Eur. Cytokine Netw. Vol. 34, n° 2, *September 2022* 10-19

RESEARCH ARTICLE

Effect of interferon-α on COVID-19 in-hospital mortality: a large-scale propensity score-matched study

Mohamad Amin Pourhoseingholi1[1*], Amirreza Rafiei Javazm[1*], Naghmeh Asadimanesh[2], Fatemeh Shojaeian[3], Mehdi Azizmohammad Looha[1], Seyed Amir Ahmad Safavi-Naini[1], Benyamin Mohammadzadeh[1,4], Parnian Jamshidi[1,4], Fatemeh Gholampoor[2], Omid Yazdani[2], Nadia Zameni[2], Zahra Azizan[2], Amirhossein Sahebkar[5,6]

[1] Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2] School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[3] Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland, US.
[4] Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[5] Applied Biomedical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
[6] Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran.
* Those authors contributed equally

Correspondence: A. Sahebkar
<amir_saheb2000@yahoo.com>

Article accepted on 22 June 2023

To cite this article: Pourhoseingholi1 MA, Javazm AR, Asadimanesh N, Shojaeian F, Looha MA, Safavi-Naini SAA, Mohammadzadeh B, Jamshidi P, Gholampoor F, Yazdani O, Zameni N, Azizan Z, Sahebkar A. The effect of interferon-alpha on COVID-19 in-hospital mortality: a large-scale propensity score-matched study. Eur. Cytokine Netw. 2023; 34(2): 10-19. doi: 10.1684/ecn.2023.0485

ABSTRACT. *Background:* Coronavirus infection can induce the production of inflammatory cytokines leading to acute respiratory distress syndrome (ARDS) and death. It is well-established that interferons (IFNs) are essential in regulating the immune response, thus their effects of IFNs on COVID-19 patients should be subject to investigation. This study aimed to investigate the effects of IFN-α alone or in combination with remdesivir in hospitalized COVID-19 patients. *Material and Methods:* A multicentre, retrospective study was conducted on COVID-19 patients admitted to three hospitals in Tehran, Iran, from March 20, 2020, to March 18, 2021. The unadjusted and adjusted effects of IFN-α on COVID-19 outcomes were investigated through propensity score matching (PSM) to achieve a 1:1 balanced dataset. *Results:* Among 4,782 patients, 3,764 were eligible for the study, including 1,704 patients (45.27%) receiving at least one treatment with IFN-α and 2,060 controls not receiving IFN-α. After PSM, 851 IFN-α patients and 851 controls were recruited in the PSM analysis with a median age of 60.8 (standard deviation [SD]: 16.2 and 60.9 [SD: 17.4]), respectively. The PSM results showed no significant difference between the survival curves of the IFN-α group and the control group (p=0.340). However, the unadjusted impact of IFN-α on the risk of mortality was statistically significant (p=0.043, hazard-ratio: 0.86; 95% confidence interval [CI]: 0.75-0.99). Also, the combination of IFN-α and remdesivir had no significant benefit (HR: 89, 95% CI: 0.74-1.34). *Conclusion:* Our findings indicate that subcutaneous administration of IFN-α, with or without remdesivir, does not have any significant impact on COVID-19 mortality and ICU admission. Future clinical trials considering the time, subtype, and form of IFN-α administration are warranted to investigate the potential therapeutic effects of IFN-α on COVID-19.

Key words: COVID-19, interferon, remdesivir, ICU, mortality

Coronavirus infection can heighten blood levels of inflammatory cytokines leading to acute respiratory distress syndrome (ARDS) [1-3]. In line with this cytokine rise, some clinical and preclinical studies revealed that disease severity and mortality mainly rely on exaggerated inflammation [4-6]. The host immune response can both limit the disease's spread and trigger a cytokine storm. Therefore, how immune cell activation could benefit COVID-19 has been a matter of debate.

Of the many inflammatory mediators, interferons (IFNs) have gained increasing attention. The crucial role of IFNs in the effective immune response against viral infections, especially coronavirus infections, is well-established. There are three major groups of IFNs: IFN-I (represented by IFN-αs and IFN-β), IFN-II (IFN-γ), and IFN-III (IFN-λ1-4) [3, 4]. In contrast to IFN-II, whose upregulation correlates with exacerbated pathology and mortality, the role of IFN-I and III remains unclear [4]. Previous studies have indicated dynamic production and opposing roles of IFNs in different anatomical sites [7]. Still, numerous investigations showed that in patients with severe COVID-19, antiviral IFN responses are delayed and/or dampened while the production of inflammatory mediators is increased [8, 9].

Aside from the challenges of comprehending the pattern of IFN expression, a major arguable point is whether

doi: 10.1684/ecn.2023.0485

IFNs pose beneficial or detrimental effects in COVID-19 patients. IFN is considered as one of the promising therapeutic agents in the treatment of COVID-19. This can be due to its antiviral effect, anti-inflammatory properties, or ability to inhibit proteases [10, 11]. According to some investigations, patients with severe COVID-19 exhibit impaired IFN responses [12-14]. Other studies reported that increased and protracted IFN production in COVID-19 patients is associated with poor clinical outcomes [2, 3]. Taken together, it seems critical to unravel the effects of IFN in COVID-19 patients. This large-scale study aimed to investigate the impact of IFN-α in hospitalized COVID-19 patients using propensity score matching (PSM).

METHODS

Study design and participants

A retrospective study was conducted between March 20, 2020, and March 18, 2021, on patients with confirmed COVID-19, admitted to three hospitals (Taleghani Hospital, Imam Hossein Hospital, and Shohadaye Tajrish Hospital) in Tehran, Iran. All hospitals are tertiary referral hospitals with distinguished COVID-19 wards. The hospital management committee could increase the number of COVID-19-dedicated wards during peaks of COVID-19. Nasopharyngeal or oropharyngeal swab samples were tested by real-time polymerase chain reaction (RT-PCR) on the first day of admission to confirm COVID-19.

Data collection and dataset

The medical team obtained all demographic and comorbidity information of the patients, as well as their triage vital signs, through the hospital's information system. The laboratory values were retrieved using Python Language Reference, version 2.7. (available at http://www.python.org). We previously investigated the epidemiological characteristics of the current database [15].

Inclusion criteria

In this study, all hospitalized patients were included, and patients who died within 24 hours of admission or who were censored were excluded from the analysis. Analyses were not conducted on patients who had missing data on demographic information, signs and symptoms, medications, or laboratory tests. In this study, patients treated with subcutaneous IFN-α in their treatment protocol were included, and the untreated group included all individuals who were not treated with IFN-α. The routinely recommended dose for adults was a subcutaneous 9,000,000-unit vial every other day for six days (a total of three doses). In patients who were capable of being discharged, IFN-α treatment was discontinued early. Also, the treatment was stopped for patients experiencing clinical conditions that were deemed dangerous based on the investigator's opinion (including a 50% decrease in creatinine clearance without any alternative explanation or liver function test abnormalities). In addition, IFN-α was not prescribed in situations involving: protocol violations that could compromise the quality of study data, new diseases that may affect the efficacy of the treatment, and patients who were not followed.

Outcome

The primary outcome was the in-hospital survival rate of COVID-19 patients, the secondary outcome was admission to the ICU, and the tertiary outcome was the number of hospitalization days. The primary objective of this study was to determine whether treatment (IFN-α alone or in combination with remdesivir) was associated with a decrease in COVID-19 mortality after matching large groups of confounders that were potentially related to treatment or death. As no information was available regarding the timing of the administration of interferon (plus remdesivir), the length of hospitalization was considered until either the patient died or the patient was discharged. Those patients who were discharged from hospital were considered as censors. Time to death or discharge from hospital was defined as survival time.

Potential confounders

Interval between symptoms and hospital referral, age, number of comorbidities, ward (Ward/ICU), sex, decreased consciousness, chest pain, hypertension (HTN), ischaemic heart disease (IHD), coronary artery bypass graft (CABG), congestive heart failure (CHF), diabetes mellitus (DM), chronic kidney disease (CKD), cancer, dialysis, transfusion (platelets or fresh frozen plasma [FFP] injection), intubation, atorvastatin, azithromycin, ceftriaxone, enoxaparin (clexane), ciprofloxacin, ipratropium bromide/albuterol (combivent), heparin, dexamethasone, imipenem, remdesivir, meropenem, oseltamivir, clopidogrel (plavix), selenium, sofosbuvir (sovodak), vancomycin, pantoprazole (pentazole), lactulose, albumin, SpO_2, pulse rate (PR), diastolic blood pressure (DBP), systolic blood pressure (SBP), respiratory rate (RR), white blood cells (WBCs), lymphocytes, blood urea nitrogen (BUN), creatinine (CR), potassium (K), magnesium (Mg), phosphorus (P), alanine transaminase (ALT), C-reactive protein (CRP), international normalized ratio (INR), pH, partial pressure of carbon dioxide (PCO_2), bicarbonate (HCO_3), D-dimer and troponin were included as variables in the model.

Statistical analysis

Descriptive statistics were reported using mean ± standard deviation (SD) or median (Q1, Q3) for numeric variables and frequency (percentage) for categorical factors. Variables significantly related to either the exposure (IFN-α alone or in combination with remdesivir) or outcome (time to death) were identified as confounders, according to the study by Zoller *et al.* [16]. To identify confounders, the relationship between potential confounders and time to event was examined using Cox regression.

Logistic regression analysis was used to determine the association between potential confounders and methylprednisolone. PSM was used to estimate the average

treatment effect in the treated population, controlling for confounding by the included covariates. The primary model involved 1:1 (exposure: control) nearest-neighbour matching without replacement with the propensity score estimated from the logistic regression of the treatment for the covariates. The caliper distance was set to less than 0.1 for the PSM to reduce systematic differences between matched treated and untreated subjects [17]. The 1:2 PSM with a caliper distance of 0.1 was used for combination therapies. The criteria were used to evaluate the matching quality, including absolute standardized mean difference (SMD), variance ratio, and Kolmogorov-Smirnov statistics. It was recommended that the absolute standardized mean difference be less than 0.1. The variance ratios are the ratios of the variances of the treated and control groups for each covariate, which is close to 1 when there is a good balance. A Kolmogorov-Smirnov test close to zero indicates a good balance. The weighted cox proportional hazard model with robust standard errors, including the subclass as a cluster, was used to estimate the hazard ratio (HR) and its standard error. The confounder-adjusted Kaplan-Meier plot was drawn using matching weights. Several approaches were used to examine the impact of interferon and combination therapy on the risk of COVID-19 death or hospitalization, including unadjusted logistic regression, full logistic regression with all covariates, and step models using backward logistic regression. All analyses were performed using R (version 4.0.2), and *p*-values less than 0.05 were regarded as statistically significant.

RESULTS

Patients' characteristics and variable selection

Among 4,782 patients with COVID-19 who were included in the study, 390 cases were excluded due to death or discharge within one day of admission, while 628 cases were dropped due to multivariate outliers. The study involved 3,764 patients, of whom 1,704 patients (45.27%) received at least one dosage treatment with IFN-α, and 2,060 patients were considered as controls. Following nearest-neighbour PSM with a caliper of less than 0.1, 1,702 matched patients were analysed (851 interferon recipients and 851 matched control individuals) (*figure 1*).

As shown in *table 1*, the data on patients' characteristics, comorbidities, treatments, and laboratory tests were documented before and after PSM. Accordingly, the mean age of patients receiving IFN-α and non-IFN-α treatments was 60.6 and 60.4 years, respectively, before PSM (p=0.733). Moreover, 1,347 (79.0%) IFN-α recipients and 862 (41.8%) non-IFN-α recipients were female.

The relationship between variables and treatment was reported. All variables that were significantly related to outcome or exposure were included in the PSM analysis (*supplementary table 1*). It was decided that some variables should be included with non-significant relationships in the analysis based on the opinions of medical experts.

Assessing balance after PSM

There was no significant association between treatment (IFN-α vs. controls) and factors to clarify the balance

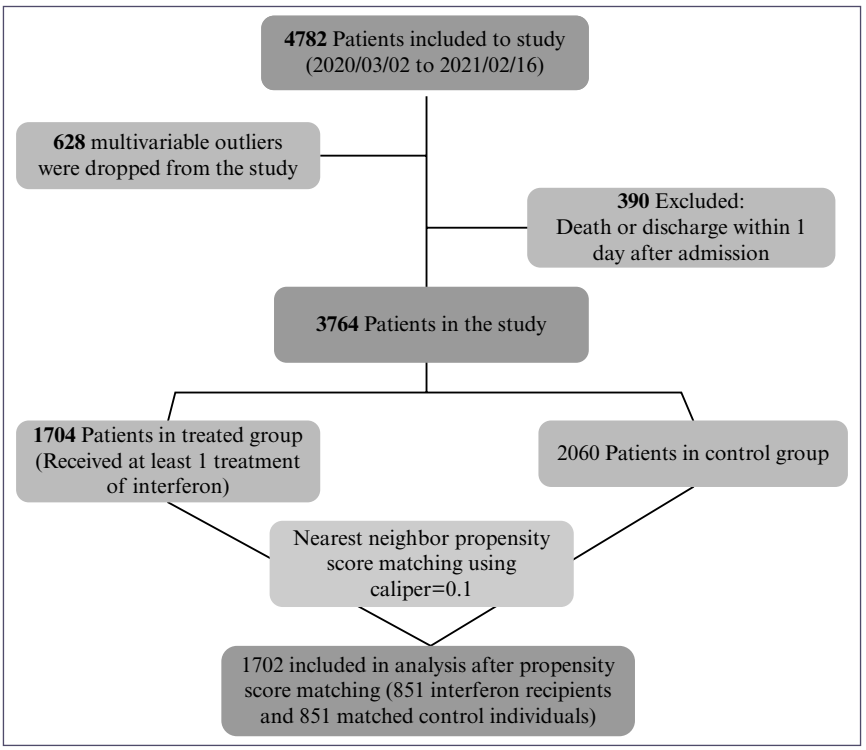

Figure 1
Patient flow diagram for propensity score matching.

Table 1.
Patients' characteristics, comorbidities, treatments, and laboratory tests before and after propensity score matching.

Variable	Level	Before propensity score matching			After propensity score matching		
		Non-interferon (N = 2060)	Inpatient interferon (N = 1706)	P value	Non-interferon (N = 851)	Inpatient interferon (N = 851)	P value
Interval between symptom & referral	...	3.2 ± 5.7	4.2 ± 5.3	<0.001	3.8 ± 6.5	3.7 ± 5.3	0.210
Age	...	60.4 ± 18.2	60.6 ± 16.2	0.733	60.9 ± 17.4	60.8 ± 16.2	0.792
No of comorbidities	...	1.3 ± 1.3	1.3 ± 1.3	0.805	1.4 ± 1.3	1.4 ± 1.3	0.689
Wards	Ward	1708 (82.9)	1347 (79.0)	0.003	689 (81.0)	683 (80.3)	0.759
	ICU	352 (17.1)	357 (21.0)		162 (19.0)	168 (19.7)	
Sex	Female	862 (41.8)	755 (44.3)	0.137	383 (45.0)	383 (45.0)	1.000
	Male	1198 (58.2)	949 (55.7)		468 (55.0)	468 (55.0)	
Decreased consciousness	No	1861 (90.3)	1603 (94.1)	<0.001	791 (92.9)	789 (92.7)	0.925
	Yes	199 (9.7)	101 (5.9)		60 (7.1)	62 (7.3)	
Chest pain	No	1856 (90.1)	1524 (89.4)	0.540	763 (89.7)	763 (89.7)	1.000
	Yes	204 (9.9)	180 (10.6)		88 (10.3)	88 (10.3)	
HTN (hypertension)	No	1316 (63.9)	1053 (61.8)	0.198	511 (60.0)	508 (59.7)	0.921
	Yes	744 (36.1)	651 (38.2)		340 (40.0)	343 (40.3)	
IHD (ischaemic heart disease)	No	1743 (84.6)	1430 (83.9)	0.592	713 (83.8)	715 (84.0)	0.947
	Yes	317 (15.4)	274 (16.1)		138 (16.2)	136 (16.0)	
CABG (coronary artery bypass graft)	No	1970 (95.6)	1635 (96.0)	0.686	822 (96.6)	817 (96.0)	0.608
	Yes	90 (4.4)	69 (4.0)		29 (3.4)	34 (4.0)	
CHF (congestive heart failure)	No	2013 (97.7)	1688 (99.1)	0.002	840 (98.7)	838 (98.5)	0.837
	Yes	47 (2.3)	16 (0.9)		11 (1.3)	13 (1.5)	
DM (diabetes mellitus)	No	1495 (72.6)	1148 (67.4)	0.001	575 (67.6)	578 (67.9)	0.917
	Yes	565 (27.4)	556 (32.6)		276 (32.4)	273 (32.1)	
CKD (chronic kidney disease)	No	1974 (95.8)	1662 (97.5)	0.005	819 (96.2)	824 (96.8)	0.596
	Yes	86 (4.2)	42 (2.5)		32 (3.8)	27 (3.2)	
Cancer	No	1951 (94.7)	1647 (96.7)	0.005	817 (96.0)	823 (96.7)	0.518
	Yes	109 (5.3)	57 (3.3)		34 (4.0)	28 (3.3)	
Dialysis	No	2010 (97.6)	1658 (97.3)	0.672	831 (97.6)	827 (97.2)	0.647
	Yes	50 (2.4)	46 (2.7)		20 (2.4)	24 (2.8)	
Platelets FFP injection	No	2018 (98.0)	1653 (97.0)	0.076	829 (97.4)	834 (98.0)	0.517
	Yes	42 (2.0)	51 (3.0)		22 (2.6)	17 (2.0)	
Intubation	No	1905 (92.5)	1582 (92.8)	0.716	787 (92.5)	789 (92.7)	0.926
	Yes	155 (7.5)	122 (7.2)		64 (7.5)	62 (7.3)	
Atorvastatin	No	1282 (62.2)	616 (36.2)	<0.001	419 (49.2)	408 (47.9)	0.628
	Yes	778 (37.8)	1088 (63.8)		432 (50.8)	443 (52.1)	
Azithromycin	No	1145 (55.6)	886 (52.0)	0.030	463 (54.4)	464 (54.5)	1.000
	Yes	915 (44.4)	818 (48.0)		388 (45.6)	387 (45.5)	
Ceftriaxone	No	931 (45.2)	753 (44.2)	0.559	390 (45.8)	378 (44.4)	0.592
	Yes	1129 (54.8)	951 (55.8)		461 (54.2)	473 (55.6)	
Enoxaparin	No	1310 (63.6)	771 (45.2)	<0.001	469 (55.1)	465 (54.6)	0.884
	Yes	750 (36.4)	933 (54.8)		382 (44.9)	386 (45.4)	
Ciprofloxacin	No	1882 (91.4)	1605 (94.2)	0.001	793 (93.2)	795 (93.4)	0.923
	Yes	178 (8.6)	99 (5.8)		58 (6.8)	56 (6.6)	
Ipratropium bromide/albuterol	No	1888 (91.7)	1502 (88.1)	<0.001	755 (88.7)	747 (87.8)	0.598
	Yes	172 (8.3)	202 (11.9)		96 (11.3)	104 (12.2)	
Dexamethasone	No	1488 (72.2)	343 (20.1)	<0.001	314 (36.9)	319 (37.5)	0.841
	Yes	572 (27.8)	1361 (79.9)		537 (63.1)	532 (62.5)	

Heparin	No	983 (47.7)	759 (44.5)	0.056	371 (43.6)	370 (43.5)	1.000
	Yes	1077 (52.3)	945 (55.5)		480 (56.4)	481 (56.5)	
Imipenem	No	1983 (96.3)	1591 (93.4)	<0.001	801 (94.1)	797 (93.7)	0.761
	Yes	77 (3.7)	113 (6.6)		50 (5.9)	54 (6.3)	
Meropenem	No	1543 (74.9)	1229 (72.1)	0.059	585 (68.7)	597 (70.2)	0.563
	Yes	517 (25.1)	475 (27.9)		266 (31.3)	254 (29.8)	
Oseltamivir	No	1648 (80.0)	1619 (95.0)	<0.001	770 (90.5)	783 (92.0)	0.303
	Yes	412 (20.0)	85 (5.0)		81 (9.5)	68 (8.0)	
Clopidogrel	No	1862 (90.4)	1583 (92.9)	0.007	773 (90.8)	778 (91.4)	0.733
	Yes	198 (9.6)	121 (7.1)		78 (9.2)	73 (8.6)	
Remdesivir	No	1911 (92.8)	1151 (67.5)	<0.001	712 (83.7)	684 (80.4)	0.088
	Yes	149 (7.2)	553 (32.5)		139 (16.3)	167 (19.6)	
Selenium	No	2033 (98.7)	1612 (94.6)	<0.001	826 (97.1)	814 (95.7)	0.155
	Yes	27 (1.3)	92 (5.4)		25 (2.9)	37 (4.3)	
Sofosbuvir	No	1852 (89.9)	1488 (87.3)	0.015	752 (88.4)	751 (88.2)	1.000
	Yes	208 (10.1)	216 (12.7)		99 (11.6)	100 (11.8)	
Vancomycin	No	1524 (74.0)	1253 (73.5)	0.784	591 (69.4)	605 (71.1)	0.491
	Yes	536 (26.0)	451 (26.5)		260 (30.6)	246 (28.9)	
Pantoprazole	No	434 (21.1)	529 (31.0)	<0.001	198 (23.3)	212 (24.9)	0.461
	Yes	1626 (78.9)	1175 (69.0)		653 (76.7)	639 (75.1)	
Lactulose	No	1717 (83.3)	1233 (72.4)	<0.001	643 (75.6)	640 (75.2)	0.910
	Yes	343 (16.7)	471 (27.6)		208 (24.4)	211 (24.8)	
Albumin	No	1830 (88.8)	1456 (85.4)	0.002	731 (85.9)	732 (86.0)	1.000
	Yes	230 (11.2)	248 (14.6)		120 (14.1)	119 (14.0)	
ASA	No	1305 (63.3)	919 (53.9)	<0.001	489 (57.5)	478 (56.2)	0.625
	Yes	755 (36.7)	785 (46.1)		362 (42.5)	373 (43.8)	
Prednisolone	No	1890 (91.7)	1527 (89.6)	0.028	775 (91.1)	767 (90.1)	0.561
	Yes	170 (8.3)	177 (10.4)		76 (8.9)	84 (9.9)	
SpO2 (saturation of peripheral oxygen)	..	91.0 (87.0, 95.0)	88.0 (83.0, 92.0)	<0.001	89.5 (84.4, 92.6)	88.5 (84.0, 92.0)	0.101
PR (pulse rate)	..	85.0 (80.0, 94.0)	85.0 (80.0, 99.5)	0.447	85.5 (79.2, 94.1)	84.5 (79.3, 99.5)	0.823
DBP (diastolic blood pressure)	..	80.0 (70.0, 80.0)	80.0 (70.0, 80.0)	0.833	79.5 (68.6, 79.9)	79.5 (68.8, 80.0)	0.862
SBP (systolic blood pressure)	..	120.0 (110.0, 130.0)	120.0 (110.0, 130.0)	0.571	119.0 (107.6, 129.4)	119.5 (109.3, 130.0)	0.473
RR (respiratory rate)	..	18.0 (17.0, 20.0)	18.0 (18.0, 21.0)	0.078	17.5 (17.0, 19.7)	17.5 (17.0, 21.0)	0.584
WBCs (white blood cells)	..	7.5 (5.3, 10.8)	6.7 (4.9, 10.4)	<0.001	7.2 (5.1, 9.9)	6.9 (4.9, 10.4)	0.236
Lymphocytes	..	16.7 (10.4, 25.0)	15.0 (10.0, 22.9)	<0.001	14.9 (10.0, 23.9)	15.1 (9.9, 22.9)	0.826
BUN (blood urea nitrogen)	..	18.0 (12.0, 26.0)	18.0 (13.0, 26.0)	0.424	17.8 (12.7, 27.0)	17.5 (12.2, 26.0)	0.251
CR (creatinine)	..	1.1 (1.0, 1.4)	1.1 (0.9, 1.4)	<0.001	1.1 (0.9, 1.4)	1.1 (0.9, 1.4)	0.313
K first (potassium)	..	4.1 (3.8, 4.4)	4.1 (3.8, 4.4)	0.796	4.0 (3.8, 4.4)	4.1 (3.8, 4.4)	0.647
Mg first (magnesium)	..	1.9 (1.7, 2.2)	1.9 (1.7, 2.1)	<0.001	1.9 (1.7, 2.1)	1.9 (1.7, 2.1)	0.647
P first (phosphorus)	..	3.4 (2.8, 4.0)	3.4 (2.9, 4.0)	0.762	3.4 (2.8, 4.0)	3.4 (2.9, 4.0)	0.883
ALT (alanine transaminase)	..	26.0 (17.0, 44.0)	30.3 (20.0, 44.0)	<0.001	27.8 (18.0, 46.7)	30.0 (18.7, 44.0)	0.206
CRP (C-reactive protein)	..	26.0 (10.0, 58.2)	38.0 (14.0, 63.0)	<0.001	30.6 (10.9, 66.1)	33.0 (12.4, 63.0)	0.578
INR first (international normalized ratio)	..	1.1 (1.0, 1.2)	1.1 (1.0, 1.2)	0.163	1.1 (1.0, 1.2)	1.1 (1.0, 1.2)	0.802
pH	..	7.4 (7.3, 7.4)	7.4 (7.3, 7.4)	0.099	7.4 (7.3, 7.4)	7.4 (7.3, 7.4)	0.944

PCO2 first (partial pressure of carbon dioxide)	..	44.1 (38.9, 49.9)	44.3 (38.9, 51.7)	0.724	44.2 (39.3, 49.8)	44.5 (38.6, 51.7)	0.714
HCO3 first (bicarbonate)	..	25.9 (23.2, 28.6)	26.2 (23.2, 29.5)	0.022	25.8 (23.3, 28.4)	26.0 (23.1, 29.5)	0.708
D-DIMER first	..	631.0 (358.0, 1500.0)	631.0 (320.0, 1210.3)	0.010	597.5 (350.8, 1435.0)	639.5 (324.0, 1210.3)	0.454
TROPONINE first	..	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)	<0.001	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)	0.146

Total patients refers to the 3,764 enrolled patients (347 patients who received methylprednisolone for COVID-19 and 3,417 patients who did not receive methylprednisolone for COVID-19), subjected to propensity score matching (PMS) in this study. Categorical variables were presented as frequency (%), and chi-square tests were used for comparisons (weighted chi-square tests were used for matched data). Variables that were normally distributed were expressed as mean ± standard deviation (SD) and compared using an independent t-test (a weighted independent t-test was used for matched data). Non-normal continuous data were summarized as medians (Q1, Q3) and compared using the Mann-Whitney U test (the weighted Mann-Whitney U test was used for matching data). P values of less than 0.05 were considered statistically significant.

of potential confounders between the IFN-α and non-IFN-α groups (*table 1*). After applying PSM, as shown in *figure 2*, all absolute SMDs of all confounders were less than 0.1, variance ratios were close to one, and Kolmogorov-Smirnov statistics were close to zero, indicating a good balance of covariate distribution between the groups.

Primary outcome

No significant difference was observed between the survival curves of the IFN-α group and those of the control group (p=0.340) (*figure 3A*). As shown in *table 2*, the unadjusted impact of receiving IFN-α on the risk of COVID-19 death was significant (HR: 0.86, 95% CI: 0.75, 0.99) compared with those who did not receive IFN-α. However, no significant impact of receiving IFN-α on the risk of COVID-19 death was observed based on the full adjusted method (HR: 1.04, 95% CI: 0.86, 1.25), stepwise model (HR: 1.03, 95% CI: 0.86, 1.24), or propensity score (PS)-matched method (HR: 0.90, 59% CI: 0.72, 1.12).

Compared with those who received other treatments, the effect of IFN-α plus remdesivir on COVID-19-related death was insignificant (HR: 88, 95% CI: 0.68, 1.15). Further, the combination of IFN-α and remdesivir had no significant impact on the risk of COVID-19 death compared with those who received IFN-α alone (HR: 89, 95% CI: 0.74, 1.34). As shown in *figures 3B,C*, there was no significant difference in survival between patients receiving combination treatments and those receiving other treatments or IFN-α alone.

Secondary outcome

Compared to those who did not receive IFN-α, the odds of ICU admission were not significantly associated with receiving interferon PSM (OR: 1.05, 95% CI: 0.82, 1.33). Neither of the combination therapies significantly impacted the odds of admission to the ICU. Accordingly, the risk of ICU admission was not significantly influenced by receiving IFN-α plus remdesivir compared to those in the control group (OR: 1.05, 95% CI: 0.81, 1.36). Similarly, IFN-α plus remdesivir, compared to IFN-α alone, had no significant impact (OR: 1.03, 95% CI: 0.77, 1.37) (*table 2*).

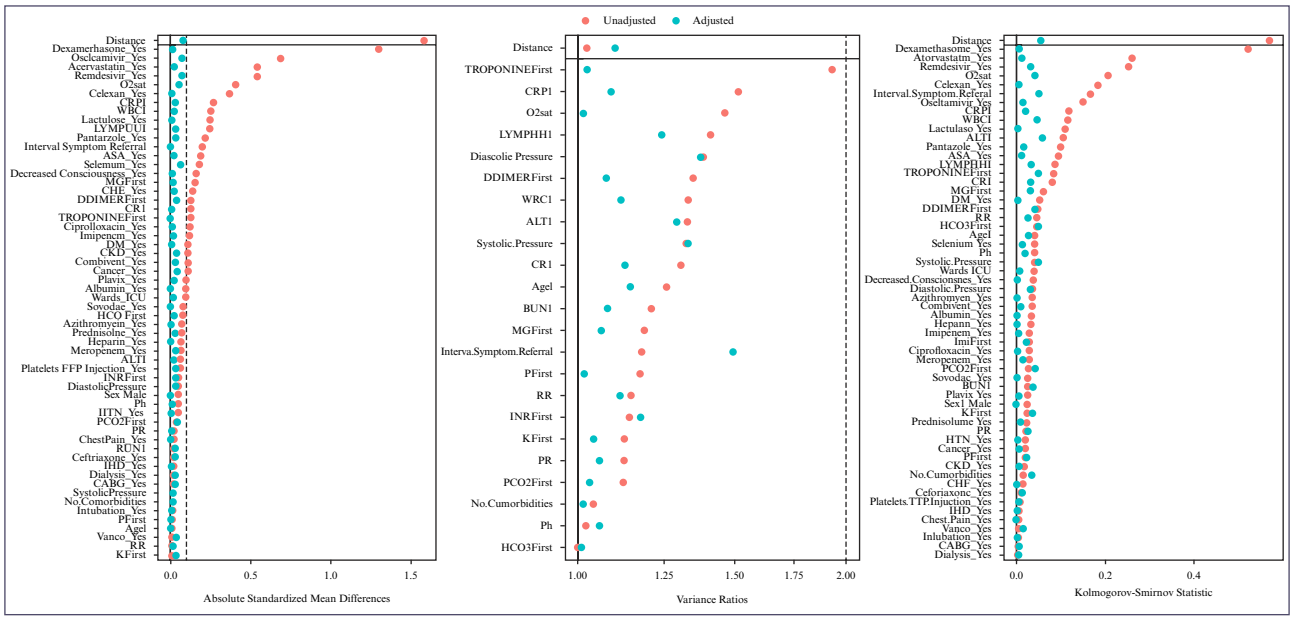

Figure 2
The standardized mean difference, variance ratio, and Kolmogorov-Smirnov statistic of covariate before (unadjusted) and after (adjusted) score matching.

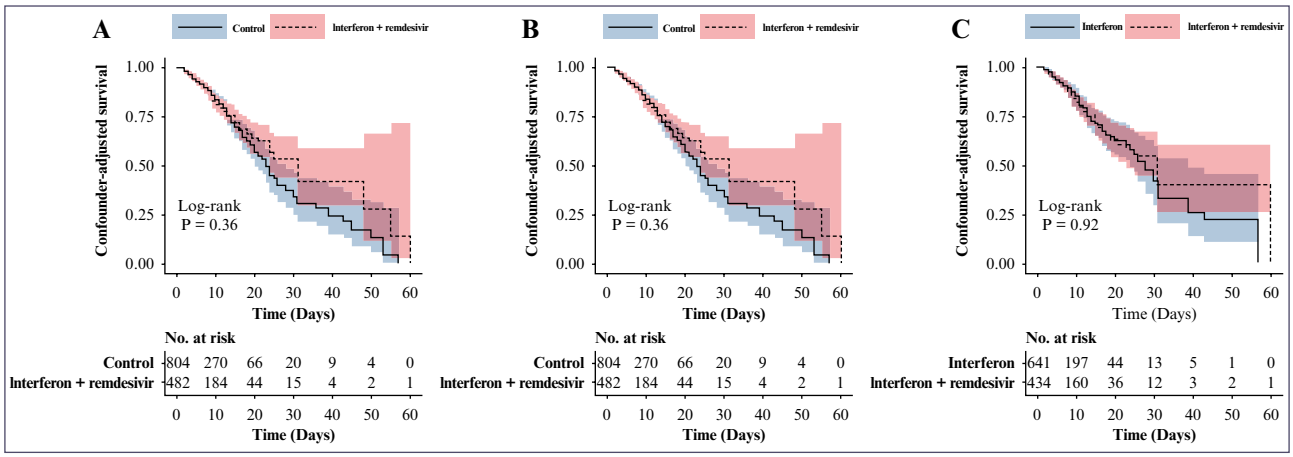

Figure 3
The confounder-adjusted survival of: IFN-*α* versus the control group (**A**), IFN-*α* plus remdesivir versus the control group (**B**), and IFN-*α* plus remdesivir versus IFN-*α* (**C**).

Table 2.
The impact of treatments on the risk of COVID-19 death and the need for ICU admission.

		Mortality		ICU admission	
	Models	**HR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**
IFN-α	Unadjusted	0.86 (0.75, 0.99)	0.043	1.38 (1.18, 1.62)	<0.001
IFN-α	Full adjusted*	1.04 (0.86, 1.25)	0.301	1.03 (0.83, 1.28)	0.774
IFN-α	Stepwise#	1.03 (0.86, 1.24)	0.721	1.04 (0.85, 1.26)	0.724
IFN-α	PS matched†	0.90 (0.72, 1.12)	0.353	1.05 (0.82, 1.33)	0.713
IFN-α + remdesivir vs. control	PS matched†	0.88 (0.68, 1.15)	0.353	1.05 (0.81, 1.36)	0.710
IFN-α + remdesivir vs. IFN-α	PS matched†	0.99 (0.74, 1.34)	0.964	1.03 (0.77, 1.37)	0.859

Note: The impact of IFN-α (plus remdesivir) on the risk of COVID-19 death was evaluated using the Cox proportional model. The impact of atorvastatin on ICU admission odds was examined using logistic regression.

*Adjusted for all variables included in the study

#Adjusted with variables after using the backward method with Wald statistic.

†Greedy nearest-neighbour matching.

Days of hospitalization

Table 3 shows a comparison of the number of days of hospitalization between the treated and untreated groups. Accordingly, the duration of hospitalization was not significantly longer in the IFN-α group compared to the control groups (7.00 vs. 6.00; $p<0.001$). However, the difference was significant for IFN-α plus remdesivir compared to controls (7.60 vs. 6.74; $p=0.004$), as well as for IFN-α plus remdesivir compared to IFN-α alone (8.00 vs. 7.00, $p<0.001$).

DISCUSSION

This large-scale multicentre retrospective study aimed to evaluate the effect of IFN-α administration on the mortality rate, ICU admission, and hospital duration of COVID-19 patients. Several other retrospective cohorts have investigated the impact of IFN-α in patients with COVID-19 [18-23]. However, some of their results are inconsistent with our study [18, 21-23]. Also, the results of three clinical trials have revealed promising effects of IFN therapy in hospitalized patients [24-26].

These conflicting results may be due to the significant heterogeneity of the studies regarding the confounding factors, time, subtype and the form of IFN administration, drug combinations, and the exclusion criteria of the study populations.

We endeavored to minimize the effect of confounders and disease severity by PSM of comorbidities, symptoms, laboratory examinations, and other treatments. Based on our PSM analysis, IFN-α does not affect COVID-19-related mortality and ICU admission. In contrast, an unadjusted analysis of mortality showed a reduction in mortality and an increase in ICU admission. This difference underlines the importance of confounders when interpreting the results of observational studies.

PSM can reduce the effect of confounding factors and change the results of retrospective studies. This statistical method was used in a retrospective cohort study by Li *et al.* [19]. In this study, the risk of 30-day mortality was not significantly different between the RBV/IFN-α group and the non-RBV/IFN-α group. Similar results were observed when a PS-matched cohort was analysed. In another retrospective study, Hao *et al.* revealed contrary results before and after the use of the PSM method, showing that

Table 3.
Total number of days of hospitalization and matched propensity score.

Exposure	Data	Level	Median (IQR)	P value
IFN-α	Total data	Non-receivers	6.00 (4.00, 10.00)	<0.001
		IFN-α	7.00 (5.00, 11.00)	
	Matched data	Non-receivers	6.07 (3.61, 10.37)	0.217
		IFN-α	6.52 (4.05. 10.36)	
IFN-α and remdesivir	Total data	Non-receivers	7.00 (4.00, 10.00)	<0.001
		IFN-α and remdesivir	8.00 (6.00, 12.00)	
	Matched data	Non-receivers	6.74 (4.28, 11.11)	0.004
		IFN-α and remdesivir	7.60 (5.06, 11.55)	
IFN-α and remdesivir	Total data	IFN-α	7.00 (4.00, 11.00)	<0.001
		IFN-α and remdesivir	8.00 (6.00, 12.00)	
	Matched data	IFN-α	6.40 (4.00, 10.35)	<0.001
		IFN-α and remdesivir	7.50 (4.97, 11.03)	

Note: The Mann-Whitney U test was used to compare the median number of hospitalization days between the groups for the total data. The weighted Mann-Whitney U test was used to analyse the median number of hospitalization days between groups for matched data.

Table 4.
Current studies investigating the effect of interferon treatment on COVID-19 patients.

Author, year, country	Type of study and method	Arms and population (*N*)	Setting and context	Finding
Our Finding	Observational with PSM	IFN-α (851) vs no IFN-α (851)	Moderate and severe	IFN-α has no to a minimal effect on COVID-19 outcome, with or without remdesivir as an add-on therapy.
Hung et al, 2020, China (36)	RCT	Lopinavir-ritonavir plus IFN-beta 1β (127) vs Lopinavir-ritonavir (86)	Mild to moderate patients	Early triple therapy (interferon plus lopinavir-ritonavir) was superior to lopinavir-ritonavir alone for decreasing LOS
Li et al, 2021, China (19)	Observational with PSM	Ribavirin or IFN-α (1281) vs neither (756)		Ribavirin/IFN-α was not associated with improved outcome
Gong, 2021, China	Retrospective cohort			

RCT: randomized clinical trial; IFN: interferon; PSM: propensity score matching.

hospitalization and reduced virus-shedding time were insignificant after PSM in patients using IFN-α2b spray inhalation treatment compared to controls [27]. Also, patients' weight has been introduced as one of the confounding factors affecting the effectiveness of COVID-19 treatment [28]. This statistical method can also help to reduce this confounding factor.

The time of IFN-α administration is another important point. Several researchers believe that IFN therapy should be used only in the early stages of infection [29-31]. In a retrospective study, IFN-α2b administered within five days of admission led to a favorable clinical response [18]. In another study that examined the combined effect of IFN-α2b and arbidol in moderate COVID-19 patients, the impact of early treatment was confirmed [22]. Contrary, Wong *et al*., in a retrospective cohort, reported that IFN-α2b does not reduce the risk of the composite outcome of death, invasive mechanical ventilation, or intensive care unit admission when administered within seven days of symptom onset, however, this risk may be increased when administered after seven days of symptom onset [32]. A possible explanation for the differences in our study results may be the lack of information on the early and late administration of IFN-α.

Different subtypes of IFN-α may have other effects and may be responsible for some of the controversies in the related trials. This study is limited to certain types of IFN, however, Li *et al*. showed no significant difference in efficacy between various subtypes of IFN-α (IFN-α2a, IFN-α2b, and IFN-α1b) [19]. We suggest future studies to compare the potential differences in this regard. Also, further studies are required to compare the effects of different forms of interferon. Pegylated IFN-α2b has been used subcutaneously in two studies. Compared to conventional IFN, polymer polyethylene glycol (PEG) conjugated to IFN has been shown to have an extended half-life; hence, a more significant antiviral effect [33]. Besides, using the intramuscular-injection form of interferon has demonstrated positive results [23]. In this study, we used the subcutaneous form of administration, which could be the reason for the conflicting results of some other studies. In the form of interferon nasal drops, it is proposed as a preventive medication for medical staff with COVID-19 [26]. In an uncontrolled exploratory study, Zhou *et al*. found that nebulized IFN-α2b accelerates viral clearance from the respiratory tract and facilitates the resolution of systemic inflammatory processes [21]. On the contrary, Hao *et al*. revealed that IFN-α2b spray inhalation does not significantly affect virus clearance among mild patients [27]. IFN-α inhalation appears to have beneficial effects on the liver during COVID-19 infection. It has been shown that IFN-α inhalation can reduce the risk of alanine aminotransferase increase in patients with or without non-alcoholic fatty liver disease [34]. More clinical trials are needed to confirm the

definitive effect of IFN-α and compare the impact of nasal drops and the subcutaneous form of IFN-α.
The discrepancy in exclusion criteria of the studies is another potential factor contributing to the controversial conclusions about the effect of IFN-α therapy on COVID-19. For example, Li *et al*. excluded patients who were intubated or were under 18 years old from their research [19], while in our study, these patients received IFN-α therapy, as shown in *table 4*.
An *in vitro* study that investigated the combined effect of IFN-α with other drugs for treating SARS-CoV-2 found that combining IFN-α and remdesivir can protect cells from viral infection more efficiently and at lower concentrations than IFN-α alone [35]. However, we did not find any significant effect of IFN-α with or without remdesivir in reducing COVID-19 fatality. Our analysis showed a significant positive impact regarding the duration of hospitalization in the IFN-α plus remdesivir group compared with the control or IFN-α alone group. We believe that this positive effect is due to remdesivir, an effective confirmed antiviral, and it is possible that interferon does not have additional antiviral properties. More clinical trials are needed to compare the effects of IFN and remdesivir, or the favourable impact of their combination as a treatment strategy for COVID-19.

Limitations

This study faces limitations that should be considered before the interpretation of results. As a first restriction, the exact dose and timing of antiviral drug administrations were unavailable. We used disease progression and mortality to measure drug efficacy. Also, the dosage of administered IFN-α was not available in this study. Due to this, we could not estimate the dose-dependent effect of IFN-α and provide a proper recommendation. In addition, our outcome was limited to in-hospital death. Although many patients received a routine protocol, a patient could have been withdrawn from the treatment because of death, discharge, or clinician opinion.

CONCLUSION

In conclusion, our findings indicate that subcutaneous administration of IFN-α, with or without remdesivir, does not have any significant impact on COVID-19 mortality and ICU admission. Future clinical trials considering the time, subtype, and form of IFN-α administration are warranted to investigate the potential therapeutic effects of IFN-α in patients with COVID-19.

DISCLOSURE.

Financial support: none. ***Conflict of interest:*** none.

Author Contributions: *Conceptualization,* **M.**A.P, S.A.A.S.N, A.R.J, A.S; *Methodology*, M.A.L, M.A.P, A.R.J; *Software and visualization*, M.A.L.; *Formal analysis*, M.A.L.; *Investigation*, N.A., F.G, N.Z, Z.A, A.S; *Resources*, M.A.P; *Data curation*, S.A.A.S.N.; *Writing original draft preparation*, A.R.J., N.A., M.A.L, F.S, B.M, P.J, O.Y.; *Literature review and revision*, B.M, P.J, A.S; *Supervision*, M.A.P., S.A.A.S.N., A.S; All authors contributed to editing and revising the original draft and have agreed to the published version of the manuscript.

Availability of data and materials. The data used in the current study are available from the corresponding author upon reasonable request. All descriptive statistics are available in the main text or supplementary files.

Ethics approval and consent to participate. The institutional review board granted ethical approval for this study, and data confidentiality was not relevant during the study. The study followed the Helsinki guideline for clinical research in humans. Patients were informed of the study purpose, enrolled voluntarily, and could exit the study at their desire.

REFERENCES

1. Raoufi M, Naini SAAS, Azizan Z, *et al.* Correlation between chest computed tomography scan findings and mortality of COVID-19 cases; a cross sectional study. *Arch Acad Emerg Med* 2020 ; 8.
2. Lee JS, Park S, Jeong HW, *et al.* Immunophenotyping of COVID-19 and influenza highlights the role of type I interferons in development of severe COVID-19. *Sci Immunol* 2020 ; 5 : eabd1554.
3. Lucas C, Wong P, Klein J, *et al.* Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature* 2020 ; 584 : 463-9.
4. Karki R, Sharma BR, Tuladhar S, *et al.* Synergism of TNF-α and IFN-γ triggers inflammatory cell death, tissue damage, and mortality in SARS-CoV-2 infection and cytokine shock syndromes. *Cell* 2021 ; 184 : 149-68.
5. Bergamaschi L, Mescia F, Turner L, *et al.* Longitudinal analysis reveals that delayed bystander CD8+ T cell activation and early immune pathology distinguish severe COVID-19 from mild disease. *Immunity* 2021 ; 54 : 1257-75.
6. Winkler ES, Bailey AL, Kafai NM, *et al.* SARS-CoV-2 infection of human ACE2-transgenic mice causes severe lung inflammation and impaired function. *Nat Immunol* 2020 ; 21 : 1327-35.
7. Sposito B, Broggi A, Pandolfi L, *et al.* The interferon landscape along the respiratory tract impacts the severity of COVID-19. *Cell* 2021 ; 184 : 4953-68.e16.
8. Galani IE, Rovina N, Lampropoulou V, *et al.* Untuned antiviral immunity in COVID-19 revealed by temporal type I/III interferon patterns and flu comparison. *Nat Immunol* 2021 ; 22 : 32-40.
9. Blanco-Melo D, Nilsson-Payant BE, Liu WC, *et al.* Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell* 2020 ; 181 : 1036-45.
10. Sujaritha J, Mathivathani K, Aravindh G, Gnanasekaran G. An overview of some drugs: lopinavir, ritonavir, chloroquine, hydroxy chloroquine and Interferon as a effective treatment against COVID-19. *Int J Res Phytochem Pharmacol* 2020 ; 10 : 20-4.
11. Rameshrad M, Ghafoori M, Mohammadpour AH, Nayeri MJD, Hosseinzadeh H. A comprehensive review on drug repositioning against coronavirus disease 2019 (COVID19). *Naunyn Schmiedebergs Arch Pharmacol* 2020 ; 393 : 1137-52.
12. Wang EY, Mao T, Klein J, *et al.* Diverse functional autoantibodies in patients with COVID-19. *Nature* 2021 ; 595 : 283-8.
13. Bastard P, Rosen LB, Zhang Q, *et al.* Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science* 2020 ; 370 : eabd4585.
14. Pairo-Castineira E, Clohisey S, Klaric L, *et al.* Genetic mechanisms of critical illness in COVID-19. *Nature* 2021 ; 591 : 92-8.
15. Hatamabadi H, Sabaghian T, Sadeghi A, *et al.* Epidemiology of COVID-19 in Tehran, Iran: a cohort study of clinical profile, risk factors, and outcomes. *Biomed Res Int* 2022 ; 2022 : 2350063. 10.1155/2022/2350063.

16. Zöller D, Wockner LF, Binder H. Automatic variable selection for exposure-driven propensity score matching with unmeasured confounders. *Biom J* 2020 ; 62 : 868-84.

17. Austin PC. Optimal caliper widths for propensity-score matching when estimating differences in means and differences in proportions in observational studies. *Pharm Stat* 2011 ; 10 : 150-61.

18. Wang N, Zhan Y, Zhu L, *et al.* Retrospective multicenter cohort study shows early interferon therapy is associated with favorable clinical responses in COVID-19 patients. *Cell Host Microbe* 2020 ; 28 :455-64. e2.

19. Li H, Xiong N, Li C, *et al.* Efficacy of ribavirin and interferon-α therapy for hospitalized patients with COVID-19: a multicenter, retrospective cohort study. *Int J Infect Dis* 2021 ; 104 : 641-8.

20. Xu P, Huang J, Fan Z, *et al.* Arbidol/IFN-α2b therapy for patients with corona virus disease 2019: a retrospective multicenter cohort study. *Microbes Infect* 2020 ; 22 : 200-5.

21. Zhou Q, Chen V, Shannon CP, *et al.* Interferon-α2b Treatment for COVID-19. *Front Immunol* 2020 ; 11 : 1061.

22. Yin P, Meng J, Chen J, *et al.* Antiviral drugs arbidol and interferon alpha-1b contribute to reducing the severity of COVID-19 patients: a retrospective cohort study. *Virol J* 2021 ; 18 : 142.

23. Pereda R, González D, Rivero HB, *et al.* Therapeutic effectiveness of interferon-α2b against COVID-19: the cuban experience. *J Interferon Cytokine Res* 2020 ; 40 : 438-42.

24. Bhushan BLS, Wanve S, Koradia P, *et al.* Efficacy and safety of pegylated interferon-α2b in moderate COVID-19: a phase 3, randomized, comparator-controlled, open-label study. *Int J Infect Dis* 2021 ; 111 : 281-7.

25. Pandit A, Bhalani N, Bhushan BLS, *et al.* Efficacy and safety of pegylated interferon alfa-2b in moderate COVID-19: a phase II, randomized, controlled, open-label study. *Int J Infect Dis* 2021 ; 105 : 516-21.

26. Meng Z, Wang T, Chen L, *et al.* The effect of recombinant human interferon alpha nasal drops to prevent COVID-19 pneumonia for medical staff in an epidemic area. *Curr Top Med Chem* 2021 ; 21 : 920-7.

27. Hao SR, Yan R, Zhang SY, *et al.* Interferon-α2b spray inhalation did not shorten virus shedding time of SARS-CoV-2 in hospitalized patients: a preliminary matched case-control study. *J Zhejiang Univ SCIENCE B* 2020 ; 21 : 628-36.

28. Rao X, Wu C, Wang S, *et al.* The importance of overweight in COVID-19: a retrospective analysis in a single center of Wuhan, China, *Medicine* 2020 ; 99 : e22766.

29. Sallard E, Lescure FX, Yazdanpanah Y, Mentre F, Peiffer-Smadja N. Type 1 interferons as a potential treatment against COVID-19. *Antiviral Res* 2020 ; 178 : 104791.

30. To KKW, Tsang OTY, Leung WS, *et al.* Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* 2020 ; 20 : 565-74.

31. Siddiqi HK, Mehra MR. COVID-19 illness in native and immunosuppressed states: a clinical–therapeutic staging proposal. *J Heart Lung Transplant* 2020 ; 39 : 405-7.

32. Wong CKH, Wan EYF, Luo S, *et al.* Clinical outcomes of different therapeutic options for COVID-19 in two Chinese case cohorts: a propensity-score analysis. *EClinicalMedicine* 2021 ; 32 : 100743. DOI: 10.1016/j.eclinm.2021.100743.

33. Wedemeyer H, Wiegand J, Cornberg M, Manns MP. Polyethylene glycol–interferon: current status in hepatitis C virus therapy. *J Gastroenterol Hepatol* 2002 ; 17 : S344-S50.

34. Huang R, Zhu L, Wang J, *et al.* Clinical features of patients with COVID-19 with nonalcoholic fatty liver disease. *Hepatology Commun* 2020 ; 4 : 1758-68.

35. Ianevski A, Yao R, Zusinaite E, *et al.* Synergistic interferon-alpha-based combinations for treatment of SARS-CoV-2 and other viral infections. *Viruses* 2021 ; 13 : 2489.

36. Hung IFN, Lung KC, Tso EYK, *et al.* Triple combination of interferon beta-1b, lopinavir–ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. *Lancet* 2020 ; 395 : 1695-704.