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ORIGINAL ARTICLE

Neuraminidase inhibitor treatment is associated with decreased mortality in COVID-19 patients: a retrospective analysis

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Aims The aim of this study was to investigate the effects of Neuraminidase inhibitors (NI) on COVID-19 in a retrospective study.

Methods and results The study included an overall COVID-19 patients ($n = 3267$) and a 1:1 propensity score-matched patients ($n = 972$). The levels of plasma *N*-acetylneuraminic acid and neuraminidase expression were further evaluated in a panel of hospitalized and 1-month post-infection recovered COVID-19 subjects. The mortality rate in the overall patients was 9.6% (313/3267) and 9.2% (89/972) in the propensity-score matched patients. The NI treatment lowered the mortality rate (5.7% vs. 10.3%) and the critically ill conversion rate (14.1% vs. 19.7%) compare to those in the non-NI group in the overall patients and evaluated in the propensity score-matched patients when applying the multivariate Cox model for adjusting imbalanced confounding factors. Furthermore, NI treatment was associated with attenuated cytokine storm levels and acute heart injury but not liver or kidney injuries. Further analysis in a small panel of patients found the levels of *N*-acetylneuraminic acid and neuraminidase (dominantly the NEU3 isoform) were elevated in the hospitalized COVID-19 subjects and recovered at the 1-month post-infection stage, suggesting increasing desialylation in COVID-19 patients.

Conclusion These results suggest that NI treatment is associated with decreased mortality in COVID-19 subjects, especially for those subjects with acute heart injury.

Keywords COVID-19 • Neuraminidase inhibitor • *N*-acetylneuraminic acid

Introduction

The coronavirus disease 2019 (COVID-19) was declared as a pandemic by the World Health Organization. Currently, there is no specific treatment for diseases caused by this new coronavirus.[1] Antiviral therapy,[2] immune-based therapy,[3] corticosteroids therapy,[4] and adjunctive therapy[5] are the main approaches for treating the symptoms. Remdesivir, which is used as an experimental broad-spectrum antiviral drug designed to target Ebola has been found effective against this new coronavirus in isolated cells, but its effects for COVID-19 are uncertain in a large-scale clinical trial.[6] Chloroquine can modulate the immune reaction in critically ill patients.[7] However, there is still a lack of sufficient clinical evidence to demonstrate the susceptibility of individuals to this coronavirus infection.

Neuraminidase inhibitors (NI, such as oseltamivir and peramivir) are indicated in the management of influenza and are not expected to be effective for the prevention or treatment of COVID-19 because coronavirus does not contain neuraminidase.[8] However,

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empirical oseltamivir treatment showed therapeutic effects in MERS-CoV infections[9,10] while the evidence of oseltamivir efficacy is inconclusive, owing to the lack of a suitable control group in other studies.[11]

N-acetylneuraminic acid (Neu5Ac) is the major form of sialic acid in mammals. Previous studies have found that serum sialic acid is associated with cardiovascular mortality.[12,13] It triggers myocardial injury *in vitro* and *in vivo* by activating the Rho-ROCK signaling pathway by binding to RhoA and Cdc42, which can be alleviated by NI treatment.[14,15] Recently, Chu *et al.* found that coronavirus could utilize sialic acid for virus attachment and entry in both human lung epithelial cells and ex vivo human lung tissue explants.[16] Additionally, sialic acid-mediated cross reactivity with host immune lectins is known to exert an immune response in different pathological stages in coronaviruses,[17] indicating that Neu5Ac plays an important role in this novel coronavirus infection.

Here, we investigated the effects of NI on COVID-19 subjects from Wuhan, China in a retrospective study. We also measured the levels of plasma Neu5Ac and neuraminidase (NEU) expression in a panel of hospitalized and 1-month post-infection recovered subjects to explore the underlying mechanism. This study aimed to evaluate the efficacy of NI based on the clinical outcomes of COVID-19 patients.

Materials and methods

Study design and patient's information

This was a retrospective study. We obtained the medical records of hospitalized patients with laboratory-confirmed COVID-19 between January 18, 2020 and April 2, 2020 at Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology. COVID-19 was diagnosed according to WHO interim guidance.[18] All patients were diagnosed using a real-time reverse transcription-polymerase-chain-reaction assay of nasal swab specimens and confirmed by chest computed tomography. The disease history were collected from the electronic medical records.

Patients aged over 18 years were admitted in this retrospective study. The inclusion of critically ill patients had to meet one of the criteria following previous works.[19] Briefly, this included (1) respiratory failure that required mechanical ventilation; (2) septic shock; (3) multi-organ failure that needed monitoring in the intensive care unit. In total, we included 3267 patients with COVID-19, which were categorized into two groups based on whether they were treated with NI (including oseltamivir and peramivir, NI group) or not (non-NI group) during hospitalization.

The study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology Wuhan, China (IRBID: TJ-IRB20200229). Written informed consent was waived by the ethics committee owing to the retrospective and anonymous characteristics of the study. The levels of Neu5Ac was further investigated in a panel of plasma samples from COVID-19 patients, which also get approved (IRBID: TJ-IRB20200336).

Data collection and endpoint definitions

The clinical data and laboratory results were exported from the electronic medical records in Tongji Hospital and checked by two study investigators independently to obtain the admission symptoms, clinical diagnosis, and treatment process for each patient. The primary outcome was defined as all-cause death during hospitalization. The secondary outcomes included acute heart injury, acute liver injury, acute kidney injury, intensive care unit, invasive mechanical ventilation, ventilator, intubation, and oxygen therapy. Of these, acute cardiac injury was defined as levels of serum cardiac Troponin I was higher than its upper limit.[20,21] Acute liver injury was defined as a serum ALT 3-fold higher than the normal upper limit.[22] The increased in serum creatinine by 26.5 μM in 48 h was regarded as the acute kidney injury.[23]

Levels of Neu5Ac quantification and expression of neuraminidase

The levels of Neu5Ac was performed in a panel of plasma samples from the COVID-19 patients by LC-MS/MS (SCIEX AB, QTRAP4500).[24] The small panel included 48 control subjects without coronavirus infection, 28 samples from hospitalized COVID-19 patients (COVID-H), and 21 samples from one month recovered COVID-19 patients (COVID-R).

The total neuraminidase (NEU) for the three groups was measured using human neuraminidase ELISA kits. The three subgroups of neuraminidase (NEU-1, NEU-3, and NEU-4) in plasma samples were identified by western blot analysis.

Statistical analysis

Statistical analysis was performed by using GraphPad Prism (version 7.0, San Diego, CA, USA) and R (version 3.5.1, R Foundation for statistical computing, Vienna, Austria). To account for the confounding covariates that predict the effects of the NI treatment, a 1:1 propensity score matching analysis was performed for appropriate comparison of the observed baseline data and its associated treatments using a logistical regression model under an R environment. The matched variables included age, sex, disease history (such as hypertension, coronary vascular disease, diabetes, COPD, chronic kidney disease, cerebral vascular disease), baseline admission indices (SpO2, respiratory rate, pulse rate, diastolic blood pressure, and systolic blood pressure), vital admission clinical symptoms (fever, cough, dyspnea, and fatigue), representative clinical laboratory index (lymphocyte count, CRP, NT-proBNP, hs-cTnI, ALT, albumin, creatinine, and D-dimer), and other common medical treatments (corticosteroids, total immuno-modulators, antibiotics, anti-fungal drugs, and other anti-viral agents) in NI and non-NI groups. The optimal caliper width was set at the level of 0.05.

The D'Agostino and Pearson omnibus normality was used to check the distribution and homoscedasticity for each dataset. Continuous variables between different groups were expressed as median and interquartile range (IQR) and analyzed using the Kruskal–Wallis test with FDR correction for the non-parametric dataset. Categorical variables between different groups were expressed as counts and percentages and analysed using the Chi-square test or Fisher exact test. Survival analysis was performed using the Kaplan Meier Curve with adjusted log-rank test in an R environment. The

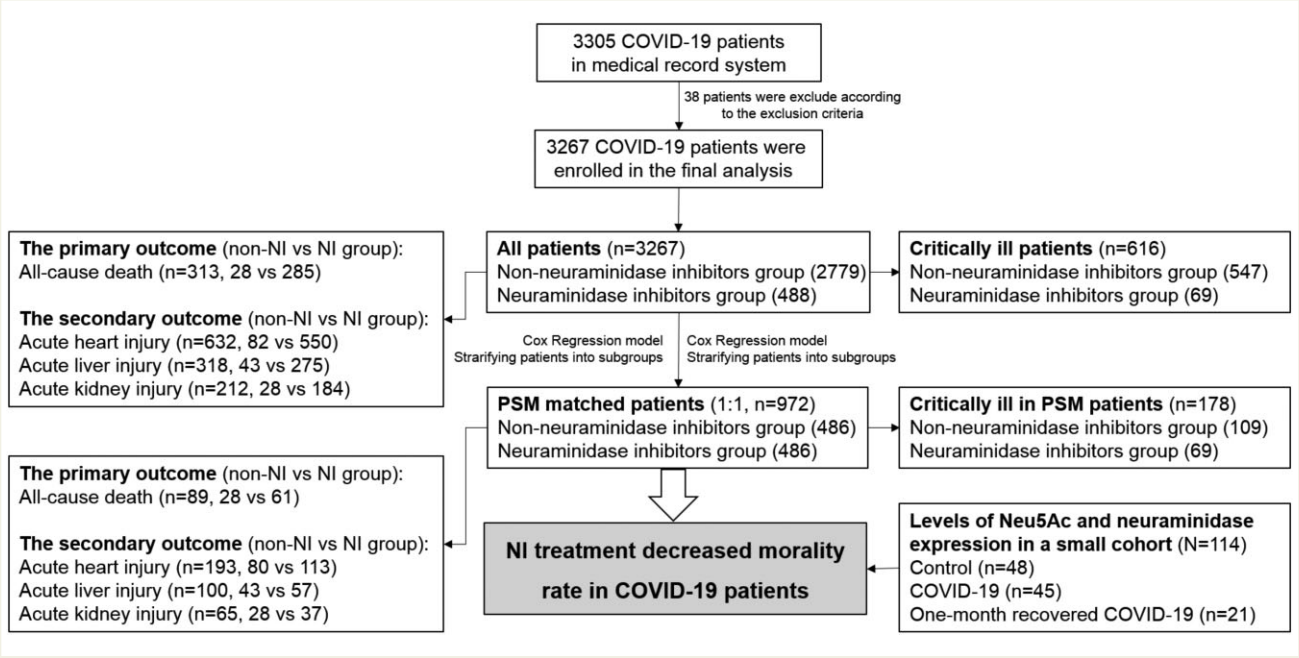

Figure 1 The flowchart of study design.

Cox proportional-hazard model was used to investigate the association between confounding variables and the clinical outcomes. Age, sex, original comorbidities (including hypertension, coronary vascular disease, diabetes, COPD, and chronic kidney disease), respiratory rate, and heart rate on admission, and baseline levels of NT-proBNP, CRP, and IL-6 were adjusted for the total patients since these variables are reported to be associated with the severity of COVID-19 in previous studies.[20,25]

Results

Demographics and general characteristics

A total of 3305 confirmed cases of COVID-19 patients in Tongji Hospital participated in this retrospective cohort study. Of these, 38 patients were excluded according to the exclusion criteria. The final patients included 3267 individuals, including 488 subjects (male, 52%) treated with NI and 2779 subjects (male 49.3%) without NI treatment (*Figure 1*). The median starting time of NI treatment was at day 1 (0–4) post admission. In the NI group, the median duration of NI treatment was 4 (2–7) days. For the oseltamivir oral medication group (p.o., 75mg, b.i.d.), the median duration was 5 (2–7) days while the median duration was 4 (4–6) days for the peramivir intravenous administration group (i.v. drop >30 min, 300 mg, q.d.). The baseline characteristics on admission for both groups after propensity score matching are shown in *Table 1*. The median ages were 62 (IQR, 49–70) and 61 (IQR, 48–70) years old in the NI and non-NI groups, respectively. These two groups showed no significant difference in any of the baseline characteristics on admission after 1:1 propensity-score matching analysis ($n = 486$ for each group, *Table 1*). The baseline characteristics for all patients in both groups are presented in *Table S1*.

The baseline laboratory test results, including the routine blood test, biochemistry, infection-related indices, and coagulation function of all patients and the propensity score-matched patients between the NI group and non-NI group are shown in *Table S2*. Most of the infection-related cytokine indices (including pro-inflammatory IL-6, IL-8, TNF-α, and anti-inflammatory IL-10) were lower in the NI-group compared to the non-NI group in the total patients ($P < 0.01$) after multiple comparison. After propensity scores-matching, the IL-8 levels remained lower in the NI group ($P = 0.009$), even though the levels of IL-6 were well matched.

All subjects received standard treatment following the National Guidelines for diagnosis and treatment program against pneumonia from new coronavirus infections (3rd version). For the overall patients, the percentage of some treatments, such as corticosteroids, immune-enhancement, antibiotics, antifungal drugs, and anti-HIV drugs showed a clear difference with P value less than 0.05 between the comparable groups (*Table S3*). In order to investigate the effects of NI treatment on COVID-19 subjects, propensity score matched analysis was carried out for the corticosteroids, total immune-modulators, antibiotics, antifungal drugs, and other antivirus drugs to balance other treatments that are associated with clinical outcomes. All other treatments except for NI were well matched in the sub-group ($n = 486$ for NI and non-NI group, respectively).

Clinical outcomes

Among the overall COVID-19 patients, 313 out of the total 3267 patients died during the hospitalization (*Table 2*). After the propensity score-matched analysis, the mortality rate in the NI group (28 out of 486 subjects, 5.8%) was clearly lower than that in the non-NI group (61 out of 486 subjects, 12.6%, $P < 0.001$).

Table 1 Baseline characteristics of patients with COVID-19

	All patients (*n* = 3267)	PSM (1:1)		
		Non-NI (*n* = 486)	NI (*n* = 486)	P
Sex, male (%)	1624 (49.7)	269 (55.3)	254 (52.3)	0.368
Age, years	62 (50–70)	62 (49–70)	61 (48–70)	0.765
Age range, years: >60 (%)	1819 (55.7)	264 (54.3)	261 (53.7)	0.897
Age range, years: <60 (%)	1448 (44.3)	222 (45.7)	225 (46.3)	0.897
Original comorbidities (%)				
Hypertension (%)	974 (30.1)	130 (26.9)	156 (32.5)	0.065
Coronary heart disease (%)	233 (7.2)	23 (4.7)	30 (6.2)	0.397
Diabetes (%)	450 (13.9)	67 (13.8)	69 (14.3)	0.916
COPD (%)	42 (1.3)	4 (0.8)	5 (1)	1.000
Cancer (%)	90 (2.8)	12 (2.5)	16 (3.3)	0.565
Chronic kidney diseases (%)	19 (0.6)	1 (0.2)	1 (0.2)	0.479
Cerebrovascular disease (%)	113 (3.5)	8 (1.6)	10 (2.1)	0.812
Vital signs				
SpO2 on admission (%)	97 (95–98)	97 (94–98)	96 (94–98)	0.751
Temperature (°C)	37 (36.3–37)	37 (36.3–37.2)	37 (36.3–37.2)	0.234
Respiratory rate, breaths/min	20 (20–22)	20 (20–23)	20 (20–22)	0.864
Pulse, beats/min	90 (80–102)	90 (80–103)	88 (80–101)	0.182
Diastolic blood pressure, mmHg	80 (72–89)	80 (72–89)	80 (73.8–89)	0.559
Systolic blood pressure, mmHg	130 (119–143)	130 (118–144)	130 (120–141)	0.319
Body Mass Index	23 (21.5–25.4)	23 (21.6–24.8)	24 (21.8–26)	0.087
Symptoms (%)				
Fever (%)	2454 (75.4)	385 (79.4)	390 (80.6)	0.735
Cough (%)	2039 (62.6)	305 (62.9)	299 (61.8)	0.759
Sputum production (%)	1039 (31.9)	151 (31.1)	168 (34.7)	0.254
Dyspnea (%)	579 (17.8)	127 (26.3)	116 (24)	0.447
Chest tightness (%)	1161 (35.7)	180 (37.1)	167 (34.5)	0.487
Diarrhea (%)	877 (26.9)	152 (31.3)	130 (26.9)	0.132
Stuffiness (%)	51 (1.6)	6 (1.2)	10 (2.1)	0.452
Fatigue (%)	1059 (32.4)	166 (34.2)	161 (33.1)	0.802
Pharynx discomfort (%)	21 (0.6)	2 (0.4)	0 (0)	0.478
Lesion of pleura in CT (%)	1175 (40.6)	188 (44)	194 (42.5)	0.685

Data were presented as median and interquartile range (Q1-Q3). Keys: COPD, chronic obstructive pulmonary disease; CT, Computed tomography; IQR, interquartile range; NI, neuraminidase inhibitors; SpO2, percutaneous oxygen saturation.

The percentage of critically ill conversion rate in the NI group (69 out of 486, 14.2%) was also lower than that in the non-NI group (109 out of 486, 22.4%, $P = 0.001$), while the hospitalization time was longer in the NI group. The mortality rate and critically ill incidence for all patients were also lower in NI treated group (*Table S4*).

When applying the multivariate Cox model for adjusting imbalanced confounding factors in both the total patients and propensity score-matched patients, the NI group was still associated with a lower risk of all-cause mortality compared to the non-NI group (adjusted HR: 2.35; 95% CI: 1.31–4.23, $P = 0.004$ in all patients, adjusted HR: 2.76; 95% CI: 1.46–5.20, $P = 0.002$ in propensity score-matched patients) compared to those without NI treatment (*Figure 2A, C*, *Table 2*). Among them, the mortality rate in the critically ill patients was also lower in the NI treatment group (*Figure 2B, D*), especially in the propensity score matched patients (adjusted HR: 1.99; 95% CI: 1.05–3.78, $P = 0.035$). Furthermore, the higher survival rate in the NI group did not present any sex difference when compared with those without NI treatment, regardless of the total patients (female: adjusted HR 3.56, 95% CI: 1.05–12.05, $P = 0.04$; male: adjusted HR 2.26, 95% CI: 1.09–4.66, $P = 0.028$) or the 1:1 propensity score matched patients (female: adjusted HR 3.80, 95% CI 1.29–11.22, $P = 0.01$; male: adjusted HR 2.89, 95% CI 1.25–6.68, $P = 0.013$) from the Kaplan–Meier survival curve and Cox regression model (*Figure S1*). It was more effective in elderly subjects that older than 60 years (*Figure S2*), which indicated that the NI treatment is associated with decreased mortality rate in elderly subjects regardless of sex in COVID-19 patients. Moreover, NI treatment was more effective for patients with higher levels of IL-6 (adjusted HR: 2.39; 95% CI: 1.33–4.28, $P = 0.004$ in all patients,

Table 2 Clinical outcomes of COVID-19 patients between NI and non-NI treatment group

		PSM (1:1)		
Clinical Outcomes	**All patients (*n* = 3267)**	**Non-NI (*n* = 486)**	**NI (*n* = 486)**	**P**
Primary outcomes				
Hospitalization time (days)	19 (11–28)	20 (12–30)	22 (15–30)	0.051
Critically-ill, n (%)	616 (18.9)	109 (22.4)	69 (14.2)	0.001
Death, n (%)	313 (9.6)	61 (12.6)	28 (5.8)	<0.001
Seconday outcomes				
Acute heart injury, n (%)	632 (19.3)	113 (23.3)	80 (16.5)	0.010
Acute liver injury, n (%)	318 (9.7)	57 (11.7)	43 (8.8)	0.170
Acute kidney injury, n (%)	212 (6.5)	37 (7.6)	28 (5.8)	0.304
Intensive care unit (ICU), n (%)	1048 (32.1)	157 (32.3)	186 (38.3)	0.060
Invasive mechanical ventilation, n (%)	413 (12.6)	79 (16.3)	64 (13.2)	0.205
Ventilator, n (%)	392 (12)	67 (13.8)	42 (8.6)	0.014
Intubation, n (%)	133 (4.1)	24 (4.9)	14 (2.9)	0.136
Oxygen therapy, n (%)	2215 (67.8)	340 (70)	318 (65.4)	0.150

Data were presented as median and interquartile range (Q1–Q3).

adjusted HR: 3.07; 95% CI: 1.58–5.96, $P = 0.001$ in propensity score matched patients) (*Figure S3*). Different from IL-6, patients with lower levels of IL-8 were ameliorated from the Kaplan–Meier survival curve (adjusted HR: 2.22; 95% CI: 1.15–4.3, $P = 0.018$ in all patients, adjusted HR: 3.10; 95% CI: 1.44–6.69, $P = 0.004$ in propensity score matched patients) (*Figure S4*), which indicated the NI treatment is beneficial for decreasing inflammatory status in COVID-19.

To further explore the secondary outcomes associated with NI treatment in COVID-19 subjects, the incidence of multi-organ failure and transfer to the intensive care unit was investigated in both unmatched and matched patients. In the group of NI treatment, the incidence of acute cardiac injury was lower and the utilization of ventilator was decrease in the propensity score-matched patients (*Table 2*, *Figure 3A, D*). The Cox regression model consistently confirmed that the risk of developing acute heart injury in COVID-19 subjects was ameliorated in the propensity score-matched patients (Figure 3D, adjusted HR 1.47, 95% CI 1.05–2.07, p = 0.027), but not for the acute liver injury and acute kidney injury (Table 2, *Figure S5*). The Kaplan-Meier survival curve and Cox regression analysis for different hs-cTnI (indicator of myocardial injury) and NT-proBNP (indicator of heart failure) levels further proved that NI treatment was more effective for patients with higher levels of hs-cTnI and NT-proBNP, regardless of the total and propensity score matched patients (*Figure 3B–C, E–F, Figure S6*). These implied that NI treatment is associated with reduced incidence of acute heart injury in COVID-19 patients.

Dynamic profile of vital signs and representative indices for COVID-19 patients with and without NI treatment

The dynamic alteration of vital signs (such as pulse, diastolic blood pressure) and representative clinical indices (such as lymphocyte count, ALT, Creatinine, hs-cTnI, NT-proBNP, D-dimer, CRP, and a panel of cytokine factors) over time since admission were analysed to evaluate the clinical manifestation that following the NI treatment in the total patients (*Figure 4*). A similar temporal pattern of vital signs and laboratory parameters were found in the propensity score-matched patients (*Figure S7*). These parameters were tracked from day 2 after admission to day 20 at an interval of 4 days and plotted by using the median value of each period. We found a lower heart rate and higher diastolic blood pressure in the NI group compared to the non-NI group during the observational period (*Figure 4A and B*, *Figure S7A*). From trajectory plots, the majority of clinical indices in the non-NI group presented a turning point on the 4th day as the dynamic curves in NI group were more flat even there was no difference at the onset of observation (day 2). For example, the indicator of immune system reaction (levels of lymphocyte count, *Figure 4D*, *Figure S7D*) were decreased and indicators of myocardial injury (hs-cTnI, *Figure 4I*, *Figure S7I*), heart failure (NT-proBNP, *Figure 4J*, *Figure S7J*), coagulation (D-dimer, *Figure 4K*, *Figure S7K*), inflammation (CRP, *Figure 4L*, *Figure S7L*), and cytokine storm (IL-6, IL-8, IL-2γ, and TNF−α, *Figure 4M-P*, *Figure S7O-7P*) were increased markedly on the 4th day in the non-NI group. However, the variation trends of these indices in the NI group were more moderate, which implied the NI treatment is related to the decelerated progression of COVID-19. Indeed, the indicators of myocardial injury, heart failure, inflammation, and cytokine storm were consistently and markedly higher in the non-NI group during the observational period, suggesting the association of NI treatment and reduced inflammatory status and incidences of myocardial injury in COVID-19 subjects. Different from the hs-cTnI and NT-proBNP, the indicators for acute liver injury (ALT, *Figure 4F*, *Figure S7F*) and acute kidney injury (creatinine, *Figure 4H*, *Figure S7H*) did not show obvious difference in comparable two groups, which is in good agreement with previous incidence of liver and kidney injuries following NI treatment (*Table 2*, *Figure S5*).

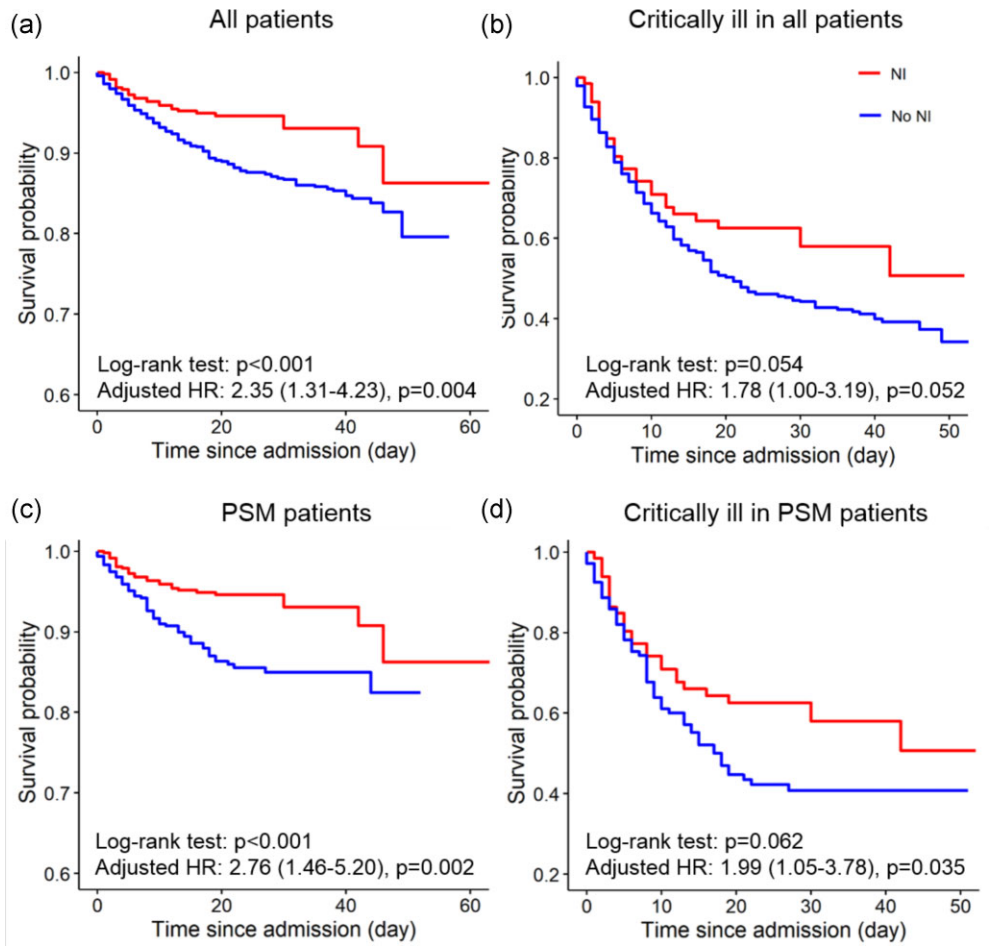

Figure 2 The Kaplan–Meier survival curve of the primary outcome for COVID-19 patients with and without NI treatment. (**A–B**) The Kaplan–Meier survival curve for all enrolled COVID-19 patients (A) and total critically ill patients (B) with (red) or without (blue) NI treatment. (**C–D**) The Kaplan–Meier survival curve for propensity score matched (C) and matched critically ill (D) COVID-19 patients.

Levels of *N*-acetylneuraminic acid and expression of neuraminidase of COVID-19 patients

To further investigate the effects of NI on COVID-19 disease progression, the main form of sialic acid, *N*-acetylneuraminic acid (Neu5Ac) was evaluated in a small panel of COVID-19 subjects. Of them, the patients in COVID-H group presented higher levels of NT-proBNP, hs-cTnI, and Neu5Ac, which recovered in 1-month post-infection (*Figure 5A*, *Table S5*), indicated that Neu5Ac was associated with coronavirus infection. To take into consideration other factors that affected the alterations of detected metabolites, FDR-corrected multinomial logistic regression was performed to minimize the profound effects of age, sex, and disease history. When compared the metabolic profile of the COVID-H vs. control group, we determined a significant increase in the levels of Neu5Ac ($P = 0.006$, OR = 14.19) when considering the profound factors.

Then the neuraminidase (NEU), a rate-limiting enzyme for breakdowning glycosides containing Neu5Ac, was measured by ELISA (*Figure 5B*) and its 3 isoforms expressed (NEU 1/3/4) in plasma were identified by western blotting (*Figure 5C, D*). In the hospitalized patients, the NEU3 is main isoform that contributes to the increased levels of total NEU. The levels of total NEU and its isoforms were elevated in the hospitalized COVID-19 subjects and recovered at the 1-month post-infection stage, which indicated increasing desialylation during the disease development of COVID-19.

Discussion and conclusions

The COVID-19 pandemic is a global public health crisis, with considerable mortality and morbidity exerting pressure on healthcare resources. In this retrospective study, we evaluated the association of NI with clinical outcomes in COVID-19 subjects. NI (such as oseltamivir, peramivir, and zanamivir) are indicated in the management of influenza. The influenza virus neuraminidase is an enzyme anchored to the viral membrane, which can cleave Neu5Ac residues that cap the ends of various glycoconjugates.[26] This process plays a crucial role in the movement of virus particles through the upper respiratory tract as well as in the release of virion progeny from

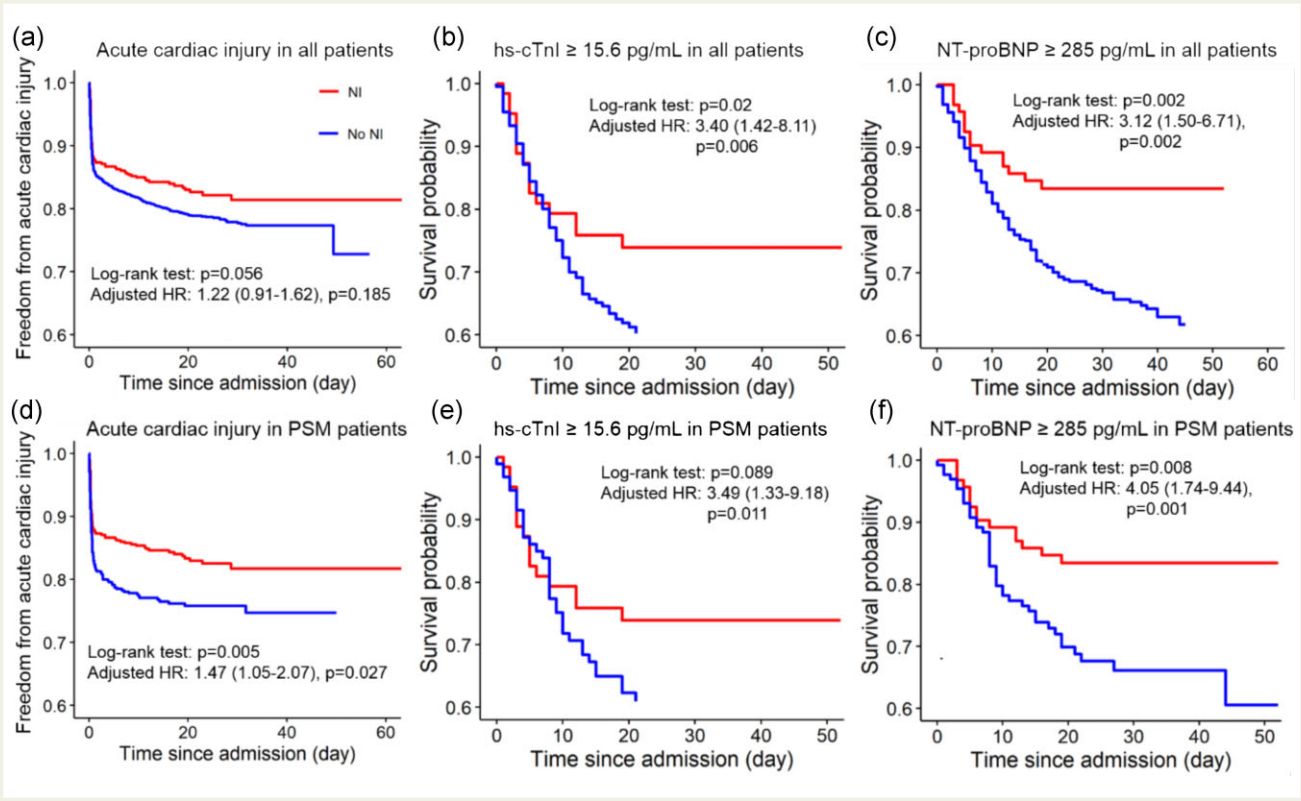

Figure 3 The Kaplan–Meier survival curve of acute heart injury for COVID-19 patients with and without NI treatment. The cut-off value was set based on the upper limit of each clinical reference range. (**A, D**) The Kaplan–Meier survival curve for all enrolled (A) and propensity score matched (D) COVID-19 patients with acute heart injury when treated with NI or not (NI group: red, non-NI group: blue). (**B, E**) The Kaplan–Meier survival curve for all enrolled (B) and propensity score matched (E) COVID-19 patients with hs-cTnI level higher than 15.6 pg/mL. (**C, F**) The Kaplan–Meier survival curve for all enrolled (C) and propensity score matched (F) COVID-19 patients with NT-proBNP level higher than 285 pg/mL.

infected cells.[27] NI are not expected to be effective for the prevention or treatment of COVID-19 because coronavirus does not contain neuraminidase and the coronaviruses cannot utilize it for the budding stage of reproduction. In addition, a previous SARS-CoV study found the effects of NI assessed to be ineffective from cytopathic endpoints utilizing cell-based assays in a SARS-CoV-infected Vero E6 cell model.[28] However, in a study on MERS-CoV infection, the empirical oseltamivir showed effective impacts[9] and was evaluated in other MERS-CoV infections.[10] Recently, Chu *et al.* identified sialic acid as a host attachment factor that restricts coronavirus infection[16] in human lung tissue, which confirmed the important role of sialic acid and neuraminidase in the disease development of COVID-19 illness. In our retrospective study, elevated levels of Neu5Ac and neuraminidase expression were demonstrated to be associated with this viral infection. The enhanced neuraminidase activity may have led to rapid release of virion progeny from infected cells demonstrated by elevated levels of plasma Neu5Ac (*Table S5*). Recently, an omics-driven study found monosialodihexosyl ganglioside enriched exosomes, which also share a Neu5Ac molecule in the tail, were also highly positively correlated with COVID-19 pathogenesis.[29] Thus, NI could serve as a competitive inhibitor of neuraminidase activity upon Neu5Ac to improve the levels of sialylation, which supported its important role in COVID-19. Moreover, NI reduced steatosis, decreased liver inflammation, and alleviated pulmonary fibrosis symptoms in experimental mouse studies.[30,31]

In line with this finding, our results suggest that the NI treatment is associated with decreased incidence of acute heart injury but not liver and kidney injuries in the matched patients. Higher levels of plasma Neu5Ac were previously reported to be associated with the risk and prognosis of cardiovascular diseases.[14,32] In addition, cardiovascular system injury has received increasing attention as it is involved in the pathophysiology of COVID-19.[33] Chen *et al.* suggested that the fulminant myocarditis should be considered for those patients who deteriorate rapidly.[34] Here, from the occurrence time of acute heart injury in all COVID-19 subjects (*Figure S8*), the majority of acute heart injury occurred within 3 days (489/632, 77.4%) or in 2 weeks (591/632, 93.5%) since admission, which in accordance with the time window of fulminant myocarditis[35] or acute myocarditis.[36] It has been reported that NI treatment has a beneficial effect on fulminant myocarditis to prevent cardiac damage and cytokine storm by the released neuraminidase.[35] In our study, NI treatment was associated with a flattened trajectory curve of the

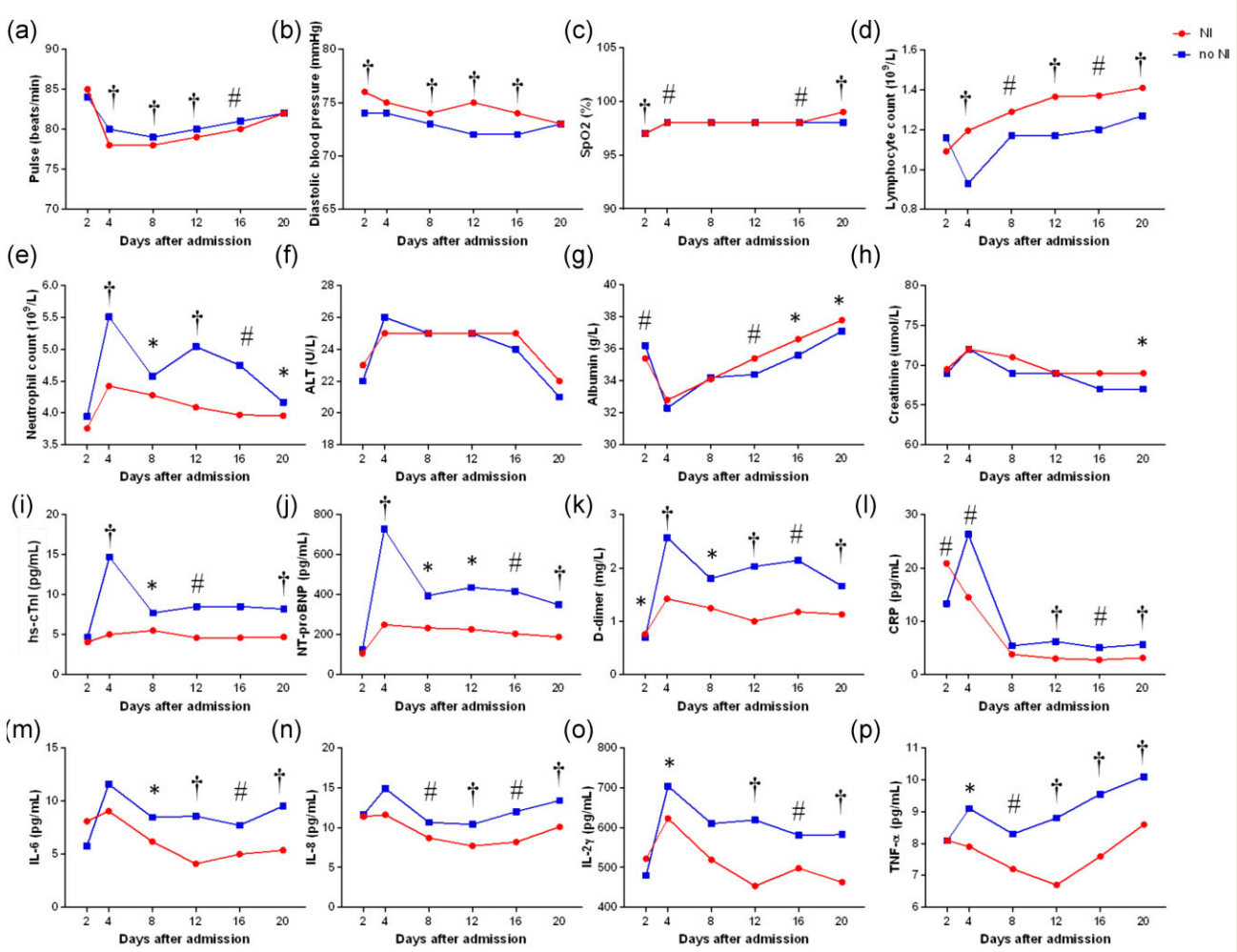

Figure 4 Dynamic profile of vital signs and representative indices for COVID-19 patients with and without NI treatment. (**A–C**) Dynamic profiles of vital signs with pulse rate (A), diastolic blood pressure (B) and SpO2 (C). (**D–E**) Dynamic profiles of representative blood routine indices with lymphocyte (D) and neutrophil count (E). (**F–J**) Dynamic profiles of representative liver, kidney and cardiac function indices with ALT (F), Albumin (G), Creatinine (H), hs-cTnI (I), NT-proBNP (J). (**K–P**) Dynamic profiles of representative inflammatory factors and coagulation function with D-dimer (K), CRP (L), IL-6 (M), IL-8 (N), IL-2γ (O), and TNF-α (P). The level of statistical significance is expresses as * $p < 0.05$, # $p < 0.01$, † $p < 0.001$.

disease progression by attenuating the myocardial injury/heart failure and immune system reaction (*Figure 4*). In addition, the lower levels of plasma cytokine factors and CRP following NI treatment suggested that NI may be helpful for ameliorating the inflammatory status in patients with COVID-19. It is worth mentioning that the utilization of cardiovascular drugs (*Table S6*), in particular, did not show obvious difference in the propensity score-matched patients between NI and non-NI group, implied the beneficial effects of NI on COVID-19 with acute heart injury.

In summary, our results suggest that NI treatment is associated with the decreased mortality in patients with COVID-19 and NI treatment is helpful for decelerating the disease progression and mainly alleviating the incidence of acute heart injury. Levels of Neu5Ac and higher expression of plasma neuraminidase, which is mainly driven by NEU-3, indicated the role of desialylation in the disease progression of COVID-19.

Study limitation

This study has several limitations in this work. First, it is ideally to have a randomized controlled trial design to investigate the effects of NI. During this retrospective study, not all clinical indices and treatment could be matched very well to look into the effects of NI treatment in the total patients. Even though the propensity-score matched analysis and Cox regression analysis were carried out to balance the confounding factors that associated with the clinical outcomes, there was still some unintended bias (such as education and profession) that could not fully corrected and it is not possible to determine if there are imbalances in unmeasured confounders. Second, since the coronavirus undergoes frequent mutation of its genome, the currently dominating variants are different from the ones common in early 2020. Besides, with increasing vaccination rates and effective government management, the pandemic was well controlled

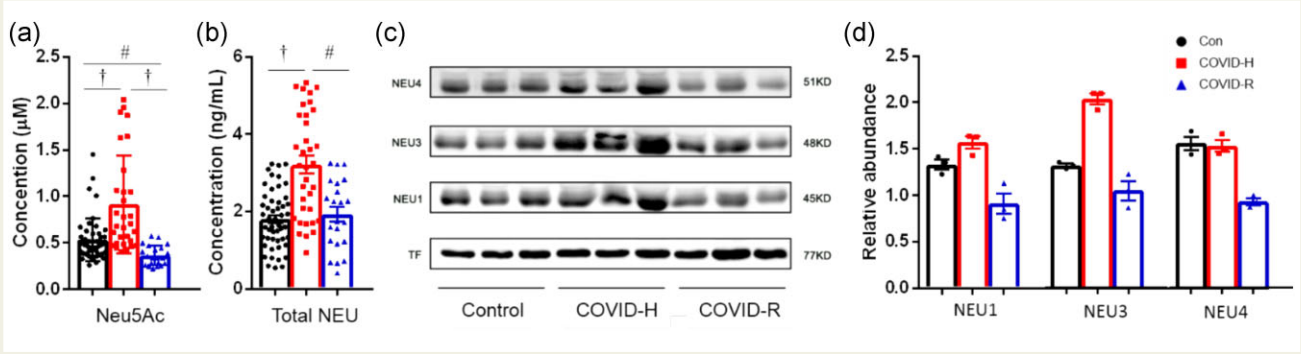

Figure 5 Levels of *N*-acetylneuraminic acid and neuraminidase expression in a small panel of patients. (**A–B**) Levels of *N*-acetylneuraminic acid (Neu5Ac) (A) and total neuraminidase expression (B) in COVID-19 subjects and 1-month recovered COVID-19 subjects ($n = 48$ in control group, $n = 28$ in COVID-H, and $n = 21$ in COVID-R group). (**C–D**) Representative western blotting plot (C) and the bar plot of relative abundance (D) of NEU-1/3/4 in plasma samples from the above three groups. The bar plots in A, B, and D were presented in mean ± SEM. Statistical significance was evaluated by one-way ANOVA test and unpaired *t*-test with FDR correction. # $P < 0.01$, † $P < 0.001$.

in China. Even for those infected patients that were mostly asymptomatic or had mild manifestations, we could not further investigate the effects of NI on patients with acute heart injury. Third, we did not have more exhaustive echocardiography examinations or endomyocardial biopsy for all suspected patients with acute heart injury because of the exceptional infectious circumstances. Fourth, both of the NI used here (oseltamivir and peramivir) inhibit the activity of neuraminidase-1 selectively. However, neuraminidase expression of NEU isoforms from hospitalized COVID-19 subjects showed the effects were mainly driven by NEU3. Further investigation with different selective NI will be carried out to explore the targeted effects on COVID-19 associated myocardial injury.

Supplementary material

Supplementary material is available at *European Heart Journal—Cardiovascular Pharmacotherapy* online.

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