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The dual role of anti-viral therapy in the treatment of Coronavirus disease 2019

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Abstract. – OBJECTIVE: Coronavirus disease 2019 (COVID-19) has become a worldwide public health emergency; unfortunately, there is currently no treatment for improving outcomes or reducing viral-clearance times in infected patients. The aim of the present study was to evaluate the efficacy of interferon (IFN) with or without lopinavir and ritonavir as antiviral therapeutic option for treating COVID-19 infection.

PATIENTS AND METHODS: The present study enrolled 148 patients that received either standard care, treatment with IFN alfa-2b, or IFN alfa-2b combined with lopinavir plus ritonavir. Viral testing was performed using Reverse-Transcription Polymerase Chain Reaction (RT-PCR).

RESULTS: There was no significant difference in the viral-clearance time at 28 days after treatment between patients receiving standard care and those receiving anti-viral treatments. However, the average viral-clearance time of patients receiving standard care (14 days) was shorter than that for patients receiving IFN alfa-2b or IFN alfa-2b combined with lopinavir plus ritonavir (15.5 or 17.5 days) ($p<0.05$). Patients treated with IFN alfa-2b within five days or IFN alfa-2b combined with lopinavir plus ritonavir after three days of symptoms exhibited shorter viral-clearance times than the other groups ($p<0.05$). Moreover, viral-clearance times were significantly longer in patients receiving standard care or anti-viral treatment 5 days after symptoms appeared than those of patients who received these treatments within five days of symptom onset ($p<0.05$).

CONCLUSIONS: Early symptomatic treatment is most critical for maximizing amelioration of COVID-19 infection. Anti-viral treatment might have complicated effect on viral-clearance.

Key Words:
Coronavirus disease 2019, Early treatment, Viral clearance, Antiviral treatment, Standard care.

Introduction

The global pandemic of novel coronavirus disease 2019 (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and began in December 2019 in Wuhan, China[1-3]. SARS-CoV-2 has since spread to >200 countries and resulted in >821,000 deaths[4]. Clinical symptoms are consistent with respiratory infections, with severities ranging from a mild illness resembling the common cold to severe viral pneumonia leading to a potentially fatal acute respiratory distress syndrome[5].

According to genetic analysis, SARS-CoV-2 is similar to SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV). Diagnosing COVID-19 requires analysis of SARS-CoV-2 RNA *via* Reverse-Transcription Polymerase Chain Reaction (RT-PCR) or genetic sequencing of respiratory or blood specimens. All tests in China are performed according to the latest guidelines for the Diagnosis and Treatment of Pneumonitis Caused by 2019-nCoV (trial seventh version) published by the Chinese government and World Health Organization[3,6]. Additionally, vascular enlargement and interlobular septal thickening are defined as common computed tomographic features of COVID-19[3,7].

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There are currently >300 active clinical treatment trials underway; however, there is no evidence from randomized clinical trials that any treatment improves outcomes in patients with either suspected or confirmed COVID-19, including treatment with remdesivir, lopinavir-ritonavir, or other antiviral therapies[8-10]. Chinese guidelines list interferons (IFNs) as an alternative treatment for this combination therapy. *In vitro*, IFNs are partially effective against coronaviruses[11], with previous studies detecting that early IFN-I administration effectively inhibits viral replication and produces positive outcomes in infected mice[12,13]. However, treatment of MERS with ribavirin or lopinavir/ritonavir in combination with IFNs have yielded no discernible effects on clinical outcomes or viral clearance[13,14]. Furthermore, no animal studies or human trials have been performed to assess IFN efficacy in treating COVID-19 infection, and it remains uncertain whether these treatments confer protection for patients already receiving these drugs for other conditions[15,16].

In the present study, we conducted a retrospective analysis to elucidate the effects of standard care and antiviral therapy on viral clearance and patient outcomes in a cohort of hospitalized COVID-19 patients.

Patients and Methods

Patients

The severity of COVID-19 infection was defined according to the Chinese management guidelines for COVID-19 (version 7.0). We excluded patients receiving other antiviral treatments and with more than two consecutive negative results upon hospitalization in order to analyze the viral-clearance effect of IFN alfa-2b with lopinavir and ritonavir. The 148 enrolled patients were assigned to three groups. Group 1 (n = 46) received only standard care, Group 2 (n = 37) received IFN alfa-2b (5 million U twice daily, atomization inhalation), and Group 3 (n = 65) received IFN alfa-2b (5 million U twice daily, atomization inhalation) with lopinavir and ritonavir (500 mg twice daily, orally), patients received anti-viral treatment until they obtained negative viral result. All 148 patients received standard care as necessary, which included supplemental oxygen, noninvasive and invasive ventilation, antibiotic agents to prevent bacterial infections, vasopressor support, renal-replacement therapy, and extracorporeal membrane oxygenation. To analyze the time-dependent effects of the treatments, 33 patients with positive or unascertainable results by the time of analysis were excluded (Figure 1).

Viral Detection

Respiratory specimen was the total RNA from nasopharyngeal swab, RT-PCR was conducted using primers and probes targeting the ORF1ab and N genes of COVID-19. Reactions and amplification conditions were performed according to manufacturer guidelines (Shanghai BioGerm Medical Technology Co. Ltd., Shanghai, China). The results were considered positive when the cycle threshold (Ct) values of both target genes exceeded 38[17].

The primary endpoint was considered as two consecutive negative RT-PCR tests due to high rates of false-negative tests. Patients with axillary

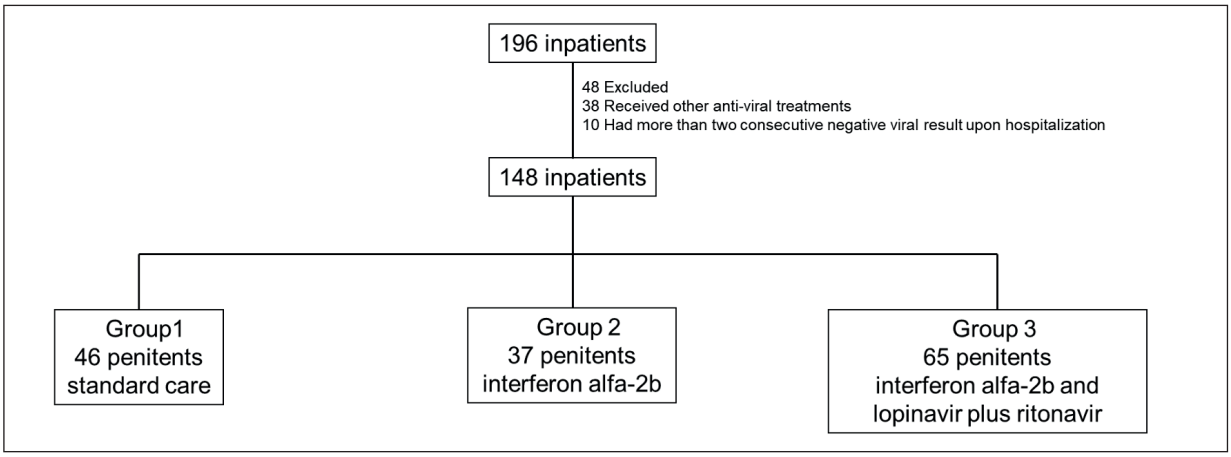

Figure 1. Patient demographics.

temperatures of at least 37.3°C were considered to have a fever. Re-positive was defined as having positive SARS-CoV-2 nucleic acids after two consecutive negative tests over a 72-h period. The viral-carrying time was defined as the time between symptoms appearing and two consecutive negative tests over a 72-h period (https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html). Exposure history was defined as exposure to patients with confirmed SARS-CoV-2 infections or a history of travel to Wuhan, China. Ventilator-associated pneumonia was diagnosed according to treatment guidelines for hospital-acquired and ventilator-associated pneumonia. Acute kidney injuries were diagnosed according to the clinical practice guidelines of the Kidney Disease Improving Global Outcomes, and acute respiratory distress syndrome was diagnosed according to the Berlin Definition.

Statistical Analysis

No calculations of statistical sample size were performed beforehand; the sample size was equal to the number of patients treated during the study period. Continuous variables are presented as the medians and interquartile ranges (IQRs) with 95% confidence intervals (CIs). Categorical variables are expressed as the percentage of patients with 95% CIs. Differences in the distribution of patient characteristics (by median-age subgroups and by presence/absence of hypertension) are reported using differences with 95% CIs.

A paired-sample *t*-test and one-way analysis of variance were used to compare nonparametric continuous variables between median-age subgroups and patients with or without hypertension. A χ^2 or Fisher's exact test was used for categorical variables, as needed. All statistical tests were two-tailed, and statistical significance was defined as a $p<0.05$. Analyses were performed using SPSS (v.9.4; SPSS Inc., Chicago, IL, USA). The analyses were not adjusted for multiple comparisons, and given the possibility of a type-I errors, the findings should be interpreted as exploratory and descriptive.

Results

Patient Demographics and Baselines

Of the 148 patients in this study, 46 received standard care (Group 1), 37 were treated with IFN alfa-2b (Group 2) and 65 were treated with IFN alfa-2b combined with lopinavir plus ritonavir (Group 3). The median age of the patients was 38 years (interquartile range [IQR]: 29-55.8 years), and 48.0% of the patients were men. There were no significant differences in demographics or in the results of baseline laboratory tests among the three groups (Table I). During the study period, 56 patients were administered noninvasive ventilation, six patients underwent invasive ventilation, and two patients received extracorporeal membrane oxygenation.

Table I. Baseline demographic and clinical characteristics of patients.

Characteristics	Group 1 (*n* = 46)	Group 2 (*n* = 37)	Group 3 (*n* = 65)
Age, median (IQR)	48.5 (45)	37 (32.5)	37 (14)
Male/Female, *n* (%)	18 (39.1)	21 (56.8)	32 (49.2)
WBC (10^9/L), median (IQR)	3.9 (2.5)	3.7 (2.2)	4.2 (2.2)
PCT (μg/L), median (IQR)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)
PLT (10^9/L), median (IQR)	219 (72.5)	194 (118.3)	203 (87)
CK (U/L), median (IQR)	63.7 (68.1)	73.1 (65.7)	81.7 (76.1)
ALT (U/L), median (IQR)	23.0 (17.4)	26.5 (25.4)	25.1 (17.7)
AST (U/L), median (IQR)	26.7 (27.4)	23.1 (14.3)	24.1 (11.6)
Temp (°C), median (IQR)	38.2 (0.6)	38.5 (1.3)	38.3 (1.1)
Diabetes mellitus, *n* (%)	5 (22.7)	2 (13.3)	1 (4.2)
Cancer, *n* (%)	1 (4.5)	0 (0)	0 (0)

Laboratory values for WBC counts were obtained from 46, 37, and 65 patients from Groups 1, 2, and 3, respectively. PCT laboratory values were obtained from 41, 22, and 21 patients from Groups 1, 2, and 3, respectively. PLT values were obtained from 46, 36, and 65 patients from Groups 1, 2, and 3, respectively. ALT values were obtained from 41, 30, and 63 patients from Groups 1, 2, and 3, respectively. AST values were obtained from 32, 28, and 61 patients from Groups 1, 2, and 3, respectively. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; PCT, procalcitonin; PLT, platelet count.

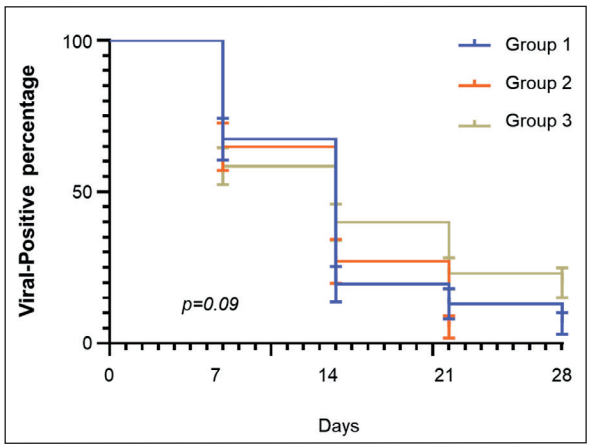

Figure 2. Viral-clearance rates of the three groups at 28 days after treatments. Of the 148 patients, 127 were virus-negative (Group 1 [blue], 93.5%; Group 2 [orange], 94.6%; and Group 3 [gray], 80%).

Combined Anti-viral Treatment Associated with Longer Viral-Clearance Time

All patients had viral-positive results at the first day of hospitalization. After 28 days of hospitalized treatment, 127 of the 148 patients were viral-negative. Furthermore, the 28-day viral-negative rate was 80% among patients treated with IFN alfa-2b combined with lopinavir plus ritonavir, which was significantly lower compared with that of patients who received only standard care (93.5%) and IFN alfa-2b (94.6%) (Figure 2). Among all patients, 23 patients had re-positive results during treatment. Patients administered IFN alfa-2b combined with lopinavir/ritonavir had a significantly higher re-positive rate (18/65; 27.7%) than that of the other two groups (Group 1: 2/46, 4.3%; and Group 2: 3/37, 8.1%), which may have been related to the lower 28-day viral-negative rate in Group 3.

Early Treatment is Critical for Ameliorating COVID-19

To evaluate the function of anti-viral treatment, we analyzed the viral-clearance time of the 3 groups. The average virus-carrying times of patients receiving standard care or IFN 2b were 14 days (IQR: 11–20), 15.5 days (IQR: 10.75–23.75) and 17.5 days (IQR: 12–25.75) ($p < 0.05$) days, respectively (Figure 3A).

When patients were administered standard care, IFN alfa-2b or IFN alfa-2b combined with lopinavir plus ritonavir at five days after symptoms appeared, the viral-clearance times were 18 days (IQR:14.5-22.0), 21 days (IQR: 14.8-28.0), or 17.5 days (IQR: 12.25-31.75) respectively, which were significantly longer than those resulting from early treatments (11.5, 11 or 17 days respectively; $p < 0.05$) (Figure 3B). Similar results were observed when treatment was administered at three days in or after three-symptom onset (**Supplementary Figure 1**).

Anti-viral Treatment Showed a Time-Dependent Bidirectional Effect in Reducing Viral-Clearance Time

When patients were administered IFN alfa-2b treatment within five days of symptom onset (fever or cough), the virus-carrying time was re-

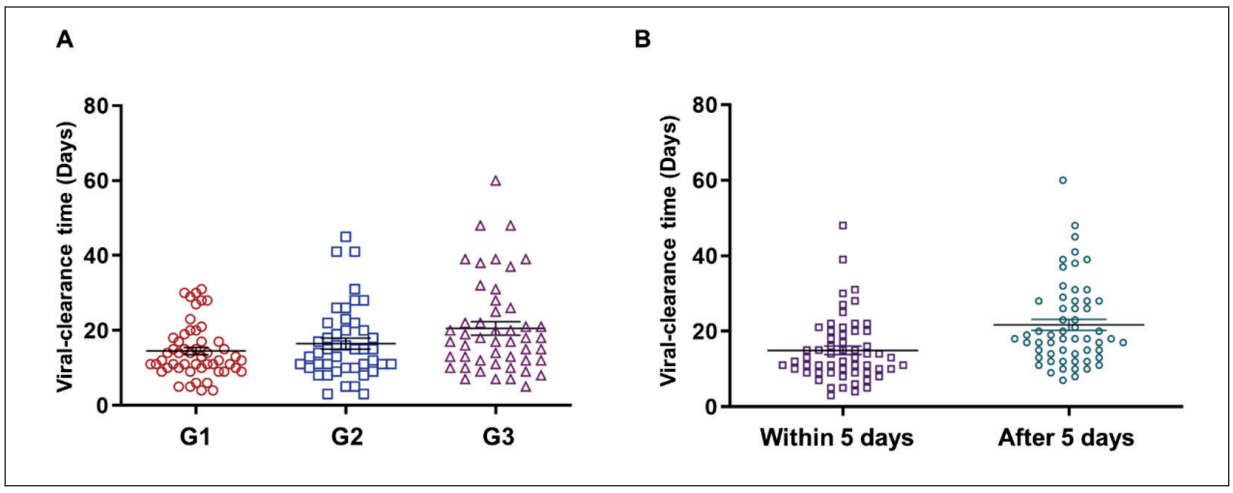

Figure 3. The effects of anti-viral treatment. A, Viral-clearance times when receiving standard care (Group 1), interferon alfa-2b (Group 2) or interferon alfa-2b combined with lopinavir plus ritonavir (Group 3). B, Viral-clearance times of the patients received treatment within or after five days of symptom onset.

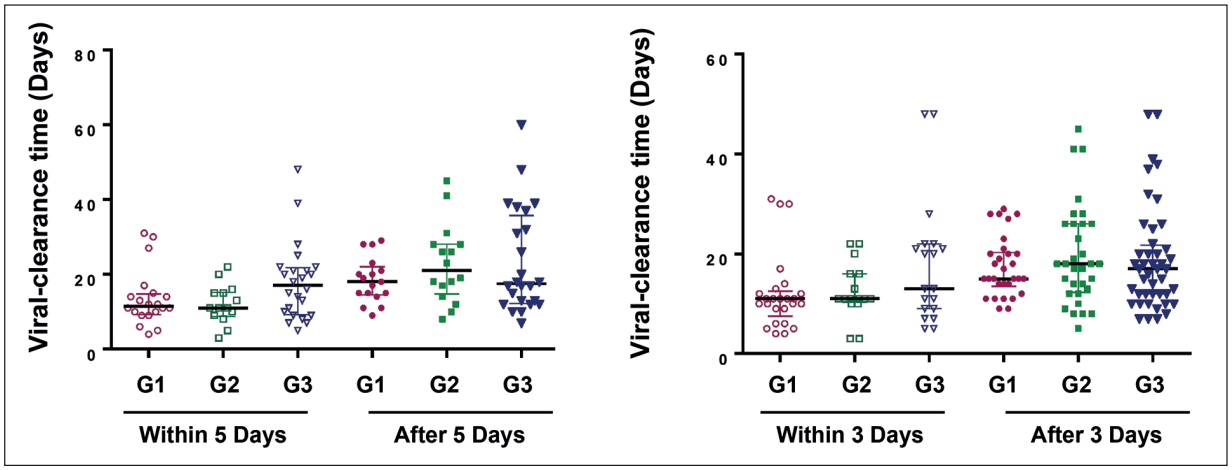

Figure 4. Time-dependent viral-clearance time for patients.

duced to 11 days (IQR: 8.75-15.25 days), which was significantly shorter than that of patients receiving only standard care and IFN alfa-2b combined with lopinavir plus ritonavir treatment (11.5 days; IQR, 9.3-14.8 days and 17 days; IQR, 9.25-21.75 days) ($p < 0.05$), on the contrary, when received late treatments with IFN alfa-2b combined with lopinavir plus ritonavir treatment (five days after the symptom onset), patients had a shorter viral-clearance time (17.5; IQR,12.25-23.75 days) than the other two groups (18 days; IQR, 14.5-22 days and 21 days; IQR, 14.75-28 days (Figure 4A).

However, patients receiving IFN alfa-2b treatment within three days of symptom onset did not display a shorter viral-clearance time compared to that of patients receiving standard care (clearance time in both: 11 days). Moreover, the IFN alfa-2b combined with lopinavir plus ritonavir treatment at three days after symptoms appeared showed a shorter viral-clearance time, which is consistent with the result of the treatment after five days (Figure 4B).

Discussion

The results of our present study indicate that no benefit was obtained from administering IFN alfa-2b combined with lopinavir plus ritonavir, which is consistent with a previous study[9]. In contrast, we found that this treatment actually led to higher re-positive rates; however, the mechanism of this unexpected outcome requires further study.

The time between symptom onset and viral clearance is crucial to analyze the effect of active antiviral agents, because the viral load of SARS-CoV-2 peaks at approximately the time of symptom onset[18]. Patients may benefit from early IFN treatment due to the functions of type-I IFN signaling related to immune activity[19]. During the response to COVID-19 infection, IFN might play an important role in cytokine storms[20], which may cause an imbalanced immune response and concomitant organ damage; however, the precise immune-response mechanism related to COVID-19 infection remains unclear.

Our present results suggest that early treatment with IFN alfa-2b or later treatment with IFN alfa-2b combined with lopinavir plus ritonavir may assist in fighting COVID-19 infection, although this effect was minimal relative to the outcomes associated with early treatment of the disease. Furthermore, the long-term prognosis of patients receiving these treatments remains unclear.

Conclusions

Anti-viral treatment might have complicated effect on viral-clearance. Early symptomatic treatment is most critical for maximizing amelioration of COVID-19 infection. Treating patients with IFN alfa-2b within five days or IFN alfa-2b combined with lopinavir plus ritonavir five days after the symptom onset may accelerate viral clearance. However, the optimal outcomes involved administration of appropriate treatments immediately upon symptom appearance. This finding had an important significance in reducing global medical consumption and patient mortality.

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Conflict of Interests

The authors declare that they have no conflict of interests.

References

1) Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical characteristics of 138 hospitalized patients with 2019 Novel Coronavirus-infected pneumonia in Wuhan, China. JAMA 2020; 323: 1061-1069.
2) Zu ZY, Jiang MD, Xu PP, Chen W, Ni QQ, Lu GM, Zhang LJ. Coronavirus Disease 2019 (COVID-19): a perspective from China. Radiology 2020; 296: E15-E25.
3) World Health Organization. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance, 2 March 2020. World Health Organization, 2020.
4) Surveillances VJCCW. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19)—China, 2020. Zhonghua Liu Xing Bing Xue Za Zhi 2020, 41: 145-151.
5) Pan L, Mu M, Yang P, Sun Y, Wang R, Yan J, Li P, Hu B, Wang J, Hu C, Jin Y, Niu X, Ping R, Du Y, Li T, Xu G, Hu Q, Tu L. Clinical characteristics of COVID-19 patients with digestive symptoms in hubei, china: a descriptive, cross-sectional, multicenter study. Am J Gastroenterol 2020; 115: 766-773.
6) Gao J, Tian Z, Yang X. Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends 2020; 14: 72-73.
7) Xia W, Shao J, Guo Y, Peng X, Li Z, Hu D. Clinical and CT features in pediatric patients with COVID infection: different points from adults. Pediatr Pulmonol 2020; 55: 1169-1174.
8) Battat R, Dulai PS, Ma C, Jairath V, Feagan BG, Sandborn WJ, Khanna R. Current endpoints of clinical trials in ulcerative colitis: are they valid? Curr Treat Options Gastroenterol 2020 Jan 4. doi: 10.1007/s11938-019-00259-w. Epub ahead of print.
9) Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Chen H, Li H, Huang H, Tu S, Gong F, Liu Y, Wei Y, Dong C, Zhou F, Gu X, Xu J, Liu Z, Zhang Y, Li H, Shang L, Wang K, Li K, Zhou X, Dong X, Qu Z, Lu S, Hu X, Ruan S, Luo S, Wu J, Peng L, Cheng F, Pan L, Zou J, Jia C, Wang J, Liu X, Wang S, Wu X, Ge Q, He J, Zhan H, Qiu F, Guo L, Huang C, Jaki T, Hayden FG, Horby PW, Zhang D, Wang C. A trial of lopinavir-ritonavir in adults hospitalized with severe Covid-19. N Engl J Med 2020; 382: 1787-1799.
10) Sanders JM, Monogue ML, Jodlowski TZ, Cutrell JB. Pharmacologic treatments for Coronavirus disease 2019 (COVID-19): a review. JAMA 2020; 323: 1824-1836.
11) Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Treatment of SARS with human interferons. Lancet 2003; 362: 293-294.
12) Falzarano D, de Wit E, Rasmussen AL, Feldmann F, Okumura A, Scott DP, Brining D, Bushmaker T, Martellaro C, Baseler L, Benecke AG, Katze MG, Munster VJ, Feldmann H. Treatment with interferon-α2b and ribavirin improves outcome in MERS-CoV-infected rhesus macaques. Nat Med 2013; 19: 1313-1317.
13) Chan JF, Yao Y, Yeung ML, Deng W, Bao L, Jia L, Li F, Xiao C, Gao H, Yu P, Cai JP, Chu H, Zhou J, Chen H, Qin C, Yuen KY. Treatment with lopinavir/ritonavir or interferon-β1b improves outcome of MERS-CoV infection in a nonhuman primate model of common marmoset. J Infect Dis 2015; 212: 1904-1913.
14) Hart BJ, Dyall J, Postnikova E, Zhou H, Kindrachuk J, Johnson RF, Olinger GG, Frieman MB, Holbrook MR, Jahrling PB, Hensley L. Interferon-β and mycophenolic acid are potent inhibitors of Middle East respiratory syndrome coronavirus in cell-based assays. J Gen Virol 2014; 95: 571-577.
15) Lee N, Allen Chan KC, Hui DS, Ng EK, Wu A, Chiu RW, Wong VW, Chan PK, Wong KT, Wong E, Cockram CS, Tam JS, Sung JJ, Lo YM. Effects of early corticosteroid treatment on plasma SARS-associated Coronavirus RNA concentrations in adult patients. J Clin Virol 2004; 31: 304-309
16) Russell CD, Millar JE, Baillie JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. Lancet 2020; 395: 473-475.
17) Yu F, Yan L, Wang N, Yang S, Wang L, Tang Y, Gao G, Wang S, Ma C, Xie R, Wang F, Tan C, Zhu L, Guo Y, Zhang F. Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients. Clin Infect Dis 2020; 71: 793-798.
18) Joynt GM, Wu WK. Understanding COVID-19: what does viral RNA load really mean? Lancet Infect Dis 2020; 20: 635-636.
19) Zhao J, Li K, Wohlford-Lenane C, Agnihothram SS, Fett C, Zhao J, Gale MJ Jr, Baric RS, Enjuanes L, Gallagher T, McCray PB Jr, Perlman S. Rapid generation of a mouse model for Middle East respiratory syndrome. Proc Natl Acad Sci U S A 2014; 111: 4970-4975.
20) Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 2020; 395: 1033-1034