.35      | 0.0553                              |
| Valvular diseases                                 | -                    | -                                   |
| Arrhythmias                                       | -                    | -                                   |
| 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     | 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 co-infection, yes                           | 1.48 0.69, 3.16      | 1.02 0.39, 2.65 0.9753             |
| Bacterial co-infection, yes                       | 0.71 0.17, 3.01      | 0.6466                              |
| PaCO2, Torr                                       | 1.37 0.63, 2.97      | 0.4245                              |
| <35                                                | Ref                  |                                      |
| 35-45                                              | -                    | -                                   |
| Lactate, mmol/L                                   | 3.12 1.37, 7.08      | 0.72 0.21, 2.43 0.5907             |
| <2                                                | Ref                  |                                      |
| ≥2                                                 | 1.05 0.37, 3.03      | 0.9255                              |
| Lymphocytes, /mm³                                 | 1.86 0.56, 6.17      | 1.19 0.22, 6.32 0.8416             |
| <500                                              | Ref                  |                                      |
| 500≤                                               | -                    | -                                   |
| D-dimer, μg/mL                                    | 13.06 2.97, 57.45    | 17.45 2.09, 146.09 0.0083          |
| <2                                                | Ref                  |                                      |
| ≥2                                                 | 13.87 3.10, 61.99    | 5.30 0.63, 44.83 0.1255            |
| KL-6, U/mL                                       | 6.39 1.28, 12.76     | <.0001                              |
| <500                                              | Ref                  | 3.11 0.90, 10.71 0.0721            |
| 500≤                                               | 6.03 2.85, 12.76     | <.0001                              |
| Ferritin, ng/mL                                   | 13.01 4.14, 40.88    | <.0001                              |
| <500                                              | Ref                  | 4.39 1.11, 17.33 0.0349            |
| 500-1,000                                         | 18.32 4.92, 68.28    | <.0001                              |
| 0.5≤,<1                                           | 3.49 0.71, 18.25     | 0.1254                              |
| 0.5≤                                               | 13.32 4.92, 68.28    | <.0001                              |
| Treatment during hospital stay                    | 2.64 1.22, 5.71      | 1.13 0.49, 2.58 0.7807             |
| Antibiotics, yes                                  | 18.32 4.92, 68.28    | <.0001                              |
| Corticosteroid use in non-respiratory failure     | 13.01 4.14, 40.88    | <.0001                              |
| Corticosteroid use in respiratory failure         | 0.91 0.42, 1.96      | 0.8012                              |

KL-6: Krebs von der Lungen-6
| Table 7 | Univariable and Multivariable Analysis of Mortality. |
|---------|--------------------------------------------------|
| **Body mass index (BMI)** | **HR** | **95%CI** | **p value** | **HR** | **95%CI** | **p value** |
| 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 |
| Age, years |  |
| ≤65 | Ref | - | - |
| 65-74 | 1.65 | 0.44, 6.16 | 0.4531 |
| 75-84 | 12.33 | 4.42, 34.41 | <.0001 |
| 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 |
| Bronchietasis | 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 |
| Atrrrythias | 1.14 | 0.15, 8.49 | 0.8967 |
| Cerebrovascular diseases | 2.02 | 0.27, 15.02 | 0.4906 |
| Neumorulcas 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 |  |
| Premorbid performance status | 9.04 | 3.95, 20.71 | <.0001 |
| ≤0 | Ref | - | - |
| 1-2 | 3.12 | 1.15, 8.47 | 0.0254 |
| 3-4 | 2.42 | 0.32, 18.23 | 0.3903 |
| Viral co-infection, yes | 1.49 | 0.63, 3.52 | 0.3672 |
| Bacterial co-infection, yes | 1.65 | 0.49, 5.56 | 0.4225 |
| PaCO₂, Torr |  |
| <35 | 2.11 | 0.83, 5.36 | 0.1151 |
| 35-45 | Ref | - | - |
| 45< | - | - | - |
| Lactate, mmol/L |  |
| ≤2 | Ref | - | - |
| 2-5 | 1.44 | 0.49, 4.23 | 0.5094 |
| Lymphocytes, /mm³ |  |
| <500 | 8.68 | 3.51, 21.48 | <.0001 |
| 500< | Ref | - | - |
| D-dimer, μg/mL |  |
| <2 | 3.70 | 1.52, 9.03 | 0.0040 |
| 2< | Ref | - | - |
| KL-6, U/mL |  |
| <500 | 4.03 | 1.71, 9.51 | 0.0015 |
| 500< | Ref | - | - |
| Ferritin, ng/mL |  |
| <500 | 12.74 | 2.85, 57.01 | 0.0009 |
| 500-1,000 | 11.65 | 2.51, 54.03 | 0.0017 |
| 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 | <.0001 |
| Corticosteroid use in respiratory failure | 16.87 | 4.42, 64.31 | <.0001 |
| 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 bacterial and 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
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 coinfected 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).

Acknowledgments

We thank our colleagues at Saitama Cardiovascular and Respiratory Center for their valuable cooperation in clinical practice.

**Funding:** This study was partially supported by a grant from Saitama Cardiovascular and Respiratory Center (16ES, 17ES, 18 ES, 19ES, 20ES).

**References**

1. Jain S, Self WH, Wunderink RG; CDC EPIC Study Team. Community-acquired pneumonia requiring hospitalization. N Engl J Med 373: 415–427, 2015.
2. Katsurada N, Suzuki M, Aoshima M, et al; Adult Pneumonia Study Group-Japan. The impact of virus on pneumonia mortality is complex in adults: a prospective multicenter observational study. BMC Infect Dis 17: 755, 2017.
3. Vaughn VM, Gandhi TN, Petty LA, et al. Empiric antibacterial therapy and community-onset bacterial coinfection in patients hospitalized with coronavirus disease 2019 (COVID-19): a multicenter cohort study. Clin Infect Dis 8: ofaa578, 2020.
4. Karaba SM, Jones G, Heisel T, et al. Prevalence of co-infection at the time of hospital admission in COVID-19 patients, a multicenter study. Open Forum Infect Dis 8: ofaa578, 2020.
5. Ishiguro T, Kobayashi Y, Uozumi R, et al. Viral pneumonia requiring differentiation from acute and progressive diffuse interstitial lung diseases. Intern Med 58: 3509-3519, 2019.
6. Ishiguro T, Takayanagi N, Yamaguchi S, et al. Etiology and factors contributing severity and mortality of community-acquired pneumonia. Intern Med 52: 317-324, 2013.
7. Louria DB, Blumenfeld HL, Ellis JT, Kilbourne ED, Rogers DE. Studies of influenza in the pandemic of 1957-1958. II. pulmonary complications of influenza. J Clin Invest 38: 213-265, 1959.
8. Mandell LA, Wunderink RG, Anaetozo A, et al. Infectious Disease Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 44: S27-S72, 2007.
9. Crotty MP, Meyers S, Hamilton N, et al. Epidemiology, co-infections, and outcomes of viral pneumonia in adults. Medicine 94: e2332, 2015.
10. Kim D, Quinn J, Pinsky B, Shah NH, Brown I. Rates of coinfection between SARS-CoV-2 and other respiratory pathogens. JAMA 323: 2085-2086, 2020.
11. Song W, Jia X, Zhang X, Ling Y, Yi Z. Co-infection in COVID-19, a cohort study. J Infect 82: 414-451, 2020.
12. Massey BW, Jayathilake K, Meltzer HY. Respiratory microbial co-infection with SARS-CoV-2. Front Microbiol 11: 2079, 2020.
13. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting charac-
teristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City Area. JAMA 323: 2052-2059, 2020.

14. Lin D, Liu L, Zhang M, et al. Co-infections of SARS-CoV-2 with multiple common respiratory pathogens in infected patients. Sci China Life Sci 63: 606-609, 2020.

15. Wang M, Wu Q, Xu M, et al. Clinical diagnosis of 8274 samples with 2019-novel coronavirus in Wuhan. medRxiv 20202327, 2020.

16. Cawcutt K, Kalil AC. Pneumonia with bacterial and viral coinfection. Curr Opin Crit Care 23: 385-390, 2017.

17. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. Nature Rev Immunol 20: 363-374, 2020.

18. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med 8: 475-481, 2020.

19. Wang L, He W, Yu X, et al. Coronavirus disease 2019 in elderly patients: characteristics and prognostic factors based on 4-week follow up. J Infect 80: 639-645, 2020.

20. Ishiguro T, Kagiyama N, Uozumi R, et al. Clinical characteristics of influenza-associated pneumonia of adults: Clinical features and factors contributing to severity and mortality. Yale J Biol Med 90: 165-181, 2017.

21. Estenssoro E, Ríos FG, Apezteguía C, et al. Registry of the Argentinian Society of Intensive Care SATI. Pandemic 2009 influenza a in Argentina a study of 337 patients on mechanical ventilation. Am J Respir Crit Care Med 182: 41-48, 2010.

22. Kiedrowski MR, Bomberger JM. Viral-bacterial co-infections in the cystic fibrosis respiratory tract. Front Immunol 9: 3067, 2018.

23. Lim YK, Kweon OJ, Kim HR, Kim TH, Lee MK. Impact of bacterial and viral coinfection in community-acquired pneumonia in adults. Diagn Microbiol Infect Dis 94: 50-54, 2009.

24. Vareille M, Kieninger E, Edwards MR, Regamey N. The airway epithelium: soldier in the fight against respiratory viruses. Clin Microbiol Rev 24: 210-229, 2011.

25. Hsu J, Santesso N, Mustafa R, et al. Antivirals for treatment of influenza: a systematic review and meta-analysis of observational studies. Ann Intern Med 156: 512-524, 2012.

The Internal Medicine is an Open Access journal distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. To view the details of this license, please visit (https://creativecommons.org/licenses/by-nc-nd/4.0/).