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Interferon-α2b spray inhalation did not shorten virus shedding time of SARS-CoV-2 in hospitalized patients: a preliminary matched case-control study*

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Abstract: Background: Currently, there are no drugs that have been proven to be effective against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Because of its broad antiviral activity, interferon (IFN) should be evaluated as a potential therapeutic agent for treatment of coronavirus disease 2019 (COVID-19), especially while COVID-19-specific therapies are still under development. Methods: Confirmed COVID-19 patients hospitalized in the First Affiliated Hospital, School of Medicine, Zhejiang University in Hangzhou, China, from January 19 to February 19, 2020 were enrolled in a retrospective study. The patients were separated into an IFN group and a control group according to whether they received initial IFN-α2b inhalation treatment after admission. Propensity-score matching was used to balance the confounding factors. Results: A total of 104 confirmed COVID-19 patients, 68 in the IFN group and 36 in the control group, were enrolled. Less hypertension (27.9% vs. 55.6%, *P*=0.006), dyspnea (8.8% vs. 25.0%, *P*=0.025), or diarrhea (4.4% vs. 19.4%, *P*=0.030) was observed in the IFN group. Lower levels of albumin and C-reactive protein and higher level of sodium were observed in the IFN group. Glucocorticoid dosage was lower in the IFN group (median, 40 vs. 80 mg/d, *P*=0.025). Compared to the control group, fewer patients in the IFN group were ventilated (13.2% vs. 33.3%, *P*=0.015) and admitted to intensive care unit (ICU) (16.2% vs. 44.4%, *P*=0.002). There were also fewer critical patients in the IFN group (7.4% vs. 25.0%, *P*=0.017) upon admission. Although complications during admission process were comparable between groups, the discharge rate (85.3% vs. 66.7%, *P*=0.027) was higher and the hospitalization time (16 vs. 21 d, *P*=0.015) was shorter in the IFN group. When other confounding factors were not considered, virus shedding time (10 vs. 13 d, *P*=0.014) was also shorter in the IFN group. However, when the influence of other factors was eliminated using propensity score matching, virus shedding time was not significantly shorter than that of the control group (12 vs. 15 d, *P*=0.206). Conclusions: IFN-α2b spray inhalation did not shorten virus shedding time of SARS-CoV-2 in hospitalized patients.

Key words: Coronavirus disease 2019 (COVID-19); Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); Propensity score match; Interferon (IFN)
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1 Introduction

As of mid-May 2020, the global epidemic of coronavirus disease 2019 (COVID-19) has infected more than 4 million people, with almost 300000 deaths, since its first outbreak in Wuhan, China in late December, 2019 (Zhu et al., 2020). Thus far, no drugs have proved effective against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Shi et al., 2020). Drugs used to treat SARS and Middle East respiratory syndrome (MERS) have been attempted as treatment for COVID-19, but have not yet yielded clinically definitive results (Li and de Clercq, 2020). Drugs that inhibit SARS-CoV-2 with various mechanisms such as inhibition of virus fusion/entry, disruption of virus replication, suppression of excessive inflammation, convalescent plasma treatment, and vaccines are still under development (Li et al., 2020).

Much more is known, in contrast, about the mechanism and clinical uses of interferon (IFN) (Ren et al., 2019). Virus infection-induced type 1 IFNs, for instance, play a central role in the clearance of virus. IFN-α can bind to a heterodimeric receptor complex consisting of IFN-α receptors 1 and 2, followed by activation of the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway and expression of IFN-response genes (Cinatl et al., 2004). IFN production in SARS-CoV-infected cells may be inhibited by the virus through inactivation of IFN regulatory factor 3 (Spiegel et al., 2005), or by viral evasion of cellular RNA detection by creating a microenvironment that is not accessible to cytoplasmic pathogen recognition receptors (Thiel and Weber, 2008). IFN could restore the dysregulated antiviral status. Based on its character of broad antiviral activity, IFN offers a potential therapeutic alternative for treatment of COVID-19 until more specific treatments are developed.

IFN has been shown to exert a protective effect against SARS-CoV infection (Haagmans et al., 2004). A delayed IFN response induced the accumulation of monocyte-macrophages and resulted in increased immunopathology and mortality in SARS-CoV-infected mice (Channappanavar et al., 2016). Subcutaneous administration of IFN-α2b has been shown to stimulate the expression of IFN-response genes (Danesh et al., 2011), and SARS-CoV is sensitive to the action of exogenous IFNs, especially IFN-β (Cinatl et al., 2003).

IFN-β1b treatment yielded improved clinical, radiological, and pathological outcomes in a MERS common marmoset model (Chan et al., 2015). In vitro, IFN-α2b showed antiviral effects against MERS-CoV, when used alone or combined with ribavirin (Falzarano et al., 2013a). In a rhesus macaque model, combined IFN-α2b-ribavirin treatment has been shown to reduce virus replication, moderate the host response, and improve clinical outcomes (Falzarano et al., 2013b). The dosage of IFN-α2b was 5 mega international units (MIU)/kg delivered subcutaneously every 16 h (Falzarano et al., 2013b). In MERS patients, ribavirin and pegylated IFN-α2a treatment improved survival rate at 14 d after disease onset, but not at 28 d (Omrani et al., 2014). The ribavirin and IFN-α2b treatment may not benefit critically ill MERS patients when diagnosed and treated late in the course of their illness (Al-Tawfiq et al., 2014). More recent studies have shown that ribavirin and IFNs (IFN-α2a, IFN-α2b, or IFN-β1a) combination did not reduce 90-d mortality or MERS-CoV RNA shedding time (Arabi et al., 2020).

Despite the uses of IFN therapy in other coronavirus infections, there is still insufficient evidence for the efficacy of IFNs, either alone or in combination with other antivirals, in COVID-19 treatment (Alhazzani et al., 2020). The World Health Organization (WHO) has launched the SOLIDARITY trial for evaluation of potential COVID-19 treatments, in which lopinavir/ritonavir and IFN-β therapy is one of the prioritized regimens. However, the effect of IFN-α2b inhalation treatment on COVID-19 has not yet been investigated. Atomization inhalation of IFN-α2b has been recommended by the National Health Commission of the People's Republic of China as a potential alternative to subcutaneous administration (National Health Commission of the People's Republic of China, 2020). IFN inhalation could reduce the adverse reactions of flu-like symptoms seen in subcutaneous methods. However, because atomization inhalation can also increase the possibility of aerosol spread of the SARS-CoV-2 virus, we used IFN-α2b spray in our center to reduce the opportunity for aerosol spread of SARS-CoV-2. This study aimed to analyze the treatment effects of IFN-α2b spray inhalation in virus clearance of SARS-CoV-2.

2 Methods

2.1 Study population and data collection

All patients enrolled in the study were hospitalized in the First Affiliated Hospital, School of Medicine, Zhejiang University in Hangzhou, China, between January 19 and February 19, 2020, with SARS-CoV-2 infections confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of sputum or nasopharyngeal swab. Demographic information, medical history, laboratory and imaging data, and treatment records and outcomes were collected for all patients. All records were summarized by two clinicians from the original electronic medical records using a standardized collection form and reviewed by a third researcher.

2.2 Study design

This is a single-center matched case-control study and the primary outcome evaluated in the study was the duration of SARS-CoV-2 virus shedding time from the respiratory tract. The study cohort consisted of confirmed SARS-CoV-2-positive patients hospitalized from January 19 to February 19, 2020; the final follow-up was March 3, 2020. The patients were separated into IFN group and control group, depending on whether they received IFN-α2b inhalation treatment on admission. In the IFN group, patients received recombinant human IFN-α2b spray at a dosage of 100000 U, four times a day, for 7 d. Other treatment measures including supportive treatments and treatment of complications were according to the Chinese national guidelines (National Health Commission of the People's Republic of China, 2020). Patients in these two groups were matched to balance the confounding factors and compared to verify the effect of IFN-α2b inhalation treatment on virus shedding time.

2.3 Study definitions and variables

SARS-CoV-2 RNA was assayed daily in respiratory specimens from each patient until the patient was discharged; only qualitative data were available. The end of virus shedding was determined by at least two consecutive negative RT-PCR results, and virus shedding time was defined as the duration from the first confirmed RT-PCR-positive result and the end of virus shedding. For patients still shedding virus at the end of the study, the time from the date of confirmed diagnosis to the final follow-up date of March 3, 2020 was used for the calculation of virus shedding time. The criteria for discharge followed the Chinese management guidelines for COVID-19 (7th edition) (National Health Commission of the People's Republic of China, 2020) in detail, including absence of fever for at least 3 d, improvement in chest computed tomography (CT) and clinical symptoms, and two consecutive negative results for SARS-CoV-2 RNA in respiratory tract samples (nasopharyngeal swab or sputum) obtained at least 24 h apart. The date of onset of illness was defined as the day when the first symptoms were noticed. Acute respiratory distress syndrome (ARDS) was defined according to WHO interim guidance (World Health Organization, 2020). Acute kidney injury was defined according to the KDIGO (kidney disease: improving global outcomes) definition (Kellum et al., 2012). Disease severity was determined as previously described (Wu and McGoogan, 2020). Briefly, a mild case was defined as slight clinical symptoms without pneumonia or with only mild pneumonia. A severe case was diagnosed by the presence of dyspnea (respiratory rate of ≥30 times/min), resting peripheral oxygen saturation of ≤93%, arterial PaO_2/FiO_2 of ≤300 mmHg (1 mmHg=133.3 Pa), and/or lung infiltrates of >50% within a 24- to 48-h period. A critical case was determined by any respiratory failure requiring mechanical ventilation, septic shock, and/or intensive care unit (ICU) admission for multiple-organ dysfunction or failure.

2.4 Statistical analysis

Continuous variables were described as median values (interquartile ranges (IQRs)) and compared by Mann-Whitney test. Categorical variables were described as frequencies (percentages) and compared by Chi-squared test or Fisher's exact test where appropriate. All inter-group comparisons were between the IFN group and the control group. A two-sided α of less than 0.05 was considered statistically significant.

Univariate Cox regressions were used to determine the association of factors in Tables 1 and 2 with prolonged virus shedding, and independent risk factors with P values of less than 0.20 were included in a multivariate Cox regression model. The probabilities for stepwise entry and removal using a forward likelihood ratio (LR) method were 0.05 and 0.10, respectively. The selected independent factors from

multivariable Cox regression model were used to perform propensity score one-to-one matching to minimize the effect of selection bias between the IFN group and the control group. The propensity score matching and balance check were performed in R (Ver. 3.6.2) using the R package "MatchIt." The Greedy matching method was used for the nearest neighbor matching, where the closest control match for each treated unit is chosen one at a time, without trying to minimize a global distance measure. All analyses except propensity score matching were carried out using SPSS 19.0 (IBM, Armonk, NY, USA).

3 Results

3.1 Patients' characteristics

A total of 104 confirmed patients were hospitalized from January 19 to February 19, 2020 and enrolled in this study. Sixty-eight patients were allocated to the IFN group, and 36 patients were allocated to the control group in this retrospective study. IFN-α2b spray inhalation was well tolerated and no obvious adverse effects, such as flu-like symptoms or leucopenia, were observed. The IFN group was younger (53.0 vs. 60.5 years) and included more females (47.1% vs. 27.8%), although these differences were not statistically significant. Fewer cases of hypertension were observed in the IFN group (27.9% vs. 55.6%, P=0.006). Wuhan travel history was comparable between the two groups. There was less dyspnea (8.8% vs. 25.0%, P=0.025) or diarrhea (4.4% vs. 19.4%, P=0.030) in the IFN group than in the control group (Table 1). Laboratory tests indicated lower levels of albumin and C-reactive protein, and higher level of sodium in the IFN group. CT lung scans were comparable between the IFN and control groups (Table 2). More patients were also treated with lopinavir/ritonavir in the IFN group (77.9% vs. 55.6%, P=0.018). Glucocorticoid dosages were lower in the

Table 1 Characteristics of 104 COVID-19 patients with and without interferon treatment

Characteristics	Unmatched group			Matched group		
	Interferon (n=68)	Control (n=36)	P	Interferon (n=32)	Control (n=32)	P
Age (year)	53.0 (41.0–62.8)	60.5 (48.3–71.5)	0.057	55.0 (41.8–65.5)	61.5 (48.3–72.8)	0.242
Female sex	32 (47.1%)	10 (27.8%)	0.057	10 (31.3%)	10 (31.3%)	>0.999
Current smoker	4 (5.9%)	4 (11.1%)	0.443	2 (6.3%)	4 (12.5%)	0.672
Coexisting condition						
Hypertension	19 (27.9%)	20 (55.6%)	0.006	13 (40.6%)	17 (53.1%)	0.316
Diabetes	5 (7.4%)	8 (22.2%)	0.057	4 (12.5%)	7 (21.9%)	0.320
Heart disease	6 (8.8%)	1 (2.8%)	0.417	3 (9.4%)	1 (3.1%)	0.613
COPD	4 (5.9%)	0	0.296	2 (6.3%)	0	0.492
Chronic renal disease	1 (1.5%)	1 (2.8%)	>0.999	1 (3.1%)	1 (3.1%)	>0.999
Travelled to Wuhan	16 (23.5%)	12 (33.3%)	0.284	9 (28.1%)	9 (28.1%)	>0.999
Symptoms						
Fever	61 (89.7%)	33 (91.7%)	>0.999	29 (0.7%)	30 (93.8%)	>0.999
Cough	43 (63.2%)	22 (61.1%)	0.831	23 (71.9%)	20 (62.5%)	0.424
Sputum production	27 (39.7%)	15 (41.7%)	0.846	15 (46.9%)	15 (46.9%)	>0.999
Myalgia	17 (25.0%)	3 (8.3%)	0.040	3 (9.4%)	3 (9.4%)	>0.999
Dyspnea	6 (8.8%)	9 (25.0%)	0.025	3 (9.4%)	9 (28.1%)	0.055
Headache	11 (16.2%)	3 (8.3%)	0.370	2 (6.3%)	3 (9.4%)	>0.999
Diarrhea	3 (4.4%)	7 (19.4%)	0.030	1 (3.1%)	6 (18.8%)	0.104
Nausea/vomiting	3 (4.4%)	4 (11.1%)	0.232	2 (6.3%)	4 (12.5%)	0.672
Hemoptysis	0	2 (5.6%)	0.118	0	2 (6.3%)	0.492
Onset to (d)						
Outpatient clinic	2 (0–5)	2 (1–5)	0.605	3 (0–5)	2 (1–5)	>0.999
Sputum PCR positive	6 (3–9)	6 (3–9)	0.595	7 (3–10)	6 (3–9)	0.348
Admission	5 (3–7)	5 (1–7)	0.497	6 (3–10)	6 (1–7)	0.173

Data are presented as median (interquartile range (IQR)) or number (percentage). P values denoted the comparison between the interferon group and the control group. COVID-19: coronavirus disease 2019; COPD: chronic obstructive pulmonary disease

Table 2 Laboratory and radiographic data of 104 COVID-19 patients with and without interferon treatment

Variable	Unmatched group			Matched group		
	Interferon (*n*=68)	Control (*n*=36)	*P*	Interferon (*n*=32)	Control (*n*=32)	*P*
Leukocytes ($\times10^9$ L^{-1})	4.8 (3.6–8.0)	5.6 (4.0–8.7)	0.194	5.5 (3.9–8.3)	5.6 (4.0–8.7)	0.819
Neutrophils ($\times10^9$ L^{-1})	3.2 (2.1–6.6)	3.8 (2.6–6.8)	0.127	3.5 (2.4–7.2)	3.6 (2.6–6.9)	0.682
Lymphocytes ($\times10^9$ L^{-1})	1.0 (0.6–1.3)	0.9 (0.5–1.3)	0.559	0.9 (0.5–1.3)	0.9 (0.5–1.3)	0.783
Platelets ($\times10^9$ L^{-1})	167 (132–219)	165 (131–188)	0.332	168 (137–224)	165 (131–188)	0.365
Hemoglobin	138 (122–151)	141 (123–155)	0.534	141 (123–153)	135 (122–148)	0.610
International normalized ratio	0.99 (0.95–1.04)	1.02 (0.96–1.07)	0.175	0.99 (0.95–1.03)	1.02 (0.96–1.08)	0.181
Albumin (g/L)	40.7 (36.6–44.2)	37.0 (33.6–42.0)	0.010	39.0 (35.5–44.3)	36.1 (33.3–42.0)	0.071
Alanine aminotransferase (U/L)	22 (15–32)	20 (14–37)	0.937	26 (19–44)	18 (14–35)	0.071
Aspartate aminotransferase (U/L)	22 (18–32)	31 (22–36)	0.084	24 (18–36)	31 (21–38)	0.409
Total bilirubin (mmol/L)	9.9 (6.5–13.4)	9.2 (5.7–14.4)	0.886	10.4 (8.4–13.6)	9.1 (5.6–14.8)	0.365
Potassium (mmol/L)	3.8 (3.5–4.1)	3.8 (3.4–4.0)	0.984	3.8 (3.5–4.1)	3.8 (3.4–4.0)	0.702
Sodium (mmol/L)	138 (136–141)	136 (134–139)	0.023	139 (135–141)	137 (135–139)	0.056
Creatinine (μmol/L)	77 (65–93)	70 (60–87)	0.087	72 (61–94)	77 (66–95)	0.286
Lactate dehydrogenase (U/L)	232 (183–312)	238 (190–308)	0.878	264 (186–359)	241 (196–312)	0.448
C-reactive protein (mg/L)	15.1 (8.2–35.6)	28.0 (8.9–56.9)	0.038	16.1 (10.1–50.3)	40.3 (8.1–63.2)	0.398
Chest CT findings						
Normal	2 (2.9%)	1 (2.8%)	>0.999	2 (6.3%)	0	0.492
Unilateral pneumonia	5 (7.4%)	2 (5.6%)	>0.999	1 (3.1%)	1 (3.1%)	>0.999
Bilateral pneumonia	24 (35.3%)	10 (27.8%)	0.437	10 (31.3%)	10 (31.3%)	>0.999
Multiple mottling and ground-glass opacity	37 (54.4%)	23 (63.9%)	0.352	19 (59.4%)	21 (65.6%)	0.606

Data are presented as median (interquartile range (IQR)) or number (percentage). COVID-19: coronavirus disease 2019; CT: computed tomography

IFN group (median, 40 vs. 80 mg/d, *P*=0.025) than in the control group, and fewer IFN-treated patients were ventilated (13.2% vs. 33.3%, *P*=0.015) or admitted to ICU (16.2% vs. 44.4%, *P*=0.002) (Table 3).

There were fewer critical patients in the IFN group (7.4% vs. 25.0%, *P*=0.017) at the time of admission. Although the complications during admission process were comparable between the two groups, the discharge rate (85.3% vs. 66.7%, *P*=0.027) was higher and hospitalization time (16 vs. 21 d, *P*=0.015) was shorter in the IFN group. The virus shedding time (10 vs. 13 d, *P*=0.014) was shorter in the IFN group as well.

3.2 Risk factors of prolonged virus shedding time

The risk factors associated with prolonged virus shedding (univariate Cox regression analysis with *P* values less than 0.2) included duration from illness onset to RT-PCR confirmation, sex, platelet count, time from onset of illness to hospital admission, temperature on admission, pregnancy, age, myalgia, sputum production, aspartate aminotransferase (AST) level, history of fever before admission, headache, IFN treatment, time from onset of symptoms to first outpatient visit, diarrhea, lymphocyte count, blood urea nitrogen level, Wuhan travel history, and lactate dehydrogenase level. All these factors, except for IFN treatment, were included in a multivariable Cox regression model to identify factors independent of prolonged virus shedding. Sputum production (hazard ratio (HR) 1.139, 95% confidence interval (CI) 1.087–1.193, *P*=0.021), myalgia (HR 2.533, 95% CI 1.131–5.671, *P*=0.024), time from illness onset to first outpatient visit (HR 0.880, 95% CI 0.787–0.985, *P*=0.026), time from illness onset to RT-PCR confirmation of virus infection (HR 1.1.42, 95% CI

Table 3 Treatment and outcomes of 104 COVID-19 patients with and without interferon treatment

Variable	Unmatched group			Matched group		
	Interferon (*n*=68)	Control (*n*=36)	*P*	Interferon (*n*=32)	Control (*n*=32)	*P*
Treatment						
Disease onset to antiviral therapy (d)	5 (3–8)	5 (1–7)	0.160	6 (2–9)	5 (1–8)	0.277
Lopinavir/ritonavir	53 (77.9%)	20 (55.6%)	0.018	22 (68.8%)	18 (56.3%)	0.302
Mechanical ventilation	9 (13.2%)	12 (33.3%)	0.015	8 (25.0%)	12 (37.5%)	0.281
CRRT	2 (2.9%)	2 (5.6%)	0.608	2 (6.3%)	2 (6.3%)	>0.999
ECMO	5 (7.4%)	6 (16.7%)	0.183	4 (12.5%)	6 (18.8%)	0.491
Glucocorticoids	41 (60.3%)	22 (61.1%)	0.935	20 (62.5%)	20 (62.5%)	>0.999
Maximum dosage (mg)*	40 (40–80)	80 (40–80)	0.025	40 (40–80)	80 (40–80)	0.101
Intravenous immunoglobin	26 (38.2%)	15 (41.7%)	0.733	16 (50.0%)	13 (40.6%)	0.451
Admission to ICU	11 (16.2%)	16 (44.4%)	0.002	7 (21.9%)	16 (50.0%)	0.019
Severity on admission						
Mild	41 (60.3%)	20 (55.6%)	0.641	18 (56.3%)	17 (53.1%)	0.802
Severe	22 (32.4%)	7 (19.4%)	0.163	10 (31.3%)	6 (18.8%)	0.248
Critical	5 (7.4%)	9 (25.0%)	0.017	4 (12.5%)	9 (28.1%)	0.120
Complications						
ARDS	27 (39.7%)	20 (55.6%)	0.122	14 (43.8%)	19 (59.4%)	0.211
Shock	1 (1.5%)	1 (2.8%)	>0.999	1 (3.1%)	1 (3.1%)	>0.999
Acute kidney injury	3 (4.4%)	1 (2.8%)	>0.999	3 (9.4%)	1 (3.1%)	0.613
Acute liver injury	4 (5.9%)	2 (5.6%)	>0.999	3 (9.4%)	2 (6.3%)	>0.999
Clinical outcomes						
Virus shedding time (d)	10 (5–15)	13 (10–23)	0.014	12 (7–20)	15 (10–23)	0.206
Discharged	58 (85.3%)	24 (66.7%)	0.027	25 (78.1%)	20 (62.5%)	0.171
Hospitalization time of discharged patients (d)	16 (11–20)	21 (15–26)	0.015	16 (12–22)	21 (15–26)	0.084

Data are presented as median (interquartile range (IQR)) or number (percentage). COVID-19: coronavirus disease 2019; CRRT: continuous renal replacement therapy; ECMO: extracorporeal membrane oxygenation; ICU: intensive care unit; ARDS: acute respiratory distress syndrome

1.011–1.289, *P*=0.033), male gender (HR 1.939, 95% CI 1.029–3.656, *P*=0.041), fever before admission (HR 0.357, 95% CI 0.124–1.029, *P*=0.056), Wuhan travelling history (HR 0.600, 95% CI 0.323–1.112, *P*=0.105), international normalized ratio (INR; HR 0.204, 95% CI 0.027–1.564, *P*=0.126), headache (HR 1.981, 95% CI 0.761–5.155, *P*=0.161), and AST level (HR 1.015, 95% CI 0.993–1.037, *P*=0.177) were factors with *P* values less than 0.2; thus, these factors were used to generate propensity scores using a multivariable logistic regression.

3.3 Effects of IFN on clinical outcomes determined using propensity score matching

A total of 32 propensity score-matched pairs were generated from the IFN and control groups. A balance check was performed, and the distribution of propensity scores was well paired. Demographic and physiological characteristics were similar between the matched groups (Tables 1 and 2). After matching, glucocorticoid dosages and rates of lopinavir/ritonavir treatment were comparable between groups, and the proportions of critically ill patients were also comparable between the groups. In the propensity score-matched IFN group, virus shedding time was not significantly shorter than that of the control group (12 vs. 15 d, *P*=0.206). Similarly, the discharge rate and hospitalization time by date of final follow-up showed no significant differences between these groups as well. After matching, there were 12 (37.5%) patients who did not use glucocorticoids in each group. The virus shedding time of patients who did not use glucocorticoids was 13 d (IQR, 6–25 d) and 12 d (IQR, 8–24 d) in the IFN group and the control group, respectively (*P*=0.932). After matching, there were 18 (56.3%) and 17 (53.1%) patients with mild cases in the IFN group and the control group, respectively. The virus shedding time of patients with mild infections

was 9 d (IQR, 5–17 d) in the IFN group vs. 12 d (IQR, 10–23 d) in the control group (P=0.089). Thus, the effect of IFN spray inhalation on virus clearance was not significant among patients with mild cases or among patients who did not use glucocorticoids in our study.

4 Discussion

In this study, we compared the clinical characteristics and outcomes of COVID-19 patients between initially treated with IFN-α2b spray inhalation (the IFN group) and a control group that did not receive IFN inhalation treatment. There were fewer patients with hypertension, dyspnea, or diarrhea in the IFN-treated group than in the control group. Fewer patients were ventilated and admitted to the ICU in the IFN-treated group than in the controls. Discharge rates, hospitalization time, and virus shedding time were superior in the IFN group compared to the controls. After propensity score matching, the baseline covariates between the IFN and control groups were well balanced. Virus shedding time was not significantly shorter than that of the control group after matching. To our best knowledge, this is the first report to describe the effects of IFN-α2b spray inhalation on virus shedding time of SARS-CoV-2.

IFN-α2b spray inhalation had several advantages. First, it is a commercially available drug that is convenient to apply, compared to subcutaneous injection or atomization inhalation. Second, the inhalation treatment can directly target the respiratory tract without systemic distribution. The side effects of IFN injection were thus minimized by using the inhalation route. In subcutaneous or intramuscular injection, side effects such as flu-like symptoms, leukocytopenia, and psychiatric symptoms can develop, sometimes necessitating dose modification or even discontinuation of the treatment (Dusheiko, 1997). In this study, obvious side effects of IFN treatment were not observed. Thus, the respiratory route is apparently a safe route for IFN delivery. Third, use of atomization inhalation methods raises concerns about the nosocomial spread of SARS-CoV-2 by droplets and aerosols (van Doremalen et al., 2020). The potential for aerosol transmission of SARS-CoV-2 in dentistry (Ge et al., 2020) and ophthalmology (Lu et al., 2020) has been discussed as matters of concern. In contrast to atomization inhalation, IFN-α2b spray inhalation avoids the potential risk of droplets and aerosol transmission in hospital.

Although initial statistical analysis suggested an effect of IFN treatment on hospitalization time and duration of viral shedding, analysis with propensity score matching indicated no statistically significant benefits of IFN on hospitalization time or virus shedding time. Several reasons might explain this discrepancy. First, the optimal treatment dose of IFN-α2b has not yet been established. The IFN-α2b dosage for treatment of hepatitis B and C is 3–6 MIU/d subcutaneously, while most clinical trials have used an approximately equivalent dose of pegylated IFN in treatment of MERS. In this study, 0.4 MIU/d IFN-α2b spray was used. Because the pharmacodynamics and pharmacokinetics of respiratory administration have never been assessed, there is no systemic index available to assess the sufficiency of treatment. Second, although the combination of IFN with lopinavir/ritonavir, ribavirin, or remdesivir is thought to improve its efficacy in MERS, remdesivir or ribavirin was not used in this study. The possibly synergistic effects between IFN and lopinavir/ritonavir cannot be evaluated by this study. Moreover, more patients were treated with lopinavir/ritonavir in the IFN group before matching, and with this factor balanced after matching, the differences in virus shedding were no longer significant. However, recent study has shown that lopinavir/ritonavir did not reduce mortality rates at 28 d (Cao et al., 2020). Thus, the disappearance of statistically significant effects after matching cannot not be related solely to the imbalance of lopinavir/ritonavir. After matching, although there were more patients admitted to ICU in the control group, the rates of critical patients and complications were comparable between groups; thus, the lack of effect of IFN treatment cannot be explained by concluding that patients in the control group had more severe cases of COVID-19.

IFN-β and lopinavir/ritonavir therapy is one of the proposed treatment regimens in the WHO SOLIDARITY trial. Previous studies on SARS and MERS have indicated that IFN-β should be the most clinically relevant IFN subtype. A recently published study has found potent antiviral activity of type I IFNs (IFN-α/β) against SARS-CoV-2 in cell lines; however,

IFN-β shows an even better antiviral effect (Mantlo et al., 2020). The effects of IFN-β should be evaluated in COVID-19.

This study has several limitations. First, the case-control design of this study may decrease its credibility. Although propensity score matching could decrease the risk of selection bias, there are limitations to this statistical method and future prospective randomized clinical trials should be conducted to verify the effect of IFN treatment. Second, the lack of statistical significance after propensity score matching might be a reflection of small sample size. A well-designed, large sample-size randomized study is needed for a more definitive evaluation of the clinical effects of this treatment protocol. Finally, the sufficiency of the dose delivered by IFN spray was not evaluated because there is currently no satisfactory index to make this assessment. Serum IFN level might be a candidate to assess the inhalation delivery efficacy; however, the pharmacodynamics and pharmacokinetics should be studied further.

In conclusion, we found that hospitalization time and virus shedding time were shorter in the IFN-α2b spray inhalation treatment group compared to controls; however, they were not statistically significant after propensity score matching. Thus, this preliminary study did not support the hypothesis of beneficial effects of IFN-α2b spray inhalation on clinical outcomes of COVID-19.

Contributors

Shao-rui HAO coordinated the work and took the lead in drafting the manuscript and interpreting. Ren YAN, Jiang-shan LIAN, and Huan CAI developed the statistical methods. Shan-yan ZHANG, Xiao-li ZHANG, Lin ZHENG, Hong-yu JIA, Jian-hua HU, Guo-dong YU, Jue-qing GU, Chan-yuan YE, Ci-liang JIN, Ying-feng LU, and Jiao-jiao XIN were participated in the collection of experimental data. Yi-da YANG and Ji-fang SHENG designed the study and reviewed the manuscript prior to submission. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. All authors have read and approved the final manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Shao-rui HAO, Ren YAN, Shan-yan ZHANG, Jiang-shan LIAN, Huan CAI, Xiao-li ZHANG, Lin ZHENG, Hong-yu JIA, Jian-hua HU, Guo-dong YU, Jue-qing GU, Chan-yuan YE, Ci-liang JIN, Ying-feng LU, Jiao-jiao XIN, Ji-fang SHENG, and Yi-da YANG declare that they have no conflict of interest.

This study was conducted in accordance with the guidelines of the Helsinki Declaration of 1975, as revised in 2008 (5), and was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China (No. IIT20200005C). Written informed consent was waived by the ethics commission for this retrospective study.

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中文概要

题　目：干扰素-α2b 喷雾吸入未缩短住院患者的 SARS-CoV-2 的脱落时间：一项初步的配对病例对照研究

目　的：分析干扰素-α2b（IFN-α2b）喷雾吸入治疗对严重急性呼吸综合征冠状病毒 2（SARS-CoV-2）在呼吸道病毒脱落时间的影响。

创新点：IFN 在 2019 冠状病毒病（COVID-19）中的治疗价值尚未得到验证。

方　法：我们进行了一项回顾性研究，纳入 2020 年 1 月 19 日至 2 月 19 日在中国杭州浙江大学医学院附属第一医院住院的 104 例确诊的 COVID-19 患者。根据入院时是否接受了初始 IFN-α2b 喷雾吸入治疗，将患者分为 IFN 组和对照组。采用倾向性得分匹配方法平衡混杂因素后，比较两组间住院时间和病毒脱落时间的差异。

结　论：IFN-α2b 喷雾吸入不能缩短 COVID-19 住院患者的住院时间和 SARS-CoV-2 的病毒脱毒时间。

关键词：2019 冠状病毒病（COVID-19）；严重急性呼吸综合征冠状病毒 2（SARS-CoV-2）；倾向性评分匹配；干扰素