Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company’s public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Impact of early interferon-β treatment on the prognosis of patients with COVID-19 in the first wave: A post hoc analysis from a multicenter cohort

Sonsoles Salto-Alejandre a,b, Zaira R. Palacios-Baena b,c,d,1, José Ramón Arribas d,e,f, Juan Berenguer f,g,h, Jordi Carratalà d,i,j,k, Inmaculada Jarrín d,1, Pablo Ryan h,m,n, Marta de Miguel-Montero d, Jesús Rodríguez-Baño b,c,d,p,q,2, Jerónimo Pachón a,b,p,q,*, for the COVID-19@Spain Study Group

a Unit of Infectious Diseases, Microbiology and Preventive Medicine, Virgen del Rocío University Hospital, Seville, Spain
b Institute of Biomedicine of Seville, Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain
c Unit of Infectious Diseases and Microbiology, University Hospital Virgen Macarena, Seville, Spain
d CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain
e Unit of Infectious Diseases, Service of Internal Medicine, Hospital Universitario La Paz, IdiPAZ, Madrid, Spain
f Instituto de Investigación Hospital Universitario La Paz, Madrid, Spain
g Service of Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Madrid, Spain
h Instituto de Investigación Sanitaria Gregorio Marañón (ISG), Madrid, Spain
i Service of Infectious Diseases, Hospital Universitario de Bellvitge, Barcelona, Spain
j Instituto de Investigación Biomédica de Bellvitge (IDIBELL), Barcelona, Spain
k Universitat de Barcelona, Barcelona, Spain
l Centro Nacional de Epidemiología, Instituto de Salud Carlos III, Madrid, Spain
m Service of Internal Medicine, Hospital Universitario Infanta Leonor, Madrid, Spain
n Department of Medicine, Universidad Complutense de Madrid, Madrid, Spain
p Fundación SEIMC/GeSIDA, Madrid, Spain
q Department of Medicine, Universidad de Sevilla, Seville, Spain

A R T I C L E   I N F O

Key words: Interferon-β Treatment SARS-CoV-2 Mortality

A B S T R A C T

Background: Interferon-β is an attractive drug for repurposing and use in the treatment of COVID-19, based on its in vitro antiviral activity and the encouraging results from clinical trials. The aim of this study was to analyze the impact of early interferon-β treatment in patients admitted with COVID-19 during the first wave of the pandemic.

Methods: This post hoc analysis of a COVID-19@Spain multicenter cohort included 3808 consecutive adult patients hospitalized with COVID-19 from 1 January to 17 March 2020. The primary endpoint was 30-day all-cause mortality. A propensity score was calculated and used to both control for confounders and perform a matched cohort analysis.

Results: Overall, 683 patients (17.9%) received early interferon-β therapy. These patients were more severely ill. Adjusted HR for mortality with early interferon-β was 1.03 (95% CI, 0.82–1.32) in the overall cohort, 0.96 (0.82–1.13) in the PS-matched subcohort, and 0.89 (0.60–1.32) when interferon-β treatment was analyzed as a time-dependent variable.

* Corresponding authors at: Institute of Biomedicine of Seville, Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.
1 E-mail addresses: jesusrb@us.es (J. Rodríguez-Baño), pachon@us.es (J. Pachón).
2 Join first author.
3 Join last author.

https://doi.org/10.1016/j.biopha.2021.112572

Available online 22 December 2021
0753-3322/© 2021 The Author(s). Published by Elsevier Masson SAS. This is an open access article under the CC BY-NC-ND license.
1. Introduction

Since the pandemic of coronavirus disease 2019 (COVID-19) beginning in December 2019, caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, more than 272 million cases and 5.3 million deaths have been reported around the world as of 16 December 2021 [1]. Compared to the other beta coronaviruses that have caused epidemics over the last two decades, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-2 exhibits higher infectivity and lower fatality; hence, its destructive and expansive nature has led to the most devastating pandemic of the century [2].

Symptomatic SARS-CoV-2 infection presents a characteristic sequence of phases, beginning with accelerated viral replication that can escape the immune system, manifesting as an influenza-like illness. Within 7–10 days from symptom onset, an inflammatory phase develops in up to 20% of infected individuals, typically heralded by an organizing pneumonia [3]. Around 5% of patients subsequently deteriorate, with immune system dysregulation and stimulation of a hyperinflammatory state leading to acute respiratory distress syndrome (ARDS), endothelial damage and microvascular injury, and hypercoagulability [4].

In the absence of an antiviral drug with proven clinical efficacy against SARS-CoV-2, physicians across the world began treating patients with agents such as hydroxychloroquine, azithromycin, lopinavir/ritonavir against SARS-CoV-2, with accelerated viral replication that can escape the immune system, manifesting as an influenza-like illness. However, coronaviruses encode interferon antagonists that actively interfere with host interferon induction and/or signaling [5]. There is evidence that the severity of COVID-19 is correlated with highly impaired type I IFN activity, characterized by no IFN-β and low IFN-α production [6]. Furthermore, it has been reported that at least 10% of patients with life-threatening pneumonia have neutralizing auto-antibodies (auto-Abs) against type I IFNs, which, like the abovementioned inborn errors, are associated with persistent blood viral load and an exacerbated inflammatory response [7]. The most important barriers to the use of type I IFNs as therapy are the lack of knowledge about timing and appropriate dosing and the increased chance of immunopathology by further stimulation of proinflammatory signals [8,9]. Promising results obtained from three randomized controlled trials with small sample sizes showed that subcutaneous injection of IFN-β in patients with moderate-to-severe COVID-19 improved clinical outcomes with no specific side effects [10–12]. However, two other multicenter randomized controlled trials, mostly in adult inpatients with mild-to-moderate COVID-19, did not show clinical efficacy of interferon treatment [13,14].

With these data, we hypothesized that early administration of IFN-β would be associated with lower mortality compared to standard treatment alone. Therefore, we conducted a post hoc study using data from the multicenter retrospective COVID-19@Spain cohort to assess the protective effect of early IFN-β treatment compared with no IFN-β administration in patients hospitalized with COVID-19 [15].

2. Methods

2.1. Study design, sites, and participants

This post hoc analysis of the multicenter retrospective COVID-19@Spain cohort included 4035 consecutive adult patients with COVID-19 confirmed by real-time polymerase chain reaction (RT-PCR) assay, hospitalized in 127 Spanish centers between 1 January and 17 March 2020 and followed for 30 days after admission. The methodology has previously been described in detail [15–18]. In summary, all data were collected using an electronic case report form (eCRF) and added to a database built with Research Electronic Data Capture (REDCap) tools hosted at the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC)/AIDS Study Group (GESIDA) Foundation [19]. The Ethics Committee for Research with Medicines of Hospital General Universitari Gregorio Marañón approved the study and waived informed consent for the collection of clinical data. Approval was also obtained at each participating center, conforming with local requirements. Hospitals in which IFN-β was not used in any patient were excluded because they would cause a cluster effect not amenable to the control. Patients who died less than 48 h after admission were excluded from the study, whether they received IFN-β or not. This analysis was reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations (Table S1) [20].

2.2. Variables and definitions

The outcome variable was 30-day all-cause mortality, and the main exposure of interest was subcutaneous administration of IFN-β, which was classified as early IFN-β treatment (EIT) if started within ≤ 3 days (day of hospital admission was considered day 0), late IFN-β treatment (LIT) if started from day 4 onward, or no IFN-β treatment (NIT) if only standard treatment (not including IFN-β) was provided.

Additional exposure variables recorded at hospital admission were demographic data, chronic underlying conditions, admission symptoms and signs, laboratory findings, and severity according to the COVID-19 SEIMC score (14) and the WHO Clinical Progression Scale [21]. Additionally, other treatments for COVID-19 and use of respiratory support during hospitalization were recorded (Table 1).

2.3. Statistical analysis

The χ² or Fisher’s exact test was used to compare categorical variables. When appropriate, continuous variables were dichotomized using data classification analysis, according to their association with mortality. Hospitals were classified into those with lower (<30%) and higher (≥30%) mortality as well as lower (<40%) and higher (≥40%) IFN-β prescription based on the 75th percentile cut-off point, and these variables were retained in the models. Cox regression was used to analyze the impact of EIT on 30-day mortality. Variables with p < 0.10 in univariate comparisons and those considered of clinical importance were entered into the multivariate models. The variables in the models were selected manually using a backward stepwise process. Interactions and collinearity were evaluated. Sensitivity analyses for 30-day mortality were performed, including changes in covariables and specific categorizations, using the variable IFN-β treatment as a time-dependent variable considered from the admission date. In addition, a propensity score (PS) for receiving EIT instead of NIT was calculated, and its ability to predict the observed data was assessed using the area under the receiver operating characteristic curve (AUROC) with a 95% confidence interval.
3. Results

In all, 4035 patients with COVID-19 included in the COVID-19@Spain cohort were eligible for analysis; 130 patients were excluded for being treated at one of 19 centers where IFN-β was not used, and 97 because they died < 48 h after hospital admission. Finally, 3808 patients were included in this study: 683 (17.9%) received early IFN-β treatment (median (IQR) days from admission, 1 (1–2)), 440 (11.6%) received late IFN-β treatment (median (IQR) days from admission, 3–7).
The study flowchart is presented in Fig. 1.

The patient characteristics are shown in Table 1. Compared to patients who underwent EIT, those in the NIT group were more frequently over 75 years old; had chronic heart, kidney, and neurological diseases; and suffered from active solid or hematologic neoplasms. Notwithstanding, they presented a significantly lower proportion of severe symptoms and signs (i.e., dyspnea, peripheral oxygen desaturation, and tachycardia), in conjunction with fewer laboratory indicators of high risk (i.e., neutrophilia, lymphopenia, thrombocytopenia, and elevated levels of D-dimer, lactate dehydrogenase, and C-reactive protein), which is consistent with a diminished prevalence of the inflammatory phase of COVID-19 on admission (142 patients in EIT and 415 in NIT; \( p = 0.001 \)). Thus, patients in the NIT group less often reached higher disease severity scores (from 6 to 9, according to the WHO Clinical Progression Scale; from 6 to 8, according to the COVID-19 SEIMC score) \([18,21]\) and did not receive as broad therapy (including remdesivir, tocilizumab, and corticosteroids) as patients in the EIT group.

### 3.1. Variables associated with EIT

The association of different variables with EIT is shown in Table 2. Patients receiving EIT more frequently had severe signs and symptoms (i.e., dyspnea, peripheral oxygen desaturation, and tachycardia), in conjunction with fewer laboratory indicators of high inflammatory severity (i.e., neutrophilia, lymphopenia, thrombocytopenia, and elevated levels of D-dimer, lactate dehydrogenase, and C-reactive protein), which is consistent with a diminished prevalence of the inflammatory phase of COVID-19 on admission (142 patients in EIT and 415 in NIT; \( p = 0.001 \)). Thus, patients in the NIT group less often reached higher disease severity scores (from 6 to 9, according to the WHO Clinical Progression Scale; from 6 to 8, according to the COVID-19 SEIMC score) \([18,21]\) and did not receive as broad therapy (including remdesivir, tocilizumab, and corticosteroids) as patients in the EIT group.

### 3.2. Mortality analysis

The mortality rates were 33.2% (227/683), 38.4% (169/440), and 23.2% (623/2685) in patients with EIT, LIT, and NIT, respectively (\( p < 0.001 \) for EIT vs. NIT) (Table 1). Univariate and multivariate analyses of variables associated with 30-day mortality are shown in Table 3. The multivariate analysis selected the following factors as being associated with mortality: age > 75 years (HR, 2.37; 95% CI, 2.00–2.81; \( p < 0.001 \)), dyspnea (HR, 1.49; 95% CI, 1.24–1.78; \( p < 0.001 \)), low peripheral capillary oxygen saturation (SpO\(_2\)) (HR, 1.55; 95% CI, 1.26–1.90; \( p < 0.001 \)), lymphocyte count < 1000/μL (HR, 1.28; 95% CI, 1.08–1.53; \( p = 0.01 \)), platelets < 150,000/μL (HR, 1.29; 95% CI, 1.08–1.53; \( p = 0.004 \)), lactate dehydrogenase > 250 U/L (HR, 1.44; 95% CI, 1.19–1.76; \( p < 0.001 \)), C-reactive protein > 100 mg/L (HR, 1.42; 95% CI, 1.19–1.69; \( p < 0.001 \)), and corticosteroids (HR, 1.32; 95% CI, 1.11–1.56; \( p = 0.002 \)). Early IFN-β treatment did not show an association with mortality. The model exhibited good predictive ability (AUROC, 0.86 (95% CI, 0.84–0.91; \( p = 0.004 \))). No important interactions were identified.

We then investigated the impact of EIT vs. NIT, including the PS for EIT (LIT patients were excluded from this analysis) (Table 3). No significant collinearity was found between PS and other variables. Similarly, no difference was observed among the patients undergoing EIT (adjusted hazard ratio (HR), 1.03 (95% CI, 0.82–1.30; \( p = 0.78 \)); AUROC for this model: 0.81 (95% CI, 0.77–0.83; \( p < 0.001 \)).

The estimations of the associations of EIT with mortality in the sensitivity analyses were consistent with the analysis of the whole cohort. When including the COVID-19 SEIMC score as a continuous variable instead of the component variables (age, dyspnea, low SpO\(_2\), and lymphocyte count), the adjusted hazard ratio for EIT was 1.08 (95% CI, 0.93–1.25; \( p = 0.32 \)) (Table S2). When excluding the covariates lopinavir/ritonavir, tocilizumab, and corticoids, the adjusted hazard ratio for EIT was 1.10 (95% CI, 0.96–1.27; \( p = 0.16 \)) (Table S3). Therefore, these treatments were not confounding factors for the association between EIT and mortality. We also studied interferon treatment as a time-dependent covariate within the entire cohort, having an adjusted hazard ratio of 0.89 (95% CI, 0.59–1.32; \( p = 0.55 \)) (Table S4).

Finally, we matched 144 pairs of patients receiving EIT or NIT based on PS. Matched subcohorts had similar exposure frequency to all variables (Table 4). Early IFN-β treatment did not show an association with mortality in this analysis (HR, 0.96 (95% CI, 0.82–1.13; \( p = 0.99 \)).

### 4. Discussion

In this post hoc analysis of a multicenter cohort from the first wave of the COVID-19 pandemic, we analyzed the association of early IFN-β administration with mortality. Patients receiving EIT more frequently had severe symptoms and signs in addition to high values of inflammatory biomarkers, and a higher proportion required respiratory and/or hemodynamic support than those receiving LIT or NIT. The crude mortality rates were 33.2%, 38.4%, and 23.2% in patients with EIT, LIT, and NIT, respectively (\( p < 0.001 \) for EIT vs. NIT) (Table 1).
and NIT, respectively. The factors independently associated with 30-day mortality were age ≥ 75 years, dyspnea, low peripheral capillary oxygen saturation, lymphopenia, thrombocytopenia, high values of lactate dehydrogenase and C-reactive protein, and the use of corticosteroids. Early IFN-β treatment did not show an association with mortality. Moreover, the analysis of 144 pairs of patients receiving EIT or NIT based on PS did not reveal an association of EIT with lower mortality.

To the best of our knowledge, this is the biggest study providing information on the effectiveness of systemic early IFN-β administration vs. standard treatment alone in patients with moderate-to-severe COVID-19 addressing the confounding effects of other potential targeted drugs. Our hypothesis, that early administration of IFN-β would be associated with lower mortality compared to standard treatment alone, is shared by the currently ongoing INTERCOP study, an open-label monocentric phase II randomized controlled trial (ClinicalTrials.gov identifier: NCT04449380) [22].

The unprecedented emergency of the COVID-19 pandemic, with no available medications of fully proven efficacy, provided a compelling reason to repurpose drugs already marketed for other indications. Among these, the use of IFN-β seemed immediately feasible for a number of reasons: (i) direct in vitro antiviral activity against SARS-CoV-2 [23]; (ii) previous encouraging experience in mice and nonhuman primate models of MERS [24,25]; (iii) promising results in reducing mortality when combined with lopinavir–ritonavir and started within seven days after symptom onset [26]; and (iv) safety in patients with ARDS, in addition to long-term consolidated evidence of tolerability as an established treatment for multiple sclerosis [27,28].

The very promising results from a Chinese multicenter randomized trial with 127 patients enrolled suggest that subcutaneous INF-β is a key component for success in shortening the viral shedding of a combined therapy that also includes lopinavir–ritonavir and ribavirin [10]. However, the analysis was confounded by the exclusion of a 34-patient subgroup (admitted ≥ 7 days after symptom onset), for whom INF-β was omitted due to concerns about proinflammatory side effects. Furthermore, critically ill patients were not eligible for the study, impeding the application of the findings to severe cases. Another single-center randomized controlled trial in Iran recruited 60 severely ill patients to evaluate the efficacy of subcutaneous INF-β. In short, the intervention group had a shorter time to clinical improvement, and their mortality rate was almost half that of the control group, although the difference was not statistically significant [11]. Including moderate patients and earlier administration of exogenous INF-β (mean time from enrollment to first dose was 5.4 days) might have yielded more substantial results and minimized the adverse effects (essentially abnormalities in liver injury biomarkers). A third single-center randomized controlled trial showed a significant decrease in mortality in patients receiving early therapy (less than 7–10 days from the onset of symptoms) with subcutaneous INF-β, but not late administration of INF-β [12].

The WHO Solidarity Trial [13], a multicenter randomized controlled trial, did not show lower mortality in the interferon group vs. control (11.8% vs. 10.5%, p = 0.11). Both groups were similar, but contrary to our study, only 6.7% (INF-β) and 6.3% (control) of patients were on ventilation support, and only 33.7% and 34.7% were hospitalized ≥ 2 days. Similarly, a multicenter randomized controlled trial by Kailil et al. did not show efficacy of INF-β combined with remdesivir compared to remdesivir alone concerning time to recovery [14]. Patients had mostly mild-to-moderate COVID-19, with only 7% in both groups requiring non-invasive ventilation or high-flow oxygen therapy.

Finally, Monk et al. assessed the efficacy and safety of inhaled INF-β vs. placebo for the treatment of patients admitted with non-severe COVID-19 (only 2 out of 98 patients requiring non-invasive ventilation or high-flow oxygen), showing a significant improvement in the clinical condition, on the basis of the WHO Ordinal Scale for Clinical Improvement, during the dosing period in the intention-to-treat population [29]. With this as background, we conducted a post hoc propensity score-adjusted study of 3808 consecutive patients with moderate-to-severe COVID-19, investigating the effectiveness of subcutaneous INF-β treatment. In this observational study, we mimicked the assignment of patients to treatment arms and the intention-to-treat analysis inherent in any randomized trial. Therefore, before performing any analysis, we defined EIT as IFN-β started ≥ 3 days from admission and excluded patients for whom the endpoint was reached in this period or those who started treatment from day 4 onward in order to avoid immortal time bias. We used a single robust primary outcome, mortality, because some


Table 3

| Variable                  | Crude Analysis | Adjusted Analysisa | EIT vs NIT, Adjusted by PSb |
|---------------------------|----------------|---------------------|-----------------------------|
|                           | HR (95% CI)    | P Value             | HR (95% CI)                 | P Value             | HR (95% CI) | P Value             |
| Male sex                  | 1.31           | < .001              | 1.31                        | < .001              | 1.31  | < .001              |
| Age > 75 years            | 2.66           | < .001              | 2.37                        | < .001              | 2.51  | < .001              |
| Obesity (BMI > 30)        | 1.29           | .004                | 1.55                        | < .001              | 1.67  | < .001              |
| Chronic heart disease     | 1.87           | < .001              | 1.55                        | < .001              | 1.67  | < .001              |
| Dyspnea                   | 1.74           | < .001              | 1.49                        | < .001              | 1.39  | .003                |
| Low SpO2 (age-adjusted)c  | 2.05           | < .001              | 1.55                        | < .001              | 1.67  | < .001              |
| Heart rate ≥ 100 bpm      | 1.15           | .06                 |                            |                    |       |                    |
| More than 7 days from symptoms onset to admission | 1.67 (54.83) | < .001              |                            |                    |       |                    |
| Neutrophil count > 7500/μL | 2.44 (24.3)   | < .001              |                            |                    |       |                    |
| Lymphocyte count < 1000/μL | 650 (64.9)    | 1.55 (1.38–1.84)   |                            |                    |       |                    |
| Platelets < 150,000/μL    | 382 (37.9)     | 1.30 (1.14–1.48)   |                            |                    |       |                    |
| D-dimer levels > 500 mg/mL | 233 (67.1)    | 1.27 (1.01–1.59)   |                            |                    |       |                    |
| Lactate dehydrogenase > 250 U/L | 458 (73.4) | 1.49 (1.25–1.78) |                            |                    |       |                    |
| C-reactive protein > 100 mg/L | 407 (44.1) | 1.87 (1.65–2.14) |                            |                    |       |                    |
| Interferon-β treatment    |                |                     |                            |                    |       |                    |
| No interferon-β treatment | 623 (61.1)    | Reference .01       | Reference .34               | Reference .34       |       |                    |
| Early interferon-β treatment | 227 (26.7)  | 1.28 (1.10–1.49)   | .001 (1.01–1.26)            | .97                 | 1.03 (1.32–1.30) | .78   |
| Late interferon-β treatment | 169 (21.3)  | 1.08 (0.91–1.28)   | .37 (1.19–1.49)             | .14                 | Excluded |
| Center with high mortality | 543 (53.3)  | 1.72 (1.52–1.95)   | < .001 (1.43–2.00)          | < .001              | 1.68  | < .001              |
| Propensity scorec         |                |                     |                            |                    |       |                    |

Data are presented as No. (%) unless otherwise indicated. Crude and adjusted HR have been calculated from imputed data.

Abbreviations: EIT, early interferon-β treatment; NIT, no interferon-β treatment; PS, propensity score; HR, hazard ratio; CI, confidence interval; BMI, body mass index; SpO2, peripheral capillary oxygen saturation.

aThe area under the receiver operating characteristic (AUROC) curve of the model was 86 (95% CI, 0.84–0.91), P = .004.
bPatients in the late interferon-β treatment group were excluded from this analysis.

cAdjusted low SpO2 ≤ 90% for patients aged > 50 years and < 93% for patients aged ≤ 50 years.
dCalculated only for patients in the early interferon-β treatment and no interferon-β treatment groups. The variables included in the propensity score were sex, age, obesity, chronic heart disease, dyspnea, low SpO2, hyperinflammation phase, neutrophil count, lymphocyte count, platelets, D-dimer, lactate dehydrogenase, C-reactive protein, lopinavir/ritonavir, tocilizumab, corticosteroids, and high-mortality hospital. The AUROC curve of the PS model was 83 (95% CI, 0.81–0.87), P < .001.

patients may be candidates for additional medical treatment but not for intensive care, owing to previous conditions. Regarding confounders, we used propensity scores in different ways to control for indication bias. In the crude analysis, the EIT group showed higher mortality, as it was administered to patients with more severe disease. After adjustment for other well-known risk mortality predictors [15,30,31], EIT was not found to be associated with mortality.

Regarding IFN treatment, studies supporting its use in COVID-19 are still scarce and certainly do not address the phase of the disease in which to start administration. Data on the increased severity of COVID-19 in patients with no endogenous IFN-β and low IFN-α production [6] or with neutralizing auto-Abs against type I IFNs [7] suggest a potential role for early IFN treatment. In addition, a cohort analysis of patients with multiple sclerosis showed that IFN administration is preventive of severe COVID-19 [32]. Other issues also have to be considered, such as the dosage and PEGylation to prolong the antiviral effect, as per the methods used in other mammals for acute and chronic viral diseases [33, 34]. An important aspect in our study is the fact that a substantial proportion of patients already had > 7 days of symptoms when admitted, and this was more frequent among those with EIT, meaning that the window of opportunity for benefiting from IFN-β treatment may have already passed when the drug was administered.

The present study has several limitations. First, controlling for confounders in any observational study can be incomplete despite all efforts. Second, a wide range of dosing regimens was used in all groups. Third, the investigators were not blinded to the exposure; however, we used a hard outcome and included consecutive cases. Fourth, our data were not specific to or complete for adverse events, and this is a crucial aspect that should be considered in more detail in future studies. Moreover, we had no access to the follow-up RT-PCR results; thus, we were unable to determine the time to a negative test or to shed further light on the effect of IFN-β on viral dynamics. Regarding the association found between the use of corticosteroids and mortality, the weaknesses are that the study was not designed to evaluate their efficacy, the late time of administration in many cases, and the probable different dosages depending on the clinical situation of the patients. Finally, the cohort proportion of patients already had > 7 days of symptoms when admitted, and this was more frequent among those with EIT, meaning that the window of opportunity for benefiting from IFN-β treatment may have already passed when the drug was administered.
was built during the first wave of the pandemic in Spain; management may have changed afterward. The strengths include the multicenter nature of participation, adequate sample size, and the use of standardized scoring systems and a clear, solid endpoint together with advanced strategies including the use of propensity score analysis. The strengths include the multicenter nature of participation, adequate sample size, and the use of standardization systems and a clear, solid endpoint together with advanced strategies including the use of propensity score analysis. The strengths include the multicenter nature of participation, adequate sample size, and the use of standardization systems and a clear, solid endpoint together with advanced strategies including the use of propensity score analysis.

In conclusion, our findings did not find an association between early interferon-β treatment and no interferon-β treatment groups. The variables included in the propensity score were sex, age, obesity, chronic heart disease, dyspnea, low SpO₂, hyperinflammation phase, neutrophil count, lymphocyte count, platelets, D-dimer, lactate dehydrogenase, C-reactive protein, lopinavir/ritonavir, tocilizumab, corticosteroids, and high-mortality hospital. The AUROC curve of the PS model was .83 (95% CI: .81–.87), P < .001.

Severity rating according to the WHO Clinical Progression Scale, ranged from 0 (not infected) to 10 (dead).

**Collaborators**

The COVID-19@Spain Study Group members.

**Funding** SEIMC-ESIDSA: Aznar Muñoz Esther, Gil Divasson Pedro. Hospital Universitario Virgen Macarena: Retamar Pilar, Valiente Adoración, López-Cortés Luis E., Sojo-Dorado Jesús, Bravo-Ferrer José, Salamanca Elena, Pérez-Palacios Patricia, Gandulfo-Moro María, Ruiz-Hueso Rocío, Moya-González Natalia, Peral Enrique, Valido-Morales Agustín, Pavón-Masa María. Hospital Universitario La Paz: Diaz Menéndez Marta, De la Calle Prieto Fernando, Arsuaga Vice- nte Marta, Ramos Ramos Juan Carlos, De Miguel Buckley Rosa, Cadi- nános Loidi Julen, Marcelo Calvo Cristina, Vasquez Manu Julia, Mora Ilario Marta, Loeches Yague Belen, Ramos Ruperto Luis, García-Rodríguez Julio, Montejano Sánchez Rocio, Diaz Pollan Beatriz. Hospital Universitario Gregorio Marañón: López Juan Carlos, Ramírez-Schacke Margarita, Gutiérrez Isabel, Tejerina Francisco, Aldamiz-Echevarría Teresa, Díez Cristina, Fanciulli Chiara, Pérez-Latorre Leire, Parras Francisco, Catalán Pilar, García-Leoni María E., Pérez-Tamayo Isabel, Puente Luis, Cedeño Jaimon. Hospital Infantil La Fe: Such-Díaz Ana, Álvaro-Alono Elena, Izquierdo-García Elsa, Torres-Macho Juan, Cuevas Guillermo, Notario Helena, Mestre-Gómez Beatriz, Jiménez-González de Buitrago Eva, Fernández-Jiménez Inés, Tebar-Martínez Ana Josefina, Brañas Fátima, Valeria Jorge, Pérez-Butragueño Mario, Muñoz-Rivas Nuria. Hospital Universitario de Bellvitge: Abelañon-Gabriela, Aros Aranay Carmen, Bergas Alba, Caervo Guillermo, Domínguez María Ángeles, Fernández-Huerta Miguel, Gudiol Carlota, Lorenzo-Estella Liubó, Jordi Pérez-Reco Sandra, Podzamczer Daniel, Pujol Miquel, Rombaus Alexander, Truller Nuria. Hospital Universitario Virgen del Rocio: Molina José, Álvarez-Marin Rocío, García-Gutiérrez Manuel, Paniagua María, Alarcón Arístides, Gil-Navarro María Victoria, Giménez Luis, Camacho-Martínez Pedro, Merino Laura, Caballero-Eraso Candela, Paradís Carmen, Valencia-Martín José, Fernández-Delgado Esperanza. Complejo Hospitalario Virgen de la Salud: Sepúlveda Berrocal Mª Antonia, Yera Bergua Carmen, Tole- dano Sierrá Pilar, Cano Llorente Verónica, Zafir Iqubal-Mirza Sadaf, Muniz Gema, Martín Pérez Inmaculada, Mozos Mirígena Ínigo, Alguacil Ana, García Buteniego María Paz. Hospital Universitario Rafael Méndez: Pelaye Ballesta Ana Isabel, Morcillo Rodríguez Elena. Hospital Universitario de Cruces: Goikoetxea Agirre Joseun, Bere- ciartua Bastarrica Elena, Guio Carrión Laura, Euba Ugarre Gorane. Hospital de Melilla: Pérez Hernández Isabel A., Román Soto Sergio. Hospital San Eloy de Barakaldo: Silvarío Fernández Rafael, Ugalde Espiniera Jon. Hospital Universitario Central de Asturias: Asensi Vicente, Rivas-Carmenado María, Suárez Pérez Lucía, Suárez Díaz Silvia. Hospital General Universitario de Alicante: Boix Vicente, Díez Martínez Marcos, Carreres Candela Melissa. Hospital Virgen de la Victoria: Gómez-Ayerbe Cristina, Sánchez-Lara Javier, Velasco Garrido José Luis, López-Jodar María, Santos González Jesús. Hospital Universitario Puerto Real: Ruiz Aragon Jesús, Virtu Peña Irene. EOXI Pontvedra e Salones: Alende Castro Vanessa, Fernandez Morales Marta. Hospital de Figueres: Vega Molpecedes Sonia, Pons Viñas Estel. Hospital Sant Jaume de Calella: del Río Pérez Oscar, Valero Rovira Silvia. Hospital del Mar: Gómez-Junyent Joan, Cañetehan Espinosa Silvia, Canea María Cecilia, Villar-Garcia Judit, Gimenez Argente Carmen, Soldado Folgado Jade, Nogués Solán Xavier, de Pablo Miró Mar, Cañador Labat Miriam. Hospital Clínico Universitario Virgen de

| Variable | Overall Cohort (N = 3368)¹ | Propensity Score-Matched Cohort (N = 288)² |
|----------|----------------------------|------------------------------------------|
| Male sex | 451 (67) | 97 (67.4) |
| Age > 75 years | 193 (28.3) | 30 (20.8) |
| Obesity (BMI >30) | 101 (16.3) | 23 (16) |
| Chronic heart disease | 138 (20.3) | 24 (16.7) |
| Dyspnea | 411 (60.8) | 93 (64.6) |
| Low SpO₂ (age-adjusted)³ | 261 (43.8) | 59 (41) |
| Heart rate > 100 bpm | 175 (27) | 40 (27.8) |
| > 7 days from onset to admission | 142 (20.8) | 29 (20.1) |
| Neutrophil count > 7500/µL | 122 (17.9) | 21 (14.6) |
| Lymphocyte count < 1500/µL | 406 (59.9) | 91 (63.2) |
| Platelets < 150,000/µL | 239 (35.3) | 48 (33.3) |
| D-dimer levels > 500 mg/mL | 192 (262.7) | 96 (66.7) |
| Lactate dehydrogenase > 250 U/L | 369 (83.1) | 115 (79.9) |
| C-reactive protein > 100 mg/L | 295 (46.3) | 66 (45.8) |
| Lopinavir/ritonavir | 635 (93.1) | 142 (98.6) |
| Tocilizumab | 150 (22.4) | 32 (22.2) |
| Corticosteroids | 260 (38.4) | 63 (43.8) |
| Deceased | 227 (33.2) | 38 (26.4) |
| Center with high mortality | 239 (35) | 50 (34.7) |

Data are presented as No. (%). P values are calculated by Cox regression.

Abbreviations: EIT, early interferon-β; NIT, no interferon-β; BMI, body mass index; SpO₂, peripheral capillary oxygen saturation.

¹Patients in the late interferon-β treatment group were excluded from this analysis.

²The Propensity score was calculated only for patients in the early interferon-β treatment and no interferon-β treatment groups. The variables included in the propensity score were sex, age, obesity, chronic heart disease, dyspnea, low SpO₂, hyperinflammation phase, neutrophil count, lymphocyte count, platelets, D-dimer, lactate dehydrogenase, C-reactive protein, lopinavir/ritonavir, tocilizumab, corticosteroids, and high-mortality hospital. The AUROC curve of the PS model was .83 (95% CI: .81–.87), P < .001.

³Age-adjusted low SpO₂ ≤ 90% for patients aged > 50 years and ≤ 93% for patients aged ≤ 50 years.

⁴Severity rating according to the WHO Clinical Progression Scale, ranged from 0 (not infected) to 10 (dead).
María, Parra Arribas Nuria, González Casanova Belen, Yagüe Agueda Raquel. Hospital Virgen del Puerto: Muñoz del Rey José Román, Jiménez Álvaro Montaña. Hospital Marina Salud de Denia: Coy Coy Javier, Poquet Català Inmaculada. Hospital Universitario de Jerez: Santos Peña Marta, Mora Delgado Juan. Hospital Reina Sofia de Tudela: Manso Gómez Tamara, Rubio Obanos Teresa. Hospital Clínico Universitario de Santiago de Compostela: Barbeito Castiñeiras Gema, Trastoy Pena Rocío. Hospital Universitario del Henares: Mao Martín Laura, Adalid Moll María, Díaz Luperenia Javier, Ruiz Grispan Martín Sebastián, Alonso Navarro Rodrigo, Ampuero Martínch José David, Galindo Martín Maria Aránzazu, Martínez Avilés Rocío, Rodríguez Leal Cristobal Manuel. Hospital Universitario Lucus Augustus: Romay Lema Eva María, Suárez Gil Roi. Hospital de Donostia: Iribarren Loyarte Jose Antonio, Bustinduy Orioñoz María Jesús, Ibaguren Pinilla Maialen, Álvarez Rodríguez Ignacio. Hospital de Urduliz Alfredo Espinosa: Arriola Marta Paula, Lartategui Iraurgi Alazne. Hospital de Mendaro: Álvarez de Castro María, Martín Mateu Cintia María. Hospital Juan Ramón Jiménez: Rodríguez Gómez Francisco, Aschert Águero Isabel. Hospital de Tortosa Virgen de la Cinta: Chamorro Martí Maria, Franch Llaser Diego. Hospital Riotinto: Zakarya-Yousef Breal Ismael, Ricardo Rodríguez María. Hospital Victoria Baja: Garcia Romero Laura, Jiménez Guardiola Carlos. Hospital Puerta de Hierro: Fernández Cruz Ana, Calderón Parra Jorge, Ramos Martínez Antonio, Múñez Rubio Elena, Vázquez Comendador José Manuel, Diego Yagüe Itziar, Ñe Rossón Palomo Esther, Blanco-Alonso Silvia, Muñoz-Gómez Ana Delgado Téllez de Cepeda Laura. Hospital Universitario de Getafe: Álvarez Franco Raquel, Martínez Cifre Blanca, Aranda Rife Elena María, Roger Zapata Daniel, Cardona Arias Andrés Felipe, Fernández de Orueta Lucía, Vates Gómez Roberto, Margueanda Contreras Pablo, Martín Rubio Irene, Monereo Alonso Alfonso. Hospital General de la Palma: Barbosa Ventura Andrés, Piñero Iván. Hospital El Bierzo: Bahamonde Carrasco Alberto, Martínez Vidal Ana. Fundación Hospital de Calahorra: Talaver Gómez Meroz, Mendoza Roy Paula. Hospital Alto Deba: Urrutia Losada Ainhoa, Arteche Ezquidal Lorea. Hospital Universitario San Juan de Alicante: Delgado Sánchez Elizabet, Esteve-Añón Pérez Pedro Jesús. Hospital de Guadarrama: Caro Bragado Sarah, Domínguez de Pablos Gema. Hospital Universitario de Jaén: Herrero Rodríguez Carmen, Liébana Martos Carmen. Hospital de Mataró: Force Samartín Luis, Arbones Laia. Hospital de Palamós: Maria Fidalgo Arantzazu, Marchena Romero José Andres. Hospital Universitario de Valme: Merchante Gutiérrez Nicolas, Espindola Gómez Reinaldo. Clínica Universitaria de Navarra-Campus Navarra: Del Pozo León José Luis. Hospital Clínica Bidentorn: Serralta Baudes Josefa, Cabrera Tejada Ginger Giorgiana. Hospital Doce de Octubre: Fernández-Ruiz Mário, Aguado Jose María, Lopez-Medrano Francisco. Hospital Universitario Ramón y Cajal: Vizcarraga Pilar, Rodríguez Domingo Mario José, Gioia Francesca, Del Campo Santos, Canton Moreno Rafael, Martín Dávila Pilar, Quereda Carmen. Hospital Universitario San Pedro: Oteo Revuelta José Antonio, Santibáñez Sáenz Paula, Cervera Acedo Cristina, Pellejero Galadriel, Blanco Ramos José R., Azcona Gutiérrez José M., García García Concepción, Alba Fernández Jorge, Ibarra Cucalón Vanaveran, Omatos Sonia, Metola Sacristán. Hospital Quiron A Coruña: Meijide Míguez Héctor, Paulos Viñas Silvia. HM Sanchinarro: Menéndez Justo, Villares Fernández Paula, Montes Andújar Lara. Hospital Francesc de Borja: Navarro Batet Álvaro, Ferrer Santolaria Anna. Complejo Hospitalario Universitario Nuestra Señora de La Candelaria: Padilla Salazar María de la Luz, Abella Vázquez Lucy, Hayek Pereyra María. Hospital Universitario HM Montepríncipe: Ruiz Fernández Andrés Javier, Barrio López Isabel. Hospital Universitario HM Puerta del Sur: Martakoush Ali. Hospital Universitario HM Torrelodones: Rojas-Vieyra Agustín. Hospital Universitario HM Madrid: García Calvo Sonia, Villarreal García-Lomas Mercedes. Hospital Don Benito-Villanueva de la Serena: Vizcaíno Callejón Marta, García María Pilar. Hospital de Viladecans: Lérida Urtega Ana, Carrasco Fons Natalia, María Sanjuan Beatriz, Martín González Lydia, Sanz Zamudio Camilo. Centro Nacional de Epidemiología: Alejos Belén, Moreno Cristina, Rava Marta, Iniesta Carlos, Izquierdo Rebeca, Suárez-García Inés, Díaz Asunción, Ruiz-Alguero Marta, Hernando Victoria.

CRediT authorship contribution statement

Sonsoles Salto-Alejandre: Conceptualization, Methodology, Software, Validation, Formal analysis, Writing – original draft, Visualization. Zaira R Palacios-Baena: Conceptualization, Methodology, Formal analysis, Supervision. Jose Ramon Arribas: Juan Berenguier: Investigation, Resources, Data curation. Jordi Carratala: Investigation, Resources, Data curation. Inmaculada Jarrín: Investigation, Resources, Data curation. Pablo Ryan: Investigation, Resources, Data curation. Marta de Miguel-Montero: Investigation, Resources, Data curation. Jesus Rodríguez-Bano: Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition. Jerónimo Pachón: Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Financial support

This work was primarily supported by Fundación SEIMC/GeSIDA [grant number COVID-19/SEIMC-FSG]. The funders had no role in study design, data collection, data analysis, data interpretation or writing of the manuscript. Additionally, JI, JB, JRA, JRB, JC, and JP provided funding for research from Plan Nacional de I+D+i 2013–2016 and Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Ministerio de Ciencia, Innovación y Universidades, cofinanced by the European Development Regional Fund “A way to achieve Europe”, Operative program Intelligent Growth 2014–2020, through the following networks: Spanish AIDS Research Network (RIS) to JI [grant number RD16/0002/0006], JB [grant number RD16/0025/0017], and JRA [grant number RD16/0025/0018] and Spanish Network for Research in Infectious Diseases (REIPI) to JRB [grant number RD16/0016/0001], JC [grant number RD16/0016/0005], and JP [grant number RD16/0016/0009]. IU [grant number CB21/13/00091], JB [grant number CB21/13/00044], JRA [grant number CB21/13/00039], JRB [grant number CB21/13/00012], and JC [grant number CB21/13/00009] also received support from the CIBER de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación, cofinanced by the European Development Regional Fund.

Conflict of interest statement

JRA declares the following advisory fees and speaker fees: GSK, MSD, Serono, Lilly, Roche. The rest of the authors declare that there are no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopharm.2021.112572.

References

[1] Johns Hopkins University of Medicine: Coronavirus resource center. [Internet]. 2021. Available from: https://coronavirus.jhu.edu/map.html.

[2] Z. Abdelrahman, M. Li, X. Wang, Comparative review of SARS-CoV-2, SARS-CoV, MERS-CoV, and influenza A respiratory viruses, Front Immunol. 11 (2020), 552909. [https://pubmed.ncbi.nlm.nih.gov/33039295/]

[3] Z. Wu, J.M. McGoogan, Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese center for disease control and prevention, JAMA 323 (13) (2020) 1239–1242, https://doi.org/10.1001/jama.2020.2648.

[4] A. Gupta, M.V. Madhavan, K. Sehgal, N. Nair, S. Mahajan, T.S. Seahawa et al., Extrapolatory manifestations of COVID-19, Nat. Med. 26 (7) (2020) 1017–1032.
