**Title:** Efficacy and safety of interferon β-1a in treatment of severe COVID-19: A randomized clinical trial

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**Abstract**

Objectives: To the best of our knowledge, there is no published study regarding use of IFN β-1a in the treatment of severe COVID-19. In this randomized clinical trial efficacy and safety of IFN β-1a has been evaluated in patients with severe COVID-19.

Methods: Forty-two patients in the interferon group received IFN β-1a in addition to the national protocol medications (hydroxychloroquine plus lopinavir/ritonavir or atazanavir/ritonavir). Each 44 micrograms/ml (12 million IU/ml) of interferon β-1a was subcutaneously injected three times weekly for two consecutive weeks. The control group consisted 39 patients that received only the national protocol medications. Primary outcome of study was time to reach clinical response. Secondary outcomes were duration of hospital stay, length of ICU stay, 28-day mortality, effect of early or late administration of IFN on mortality, adverse effects and complications during the hospitalization.

Results: Between 29th February to 3rd April 2020, 92 patients were recruited that finally 42 patients in the IFN group and 39 patients in the control group completed the study. As primary outcome, time to the clinical response was not significantly different between the IFN and the control groups (9.7 ± 5.8 vs. 8.3 ± 4.9 days respectively, P=0.95). On day 14, 66.7% vs. 43.6% of patients in the IFN group and the control group were discharged, respectively (OR= 2.5; 95% CI: 1.05- 6.37). The 28-day overall mortality was significantly lower in the IFN than the control group (19% vs. 43.6% respectively, p= 0.015). Early administration significantly reduced mortality (OR=13.5; 95% CI: 1.5-118).

Conclusion: Although IFN did not change time to reach the clinical response, adding it to the national protocol significantly increased discharge rate on day 14 and decreased 28-day mortality.

**Key words:** COVID-19, Interferon, Clinical response, Mortality

## Introduction

Severe acute respiratory syndrome -coronavirus-2 (SARS-CoV-2) is a novel virus that has been introduced as the first pandemic of the century, since its first detection in late December, 2019 in China (1). The disease is named as corona virus disease 2019 (COVID-19) and manifests with dyspnea, fever, cough, myalgia and other flu-like symptoms (2). However, it can progress to more severe disease and cause acute respiratory distress syndrome (ARDS), organ failure and death (3). In the absence of definite treatment, the disease has caused more than 500,000 deaths worldwide in nearly six months (4).

Some antivirals are being examined in treatment of COVID-19. However, the results are not sound. Data in terms of efficacy of lopinavir-ritonavir were promising at first but recent randomized trial failed to show benefits, especially in later stages of the disease (5). Hydroxychloroquine is another available choice that has been included in many national protocols (6). However, it has also been advised to restrict the administration of this drug for clinical trials due to its questionable efficacy and risk of adverse effects (7). The race is still on to find an effective treatment for COVID-19. Most of the experience came from other corona virus epidemics; severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) (8).

Interferon (IFN) subtypes were previously examined in treatment of SARS and MERS. Primary in-vitro experience showed antiviral effects of IFNs, especially IFN-β and IFN-γ on SARS-CoV (9). Also same results were reported for IFN-β against MERS-CoV (10, 11). Later, in an animal model, higher antiviral activity of IFN-β compared to lopinavir-ritonavir was shown against MERS-CoV (12). The efficacy of IFN-β on MERS is still being investigated (13).

The antiviral effect of IFNs express through activating interferon-stimulated genes (ISGs) (14). Additionally, by decreasing the vascular leakage, IFN β-1a improved ARDS complications, regardless of its antiviral properties (15). Also the higher expression of a protein named CD-73 could lead to better prognosis in ARDS. However, this data was not replicated in a later trial (16).

To the best of our knowledge, there is no published study regarding use of IFN β-1a in the treatment of severe COVID-19. In this randomized clinical trial efficacy and safety of IFN β-1a has been evaluated in patients with severe COVID-19.

## Methods

### Study design

This open-label randomized clinical trial was conducted to assess efficacy and safety of IFN-β-1a in treatment of adult (aged ≥ 18 years old) patients diagnosed with COVID-19. Patients were admitted during 29th February to 3rd April 2020 in Imam Khomeini Hospital Complex, as main central hospital in Tehran, capital of Iran.

### Eligibility criteria

The diagnosis of COVID-19 was according to either a positive Real-Time Polymerase Chain Reaction (RT-PCR) of the deep nasopharyngeal secretions or clinical signs/symptoms plus

imaging findings highly suspicious for COVID-19. RNA extraction was performed using the viral nucleic acid extraction kit (Cat. No. YVN50/YVN100) provided by RBC Bioscience, Taipei, Taiwan. After processing of nasopharyngeal samples, rapid RT-PCR was performed by Novel Coronavirus (2019-nCOV) nucleic acid diagnostic kit (PCR-Fluorescence Probing) of Sansure Biotech (S3102E) (Changsha, China) according to instruction of manufacturer.

Cough, fever, myalgia, dyspnea, headache, chills, anorexia, gastrointestinal problems, and chest discomfort were considered symptoms of the disease. More than 50% involvement of the field with typical findings of the COVID-19 including peripheral, bilateral ground glass opacities (GGO) or multifocal GGO of rounded morphology with or without consolidations or crazy paving was defined as a positive CT scan.

Patients with severe COVID-19 were included (17). These patients had at least one of the following conditions: (a) hypoxemia (need for non-invasive or invasive respiratory support to provide capillary oxygen saturation above 90%) (b) Hypotension (systolic blood pressure less than 90 mmHg or vasopressor requirement) (c) renal failure secondary to COVID-19 (according to KDIGO definition) (18) (d) neurologic disorder secondary to COVID-19 (decrease of 2 or more scores in Glasgow Coma Scale) (e) thrombocytopenia secondary to COVID-19 (platelet count less than 150000 /mm3) (f) severe gastrointestinal symptoms secondary to COVID-19 (vomiting/diarrhea that caused at least mild dehydration).

Allergy to IFNs, receiving IFNs for any other reasons, severe depression, previous suicide attempts, alanine amino transferase (ALT)> 5× the upper limit of the normal range and pregnant women were defined as the exclusion criteria of the study.





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Ethics Committee of Tehran University of Medical Sciences approved the study (approved ID: IR.TUMS.VCR.REC.1398.1052). Also the protocol of trial was registered (Registered ID: IRCT20100228003449N28). The protocol of study was explained to the patients and written informed consents were obtained from them or from their next-of-kin.

Sample size and randomization

Due to lack of similar study at the start of this trial, for producing significant difference, the estimated time to clinical response was assumed. By estimating 15 days to reach clinical response and to detect a difference of 5 days, sample size was estimated. A number of 46 patients was estimated for each group.

Randomization was performed for all patients. Allocation ratio of 1:1 was accounted between IFN and control group in randomization. But the patients had to receive at least four injections of IFN to be included in the final analysis. The randomization was performed by permuted block randomization and block sizes of 2, 4 and 6 used randomly. The statistician prepared a list of numbers for randomization. Sequentially numbered opaque envelopes were prepared according to the list and envelopes were delivered to the clinical investigators. The statistician that performed randomization and analysis was unaware regarding to treatment and follow-up of patients.

Treatment protocols

Patients in the IFN group received IFN β-1a in addition to the national protocol medications. Each 44 micrograms/ml (12 million IU/ml) of interferon β-1a (ReciGen®, CinnaGen Co., Iran) was subcutaneously injected three times weekly for two consecutive weeks. The control group received only the standard of care. The standard of care (the hospital protocol) consisted of

hydroxychloroquine (400 mg BD in first day and then 200 mg BD) plus lopinavir/ritonavir (400/100 mg BD) or atazanavir/ritonavir (300/100 mg daily) for 7-10 days. Also primary care, respiratory support, fluid, electrolytes, analgesic, antipyretic, corticosteroid and antibiotic were recommended in the hospital protocol if indicated. The duration of study was two weeks and the patients were followed for four weeks.

Demographic data, baseline characteristics and laboratory data were recorded for each patient. APACHE II score at the time of ICU admission was calculated for critically ill patients. All patients were daily monitored in terms of vital signs, medical interventions and clinical conditions during the study course.

Need for respiratory support (invasive, non-invasive or none-required) were assessed by a physician regularly. Patients were assessed to fit in one of the six-category ordinal scale at days 0, 7, 14 and 28 of the inclusion (19). If discharged, patient was followed up by phone. Readmission was surveyed until 3rd May.

Outcomes assessment

Primary outcome of study was time to reach clinical response. Clinical response was defined according to the six-category ordinal scale (19). This scale classifies patients in six categories according to the severity of the viral pneumonia. The six categories are: (1) discharge (2) hospital admission, not requiring oxygen (3) hospital admission, requiring oxygen (4) hospital admission, requiring non-invasive positive pressure ventilation (5) hospital admission requiring invasive mechanical ventilation (6) death. Time to clinical response was considered days required to at least two scores improvement in the scale or patient's discharge, each that occurred sooner. Secondary outcomes were duration of mechanical ventilation, duration of hospital stay, length of ICU stay, 28-day mortality, effect of early or late (before or after 10 days of the onset

of symptoms) administration of IFN on mortality, adverse effects and complications during the hospitalization. Following adverse effects of the antiviral regimen/IFN β-1a and complications during the hospitalization course were assessed: gastrointestinal (nausea, vomiting, diarrhea, abdominal pain, pancreatitis), anaphylaxis and allergic reactions (rash, urticaria, angioedema, bronchospasm, and dyspnea related to medication administration), IFN injection-related reaction (skin erythema and necrosis, chills, fever, and flu-like symptoms after injection), neuropsychiatric (sleep disorder, psychosis, agitation, depression, and mania), renal impairment (according to KDIGO definition) (18), hepatic impairment (hepatic aminotransferases serum levels raised more than three times the upper limit of normal or serum total bilirubin above 2 mg/dL) (20), indirect hyperbilirubinemia (direct bilirubin level less than 15% of the total bilirubin) (21), incidence of thromboembolism (deep vein thrombosis or pulmonary thromboembolism), incidence of nosocomial infections, diagnosis of septic shock (according to surviving sepsis campaign guideline) (22). The Naranjo scale was used for evaluation of adverse effects of IFN. In this standard scale several items including previous same reports, relationship with starting the agent, improvement after discontinuation, challenge result, alternative causes, data of drug assay, dose-dependency and patient' history of same reaction were considered. Total score >8, 5-8 and 1-4 were considered as definite, probable and possible correlation between use of INF and the adverse drug reaction respectively (23).

Statistical analysis

The quantitative variables were reported as mean ± standard deviation (SD) if they had normal distributions or as median with Inter Quartile Range (IQR) if they did not pass the normality test. The qualitative ones were reported as number (percentage). For comparing the quantitative

variables, t-test or Mann-Whitney test was used. Otherwise, was applied. The qualitative variables were compared by Chi-square test.

The analysis was performed on per-protocol and patients who did not receive at least four doses of IFN were not included. The Kaplan-Meier plot and log- rank test were used to compare the days to reach the clinical response and survival time between the groups. The HR and 95% CI for clinical improvement and death were estimated by Cox proportional hazards model. The odds ratio was also calculated for patients who received IFN early vs. late. The multiple logistic regression model was performed for the variables that were significantly different between the groups according to the univariate analysis. In multiple logistic regression model corticosteroid and immunoglobulin were adjusted as confounding factors and the adjusted odds ratio was reported. SPSS software (version 24) was used for statistical analyses.

## Results

Patients and baseline features

During the study period, 139 patients were screened, of whom 103 subjects were eligible. Considering dropped-outs, finally 81 patients (42 in the IFN and 39 in the control group) completed the treatment for further analysis (figure 1). Males were 54.3% of patients. The mean ± SD of age in the IFN and control groups was 56.0 ± 16 and 59.5 ± 14 years respectively. Fifty-two (64.19%) patients had positive nasopharyngeal RT-PCR for SARS-CoV-2 and 29 (35.81%) patients were diagnosed according to the clinical signs/symptoms along with the imaging findings. Hypertension (38.3%), cardiovascular diseases (28.4%), diabetes mellitus (27.2%), endocrine disorders (14.8%), and malignancy (11.1%) were common baseline diseases. Endocrine disorders were dyslipidemia and hypothyroidism. There was no significant difference in terms of demographic data and baseline diseases between the groups. The most frequent chief

complaints of patients were cough, fever and dyspnea (table 1). APACHE II score at the time of ICU admission was not significantly different between two groups (15.3 ± 4 in the IFN group vs. 14.5 ± 3 in the control group, p= 0.79)

Vital signs and laboratory data

Median time from the onset of symptoms (according to patients' reports) to the administration od IFN was 10 days (IQR: 8-13). The vital signs at the time of hospital admission were not statistically different, except respiratory rate that was significantly higher in the IFN group (22 vs. 20 respectively, p= 0.009). Comparing the baseline laboratory data at the time of hospital admission revealed that median of blood urea nitrogen level was higher in the IFN than the control group. Also median INR value was significantly higher in the control group than the IFN group. Other laboratory findings were comparable (table 2)

Treatment strategies

Hydroxychloroquine is the main medication in Iran's national protocol for the treatment of COVID-19. lopinavir/ritonavir or atazanavir/ ritonavir may be added to hydroxychloroquine in severe cases. The antiviral regimens were not significantly different between two groups. All antivirals were continued for 10 days. Deep vein thrombosis prophylaxis and stress ulcer prophylaxis were considered for patients if indicated. Based on patients' clinical conditions, azithromycin, intravenous ascorbic acid, antibiotics, intravenous immunoglobulin or a corticosteroid was added to the antiviral regimens. A corticosteroid (methyl prednisolone, hydrocortisone or dexamethasone) was administered for 61.9% and 43.6% of patients in the IFN and the control groups, respectively. The corticosteroid dose was equivalent to 250mg methylprednisolone daily for 3 days. Also 35.7% and 25.6% of patients in the IFN and the control group received intravenous immunoglobulin (IVIG) respectively. The dose of IVIG was

5 grams daily for 3 days. Supportive care modalities and administered medications are summarized in table 3.

Outcomes and complications

As primary outcome, time to the clinical response was not significantly different between the IFN and the control groups (9.7 ± 5.8 vs. 8.3 ± 4.9 days respectively, P=0.95) which is shown in the Kaplan-Meier plot (figure 2). Also the log rank test revealed that there was no statistically significant difference between the groups, considering time to clinical response (HR= 1.10; 95% CI: 0.64-1.87, p= 0.72).

The six-category ordinal scale was assessed at days 0, 7, 14 and 28 (table 4). On day 0, there was no significant difference between the groups in terms of the components of this scale. On day 7 of therapy, 19% of patients in the INF group were discharged and with no death. At this time, 28% of patients in the control group were discharged and 25% died. However the difference was not statistically considerable (OR= 0.60; 95% CI: 0.21- 1.69). On day 14, the results were statistically notable and 66.7% vs. 43.6% of patients in the IFN group and the control group were discharged, respectively (OR= 2.5; 95% CI: 1.05- 6.37). When administration of IVIG and corticosteroid was considered as probable confounding factors, the adjusted odds ratio was 4.05 (95% CI: 1.42-11.55).

Regarding the time of INF initiation, the analysis showed that early administration significantly reduced mortality (OR=13.5; 95% CI: 1.5-118). However, late administration of INF did not show significant effects (OR=2.1; 95% CI: 0.48-9.6).

Other secondary outcomes such as duration of hospital stay, length of ICU stay and duration of mechanical ventilation were not statistically different. However, more patients were extubated in

the IFN group (p=0.019). Additionally, the 28-day overall mortality was significantly lower in the IFN than the control group (19% vs. 43.6% respectively, p= 0.015). In multivariate analysis, the effect of IFN on reduction of mortality was shown (OR=2.95; 95% CI: 1.08 to 8.03). When the model was adjusted for administration of IVIG and corticosteroid as confounding factors, the effect not only remained but also became stronger (OR=6.65; 95% CI :1.67 to 26.45). Kaplan Meier plot for assessment of survival was also added (figure 3, HR= 0.375; 95% CI: 0.16-0.87, p= 0.024).

Complications during the hospitalization course, incidence of organ failure and adverse effects were not different between the groups. Injection-related side effects (fever, chills, myalgia and headache in few hours after injection of IFN) happened in 8 (19%) patients (table 5).

Hypersensitivity reaction occurred in one patient who received IFN. The reaction was presented with maculopapular rash on trunk and both upper and lower limbs. However, the patient was taking herbal medicine for cough consisting thyme and honey, and other medications like hydroxychloroquine and lopinavir-ritonavir concomitantly. Interferon was discontinued after fourth dose and rashes begun to disappear within three days. According to the Naranjo score, the reaction was possibly due to IFN.

Neuropsychiatric problems were detected in 4 patients in the IFN group. Two cases experienced severe agitation and two cases complained from mood swings (mostly depression). One of the patients with mood swings had history of mild depressive disorder in past years. Out of 4 cases, two patients were being in the hospital for nearly 1 month. Neuropsychiatric side effects of IFN are unlikely to happen in short-term use. All patients received psychiatric consult. According to the Naranjo score, IFN possibly and probably caused neuropsychiatric problems in three and one patients, respectively.

**Discussion**

Present study was first randomized open-label controlled trial that assessed the efficacy and safety of IFN β-1a in treatment of patients with diagnosis of severe COVID-19. Time to reach the clinical response did not change following adding IFN to the national protocol medications. However, IFN significantly improved discharge rate on day 14. Also 28-day mortality was significantly lower in the IFN group. Patients who received IFN in early phase of the disease experienced significantly more benefits from the treatment. Some injection-related adverse effects of IFN occurred and all were tolerable.

Still no effective therapy has been introduced for COVID-19. Some anti-inflammatory agents and cytokine release inhibitors like corticosteroids and tocilizumab (acting against IL-6) have been proposed (24, 25). However, increasing risk of secondary infections, activation of latent tuberculosis and other adverse effects are the serious concerns (26). Lopinavir-ritonavir did not improve time to the clinical improvement and mortality (5). Efficacy of other therapeutic modalities like convalescent plasma is not clear (27).

Among coronaviruses family, the efficacy of IFNs at first was reported in SARS (28). After subsidence of the SARS epidemic, IFN was again proposed for treatment of another coronavirus, MERS. However, different subtypes of IFN (alpha and beta) in combination with ribavirin did not show significant efficacy in critically ill patients with MERS (29). Due to promising primary effects of IFN β-1 in MERS, a trial for evaluating the efficacy is still running (13). Thus, IFNs, especially type I, are still interesting options for recent epidemics. One study evaluated the effects of IFN β-1b in combination with lopinavir-ritonavir and ribavirin on mild to moderate COVID-19 (30). Also nebulized IFN α-2b in combination with oral arbidol was examined in treatment of the disease (31).

314 IFNs may have multifunctional role in the pathogenesis of SARS-CoV-2. IFNs are natural
315 cytokines that are produced in response to viral infections. They can activate interferon-
316 stimulated genes (ISGs) and increase expression of angiotensin converting enzyme inhibitor 2
317 (ACE2). Although upregulation of ACE2 might increase risk of SARS-CoV-2 infection but by
318 inactivating angiotensin II, it might protect lung host cells from further injury (32). Viral
319 infections including SARS-CoV-2 can suppress production of IFN types 1 and 3 but induce
320 production of IL-6 and TNF. IFN type 1 might remarkably decline viral replication (33).
321 Interestingly, SARS-CoV showed ability to act against the effects of IFNs (34, 35). SARS-CoV
322 encoded synthesis a family of proteins, Open Reading Frame (ORF) that inhibits STAT1
323 transporter to enter the nucleus and block the interferon signaling (35). However, recently it has
324 been shown that function of some proteins in this family (ORF6 and ORF3b) had changed in
325 SARS-CoV-2. This may has changed pathogenesis of SARS-CoV-2 and its interaction with
326 IFN (36).

327 Beside the antiviral effects of IFNs, potential role of IFN β-1a in improving ARDS
328 complications was proposed. Expression of CD-73 proteins in lung cells and decrease in vascular
329 leakage in ARDS and subsequent mortality was reported following treatment with intravenous
330 IFN β-1a (15). However, the results were not repeated in the next larger trial (16). This may be
331 related to extensive use of glucocorticoids in the latter trial that can interfere with effect of IFN.
332 The antagonist effects of corticosteroids are considerable (37).

333 The mean age of patients in present study was 57± 15 years. The mean or median of age values
334 were different in published studies from 46 to 65 years (38, 39). The male gender was dominant,
335 resembling other studies in COVID-19 (5, 39). Although gender difference was evident in many
336 studies of COVID-19, it did not affect outcomes. However, in a study, critically ill males had

higher mortality (40). This difference was first explained by increase in expression of ACE2 (the receptor for entrance of SARS-CoV2 to cell) in Asian men (41). Later, the issue was contributed to higher rate of cigarette smoking in Asian men compared to Asian women. However, both hypotheses should be confirmed in future studies. At present, neither gender nor smoking is certainly correlated to severity of COVID-19 (42).

Baseline vital signs and laboratory data were almost comparable between the groups, with some exceptions. The respiratory rate was significantly higher in the IFN group. Also mean of blood urea nitrogen level was higher in this group. This may be due to higher rate of diarrhea as initial symptoms of COVID-19 in this group in comparison to the control one (19% vs 5.1%). Diarrhea may cause dehydration and lead to higher blood urea nitrogen.

Two patients in the control group were under treatment with warfarin which may describe the higher INR mean value in this group. Before interpretation the laboratory results, it should be noted that serum levels of LDH, CPK and troponin were not measured for all included patients.

As primary outcome of the study, time to reach the clinical response was not significantly different between the groups. Considering the dysregulated inflammatory response in the pathogenesis of the late phase of COVID-19, it is not surprising that antivirals might not have immediate effect in relieving main symptoms at this stage. In the study of Hung et al, as a secondary outcome clinical improvement occurred significantly faster in IFN combination therapy group (lopinavir-ritonavir + interferon β-1b + ribavirin) compared with the control group i.e. 7 vs. 12 days (30). However, it should be noticed that most of the patients in this study had mild disease. Also remdesivir shortened time to recovery in mild cases i.e. 11 days in comparison to 15 days in the placebo group. The definition of time to recovery was somehow

359 different from our study (43). In severe cases, remdesivir showed better results in 5-day than 10-
360 day course. However, this arm of the study did not have control group (44).

361 Length of ICU and hospital stay and duration of mechanical ventilation were not statistically
362 different between the groups like the other interferon trial (30). However, according to the six-
363 category ordinal scale, more patients were discharged following IFN therapy at day 14. This
364 scale was also used in the study of remdesivir, but same results were not detected (39). One of
365 the remarkable findings in our study was decrease in 28-day mortality in IFN group that was not
366 achieved in other studies about COVID-19 (30, 39). Neither in early use (within 10 days of onset
367 of the symptoms), nor in late phase (10 days after onset of the symptoms), remdesivir
368 significantly change mortality in patients with severe COVID-19 (39). Although effect of INF in
369 decreasing mortality of patients with severe COVID-19 is surprising, the results should be
370 interpreted with caution and considering limitations of the study. Four patients in the INF group
371 died before receiving fourth dose of INF. According to the protocol of study, these patients were
372 excluded and were not included in the final analysis for assessing mortality. Further studies are
373 needed to confirm the results.

374 Same as previous reports mortality rate in our patients in the control group was high. In patients
375 with severe COVID-19, mortality rate has been reported between 62-81% (45-46). About half of
376 the included patients had severe conditions and were transferred to the ICU. Mostly were
377 intubated and were under mechanical ventilation.

378 The clinical course of COVID-19 is divided into early viral-replication phase and late cytokine-
379 release phase (47). It was suggested that early administration of antiviral medications (within 7-
380 10 days of onset of the symptoms) might improve outcome of patients with COVID-19 (39).
381 Additionally, early administration of IFNs was recommended in treatment of MERS (48). Early

administration of antiviral agents in the viral infections can accelerate viral clearance and postpone neutrophil infiltration. Early administration of IFN β-1a, even in severely ill mechanically ventilated patients led to higher survival rate. Late administration did not show more benefits.

Regarding safety of IFN therapy in patients with COVID-19, injection-related reactions including fever, chills, headache and fatigue (early after injection) were detected in 19% of the patients. All of these symptoms responded to the supportive therapy (acetaminophen) and change in the time of injection to late night. Erythema or injection site reaction or any reaction that caused treatment interruption was not reported. Considering the duration of the intervention, incidence rate of INF adverse reactions was lower than that was reported in patients with multiple sclerosis (49). However, it should be accounted that some patients in our study were under mechanical ventilation and exact evaluation of these reactions was not feasible. As a component of the supportive care in COVID-19, most patients received analgesic and antipyretic concomitant with antiviral agents and IFN. These medications might mask the adverse reactions of IFN, too.

Nausea, vomiting and abdominal pain were the most common gastrointestinal complications in our patients and the incidence rates were not different between the groups. Although two cases experienced slight elevation in serum amylase and lipase levels, in further evaluations, no pancreatitis was confirmed. COVID-19 can cause several gastrointestinal symptoms. However, gastrointestinal symptoms that started after the hospital admission may be related to the medications. The incidence rates of AKI and hepatic impairment were not significantly different between the IFN and the control groups. Both renal and liver injuries can be COVID-19 associated organ dysfunction (50). Also nephrotoxicity of medications like antibiotics and

furosemide (that were frequently prescribed in our patients) and hepatotoxicity of antiviral agents should be taken into account. No case of hepatotoxicity that led to discontinuation of interferon was detected. Indirect hyperbilirubinemia is one of adverse effects of atazanavir-ritonavir (51).

This study had some limitations. In this open-label randomized clinical trial both patients in general, intermediate wards and intensive care unit were recruited. Most of the general wards in fact were intermediate wards, but the accurate classification was not possible due to special and emergent conditions. Due to restrictions in each pandemic event and low experiences, diagnosis of COVID-19 was according to either positive RT-PCR or signs/symptoms plus imaging findings highly suggestive for the disease. Also assessing viral load, follow-up PCR and imaging was not possible due to limitations and emergent condition.

## Conclusion

Although INF did not change time to reach the clinical response, added to the national protocol, it significantly increased discharge rate on day 14 and decreased 28-day mortality. Improved survival rate was significant when patients received IFN β-1a in the early phase of the disease. Adverse effects of IFN β-1a were injection-related, neuropsychiatric problems and hypersensitivity reaction that all were tolerable and resolved during the follow-up period.

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## References


1. Wang C, Horby PW, Hayden FG, Gao GF. 2020. A novel coronavirus outbreak of global health concern. Lancet 395(10223):470-473.
2. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. 2020. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 395(10229): 1054-1062.
3. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, Zhang Y, Song J, Wang S, Chao Y, Yang Z, Xu J, Zhou X, Chen D, Xiong W, Xu L, Zhou F, Jiang J, Bai C, Zheng J, Song Y. 2020. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med e200994. doi:10.1001/jamainternmed.2020.0994.
4. https://covid19.who.int/?gclid=Cj0KCQjwl4v4BRDaARIsAFjATPmRcsB7VPlJAxJMhXU5bnYKH2jMgjcLtozUR_fRAeH7rKbf_PV7IbIaAuU6EALw_wcB. Access date on 6th July 2020.
5. 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. 2020. A trial of Lopinavir-Ritonavir in adults hospitalized with severe Covid-19. N Engl J Med 382(19):1787-1799.
6. Gao J, Tian Z, Yang X. 2020. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends 14(1):72-73.
7. Meyerowitz EA 1, Vannier AGL, Friesen MGN, Schoenfeld S, Gelfand JA, Callahan MV, Kim AY, Reeves PM, Poznansky MC. 2020. Rethinking the role of hydroxychloroquine in the treatment of COVID-19. FASEB J 34(5):6027-6037.

8. Cunningham AC, Goh HP, Koh D. 2020. Treatment of COVID-19: old tricks for new challenges. Crit Care;24(1):91.
9. Scagnolari C, Vicenzi E, Bellomi F, Stillitano MG, Pinna D, Poli G, Clementi M, Dianzani F, Antonelli G. 2004. Increased sensitivity of SARS-coronavirus to a combination of human type I and type II interferons. Antivir Ther 9(6):1003-1011.
10. Chan JF, Chan KH, Kao RY, To KK, Zheng BJ, Li CP, Li PT, Dai J, Mok FK, Chen H, Hayden FG, Yuen KY. 2013. Broad-spectrum antivirals for the emerging Middle East respiratory syndrome coronavirus. J Infect 67(6):606-616.
11. Hart BJ, Dyall J, Postnikova E, Zhou H, Kindrachuk J, Johnson RF, Olinger GG, Frieman MB, Holbrook MR, Jahrling PB, Hensley L. 2014. Interferon-β and mycophenolic acid are potent inhibitors of Middle East respiratory syndrome coronavirus in cell-based assays. J Gen Virol 95(Pt 3):571-577.
12. Sheahan TP, Sims AC, Leist SR, Schäfer A, Won J, Brown AJ, Montgomery SA, Hogg A, Babusis D, Clarke MO, Spahn JE, Bauer L, Sellers S, Porter D, Feng JY, Cihlar T, Jordan R, Denison MR, Baric RS. 2020. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 11(1):222. doi:10.1038/s41467-019-13940-6.
13. Arabi YM, Alothman A, Balkhy HH, Al-Dawood A, AlJohani S, Al Harbi S, Kojan S, Al Jeraisy M, Deeb AM, Assiri AM, Al-Hameed F, AlSaedi A, Mandourah Y, Almekhlafi GA, Sherbeeni NM, Elzein FE, Memon J, Taha Y, Almotairi A, Maghrabi KA, Qushmaq I, Al Bshabshe A, Kharaba A, Shalhoub S, Jose J, Fowler RA, Hayden FG, Hussein MA, And the MIRACLE trial group. 2018. Treatment of Middle East Respiratory Syndrome with a combination of lopinavir-ritonavir and interferon-β1b (MIRACLE trial): study protocol for a randomized controlled trial. Trials 19(1):81. doi:10.1186/s13063-017-2427-0.
14. Sallard E, Lescure FX, Yazdanpanah Y, Mentre F, Peiffer-Smadja N. 2020. Type 1 interferons as a potential treatment against COVID-19. Antiviral Res 178:104791. doi:10.1016/j.antiviral.2020.104791
15. Bellingan G, Maksimow M, Howell DC, Stotz M, Beale R, Beatty M, Walsh T, Binning A, Davidson A, Kuper M, Shah S, Cooper J, Waris M, Yegutkin GG, Jalkanen J, Salmi M, Piippo I, Jalkanen M, Montgomery H, Jalkanen S. 2014. The effect of intravenous

488 interferon-beta-1a (FP-1201) on lung CD73 expression and on acute respiratory distress
489 syndrome mortality: an open-label study. Lancet Respir Med 2(2):98-107.
490 16. Ranieri VM, Pettilä V, Karvonen MK, Jalkanen J, Nightingale P, Brealey D, Mancebo J,
491 Ferrer R, Mercat A, Patroniti N, Quintel M, Vincent JL, Okkonen M, Meziani F, Bellani
492 G, MacCallum N, Creteur J, Kluge S, Artigas-Raventos A, Maksimow M, Piippo I, Elima
493 K, Jalkanen S, Jalkanen M, Bellingan G, INTEREST Study Group. 2020. Effect of
494 intravenous interferon β-1a on death and days free from mechanical ventilation among
495 patients with moderate to severe acute respiratory distress syndrome: a randomized
496 clinical trial. JAMA 10.1001/jama.2019.22525. doi:10.1001/jama.2019.22525
497 17. Wu Z, McGoogan JM. 2020. Characteristics of and important lessons from the
498 coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314
499 cases from the Chinese Center for Disease Control and Prevention. JAMA
500 10.1001/jama.2020.2648. doi:10.1001/jama.2020.2648
501 18. Khwaja A. 2012. KDIGO clinical practice guidelines for acute kidney injury. Nephron
502 Clin Pract 120(4):c179-184.
503 19. Peterson RL, Vock DM, Powers JH, Emery S, Cruz EF, Hunsberger S, Jain MK, Pett S,
504 Neaton JD, INSIGHT FLU-IVIG Study Group. 2017. Analysis of an ordinal endpoint for
505 use in evaluating treatments for severe influenza requiring hospitalization. Clin Trials
506 14(3):264-276.
507 20. Lescot T, Karvellas C, Beaussier M, Magder S. 2012. Acquired liver injury in the
508 intensive care unit. Anesthesiology 117(4):898-904.
509 21. Singh A, Jialal I. 2020. Unconjugated Hyperbilirubinemia. In: StatPearls. Treasure Island
510 (FL): StatPearls Publishing.
511 22. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A,
512 Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC,
513 Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche JD, Coopersmith C, De Backer
514 DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad
515 DR, Kleinpell RM, Koh Y, Lisboa TC, Machado FR, Marini JJ, Marshall JC, Mazuski JE
516 , McIntyre LA, McLean AS, Mehta S, Moreno RP, Myburgh J, Navalesi P, Nishida O,
517 Osborn TM, Perner A, Plunkett CM, Ranieri M, Schorr CA, Seckel MA, Seymour CW,
518 Shieh L, Shukri KA, Simpson SQ, Singer M, Thompson BT, Townsend SR, Van der Poll

T, Vincent JL, Wiersinga WJ, Zimmerman JL, Dellinger RP. 2016. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock. Intensive Care Med 43:304–377.

23. Naranjo CA, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA, Janecek E, Domecq C, Greenblatt DJ. 1981. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther 30(2):239-245.
24. Luo P, Liu Y, Qiu L, Liu X, Liu D, Li J. 2020. Tocilizumab treatment in COVID-19: A single center experience. J Med Virol 92(7):814-818.
25. Zhang W, Zhao Y, Zhang F, Wang Q, Li T, Liu Z, Wang J, Qin Y, Zhang X, Yan X, Zeng X, Zhang S. 2020. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China. Clin Immunol 214:108393. doi:10.1016/j.clim.2020.108393.
26. Zhang S, Li L, Shen A, Chen Y, Qi Z. 2020. Rational use of tocilizumab in the treatment of novel coronavirus Pneumonia. Clin Drug Investig 40(6):511-518.
27. Roback JD, Guarner J. Convalescent plasma to treat COVID-19: possibilities and challenges. JAMA 2020;10.1001/jama.2020.4940. doi:10.1001/jama.2020.4940.
28. Cinat J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. 2003. Treatment of SARS with human interferons. Lancet 362(9380):293-294.
29. Arabi YM, Shalhoub S, Mandourah Y, Al-Hameed F, Al-Omari A, Al Qasim E, Jose J, Alraddadi B, Almotairi A, Al Khatib K, Abdulmomen A, Qushmaq I, Sindi AA, Mady A, Solaiman O, Al-Raddadi R, Maghrabi K, Ragab A, Al Mekhlafi GA, Balkhy HH, Al Harthy A, Kharaba A, Gramish JA, Al-Aithan AM, Al-Dawood A, Merson L, Hayden F, Fowler R. 2020. Ribavirin and interferon therapy for critically ill patients with middle east respiratory syndrome: a multicenter observational study. Clin Infect Dis 70(9):1837-1844.
30. Hung IF, Lung KC, Tso EY, Liu R, Chung TWH, Chu MY, Ng YY, Lo J, Chan J, Tam AR, Shum HP, Chan V, Wu AKL, Sin KM, Leung WS, Law WL, Lung DC, Sin S, Yeung P, Yip CCY, Zhang RR, Fung AYF, Yan EYW, Leung KH, Ip JD, Chu AWH, Chan WM, Ng ACK, Lee R, Fung K, Yeung A, Wu TC, Chan JWM, Yan WW, Chan WM, Chan JFW, Lie AKW, Tsang OTY, Cheng VCC, Que TL, Lau CS, Chan KH, To KKW, Yuen KY. 2020. 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 395(10238):1695-1704.

31. Zhou Q, Chen V, Shannon CP, Wei XS, Xiang X, Wang X, Wang ZH, Tebbutt SJ, Kollmann TR, Fish EN. 2020. Interferon-α2b treatment for COVID-19. Front Immunol 11:1061. doi: 10.3389/fimmu.2020.01061.
32. Su S, Jiang S. 2020. A suspicious role of interferon in the pathogenesis of SARS-CoV-2 by enhancing expression of ACE2. Signal Transduct Target Ther 5(1):71. doi:10.1038/s41392-020-0185-z.
33. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Møller R, Jordan TX, Oishi K, Panis M, Sachs D, Wang TT, Schwartz RE, Lim JK, Albrecht RA, tenOever BR. 2020. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell 181(5):1036-1045.
34. Kopecky-Bromberg SA, Martínez-Sobrido L, Frieman M, Baric RA, Palese P. 2007. Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists. J Virol 81(2):548-557.
35. Frieman M, Yount B, Heise M, Kopecky-Bromberg SA, Palese P, Baric RS. 2007. Severe acute respiratory syndrome coronavirus ORF6 antagonizes STAT1 function by sequestering nuclear import factors on the rough endoplasmic reticulum/Golgi membrane. J Virol 81(18):9812-9824.
36. Lokugamage KG, Schindewolf C, Menacher VD. 2020. SARS-CoV-2 sensitive to type I interferon pretreatment. BioRxiv https://doi.org/10.1101/2020.03.07.982264.
37. Flammer JR, Dobrovolna J, Kennedy MA, Chinenov Y, Glass CK, Ivashkiv LB, Rogatsky I. 2010. The type I interferon signaling pathway is a target for glucocorticoid inhibition. Mol Cell Biol 30(19):4564-4574.
38. Chen X, Zheng F, Qing Y, Ding S, Yang D, Lei C, Yin Z, Zhou X, Jiang D, Zuo Q, He J, Lv J, Chen P, Chen Y, Peng H, Li H, Xie Y, Liu J, Zhou Z, Luo H. 2020. Epidemiological and clinical features of 291 cases with coronavirus disease 2019 in areas adjacent to Hubei, China: a double-center observational study. medRxiv https://doi.org/10.1101/2020.03.03.20030353
39. Wang Y, Zhang D, Du G, Du R, Zhao J, Jin Y, Fu S, Gao L, Cheng Z, Lu Q, Hu Y, Luo G, Wang K, Lu Y, Li H, Wang S, Ruan S, Yang C, Mei C, Wang Y, Ding D, Wu F, Tang

X, Ye X, Ye Y, Liu B, Yang J, Yin W, Wang A, Fan G, Zhou F, Liu Z, Gu X, Xu J, Shang L, Zhang Y, Cao L, Guo T, Wan Y, Qin H, Jiang Y, Jaki T, Hayden FG, Horby PW, Cao B, Wang C. 2020. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicenter trial. Lancet 395(10236):1569-1578.

40. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S, Shan Y. 2020. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med 8(5):475-481.
41. Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. 2020. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. bioRxiv http://doi.org.10.1101/2020.01.26.919985.
42. Cai H. 2020. Sex difference and smoking predisposition in patients with COVID-19. Lancet Respir Med 8(4):e20. doi:10.1016/S2213-2600(20)30117-X
43. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, Hohmann E, Chu HY, Luetkemeyer A, Kline S, de Castilla DL, Finberg RW, Dierberg K, Tapson V, Hsieh L, Patterson TF, Paredes R, Sweeney DA, Short WR, Touloumi G, Lye DC, Ohmagari N, Oh MD, Ruiz-Palacios GM, Benfield T, Fätkenheuer G, Kortepeter MG, Atmar RL, Creech CB, Lundgren J, Babiker AG, Pett S, Neaton JD, Burgess TH, Bonnett T, Green M, Makowski M, Osinusi A, Nayak S, Lane HC, ACTT-1 Study Group Members. Remdesivir for the treatment of Covid-19 - preliminary report. N Engl J Med. http://org.doi.10.1056/NEJMoa2007764.
44. Goldman JD, Lye DCB, Hui DS, Marks KM, Bruno R, Montejano R, Spinner CD, Galli M, Ahn MY, Nahass RG, Chen YS, SenGupta D, Hyland RH, Osinusi AO, Cao H, Blair C, Wei X, Gaggar A, Brainard DM, Towner WJ, Muñoz J, Mullane KM, Marty FM, Tashima KT, Diaz G, Subramanian A, GS-US-540-5773 Investigators. 2020. Remdesivir for 5 or 10 days in patients with severe Covid-19. N Engl J Med http://org.doi:10.1056/NEJMoa2015301.
45. Weiss P, Murdoch DR. 2020. Clinical course and mortality risk of severe COVID-19. Lancet 395(10229): 1014–1015.

46. Arentz M, Yim E, Klaff L, Lokhandwala S, Riedo FX, Chong M, Lee M. 2020. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington State. JAMA 323(16):1612-1614.
47. Cao W, Li T. 2020. COVID-19: towards understanding of pathogenesis. Cell Res 30(5):367-369.
48. Channappanavar R, Fehr AR, Zheng J, Wohlford-Lenane C, Abrahante JE, Mack M, Sompallae R, McCray PB, Meyerholz DK, Perlman S. 2019. IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes. J Clin Invest 130(9):3625-3639.
49. Hu X, Miller L, Richman S, Hitchman S, Glick G, Liu S, Zhu Y, Crossman M, Nestorov I, Gronke RS, Baker DP, Rogge M, Subramanyam M, Davar G. 2012. A novel PEGylated interferon beta-1a for multiple sclerosis: safety, pharmacology, and biology. J Clin Pharmacol 52(6):798-808.
50. Wang T, Du Z, Zhu F, Cao Z, An Y, Gao Y, Jiang B. 2020. Comorbidities and multi-organ injuries in the treatment of COVID-19. Lancet 395(10228):e52. doi:10.1016/S0140-6736(20)30558-4.
51. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012-. Atazanavir. Available from: https://www.ncbi.nlm.nih.gov/books/NBK547918/. Access date: 5th July 2020.

640 Table 1- Baseline characteristics of patients

| Variable | IFN group (42) | Control group (39) |
|---|---|---|
| Age (mean ± SD) (year) | 56.50 (47.25-67.25) | 61.00 (50.00-70.00) |
| Male | 22 (52.38) | 22 (56.4) |
| Female | 20 (47.61) | 17 (43.58) |
| BMI ($kg/m^2$), (mean ± SD) | 25.00 (23.00-29.00) | 25.00 (22.00-29.00) |
| Baseline diseases: n (%) | | |
| Any comorbidity | 32 (76.19) | 31 (79.48) |
| Hypertension | 15(35.71) | 16(41.02) |
| Diabetes mellitus | 13(30.95) | 9(23.07) |
| Ischemic heart disease | 11(26.19) | 12(30.76) |
| Endocrine disorder | 6(14.28) | 6(15.38) |
| Malignancy | 4(9.52) | 5(12.82) |
| Neuropsychiatric disorders | 3(7.14) | 2(5.12) |
| Hematologic Disorder | 2 (4.76) | 0 |
| Rheumatoid disorder | 1 (2.38) | 2 (5.12) |
| Renal disease | 1(2.38) | 2(5.12) |
| Liver disease | 1(2.38) | 2 (5.12) |
| Rheumatoid Arthritis | 1(2.38) | 1(2.56) |
| Asthma | 1(2.38) | 0 |
| Transplantation | 1(2.38) | 0 |
| COPD | 0 | 1(2.56) |

| Symptoms at admission: n (%) | | |
|---|---|---|
| Cough | 37 (88.09) | 25(64.10) |
| Fever | 30(71.42) | 20(51.28) |
| Dyspnea | 29(69.04) | 27(69.23) |
| Myalgia | 16 (38.09) | 20(51.28) |
| Chills | 16 (38.09) | 5 (12.82) |
| Anorexia | 12(28.57) | 6 (15.38) |
| Diarrhea | 8 (19.04) | 2(5.12) |
| Malaise | 6 (14.28) | 8 (20.51) |
| Nausea/Vomiting | 4(9.52) | 10(25.64) |
| Headache | 3 (7.14) | 4 (10.25) |
| Chest discomfort | 1 (2.38) | 4 (10.25) |
| Duration of symptoms before admission (mean ± SD) | 8.33 ± 4.5 | 6.57 ± 3.6 |

641 BMI: Body Mass Index, COPD: Chronic Obstructive Pulmonary Disease, SD: Standard
642 Deviation

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652 Table 2- Initial vital signs and laboratory data

| Variable | IFN group, Median (IQR) | Control group, Median (IQR) |
|---|---|---|
| Temperature (°C) | 37.30 (36.70-38.72) | 37.25 (36.97-38.00) |
| Heart rate (beats /minute) | 95 (83-105) | 92 (80-101) |
| Respiratory rate (breath per minute) | 22 (19-29) | 20 (18-24) |
| Systolic blood pressure (mm Hg) | 120 (117-130) | 120 (110-130) |
| $SPO_2$ (%) | 89 (83-90) | 87 (84-90) |
| White Blood Cell (cells /µl) | 7200.00 (5175.00-9250.00) | 6650.00 (4900.00-9550.00) |
| Acute Lymphocyte count (cells /µl) | 1017.00 (689.00-1321.25) | 850.00 (650.00-1150.00) |
| Hemoglobin(g/dl) | 13.10 (11.45-14.50) | 13.40 (11.97-14.72) |
| Platelet count (cells $\times 10^3$/ µl) | 187.50 (160.50- 281.50) | 196.00 (136.25-245.00) |
| Blood Urea Nitrogen (mg/dl) | 28.50 (22.00-48.50) | 15.00 (11.00-21.00) |
| Creatinine(mg/dl) | 1.10 (0.97- 1.30) | 1.10 (0.90-1.30) |
| Sodium(meq/l) | 139.00 (136.00- 141.25) | 138.00 (134.00-140.00) |
| Potassium(meq/l) | 4.10 (3.90-4.62) | 4.10 (3.80-4.40) |
| Calcium(mg/dl) | 8.00 (7.70-8.50) | 8.10 (7.80-8.50) |
| Phosphorus(mg/dl) | 3.15 (2.70-3.95) | 2.80 (2.50-3.10) |
| Magnesium(mg/dl) | 2.05 (1.80-2.27) | 2.00 (1.77-2.12) |
| Aspartate aminotransferase(u/l) | 40. (28-65) | 43 (30-58) |

| | | |
|---|---|---|
| Alanine aminotransferase(u/l) | 36 (24-52) | 33 (26-54) |
| Alkaline phosphatase(u/l) | 158 (115-203) | 152 (118-202) |
| Total bilirubin(mg/dl) | 0.60 (0.40-1.20) | 0.8 (0.50-1.02) |
| International Normalized Ratio (INR) | 1.06 (1.02-1.12) | 1.10 (1.05-1.20) |
| C-reactive protein(mg/dl) | 149 (91-20) | 122 (45-187) |
| Erythrocyte sedimentation rate(mm/h) | 78 (58-87) | 65 (45-81) |
| Lactate dehydrogenase(u/l)* | 642 (454-899) | 758 (582-870) |
| Creatine phosphokinase(u/l)* | 146 (69-334) | 139 (100-233) |
| Troponin-I(ng/l)* | 5.80 (1.70-13.90 | 4.50 (1.50-20.40) |

* Lactate dehydrogenase was measured for 66% and 58% of patients in the IFN and control groups, respectively. Creatinine phosphokinase was measuredin 42% and 58% of patients in the IFN and control groups, respectively. Troponin was measured in 35% and 69% of patients in the IFN and control groups, respectively.

669 Table 3- Supportive care interventions and medications

| Variable | IFN group (42) | Control group (39) |
|---|---|---|
| Time from starting the symptoms to start of the interventions (Mean ± SD) | 11.70 ± 5.71 | 9.31 ± 4.45 |
| ICU admission (%) | 19 (45.23) | 23 (58.97) |
| Respiratory support: n (%) | | |
| Nasal cannula | 2 (4.76) | 1 (2.56) |
| Face mask | 24 (57.14) | 21 (53.84) |
| NIPPV | 1 (2.38) | 0 |
| IMV | 15 (35.71) | 17 (43.58) |
| Medications: n (%) | | |
| Hydroxychloroquine | 40 (95.23) | 39(100.0) |
| Antiviral regimen<br>Atazanavir/ritonavir<br>Lopinavir/ritonavir | 42 (100)<br>17 (40.47)<br>25 (59.52) | 39 (100)<br>19 (48.71)<br>20 (51.28) |
| Azithromycin | 8 (19.04) | 5 (12.82) |
| Vitamin C | 13 (30.95) | 12 (30.76) |
| Broad spectrum antibiotics | 33 (78.57) | 27 (69.23) |
| Diphenhydramine | 17 (40.47) | 26 (66.66) |
| Antiemetic | 11 (26.19) | 6 (15.38) |
| Opioid | 14 (33.33) | 17 (43.58) |

| | | |
|---|---|---|
| Stress ulcer prophylaxis | 42 (100) | 39 (100) |
| Deep vein thrombosis prophylaxis | 41 (97.61) | 37 (94.87) |
| Statins | 9 (21.42) | 6(15.38) |
| ARBs | 10 (23.80) | 4(10.25) |
| Beta-blockers | 8 (19.04) | 2 (5.12) |
| Calcium channel blockers | 7 (16.66) | 5 (12.82) |
| ACEIs | 1 (2.38) | 2(5.12) |
| Corticosteroid | 26 (61.90) | 17 (43.58) |
| Immunoglobulin | 15 (35.71) | 10 (25.64) |

ACEI: Angiotensin Converting Enzyme Inhibitor, ARB: Angiotensin Receptor Blocker, IMV: Invasive Mechanical Ventilation, NIPPV: Non-Invasive Positive Pressure Ventilation, SD: Standard Deviation

683 Table 4- Findings based on the six-category scale at days 0, 7, 14, 28

| Parameter | INF group (n=42) | Control group (n=39) | OR (if was calculated) |
|---|---|---|---|
| Day 0, n (%) of patients | | | |
| 1-Discharge | - | - | |
| 2-Hospital admission not requiring supplemental oxygen | 1 (2.38%) | 0 | |
| 3-Hospital admission, requiring supplemental oxygen | 29 (69.04%) | 27 (69.23%) | |
| 4-Hospital admission, requiring high-flow nasal cannula or non-invasive mechanical ventilation | 3 (7.14%) | 1 (2.56%) | |
| 5-Hospital admission, requiring invasive mechanical ventilation | 9 (21.42%) | 11 (28.20%) | |
| 6- Death | - | - | |
| Day 7, n (%) of patients | | | |
| 1-Discharge | 8 (19.04%) | 11 (28.20%) | OR: 0.60 (0.21- 1.69) |
| 2-Hospital admission not requiring supplemental oxygen | 2 (4.76%) | 0 | |
| 3-Hospital admission, requiring supplemental oxygen | 21 (50.00%) | 12 (30.76%) | |
| 4-Hospital admission, requiring high-flow nasal cannula or non-invasive mechanical ventilation | 1 (2.38%) | 0 | |

| | | | |
|---|---|---|---|
| 5-Hospital admission, requiring invasive mechanical ventilation | 10 (23.80%) | 6 (15.38%) | |
| 6-Death | 0 | 10 (25.64%) | |
| Day 14, n (%) of patients | | | |
| 1-Discharge | 28 (66.66%) | 17 (43.58%) | OR: 2.5 (1.05- 6.37) |
| 2-Hospital admission not requiring supplemental oxygen | 1 (2.38%) | 0 | |
| 3-Hospital admission, requiring supplemental oxygen | 5 (11.90%) | 6 (15.38%) | |
| 4-Hospital admission, requiring high-flow nasal cannula or non-invasive mechanical ventilation | 1 (2.38%) | 0 | |
| 5-Hospital admission, requiring invasive mechanical ventilation | 3 (7.14%) | 2 (5.12%) | |
| 6-Death | 4 (9.52%) | 14 (35.89%) | |
| Day 28, n (%) of patients | | | |
| 1-Discharge | 31 (73.80%) | 23 (58.97%) | OR: 1.96 (0.76- 5.01) |
| 2-Hospital admission not requiring supplemental oxygen | 2 (4.76%) | 0 | |
| 3-Hospital admission, requiring supplemental oxygen | 1 (2.38%) | 0 | |
| 4-Hospital admission, requiring high-flow nasal | 0 | 0 | |

| | | | |
|---|---|---|---|
| cannula or non-invasive mechanical ventilation | | | |
| 5-Hospital admission, requiring invasive mechanical ventilation | 0 | 1 (2.56%) | |
| 6-Death | 8 (19.04%) | 15 (38.46%) | |

699 Table 5- Outcomes of the study

| Outcome, mean ± SD or n (%) | IFN group (42) | Control group (39) | p-value |
|---|---|---|---|
| Time from starting the interventions to the clinical response (days) | 9.74 ± 5.8 | 8.39 ± 4.9 | 0.95 |
| Required invasive mechanical ventilation | 15 (35.71%) | 17 (43.58%) | 0.30 |
| Extubation rate[¥] (%) | 8 (53.33%) | 2 (11.76%) | 0.019 |
| Duration of mechanical ventilation (days) | 10.86 ± 5.38 | 7.82 ± 7.84 | 0.47 |
| Duration of ICU stay (days) | 7.71 ± 8.75 | 8.52 ± 7.48 | 0.42 |
| Duration of hospital stay (days) | 14.80 ± 8.45 | 12.25 ± 7.48 | 0.69 |
| Death in hospital (%)<br>- Death in general wards*<br>- Death in ICU[§] | 8 (19.04%)<br>0<br>8 (42.10%) | 16 (41.02%)<br>2 (12.50%)<br>14 (60.86%) | 0.027<br>0.17<br>0.14 |
| 28-day mortality: n (%) | 8 (19%) | 17 (43.6%) | 0.015 |
| Complications : n (%) | | | |
| Acute kidney injury | 12 (28.57%) | 11 (28.20%) | 0.58 |
| Nosocomial infections | 11 (26.19%) | 5 (12.82%) | 0.09 |
| Septic shock | 10 (23.80%) | 7 (17.94%) | 0.35 |
| Hepatic failure | 5 (11.90%) | 9 (23.07%) | 0.15 |
| Deep vein thrombosis/pulmonary thromboembolism | 1 (2.38%) | 0 | 0.51 |
| Adverse events : n (%) | | | |

| | | | |
|---|---|---|---|
| Hypersensitivity reactions | 1 (2.38%) | 0 | 0.51 |
| IFN-related injection reactions | 8 (19.04%) | 0 | - |
| Neuropsychiatric problems | 4 (9.52%) | 0 | 0.06 |
| Indirect hyperbilirubinemia | 1 (2.38%) | 1 (2.56%) | 0.73 |

*22 patients in IFN group and 16 patients in control group were in general ward.

§ 19 patients in IFN group and 23 patients in control group were in intensive critical unit.

¥ The percentage of extubated patients was calculated according to number of intubated

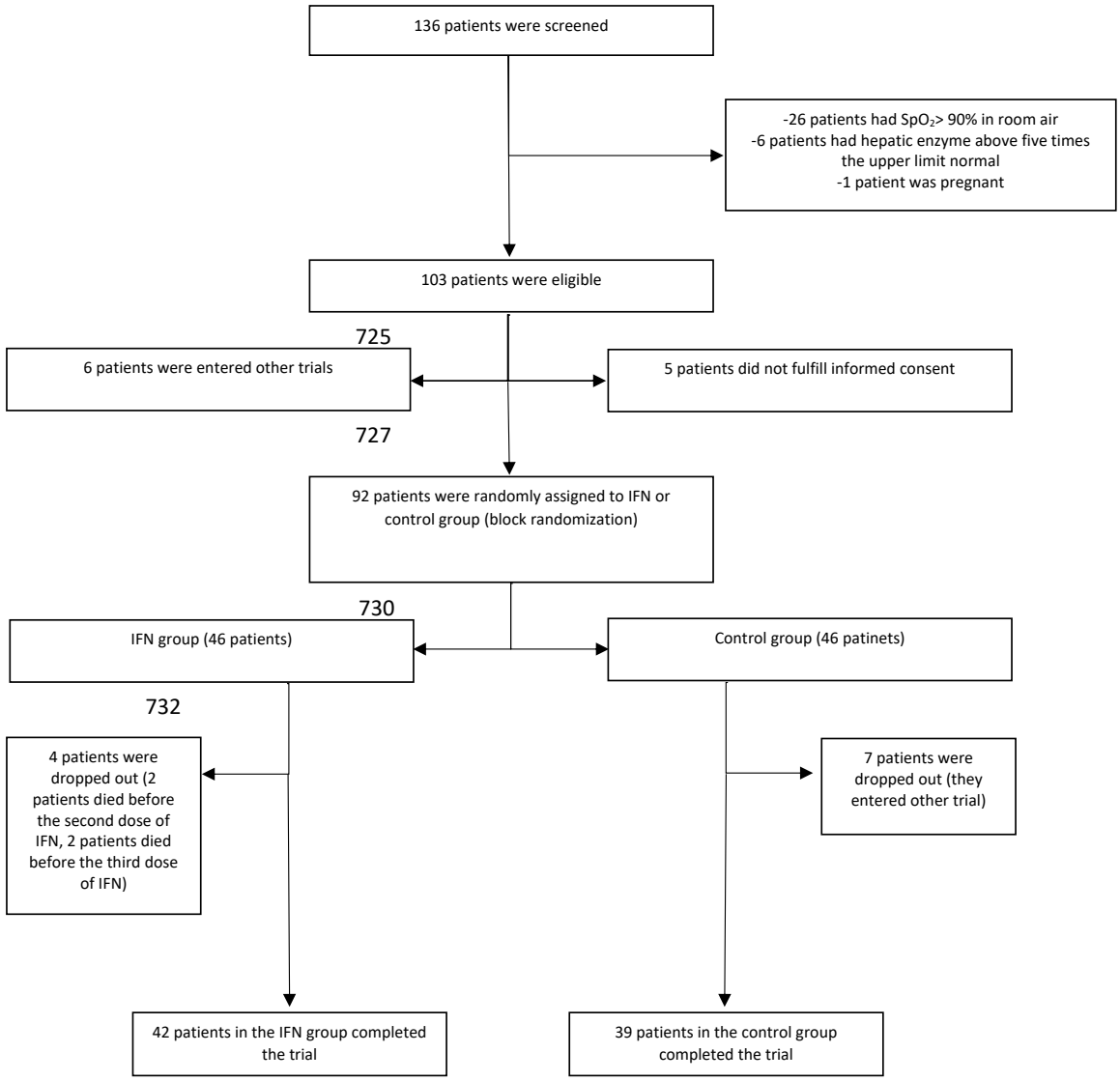
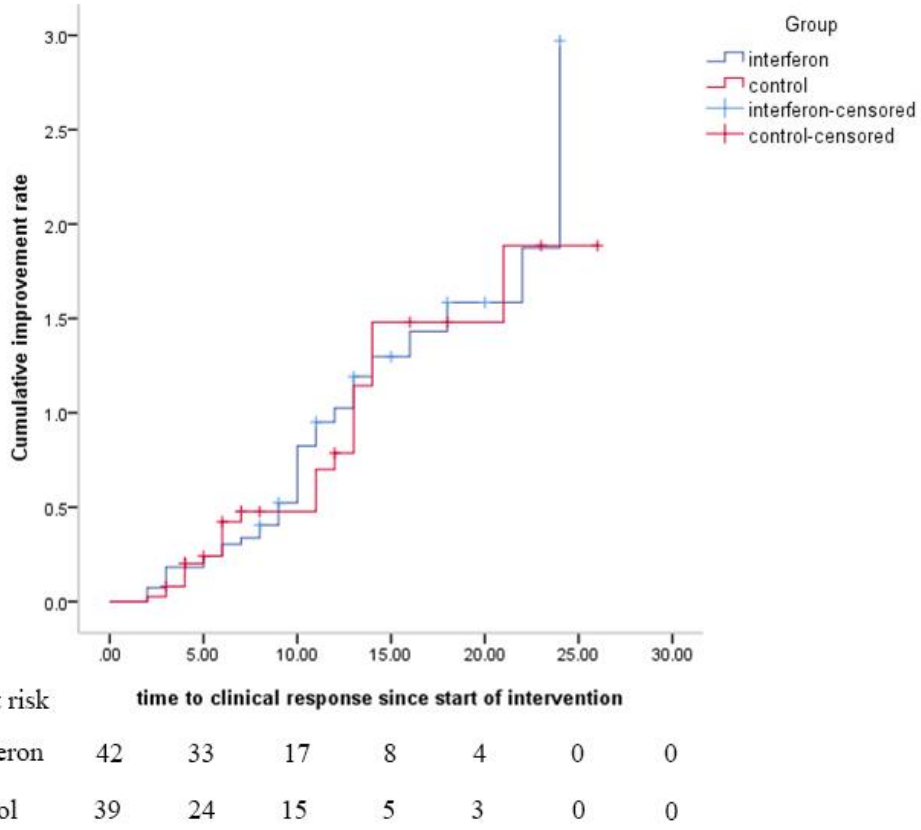



Figure 1: Consort flowchart of the study

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745 Figure 2- Kaplan Meier plot for time to clinical response. Hazard Ratio was calculated as 1.10

746 with 95% CI 0.64-1.87.

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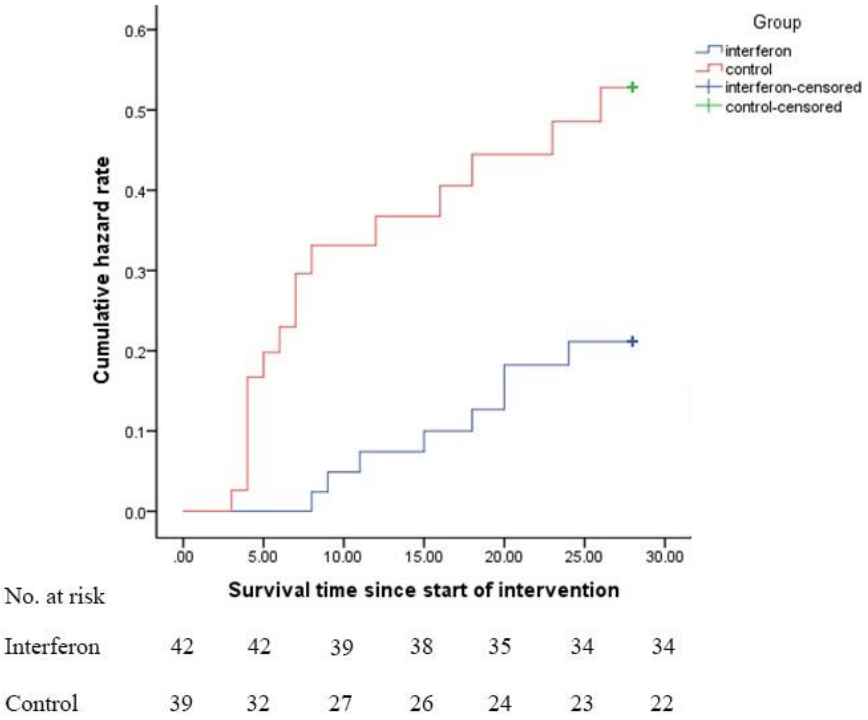


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755 Figure 3- Kaplan Meier plot for survival. Hazard Ratio was calculated as 0.375 with 95% CI
756 0.16- 0.87.
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