

Influenza A virus exposure may cause increased symptom severity and deaths in coronavirus disease 2019

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Abstract

Background: The coronavirus disease 2019 (COVID-19) outbreak occurred during the flu season around the world. This study aimed to analyze the impact of influenza A virus (IAV) exposure on COVID-19.

Methods: Seventy COVID-19 patients admitted to the hospital during January and February 2020 in Wuhan, China were included in this retrospective study. Serum tests including respiratory pathogen immunoglobulin M (IgM) and inflammation biomarkers were performed upon admission. Patients were divided into common, severe, and critical types according to disease severity. Symptoms, inflammation indices, disease severity, and fatality rate were compared between anti-IAV IgM-positive and anti-IAV IgM-negative groups. The effects of the empirical use of oseltamivir were also analyzed in both groups. For comparison between groups, *t* tests and the Mann-Whitney *U* test were used according to data distribution. The Chi-squared test was used to compare disease severity and fatality between groups.

Results: Thirty-two (45.71%) of the 70 patients had positive anti-IAV IgM. Compared with the IAV-negative group, the positive group showed significantly higher proportions of female patients (59.38% vs. 34.21%, $\chi^2 = 4.43$, $P = 0.035$) and patients with fatigue (59.38% vs. 34.21%, $\chi^2 = 4.43$, $P = 0.035$). The levels of soluble interleukin 2 receptor (median 791.00 vs. 1075.50 IU/mL, $Z = -2.70$, $P = 0.007$) and tumor necrosis factor α (median 10.75 vs. 11.50 pg/mL, $Z = -2.18$, $P = 0.029$) were significantly lower in the IAV-positive group. Furthermore, this group tended to have a higher proportion of critical patients (31.25% vs. 15.79%, $P = 0.066$) and a higher fatality rate (21.88% vs. 7.89%, $P = 0.169$). Notably, in the IAV-positive group, patients who received oseltamivir had a significantly lower fatality rate (0 vs. 36.84%, $P = 0.025$) compared with those not receiving oseltamivir.

Conclusions: The study suggests that during the flu season, close attention should be paid to the probability of IAV exposure in COVID-19 patients. Prospective studies with larger sample sizes are needed to clarify whether IAV increases the fatality rate of COVID-19 and to elucidate any benefits of empirical usage of oseltamivir.

Keywords: Influenza A; Coronavirus disease 2019; Inflammation biomarker; Fatality rate

Introduction

The coronavirus disease 2019 (COVID-19) outbreak that has been ongoing since the end of 2019, caused by the novel coronavirus (2019-nCoV), has now become a global public health emergency. It is known that the period from December to February is also the epidemic season of influenza in the northern hemisphere. According to weekly reports from the Chinese National Influenza Center, the positivity rate for influenza virus tests in patients with flu-like symptoms ranged from 19.3% to 44.9% at the end of 2019, and most (77.1–80.3%) were influenza A virus (IAV).^[1] It is already known that both IAV and 2019-nCoV infections can cause flu-like symptoms, pneumonia, and even death. However, it is not clear what impact IAV

might have on the clinical characteristics and prognosis of COVID-19 patients. To better understand this issue, we compared anti-IAV IgM-positive and -negative COVID-19 patients.

Methods

Ethical approval

The study conformed to the requirements of the *Declaration of Helsinki* and was approved by the Ethics Committee of Peking University First Hospital (No. 2020-098). The analysis used anonymous clinical data to protect privacy.

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Study design and patient recruitment

This was a retrospective cohort study of patients with COVID-19 admitted to an isolation ward in Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology in Wuhan, China. Diagnoses of COVID-19 were confirmed by reverse transcription-polymerase chain reaction (RT-PCR) of pharyngeal or nasopharyngeal swabs and chest computed tomography results based on the diagnosis and treatment protocol from the National Health Commission of the People's Republic of China.^[2] The inclusion criteria included a confirmed diagnosis of 2019-nCoV infection and clinical outcome before recruitment. The exclusion criteria included lacking IAV IgM results and co-infection with other pathogens. Eighty-two patients admitted to our ward from January 28 to February 25, 2020 were recruited, although 12 were excluded upon suspicion of co-infection with other pathogens. Of these, four were positive for anti-mycoplasma IgM, seven were positive for anti-influenza B IgM, and one was positive for anti-respiratory syncytial virus (RSV) IgM. The remaining 70 patients were enrolled in this study.

Laboratory tests

Serum tests for respiratory pathogen IgM antibodies were performed on admission, which included IAV, influenza B, parainfluenza virus, mycoplasma, chlamydia, RSV, adenovirus, and legionella. The tests were performed as per the manufacturer's instructions using Respiratory Tract Pathogen IgM kits (Euroimmun, Luebeck, Germany) based on an indirect immunofluorescence technique. To eliminate the impact of other pathogens, patients with positive IgM results for pathogens other than IAV were excluded. The IAV-positive and IAV-negative groups were divided accordingly.

Laboratory tests for inflammation biomarkers including interleukins (ILs), tumor necrosis factor α (TNF α), ferritin, D-dimer, C-reactive protein, and erythrocyte sedimentation rate were also performed on admission.

Disease severity

According to the national protocol mentioned earlier,^[2] patients were divided into common, severe, and critical types. Specifically, patients with respiratory failure requiring mechanical ventilation, septic shock, and/or multiple organ dysfunction or failure that demanded the intensive care unit treatment were defined as critical. Patients with respiratory frequency ≥ 30 /min, blood oxygen saturation $\leq 93\%$ at room air, and/or partial pressure of arterial oxygen to fraction of inspired oxygen ratio ≤ 300 mmHg were defined as severe. All other patients were considered to be a common disease type.

Data collection

Patients' basic information, symptoms, and medical history were collected using a medical record form on admission. Symptoms included fever, cough, sore throat, headache, shortness of breath, hemoptysis, myalgia, and diarrhea. All data including basic information, symptoms, medications, lab results, indices for disease severity, and

fatality were collected from the hospital information system and inputted into a database. Data input was completed independently by two authors to check for possible mistakes.

Statistical analysis

SPSS Statistics for Windows (Version 22.0; IBM Corp., Armonk, NY, USA) was used for statistical analyses. Continuous data with normal distribution are expressed as mean \pm standard deviation. As all of the inflammation biomarkers were non-normally distributed (checked by Kolmogorov-Smirnov test), they were expressed as medians and ranges. We compared symptoms, inflammation biomarkers, disease severity, and fatality rates between IAV-negative and IAV-positive groups. As oseltamivir was used empirically in both groups before admission, we further analyzed the effects of oseltamivir on fatality rates in both groups. As for the comparison between groups, *t* tests were used for continuous data with normal distribution, and the Mann-Whitney *U* test was used for continuous data that were not normally distributed. For the comparison of categorical variables between groups, such as sex, disease severity, and fatality, the Chi-squared test and Fisher Exact test were used. $P < 0.05$ was considered statistically significant.

Results

Thirty-two (45.71%) of the 70 COVID-19 patients were positive for anti-IAV IgM. The baseline characteristics, symptoms, and inflammation indices are summarized in Table 1. In the IAV-positive group, there was a significantly higher proportion of female patients (59.38% *vs.* 34.21%, $\chi^2 = 4.43$, $P = 0.035$). Fever and cough were the most common symptoms in both groups. A significantly higher proportion of patients complaining of fatigue were found in the IAV-positive group (59.38% *vs.* 34.21%, $\chi^2 = 4.43$, $P = 0.035$), but otherwise, symptoms were not different between the two groups despite a tendency toward a higher proportion of patients for most symptoms in the IAV-positive group. Inflammation indices were also generally similar; however, the levels of soluble IL-2 receptor (sIL-2R) and TNF α were slightly but significantly lower in the IAV-positive group.

The distribution of disease severity between the two groups is shown in Figure 1. There was a higher proportion of both common and critical type patients in the IAV-positive group, but this difference did not achieve statistical significance (21.88% *vs.* 10.53%, 31.25% *vs.* 15.79%; $P = 0.066$). As shown in Figure 2, oseltamivir did not affect the distribution of disease severity in the IAV-negative group. However, in the IAV-positive group, oseltamivir seemed to reduce the proportion of critical patients, although this was not statistically significant (15.38% *vs.* 42.11%, $P = 0.131$).

Similarly, there was a tendency toward a higher fatality rate in the IAV-positive group compared with the negative group (21.88% *vs.* 7.89%, $P = 0.169$). As shown in Table 2, when we analyzed the effects of oseltamivir in the IAV-positive group, we found a significantly lower fatality

Table 1: Baseline characteristics, symptoms, and inflammation indices of IAV-negative and IAV-positive COVID-19 patients.

Variables	Total (n = 70)	IAV-negative (n = 38)	IAV-positive (n = 32)	Statistics	P
Age (years)	63.4 ± 13.5	64.0 ± 14.3	62.8 ± 12.8	0.39*	0.697
Female	32 (45.71)	13 (34.21)	19 (59.38)	4.43 [†]	0.035
First symptom to admission (days)	11.36 ± 4.16	10.76 ± 3.12	12.06 ± 5.09	-1.26*	0.214
Fatigue	32 (45.71)	13 (34.21)	19 (59.38)	4.43 [†]	0.035
Lymphocytes (×10 ⁹ /L)	0.85 (0.65, 1.22)	0.83 (0.62, 1.27)	0.89 (0.66, 1.19)	-0.45 [‡]	0.654
sIL-2R (U/mL)	921.50 (658.00, 1463.50)	1075.50 (716.25, 1491.25)	791.00 (567.25, 1565.50)	-2.70 [‡]	0.007
TNFα (pg/mL)	11.30 (9.15, 14.52)	11.50 (9.33, 17.32)	10.75 (7.98, 13.03)	-2.18 [‡]	0.029

Data are presented as mean ± standard deviation, n (%) or median (P25, P75). * *t* values. [†] χ^2 values. [‡] *Z* values. The Chi-squared test showed that there were significantly higher proportions of female patients and patients with fatigue in the IAV-positive group. The Mann-Whitney *U* test showed that the levels of sIL-2R and TNFα were significantly lower in the IAV-positive group. IAV: Influenza A virus; COVID-19: Coronavirus disease 2019; sIL-2R: Soluble interleukin 2 receptor; TNFα: Tumor necrosis factor α.

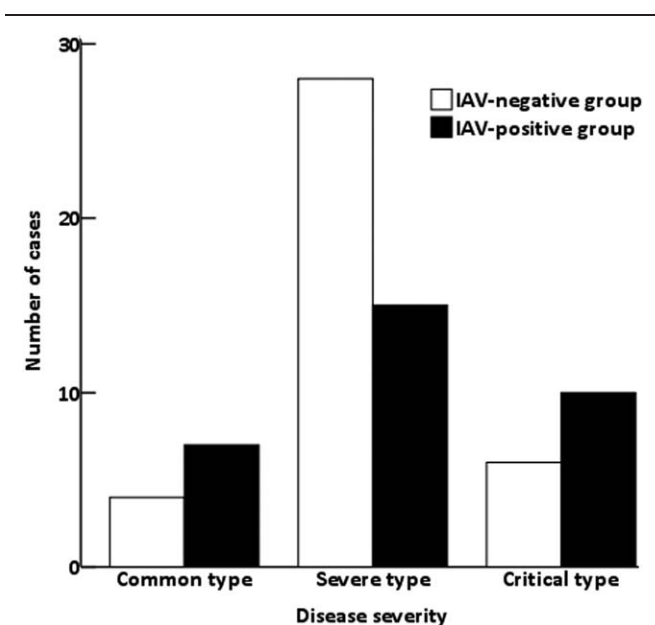


Figure 1: Distribution of disease severity in IAV-negative and IAV-positive groups. The Fisher Exact test was used to compare the severity between IAV-negative and IAV-positive groups. In the IAV-positive group, the proportion of critical type patients tended to be higher, but with no statistical significance (31.25% vs. 15.79%, $P=0.066$). Common type: Patients with pneumonia but with no signs of severe type or critical type. Severe type: Patients with respiratory frequency ≥ 30 /min, blood oxygen saturation $\leq 93\%$ at room air, and/or partial pressure of arterial oxygen to fraction of inspired oxygen ratio ≤ 300 mmHg. Critical type: Patients with respiratory failure that required mechanical ventilation, septic shock, and/or multiple organ dysfunction or failure that demanded the intensive care unit treatment. IAV: Influenza A virus; COVID-19: Coronavirus disease 2019.

rate in patients who had received oseltamivir before admission compared with those who had not (0 vs. 36.84%, $P=0.025$). In the IAV-negative group, however, no significant difference in fatality rate was observed.

Discussion

In this study, we investigated the impact of anti-IAV IgM positivity on COVID-19 patients. Of the 70 COVID-19 patients enrolled, 45.71% were positive for anti-IAV IgM, which was unexpectedly high. To our knowledge, this is a rare report regarding the IAV exposure rate in patients with 2019-nCoV-induced pneumonia in China. Co-

infection with IAV and seasonal human coronavirus has been reported by Lu *et al.*^[3] In their study, 157 patients with acute respiratory tract infections were diagnosed with human coronavirus infection by RT-PCR of nasopharyngeal swab samples. Among them, 18 patients (11.46%) were co-infected with IAV. This means that the co-infection with coronavirus and IAV might be a common occurrence. However, the high proportion of anti-IAV IgM positivity in our study might be the result of selection bias since COVID-19 patients in our ward were relatively more severe. IgM has been proven to be a reliable index for the retrospective diagnosis of influenza A, although it might last for 2 to 3 months after acute infection.^[4,5] Therefore, positive anti-IAV IgM indicates recent exposure to IAV, which means the patient either suffered co-infection with IAV and 2019-nCoV or two consecutive infections within a short period. As the exact rate of acute co-infection in COVID-19 patients is unknown, further investigations using RT-PCR should be made.

Although symptoms between the two groups were generally similar, the IAV-positive group tended to have more complaints and worse fatigue than the negative group. This indicated the COVID-19 patients with recent IAV exposure may experience more severe systemic symptoms. However, it remains difficult for clinicians to distinguish patients with influenza and COVID-19 based solely on symptoms during the flu season.

It has been reported that inflammation biomarkers, such as sIL-2R, TNFα, and IL-6, are elevated during both 2019-nCoV and IAV infection.^[6,7] Theoretically, these biomarkers should be higher in the IAV-positive group. However, sIL-2R and TNFα were significantly lower in this group. Peripheral blood levels of sIL-2R produced by activated T cells have been found to reflect the level of T-cell activation,^[8] but there was no statistically significant difference in lymphocyte counts between the two groups. This might indicate different inflammatory mechanisms and complicated cytokine interactions involved in co-infection with 2019-nCoV and IAV. Conversely, the results might have been biased due to the retrospective nature of this study.

Severity distribution and fatality rates were not significantly different between the two groups. However, in the

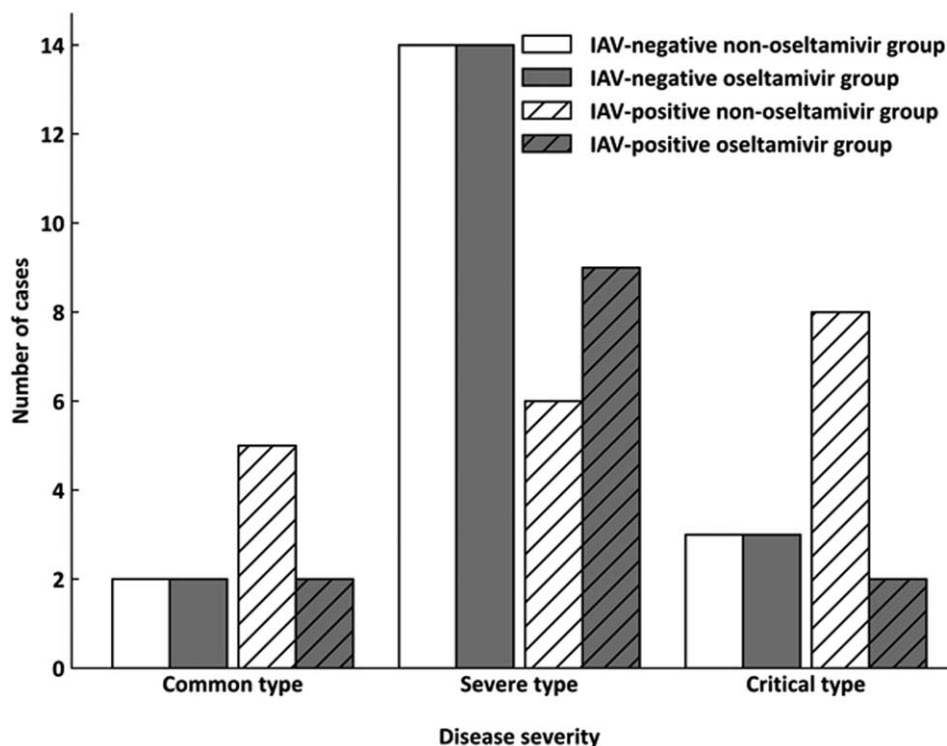


Figure 2: Distribution of disease severity in oseltamivir and non-oseltamivir group stratified by anti-IAV IgM positivity. The Fisher Exact test was used to compare the severity and fatality rate between oseltamivir and non-oseltamivir group, stratified by anti-IAV IgM. Common type: COVID-19 patients with pneumonia but with no signs of severe type or critical type. Severe type: Patients with respiratory frequency ≥ 30 /min, blood oxygen saturation $\leq 93\%$ at room air, and/or partial pressure of arterial oxygen to fraction of inspired oxygen ratio ≤ 300 mmHg. Critical type: Patients with respiratory failure that required mechanical ventilation, septic shock, and/or multiple organ dysfunction or failure that demanded the intensive care unit treatment. IAV: Influenza A virus; IgM: immunoglobulin M; COVID-19: Coronavirus disease 2019.

Table 2: Disease severity and fatality rate of anti-IAV IgM-positive COVID-19 patients.

Variables	Total (n = 32)	Oseltamivir (n = 13)	Non-oseltamivir (n = 19)	P
Disease severity				0.131
Common type	7 (21.88)	2 (15.38)	5 (26.32)	–
Severe type	15 (46.88)	9 (69.23)	6 (31.58)	–
Critical type	10 (31.25)	2 (15.38)	8 (42.11)	–
Fatality	7 (21.88)	0 (0)	7 (36.84)	0.025

Data are presented as *n* (%). The Fisher Exact test was used to compare severity and fatality rate between groups. In COVID-19 patients with positive anti-IAV IgM, patients who received oseltamivir tended to have a lower proportion of critical patients. Patients who received oseltamivir had a significantly lower fatality rate than those who did not. Common type: Patients with pneumonia but with no signs of severe type or critical type. Severe type: Patients with respiratory frequency ≥ 30 /min, blood oxygen saturation $\leq 93\%$ at room air, and/or partial pressure of arterial oxygen to fraction of inspired oxygen ratio ≤ 300 mmHg. Critical type: Patients with respiratory failure that required mechanical ventilation, septic shock, and/or multiple organ dysfunction or failure that demanded the intensive care unit treatment. IAV: Influenza A virus; IgM: immunoglobulin M; COVID-19: Coronavirus disease 2019; –: No data.

early stages of the pandemic before we had an understanding of this disease, oseltamivir was often prescribed empirically by clinicians to treat possible influenza, which may have been COVID-19. Thirty-two patients (45.71%) received oseltamivir before admission without a definite diagnosis of influenza A. After admission to hospital, 19 were found to be anti-IAV IgM-negative. So we further analyzed the effects of oseltamivir on disease severity and fatality. We noticed a tendency toward a higher proportion of critical patients and a higher fatality rate in the IAV-positive group, although this was not statistically significant. However, further analysis indicated that, in the IAV-positive group, prior oseltamivir usage significantly decreased the fatality rate. Other than oseltamivir, we

found no difference in the usage of other medications (including glucocorticoids, antibiotics, intravenous immunoglobulin, and other antiviral drugs) between oseltamivir and non-oseltamivir groups, which supported our hypothesis that concurrent IAV infection might worsen the patient's condition and cause more deaths in COVID-19 patients. Moreover, these findings are very important for guiding clinical decisions in the treatment of COVID-19 during the flu season.

There are several limitations in this study. Firstly, it is a retrospective study with a small sample size. Selection bias might have caused the observed imbalance for sex between the IAV-positive and IAV-negative groups. This selection

bias for sex might be the result of a higher susceptibility of adult females to certain subtypes of IAV.^[9] Inflammation biomarkers were generally lower in females in this study, therefore a higher proportion of females in the IAV-positive group might explain the lower sIL-2R and TNF α levels observed in this group. Selection bias might also play a role in the results observed for symptoms, disease severity, and fatality rate. However, as reported by the Chinese Center for Disease Control and Prevention, males had a higher fatality rate than females in COVID-19.^[10] The IAV-positive group had both a higher proportion of females and a higher fatality rate, which means that the sex difference was unlikely to have biased the fatality rate results. Other unknown factors caused by selection bias might have influenced our results. The sample size of this study is insufficient for the logistic regression analysis of death. Therefore, prospective studies with larger sample sizes are needed in the future.

Secondly, IAV infection was not confirmed by RT-PCR due to limited resources. Anti-IAV IgM detection in this study was qualitative, and so could not distinguish between acute infection and recent exposure. It is therefore not clear whether the observed effects were caused by co- or successive infections. As most of the patients did not report prior episodes of flu-like symptoms, we believe most of the cases in the anti-IAV IgM-positive group were suffering from co-infections. According to the results of this study, we highly recommend simultaneous RT-PCR for 2019-nCoV and IAV in both clinical and research works. Moreover, RT-PCR from swabs cannot confirm which virus caused observed pneumonia. Hence, in future studies, lower respiratory tract samples including sputum and bronchoalveolar lavage fluid might be useful.

In conclusion, during the flu season, greater attention should be paid to the probability of IAV exposure in COVID-19 patients. The present study shows the impact of IAV exposure on COVID-19 in a small cohort. Prospective studies with larger sample sizes are needed to clarify whether IAV increases the fatality rate of COVID-19 and to elucidate the benefits of anti-influenza treatment in these patients.

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Conflicts of interest

None.

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