Clinical features, immune response, and virological factors associated with SARS-CoV-2 infection outcomes

TESIS DOCTORAL

DEPARTAMENTO DE MEDICINA
UNIVERSIDAD DE SEVILLA

Doctoranda:
Sonsoles Salto Alejandre

Directores:
Javier Sánchez Céspedes
María Elisa Cordero Matía

Tutora:
María Elisa Cordero Matía

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PRODUCCIÓN CIENTÍFICA RESULTANTE DE LA TESIS DOCTORAL

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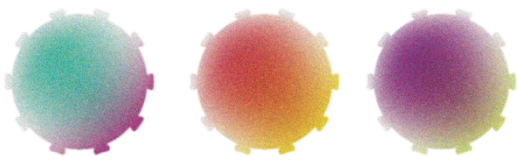


INTRODUCTION

1. INTRODUCTION

Timeframe and magnitude of the COVID-19 pandemic

In December 2019, the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causative agent of coronavirus disease 2019 (COVID-19), emerged in China[1]. It spread globally, becoming a public health emergency and a pandemic of historic dimensions[2]. 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[3]. Beyond what is directly attributable to it, the pandemic has caused extensive collateral damage that has led to losses of lives and livelihoods. In this regard, Msemburi *et al*. reported a comprehensive and consistent measurement of the impact of the COVID-19 pandemic by estimating excess deaths, by month, for 2020 and 2021 (Figure 1)[4]. Spain has been one of the most affected countries in the world in terms of absolute number of diagnosed cases and deaths *per capita* [5], causing a dramatic decline in donations and transplantation procedures per day, with mean numbers dropping from 7.2 to 1.2 and 16.1 to 2.1, respectively[6]. At present, the COVID-19 pandemic keeps causing an important burden of worldwide disease, with more than 757 million cases and 6.8 million deaths reported by WHO as of February 23th, 2023[7].

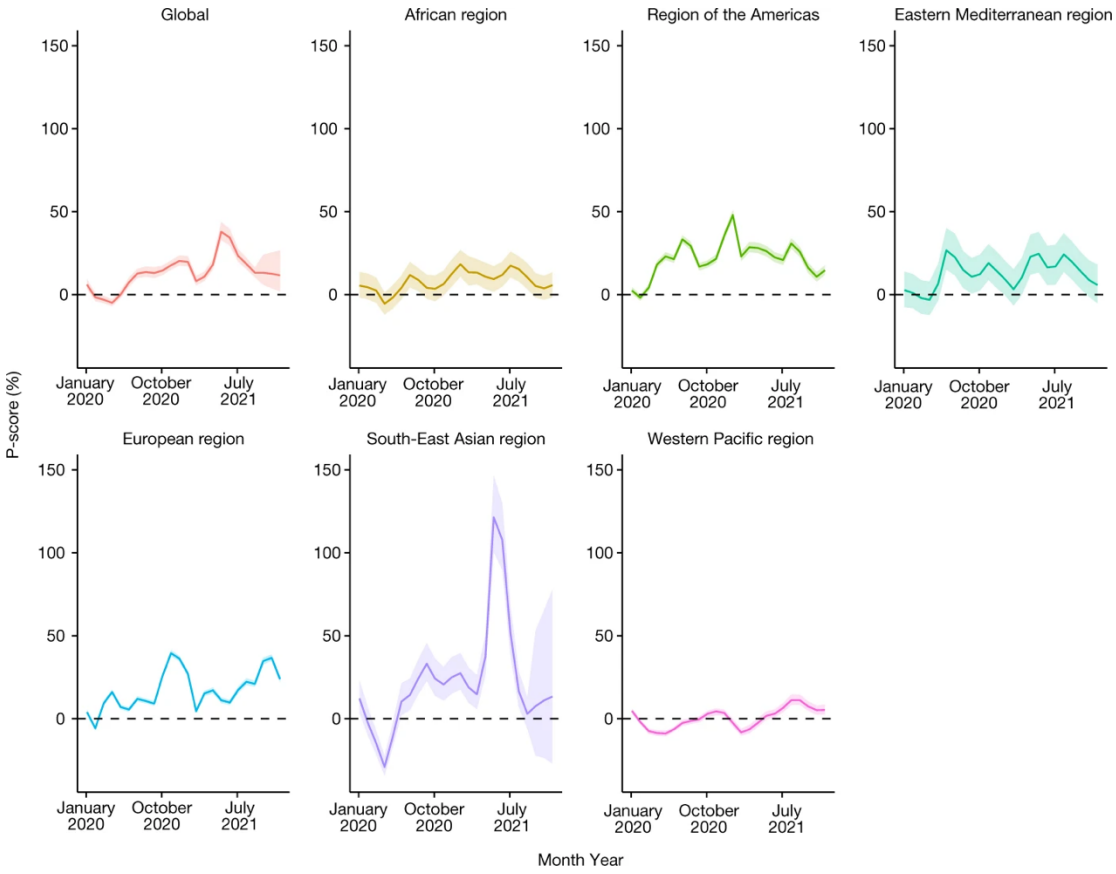


Fig. 1. *Global and WHO region P-scores (excess deaths relative to expected deaths): Monthly estimates of P-scores, expressed as a percentage, aggregated globally and for the six WHO regions for the period January 2020 to December 2021. All plots show the mean estimates and the 95% uncertainty intervals.*

The different vaccination rates across countries[7] and the emergence of SARS-CoV-2 variants of concern (VOC) facilitate the virus evasion of natural and acquired immunity, as well as the continuous community transmission. The global scientific community has collectively risen to the challenge of COVID-19 by innovating and deploying in real time a wide range of collaborative tools and platforms to facilitate epidemic nowcasting, including the COVID-19 dashboards by Johns Hopkins University (Figure 2)[8]. As healthcare systems and hospitals around the world prepare for a rising and maintained incidence of COVID-19, important questions on the natural history of the disease, susceptibility of

immunocompromised populations (particularly solid organ transplant recipients (SOTRs) severity risk factors, and specific management of antivirals and immunomodulatory drugs remain unanswered[9].

There is a growing number of reports trying to identify the factors associated with critical illness or demise. Some of them included a vast number of cases (up to 20 133 participants in a recent publication from the UK)[10], and identified that male older patients with a variety of comorbidities were more likely to die during hospitalization[10–12]. Most of these factors are, however, already well-known predictors of worse prognosis in community-acquired pneumonia, components of the prediction rule developed by Fine et al. in 1997[13]. In April 2020, Richardson et al. also analyzed the main clinical characteristics of COVID-19 inpatients in the New York City Area[14], confirming older age and male sex as important demographic factors, pointing out the high frequency of underlying chronic diseases, and reporting a mortality rate of 21%. Generally, studies of this pandemic are only descriptive, and not useful in assessing the rates of unfavourable clinical outcomes or the risk factors associated, because they were released when most patients (up to 93.6%)[15] were still hospitalized.

COVID-19 predictive models are becoming increasingly frequent in medical literature, as they serve as a basis for clinical decision-making. Nevertheless, many of them are notably biased, non-validated, or present a development lacking in clarity[16,17]. Given this scenario, we developed an effective prognostic tool composed of these five features, with high sensitivity and specificity to accurately discriminate individuals that might develop critical disease or die, from those with a favorable course. Our predictive equation was designed to reduce the

uncertainty involving this new illness and to foster more appropriate use of available resources in its management. To our knowledge, this was the first-ever predictive rule for adverse events to guide prompt therapeutic decisions and level of care at hospital admission of COVID-19 patients during early stage of outbreak. In comparison with previous studies for assessing COVID-19 severity risk factors coefficients, our prediction model had distinctive strengths. The independent variables were explicitly defined and could be readily analyzed at the time of hospital admission. Thus, patients could be assigned a probability of disease progression or fatality based on information from the initial history and quickly available laboratory examinations. Also, the accuracy and generalizability of our model was supported by its rigorous derivation and internal validation. Unlike the rest of prognosis models and risk factors assessment series published[12,13,18–20], that included already well-established and globally accepted clinical predictors of severity, we opted for incorporating exclusively the explanatory variables that, presumably, were directly related to the pathogenesis of COVID-19. In this respect, the reasonable biological plausibility of hypoxemia, thrombocytopenia, neutrophilia, and high levels of lactate dehydrogenase (LDH) and C-reactive protein (CRP), coupled with their important role in disease progression, made our selected variables of great interest for further research on SARS-CoV-2 damaging mechanisms and therapeutic targets for the infection it causes.

For coping with the best clinical attention to COVID-19 patients it is crucial to perform prognosis estimations at the first clinical evaluation, offering personalized attention based on early and easily detectable predictors that support decision making, guide level of care, and optimize the allocation of health resources.

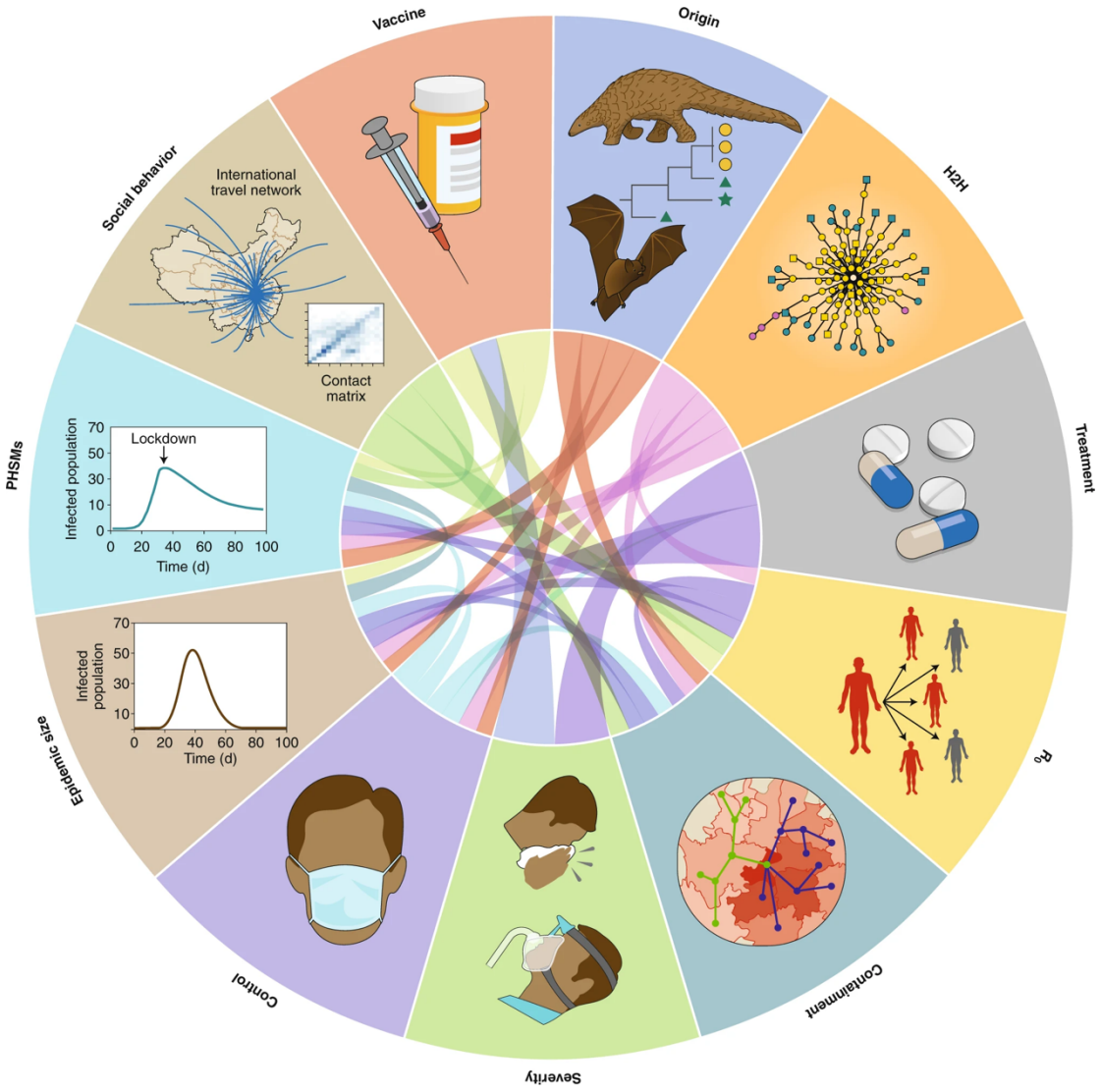


Fig. 2. *Main targets for epidemic nowcasting. The main targets of epidemic nowcasting are shown, highlighting the interconnectivity between all the elements. H2H, human to human.*

Clinical features of COVID-19

The clinical spectrum of COVID-19 ranges from asymptomatic disease to pneumonia, life-threatening complications, and ultimately death[15,21,22]. Symptomatic SARS-CoV-2 infection presents a characteristic sequence of phases (Figure 3)[23], 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[24]. 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[6].

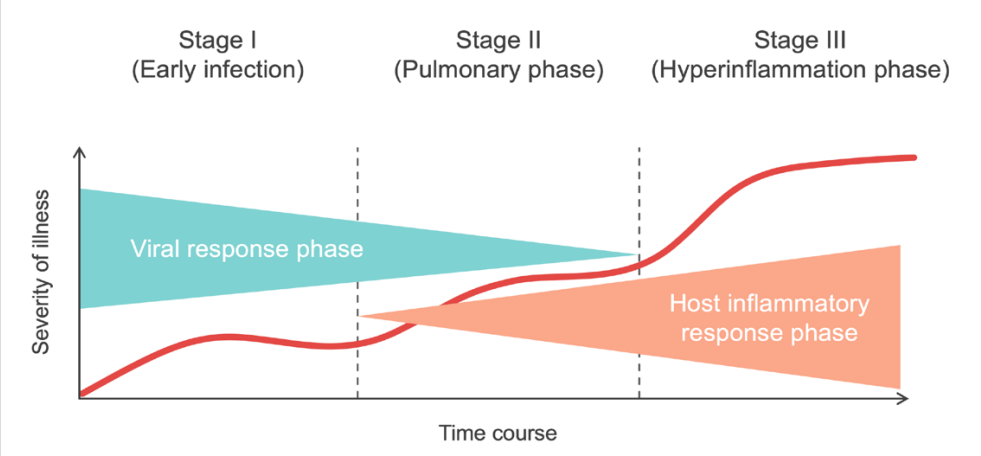


Fig. 3. *The natural history of COVID-19: viral phase and inflammatory phase.*

The Clinical Characterization and Management Working Group led the initiative of developing a common minimal outcome set, established by WHO as a component of the research and development roadmap process in response to COVID-19 (Figure 4)[25]. The scale provides a measure of illness severity across a range from 0 (not infected) to 10 (dead) with data elements that are rapidly obtainable from clinical records. Modelling in other disease states has shown that distinction is greater when seven or more classes are used, particularly at the lower range of disease severity[26]. This spectrum, from the absence of infection to death, enables the scale to be used across a broad range of studies. Clinical and virological absence of infection is suggestive of a cure for patients who are initially infected or suggestive of a misdiagnosis for those individuals included in a trial. The WHO Clinical Progression Scale can also function as the entry criterion for patients in a vaccine trial. At the other end of the severity spectrum, the scale recognizes that mechanical ventilation provides support that is survivable, although that probability is affected by both the severity of respiratory failure and the development of additional physiological organ dysfunction.

Patient State	Descriptor	Score
Uninfected	Uninfected; no viral RNA detected	0
Ambulatory mild disease	Asymptomatic; viral RNA detected	1
	Symptomatic; independent	2
	Symptomatic; assistance needed	3
Hospitalised: moderate disease	Hospitalised; no oxygen therapy*	4
	Hospitalised; oxygen by mask or nasal prongs	5
Hospitalised: severe diseases	Hospitalised; oxygen by NIV or high flow	6
	Intubation and mechanical ventilation, pO_2/FiO_2 ≥150 or SpO_2/FiO_2 ≥200	7
	Mechanical ventilation pO_2/FIO_2 <150 (SpO_2/FiO_2 <200) or vasopressors	8
	Mechanical ventilation pO_2/FiO_2 <150 and vasopressors, dialysis, or ECMO	9
Dead	Dead	10

Fig. 4. *WHO clinical progression scale: ECMO=extracorporeal membrane oxygenation. FiO2=fraction of inspired oxygen. NIV=non-invasive ventilation. pO2=partial pressure of oxygen. SpO2=oxygen saturation. *If hospitalized for isolation only, record status as for ambulatory patient.*

Risk factors for severe disease in the general population include older age and comorbidities (Figure 5)[27], but the impact of chronic immunosuppression related to transplantation on COVID-19 is not well known. Despite widespread concern that COVID-19 clinical phenotypes may be more severe among SOTRs due to a poorer inflammatory response and greater organ injury, data on this population are limited to a few case series and generally small retrospective cohorts[28–37].

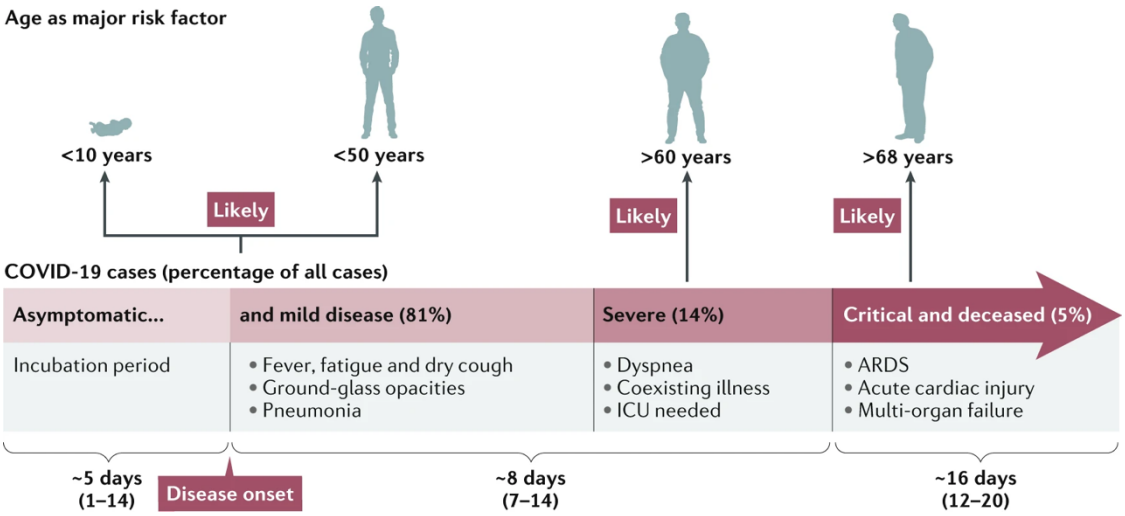


Fig. 5. *Clinical features of COVID-19. Typical symptoms of coronavirus disease 2019 (COVID-19) are fever, dry cough and fatigue and in severer cases dyspnea. Many infections, particularly in children and young adults, are asymptomatic, whereas older people and/or people with co-morbidities are at higher risk of severe disease, respiratory failure and death. The incubation period is ~5 days, severe disease usually develops ~8 days after symptom onset and critical disease and death occur at ~16 days. ARDS, acute respiratory distress syndrome; ICU, intensive care unit.*

Virological factors associated with clinical outcomes

Globally, as of 26 February 2023, over 4.8 million new cases and over 39 000 deaths were reported in the last 28 days (30 January to 26 February 2023), a decrease of 76% and 66%, respectively, compared to the previous 28 days (Figure 6)[38]. Similarly, a total of 42 258 new hospitalizations and 1619 new ICU admissions were reported. This represents a reduction in both new hospitalizations and ICU admissions of 83% and 49%, respectively, compared to the previous 28 days.

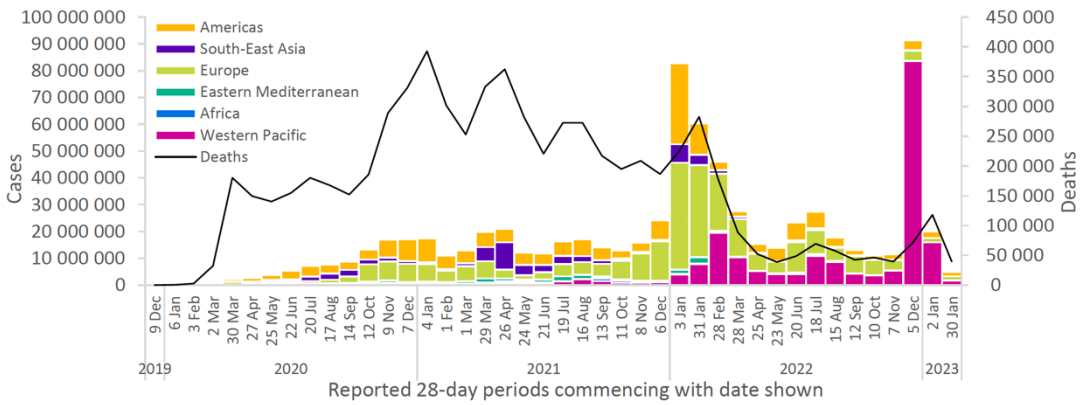


Fig. 6. *COVID-19 cases reported by WHO Region, and global deaths by 28-day intervals, as of 26 February 2023.*

In this period, 60 559 SARS-CoV-2 sequences were shared through GISAID. Among these, 60 521 sequences (99.9%) were the Omicron variant of concern (VOC). There has been an increasing trend in the proportions of recombinant lineages. In epidemiological week 6 (6 to 12 February 2023), pooled recombinant variant sequences accounted for 41.5% (7748 sequences) of sequences, which has increased from 18.7% (8919 sequences) in week 2 (9 to 15 January 2023). Most of these recombinant variants in week 6 were XBB.1.5 (32.6% of all sequences). In addition, recombinant variant XBF accounted for 1.2% of all sequences. During the same reporting period, Omicron BA.5 and its descendent lineages accounted for 31.8% of all shared sequences (5936 sequences), a reduction as compared to 61.8% in week 2 (9 to 15 January 2023). The prevalence of Omicron BA.2 and its descendent lineages remained stable (13.7% as compared to 13.9% in week 2, 2023). Unassigned sequences (all presumably Omicron awaiting descendent lineage assignment) accounted for 12.9% of the shared sequences in week 6. Omicron BA.1, BA.3 and BA.4 variants and their descendent lineages accounted for <1% prevalence in week 6.

Different studies have addressed the possible association between the viral load in nasopharyngeal (NP) swabs and the clinical outcomes. Some studies have reported that a high number of virus copies in NP swabs, mainly defined as a cycle threshold (Ct) < 25 or < 22 in the real-time polymerase chain reaction (RTPCR), was an independent risk factor for intubation and/or death[39–42]. However, other studies have not found independent association between low Ct values and critical care admission or death[43,44]. In short, the real impact of initial SARS-CoV-2 viral load in NP swabs on COVID-19 patients' outcomes has not been fully elucidated, and this issue remains controversial[45].

On the other hand, positive RNAemia at hospital admission has been associated with COVID-19-related mortality in different studies[43–49], as well as longer duration of RNAemia[50]. Moreover, visualization of virus particles in plasma indicates that SARS-CoV-2 RNAemia can be explained, at least in part, by viremia[51]. Several specific underlying conditions has been associated with the presence of SARS-CoV-2 RNAemia, i.e., active neoplasia or transplantation[52–54]. However, immunocompromised patients are usually underrepresented in studies.

Immune response to COVID-19

The infection by SARS-CoV-2 elicits an innate and specific cellular and humoral immune response[55]. Interferons (IFN), a wide class of cytokines, are key in the innate immune response during the acute phase of the viral infection, as seen with plasmacytoid dendritic cells expressing high concentrations of type I and III IFNs in COVID-19 patients[56,57]. In acute COVID-19 and convalescent patients, intracellular cytokine staining after stimulation with SARS-CoV-2 peptide pools,

as an independent measurement of specific CD8+ T-cells activation against the infection, have showed significant IFN-γ increases, compared with unexposed people, and without differences in expression in both phases of the disease, which is associated with viral elimination[58,59]. However, it is also known that coronaviruses encode IFN antagonists that interfere with host IFN induction and/or signaling (Figure 7)[60].

Several studies have reported that lower levels of systemic type I IFN and IFN signatures are associated with severe COVID-19[61–63]. A longitudinal study carried out in 32 patients with mild/moderate to critical COVID-19, with the serum IFN levels determined after 8-10 days from symptoms onset, showed an association between type I IFN response impairment and both persistent RNAemia and an exacerbated inflammatory response[61]. In a cohort of 26 critically ill patients with COVID-19, with longitudinal follow up, negative IFN-α2 was associated with the need of intensive care unit (ICU) admission, but not with mortality[62]. Moreover, it has been reported that at least 10% of patients with life-threatening COVID-19 have neutralizing autoantibodies against type I IFNs[63].

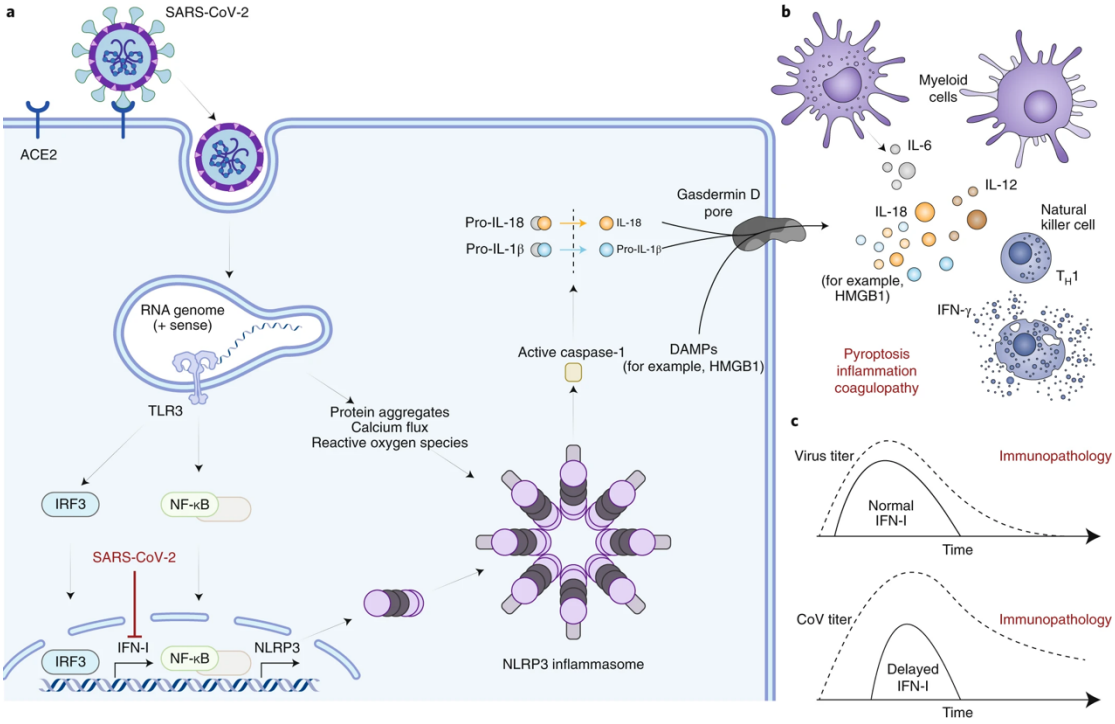


Fig. 7. *Coronavirus recognition and immune response. a, SARS-CoV-2 viruses bind to the ACE2 receptor for cell entry. Viral RNA is recognized by TLR3, which triggers transcriptional responses and cytoplasmic changes that activate the NLRP3 inflammasome. This leads to cleavage of precursor IL-1β (pro-IL-1β), pro-IL-18 and gasdermin D, allowing secretion of IL-1β and IL-18. These changes collectively induce pyroptosis, inflammation and coagulopathy. b, Secreted IL-18 together with IL-12 from myeloid cells stimulate TH1 immunity and natural killer cells to secrete IFN-γ. c, A key feature of coronaviruses (MERS-CoV, SARS-CoV) is a capability to inhibit and delay the type I IFN response, leading to increased viral replication and severe immunopathology.*

However, a study that included 50 patients showed higher IFN-α plasma levels in COVID-19 patients with more severe disease[64]. A temporal analysis in 32 patients hospitalized for COVID-19 pneumonia showed higher levels of IFN-λ1 and IFN-α in critically *vs*. noncritical patients, between 1 to 3 days after hospital admission[65]. Other study serially analyzed the immune responses in 113 patients with moderate (n=80) or severe (n=33) COVID-19 showing higher IFN-α2 levels in dead patients and in those requiring ICU admission[66]. A possible explanation for these apparent contradictory findings, of high or low-IFN signal correlating with

COVID-19 severity, could be the time elapsed between SARS-CoV-2 infection and the IFN levels and signal analysis measurement[67]. If this interval is long, a positive correlation between IFN levels and disease severity could be a consequence of the presence of higher viral loads and RNAemia. Additionally, in the case of type I IFN, the absence of its induction and/or signature, or their high levels, may be explained by the precise inflammatory state of the patient.

Treatment scenario

The magnificent research work on developing an effective COVID-19 vaccine has resulted in several safe and effective options[56,68,69]. However, there is still a need to focus on developing potential drug candidates for treating patients with severe clinical symptoms. During the COVID-19 public health emergency, the Food and Drug Administration (FDA) issued Emergency Use Authorization (EUA) for various new drugs and medical products without full FDA approval. Currently, the primary treatments for the disease are antiviral drugs, immunomodulators, neutralizing antibody, and cell and gene therapies (Figure 8)[70–72].

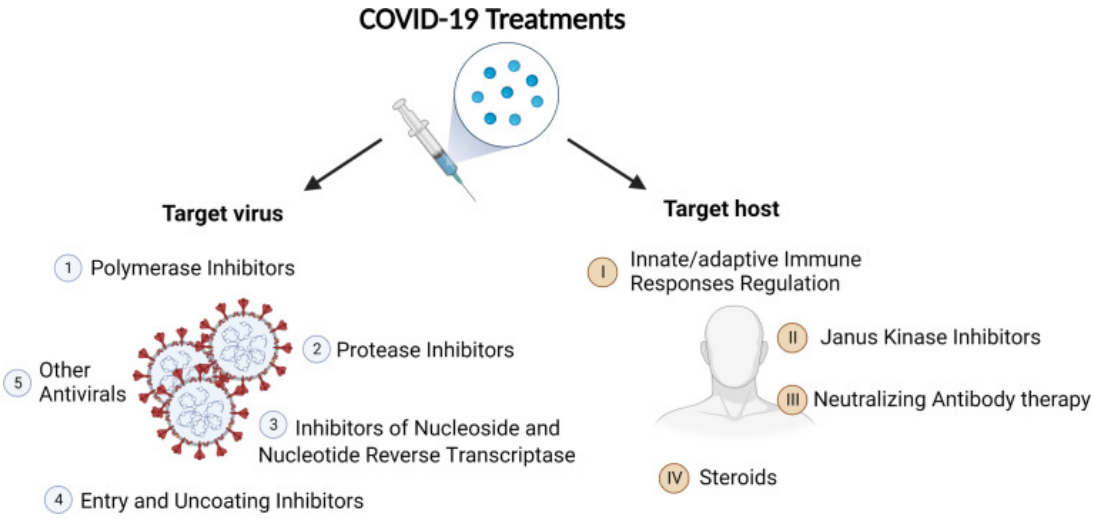


Fig. 8. *Category of COVID-19 treatments. On the basis of their targets, the treatments can be divided into two big categories: antiviral agents and therapies targeting host.*

Remdesivir is a nucleotide prodrug, and its active metabolite can inhibit the activity of RNA polymerases, which is a key enzyme for the replication of many viruses, including coronaviridae. Remdesivir showed antiviral effect on SARS-CoV-2[73,74], and it was approved by FDA for treating COVID-19. However, the clinical antiviral effect of remdesivir against SARS-CoV-2 remains controversial. One clinical trial demonstrated that remdesivir outperforms placebo. The recovery time of adults hospitalized with COVID-19 and lower respiratory tract infection is shortened after receiving remdesivir treatment[75]. Whereas other studies including a multicenter trial conducted in 10 hospitals in Hubei, China, showed that there was no statistically significant difference in the clinical status of patients with COVID-19 receiving remdesivir compared with standard care[76–80].

Lopinavir/ritonavir is marketed as a combination product. A clinical trial that enrolled 176 hospitals in the UK (1,616 patients were assigned to lopinavir/ritonavir group and 3,424 patients to the usual care group) reported

that no efficacy was observed in hospitalized patients with COVID-19 treated with lopinavir/ritonavir[81].

Nirmatrelvir is an inhibitor of the SARS-CoV-2 main protease (Mpro) enzyme[82]. A phase 2–3 clinical trial was performed in symptomatic, unvaccinated, non-hospitalized adults at high risk for progression to severe COVID-19. In this study, 1,120 patients received nirmatrelvir plus ritonavir therapy and 1,126 patients received placebo. Symptomatic patients with COVID-19 treated with nirmatrelvir plus ritonavir had an 89% lower risk of developing severe COVID-19 than placebo[83].

Molnupiravir is a small-molecule ribonucleoside prodrug of N-hydroxycytidine and has activity against coronaviruses including SARS-CoV-2[84]. Molnupiravir reduced the risk of hospital admission or death by approximately 50% in non-hospitalized adults with mild-to-moderate COVID-19 who were at risk for poor outcomes[85]. To evaluate the efficacy and safety of treatment with molnupiravir in non-hospitalized, unvaccinated adults with mild-to-moderate COVID-19, a phase 3 clinical trial was conducted. Study results suggested that the risk of hospitalization or death of unvaccinated adults with COVID-19 could be reduced by early treatment with molnupiravir[86].

Azithromycin is a synthetic macrolide antibiotic with a broad range of antibacterial, anti-inflammatory, and antiviral properties[87]. A prospective, randomized superiority trial done at 19 hospitals in the UK reported that adding azithromycin to standard care treatment did not reduce the risk of subsequent hospital admission or death in patients with mild-to-moderate COVID-19[88]. Moreover, another study also showed that the routine use of azithromycin did not

reduce the recovery time or risk of hospitalization for people who were suspected with COVID-19[89].

Hydroxychloroquine and chloroquine, used to treat malaria and rheumatologic conditions, have been suggested as potential treatments for COVID-19. Currently, at least 80 trials of chloroquine, hydroxychloroquine, or both, sometimes in combination with other drugs, are registered worldwide[90]. In one study of 1,561 patients with COVID-19 treated with hydroxychloroquine and 3,155 in usual care, hydroxychloroquine did not lower patient mortality compared with usual care[91]. Moreover, hydroxychloroquine did not provide significant improvement in symptom severity for early, mild COVID-19 outpatients[92], and could not prevent symptomatic infection after SARS-CoV-2 exposure[93,94].

Convalescent plasma from patients who recovered from infection was adopted to treat severe patients. In 2019, the first peer-reviewed study about the effect of convalescent plasma was carried out in China. Compared with patients who received standard treatment, 103 patients with severe COVID-19 did not show a statistical difference after transfusion of convalescent plasma[95]. At the same time, another trial with 1,181 patients across 23 sites in the US was carried out with the opposite conclusion. This study found that 37 COVID-19–related hospitalization occurred in 589 patients who received control plasma, whereas only 17 of the 592 patients who were infused with convalescent plasma showed disease progression, leading to hospitalization, which means convalescent plasma greatly reduced the hospitalization risk[96].

Although convalescent plasma showed partial effectiveness in selected patients, its potential is still controversial. In addition, only part of plasma antibodies will

be neutralizing, and those non-neutralizing antibodies will bind to non-spike protein viral antigens, which will sabotage antibody reactions to further cause tissue damage. Indeed, in some convalescent plasma trials, allergic responses and lung damage occurred. Furthermore, the antibody titer in convalescent plasma is low and the resource of the blood is constrained. All these disadvantages restricted the application of convalescent plasma therapy in clinics. In contrast, monoclonal/polyclonal antibodies therapy, as another type of passive immunotherapy, can precisely target the neutralizing sites, and they can be massively produced and easily scalable, which conquers all the disadvantages of convalescent plasma. So far, the only neutralizing monoclonal antibody issued by FDA for emergency use is bebtelovimab. Iketani et al. confirmed three sublineages of Omicron showed resistance to 17 neutralizing antibodies except for bebtelovimab[97,98]. Westendorf et al. were able to isolate bebtelovimab through a high-throughput B cell screening pipeline. The authors uncovered the LY-CoV1404 epitope is highly conserved in contact residues, which is why they still show neutralizing activity against omicron variance[99,100]. The efficacy of bebtelovimab was also confirmed by another study conducted by Wang et al. and published 1 month ago[101,102]. In this study, they also identified that the Omicron variance showed more transmissible and more evasive to antibodies. In an ongoing study, continuation of the Cochran work[103], SOTR who received bebtelovimab early, within 2 days after COVID-19 diagnosis, had significantly lower risk for COVID-19 related hospitalization compared to those who received bebtelovimab later, or not at all. Patients who did not receive bebtelovimab were more likely to be hospitalized for any cause and had higher all-cause mortality. Although bebtelovimab is no longer authorized in the US, we hope that this study

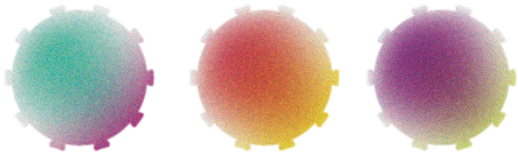
will call attention to the need for future studies to determine the optimal timing of future COVID-related therapies for high-risk immunocompromised patients. In addition, this highlights the need to devise and implement systems of care that facilitate rapid symptom reporting, diagnosis, and treatment for the most vulnerable patients.

Baricitinib is a JAK1/JAK2 inhibitor that blocks cytokine and growth factor receptor stimulation, thereby reducing downstream immune cell function[104]. Numerous studies have established the potency of baricitinib in hospitalized participants with COVID-19. Improved oxygenation and reduced levels of systemic inflammatory cytokines have been reported in patients with COVID-19 treated with baricitinib[105–107]. In May 2022, baricitinib was approved by the US FDA for the treatment of hospitalized adults with COVID-19 requiring supplemental oxygen, mechanical ventilation, or ECMO.

The latest research shows that SARS-CoV-2 infection can motivate the immune system and ignite inflammation, which occasionally causes lethal cytokine storm. Corticosteroids have been used to treat inflammation related diseases in the last decade, such as rheumatoid arthritis and asthma. However, the trials of corticosteroids in patients with COVID were not encouraged in the beginning considering their function in suppressing the immune system. After several randomized clinical trials, corticosteroids have been proved to be able to improve survival in severe COVID-19[108].

Knowing the role of the inflammatory response in the development of severe complications, it is likely that developing a compound with both antiviral and immunomodulatory effects would be the most powerful approach to combat

COVID-19. Interferons are crucial not only for antiviral immunity but also to dampen the innate response, preventing damage from pathogen-induced inflammation, and 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[109]. 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[110]. 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[111,112]. 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[113].



FOUNDATION

2. FOUNDATION

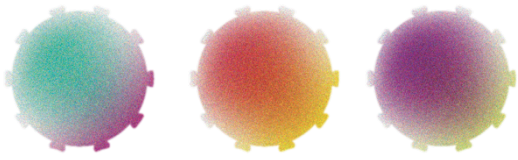
The severity of the COVID-19 pandemic has unleashed an unprecedented scientific activity focused, firstly, on the search for effective treatments to alleviate the effects of the disease and block the replication of the virus, to develop preventive vaccines, and on better understanding the pathogenesis and epidemiology of COVID-19, as well as in identifying prognostic factors that allow classifying those patients with a higher risk of ICU admission or death. Current information on risk factors for unfavourable COVID-19 outcomes in hospitalized patients are focused on patients' demographics, comorbidities, severity of the symptoms and laboratory findings. While there is abundant information regarding risk factors associated with unfavorable outcome in the general population, these are scarce for the immunosuppressed patients.

There is a lack of robust studies to define the SARS-CoV-2 infection behavior in the very variable population of immunosuppressed patients, regarding the kinetics of viral infection and immune clearance, the degree and time of viral shedding, the disease severity risk factors, and clinical manifestations and outcomes. Moreover, recommendations for a better management of the disease in these patients are urgently needed. Recent data suggest that patients with primary and secondary immunodeficiencies, including malignancies and SOT, may be at increased risk of severe COVID-19 disease and death, but new prospective and controlled studies are needed to determine the attributable risk of immunocompromising conditions and therapies on COVID-19 disease prognosis. Moreover, the relationship between COVID-19 disease outcomes, SARS-CoV-2 kinetics, and the innate and specific immune responses has not been fully elucidated. In addition, mutations of

SARS-CoV-2 virus have emerged, with VOC disseminated in several world areas. Independently of the increased dissemination ability, some VOC have presumed higher severity or possible reduction of vaccine effectiveness, without data on its impact in immunosuppressed patients and on the neutralizing activity of antibodies after the infections by the Wuhan original lineage.

A profound knowledge of the epidemiology, immune response, and clinical course of SARS-CoV-2 infection in this group of patients would allow for the establishment of proper diagnostic strategies, and accurate prophylactic and therapeutic approaches focused on possible complications, decrease of hospital admission and secondary mortality. In addition, clinical algorithms regarding the adjustment of immunosuppressive medication, antiviral treatment, need for hospital admission, and duration of isolation measures should also be addressed.

The general objective of this thesis is to study the incidence of COVID-19 in patients with immunosuppression, to analyze the SARS-CoV-2 infection and immune response dynamics in these patients, and to determine the impact of COVID-19 on clinical outcomes, as well as the risk factors associated with the unfavorable outcomes.



HYPOTHESIS AND AIMS

3.1. HYPOTHESIS

Article 1 hypothesis

1. COVID-19 clinical phenotypes may be more severe among solid organ transplant recipients (SOTRs) due to a poorer inflammatory response and greater organ injury.
2. Initial and easy-to-collect clinical and inflammatory data may identify SOTRs with poorer outcomes.

Article 2 hypothesis

1. A positive correlation between types I and II of interferon (IFN) levels and disease severity could be a consequence of the presence of higher viral loads and RNAemia.
2. In the case of type I IFN, the absence of its induction and/or signature, or their high levels, may be explained by the precise inflammatory state of the patient.

Article 3 hypothesis

1. There might be an association between initial SARS-CoV-2 viral load in nasopharyngeal swabs with COVID-19 patients' clinical severity.
2. Higher nasopharyngeal SARS-CoV-2 viral load may predict the mortality in COVID-19 adult patients.

Article 4 hypothesis

1. Early administration of IFN-β could be associated with lower COVID-19-related mortality compared to standard treatment alone.

3.2. AIMS

Article 1 aims

1. To describe the clinical characteristics, treatments, and predictors of unfavorable clinical outcomes hospitalized SOTRs adults with COVID-19.

Article 2 aims

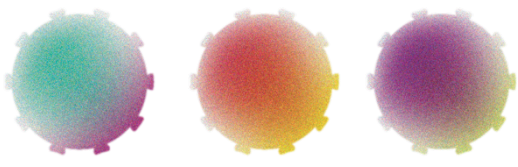
1. To assess, by days from the symptom onset, the clinical usefulness of plasma IFN-α, IFN-γ, and RNAemia at hospital admission as rapid biomarkers of unfavourable outcomes, both in SOTRs and in no SOTRs hospitalized for COVID-19.

Article 3 aims

1. To analyzed if the viral load of SARS-CoV-2 in NP swabs is associated with the disease severity.
2. To assess the ability of NP SARS-CoV-2 viral load at the first hospital evaluation to predict unfavorable clinical outcomes.

Article 4 aims

1. To assess the protective effect of early IFN-β treatment compared with no IFN-β administration in patients hospitalized with COVID-19.



METHODS AND RESULTS

4. METHODS AND RESULTS

Presentation of the articles

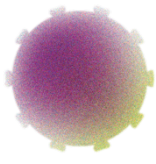
The first article *Risk factors for unfavorable outcome and impact of early post-transplant infection in solid organ recipients with COVID-19: A prospective multicenter cohort study* published in PLoS ONE (doi: 10.1371/journal.pone.0250796)[114] analyzes the characteristics and predictors of unfavorable outcomes in SOTRs with COVID-19. This is a prospective observational cohort study of 210 consecutive SOTRs hospitalized with COVID-19 in 12 Spanish centers from 21 February to 6 May 2020. Data pertaining to demographics, chronic underlying diseases, transplantation features, clinical, therapeutics, and complications were collected. The primary endpoint was a composite of ICU admission and/or death, and logistic regression analyses were performed to identify the factors associated with these unfavorable outcomes.

To gain insight in the innate immune response in SOTRs with COVID-19, compared with no SOT patients, we conducted a second article *Serum IFN-γ and RNAemia temporal profiles as biomarkers of severe COVID-19 in solid organ transplant and immunocompetent patients* published in Journal of Infection (doi: 10.1016/j.jinf.2023.01.019)[115] addressing the IFN serum levels and RNAemia detection at hospital admission and by days from symptoms onset, as well as their association with unfavorable clinical outcomes (death and/or invasive mechanical ventilation [IMV]). This was a multicenter prospective observational cohort study, including 47 SOTRs and 408 non SOTRs consecutive adult inpatients with confirmed COVID-19 and available samples for IFN-α/IFN-γ serum levels and RNAemia determinations, from January 6th, 2020, to August 13th, 2021. Data were

separately analyzed for SOTRs and non SOTRs. In addition, we performed a matched cohort analysis in which patients undergoing transplantation were paired with those from the non SOTRs cohort (1:2) according to their propensity score (PS) to control for residual confounders. IFN-α and IFN-γ levels were analyzed as discrete (undetectable and detectable) and continuous (pg/mL) variables. Multivariate Cox regression and logistic regression analyses were performed to identify factors independently associated with 30-day all-cause mortality and unfavorable clinical outcomes.

For coping with the best clinical attention to COVID-19 patients it is crucial to perform prognosis estimations at the first clinical evaluation, offering personalized attention based on early and easily detectable predictors that support decision making, guide level of care, and optimize the allocation of health resources. In this regard, different studies have addressed the possible association between viral load in NP swabs and clinical outcomes. However, this issue remains controversial. In our third article *SARS-CoV-2 viral load in nasopharyngeal swabs is not an independent predictor of unfavorable outcome* published in Scientific Reports (doi: 10.1038/s41598-021-92400-y)[116] we present a prospective cohort study including 321 adult patients with confirmed COVID-19. Quantitative Synthetic SARS-CoV-2 RNA cycle threshold values were used to calculate the viral load in $\log_{10}$ copies/mL. Disease severity at the end of follow-up was categorized into mild, moderate, and severe. Primary endpoint was a composite of ICU admission and/or death, for which univariable and multivariable logistic regression analyses were performed.

Since IFN-β seemed to be 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, we elaborated a fourth article *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* published in Biomedicine & Pharmacotherapy (doi: 10.1016/j.biopha.2021.112572)[117] that analyzed the impact of early IFN-β treatment in patients admitted with COVID-19 during the first wave of the pandemic. This was post hoc analysis of a COVID-19@Spain multicenter cohort including 3808 consecutive adult patients hospitalized with COVID-19 from 1 January to 17 March 2020. The primary endpoint was 30-day all-cause mortality, and the main exposure of interest was subcutaneous administration of IFN-β, defined as early if started ≤ 3 days from admission. Multivariate logistic and Cox regression analyses were conducted to identify the associations of different variables with receiving early IFN-β therapy and to assess its impact on 30-day mortality. A PS was calculated and used to both control for confounders and perform a matched cohort analysis.



ARTICLE 1

PLOS ONE

RESEARCH ARTICLE

Risk factors for unfavorable outcome and impact of early post-transplant infection in solid organ recipients with COVID-19: A prospective multicenter cohort study

Sonsoles Salto-Alejandre[1,2], Silvia Jiménez-Jorge[1,2], Nuria Sabé[3], Antonio Ramos-Martínez[4], Laura Linares[5], Maricela Valerio[6], Pilar Martín-Dávila[7], Mario Fernández-Ruiz[8], María Carmen Fariñas[9], Marino Blanes-Juliá[10], Elisa Vidal[11], Zaira R. Palacios-Baena[2,12], Román Hernández-Gallego[13], Jordi Carratalá[3], Jorge Calderón-Parra[4], María Ángeles Marcos[5], Patricia Muñoz[6,14], Jesús Fortún-Abete[7], José María Aguado[8], Francisco Arnaiz-Revillas[9], Rosa Blanes-Hernández[10], Julián de la Torre-Cisneros[11], Luis E. López-Cortés[2,12], Elena García de Vinuesa-Calvo[13], Clara M. Rosso[2,15], Jerónimo Pachón[2,16]*, Javier Sánchez-Céspedes[1,2], Elisa Cordero[1,2,16], on behalf of The COVIDSOT Working Team[¶]

1 Unit of Infectious Diseases, Microbiology, and Preventive Medicine, Virgen del Rocío University Hospital, Seville, Spain, **2** Institute of Biomedicine of Seville (IBiS), Virgen del Rocío University Hospital/CSIC/University of Seville, Seville, Spain, **3** Service of Infectious Diseases, Bellvitge University Hospital, Bellvitge Biomedical Research Institute (IDIBELL), University of Barcelona, Hospitalet de Llobregat, Barcelona, Spain, **4** Unit of Infectious Diseases, Microbiology, and Preventive Medicine, Puerta de Hierro University Hospital, Madrid, Spain, **5** Service of Infectious Diseases, Clinic University Hospital, Barcelona, Spain, **6** Service of Clinical Microbiology and Infectious Diseases, Sanitary Research Institute, Gregorio Marañón University Hospital, Madrid, Spain, **7** Service of Infectious Diseases, Ramón y Cajal University Hospital, Madrid, Spain, **8** Unit of Infectious Diseases, 12 de Octubre University Hospital, 12 de Octubre Hospital Research Institute (i+12), Madrid, Spain, **9** Service of Infectious Diseases, Marqués de Valdecilla University Hospital, Marqués de Valdecilla-IDIVAL, University of Cantabria, Santander, Spain, **10** Section of Infectious Diseases, La Fe University Hospital, Valencia, Spain, **11** Service of Infectious Diseases, Reina Sofía University Hospital, Maimónides Biomedical Research Institute of Córdoba (IMIBIC), Córdoba, Spain, **12** Unit of Infectious Diseases, Microbiology, and Preventive Medicine, Virgen Macarena University Hospital, Seville, Spain, **13** Unit of Kidney Transplant, Service of Nefrology, Badajoz University Hospital, Extremadura, Spain, **14** CIBERES (CB06/06/0058), Department of Medicine, Faculty of Medicine, Complutense University, Madrid, Spain, **15** Unit of Clinical Investigation and Clinical Trials, Virgen del Rocío University Hospital, Seville, Spain, **16** Department of Medicine, University of Seville, Seville, Spain

¶ Membership of the authors belonging to The COVIDSOT Working Team is listed in the Acknowledgments.
* pachon@us.es

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Abstract

The aim was to analyze the characteristics and predictors of unfavorable outcomes in solid organ transplant recipients (SOTRs) with COVID-19. We conducted a prospective observational cohort study of 210 consecutive SOTRs hospitalized with COVID-19 in 12 Spanish centers from 21 February to 6 May 2020. Data pertaining to demographics, chronic underlying diseases, transplantation features, clinical, therapeutics, and complications were collected. The primary endpoint was a composite of intensive care unit (ICU) admission and/or death. Logistic regression analyses were performed to identify the factors associated with these unfavorable outcomes. Males accounted for 148 (70.5%) patients, the median age was 63 years, and 189 (90.0%) patients had pneumonia. Common symptoms were fever,

Data Availability Statement: All relevant data are within the paper and its Supporting information files.

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Competing interests: The authors have declared that no competing interests exist.

cough, gastrointestinal disturbances, and dyspnea. The most used antiviral or host-targeted therapies included hydroxychloroquine 193/200 (96.5%), lopinavir/ritonavir 91/200 (45.5%), and tocilizumab 49/200 (24.5%). Thirty-seven (17.6%) patients required ICU admission, 12 (5.7%) suffered graft dysfunction, and 45 (21.4%) died. A shorter interval between transplantation and COVID-19 diagnosis had a negative impact on clinical prognosis. Four baseline features were identified as independent predictors of intensive care need or death: advanced age, high respiratory rate, lymphopenia, and elevated level of lactate dehydrogenase. In summary, this study presents comprehensive information on characteristics and complications of COVID-19 in hospitalized SOTRs and provides indicators available upon hospital admission for the identification of SOTRs at risk of critical disease or death, underlining the need for stringent preventative measures in the early post-transplant period.

Introduction

In December 2019, the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causative agent of coronavirus disease 2019 (COVID-19), emerged in China [1]. It spread globally, becoming a public health emergency and a pandemic of historic dimensions [2]. Spain has been one of the most affected countries in the world in terms of absolute number of diagnosed cases and deaths per capita [3], causing a dramatic decline in donations and transplantation procedures per day, with mean numbers dropping from 7.2 to 1.2 and 16.1 to 2.1, respectively [4].

The clinical spectrum of COVID-19 ranges from asymptomatic disease to pneumonia, life-threatening complications, and ultimately death [5–7]. Risk factors for severe disease in the general population include older age and comorbidities [8], but the impact of chronic immunosuppression related to transplantation on COVID-19 is not well known. Despite widespread concern that COVID-19 clinical phenotypes may be more severe among solid organ transplant recipients (SOTRs) due to a poorer inflammatory response and greater organ injury, data on this population are limited to a few case series and generally small retrospective cohorts [9–25].

As hospitals around the world prepare for a rising and maintained incidence of COVID-19, important questions on the natural history of the disease, susceptibility of SOTRs, severity risk factors, and transplant specific management of antivirals and immunosuppressants remain unanswered [26]. This multicenter study aimed to shed light on said matters, presenting the clinical characteristics, treatments, and predictors of unfavorable outcomes (intensive care unit (ICU) admission and/or death) in 210 consecutively hospitalized adult SOTRs with COVID-19.

Materials and methods

Design and patients

We conducted a nationwide prospective observational cohort study (S1 Table for STROBE checklist) within the Spanish Network for Research in Infectious Diseases (REIPI) and the Group for the Study of Infection in Transplantation and the Immunocompromised Host (GESITRA-IC). Investigators from the 12 participating centers from different regions of Spain were asked to include all consecutive SOTR adults hospitalized with confirmed COVID-19 by real-time polymerase chain reaction (RT-PCR) assay for SARS-CoV-2 in respiratory samples,

from 21 February to 6 May 2020. The baseline was the date of hospital admission, and the follow-up censoring date was 6 June 6 2020. The study protocol was approved by the Ethics Committee of Virgen del Rocío and Virgen Macarena University Hospitals (C.I. 0842-N-20), as well as by the proper institutional review board of each participating center (individual codes are listed in the Supporting Information), and complied with the Helsinki Declaration. Written informed consent was established as a mandatory requirement for all patients.

Data collection

The data source was the electronic medical record system. Anonymized data were collected using an electronic Case Report Form (eCRF) and added to a database specifically designed for this study built using Research Electronic Data Capture (REDCap) tools [27]. The registered variables included demographics, comorbidities, transplant type and date, signs and symptoms at admission, baseline laboratory tests and chest X-ray findings, complications during hospitalization, management of immunosuppression, therapeutics with purported activity against COVID-19, adjunctive strategies to modulate the host inflammatory response, and clinical outcomes.

Event of interest

The clinical outcomes of patients after 30 days follow-up were categorized into favorable (full recovery and discharged or stable clinical condition) and unfavorable (admission to ICU or death). For patients who were discharged and subsequently readmitted during the study period, only the first hospital admission episode was considered for purposes of analysis. The primary endpoint was the occurrence of an unfavorable outcome, that is, a composite of ICU admission and/or death.

Statistical approach

A descriptive analysis of all obtained data was performed. Categorical variables were presented as n (%) and continuous variable as mean (standard deviation (SD)) or median (interquartile range (IQR)) according to the normality of the distribution. We used the χ^2-test, Yates' Correction for Continuity, Student's *t*-test, or Welch's *t*-test to compare between-group differences, as appropriate.

To examine factors associated with unfavorable clinical outcomes, quantitative variables were dichotomized based on normal ranges and in the cut-offs associated with unfavorable outcomes in the general population [28], after addressing their effects as continuous. Univariable and multivariable logistic regression analyses were performed, and bivariate relationships between all predictors were thoroughly explored to account for potential confounding, collinear, and interaction effects.

For obtaining a reduced set of variables from the predictors identified in the univariable analysis, a multivariable analysis was carried out using three criteria to achieve the most accurate model: relevance to clinical situation, statistical significance ($P < 0.10$), and adequate number of events to allow for meaningful analysis. An automated backward stepwise selection was used for exclusion of variables utilizing a 5% probability threshold [29]. Gender, presence of comorbidities, lung transplantation, and immunosuppression regimens with high doses of mofetil mycophenolate ($\geq$1080 mg/day) or prednisone ($\geq$20 mg/day) appeared as possible confounders and were therefore included in the final model for adjustment. White blood cell count and oxygen saturation were excluded to prevent collinearity, since neutrophil count and respiratory rate were part of the model. We found no clinically meaningful interactions among

the potential ones examined (sex and inflammatory markers, age, and immunity response), which were not therefore included in the model as a term.

Although there are no defined well-validated measures of immunosuppression intensity, we performed a univariable analysis to specifically assess the following as possible surrogates in accordance with prior studies: earlier time post-transplant, thoracic (lung or heart) compared to non-thoracic graft, receipt of augmented mofetil mycophenolate and prednisone dosages, and higher number of baseline maintenance immunosuppressive agents [12, 30, 31]. To further ascertain the impact of a shorter interval between transplantation and COVID-19 diagnosis, as well as the type of transplant received, on unfavorable outcome, we carried out a sensitivity analysis where the roles of the dependent and independent variables were inverted.

Analyses were done using the software package SPSS (Version 26.0. Armonk, NY: IBM Corp.). All *P*-values were derived from two-tailed tests, and those <0.05 were considered statistically significant.

Results

Patients' characteristics and clinical presentation

The cohort included 210 hospitalized adult SOTRs in which SARS-CoV-2 was detected by RT-PCR from nasopharyngeal swabs (97.6%), sputum (1.9%), and endotracheal aspirate (0.5%). One hundred eight (51.4%) patients were kidney recipients, 50 (23.8%) were liver, 33 (15.7%) were heart, 15 (7.1%) were lung, and 4 (1.9%) were kidney–pancreas recipients. The median time from transplant to COVID-19 diagnosis was 6.6 (IQR 2.8–13.1) years. Six (2.9%) patients were in the first month posttransplant, 12 (5.7%) in the first three months, 18 (8.6%) in the first six months, and 29 (13.8%) in the first-year posttransplant. The median admission date was 25 March 2020, with little variability between centers (IQR from March 18 to April 1). Median length of hospitalization was 13 (IQR 7–19) days. Sixty-three (30.0%) patients experienced an unfavorable outcome at final follow-up, and 147 (70.0%) patients had a favorable course of the disease. Patients' characteristics, of the total cohort and categorized by clinical outcome, are shown in Table 1.

In brief, males accounted for 148 (70.5%) patients, the median age was 63 (IQR 51–71) years, and 28.6% were $\geq$70 years old. The age distribution of patients stratified by clinical outcome is shown in Fig 1. Age $\geq$70 years ($P = 0.001$) and shorter time from transplantation ($P = 0.048$) were associated with a poor clinical result, unlike other baseline demographics including sex or type of graft. At least one comorbidity was present in 85.2% patients, the most common being chronic kidney disease (35.2%), followed by diabetes mellitus (33.3%) and chronic cardiopathy (25.7%), all of which were more prevalent in the unfavorable outcome group. The median duration of symptoms before hospitalization was six (IQR 3–10) days, and the most common symptoms were fever (66.7%), cough (65.2%), gastrointestinal disturbances (41.0%), and dyspnea (38.6%). Dyspnea upon presentation was associated with unfavorable outcomes ($P < 0.001$), while other initial symptoms were analogous between groups. Similarly, there were no differences among baseline immunosuppression, where triple therapy was the preferred maintenance regimen, and the subsequent clinical evolution of COVID-19.

Chest X-ray, hemodynamic, and laboratory findings

One hundred eighty-nine (90.0%) SOTRs had abnormal chest X-ray images: 85.7% within the favorable and 100% in the unfavorable outcome groups ($P = 0.002$). Patients with unfavorable clinical outcomes had higher respiratory rate ($P < 0.001$) and lower capillary oxygen saturation ($P = 0.03$) on initial presentation than those with a favorable disease course. We also found between-group differences regarding the baseline laboratory values. In terms of blood

Table 1. Demographics, comorbidities, clinical data, and baseline immunosuppression in all patients and by clinical outcome at final follow-up.

	All (n = 210)	Favorable Outcome (n = 147)	Unfavorable Outcome (n = 63)	*P*-value
Age in years, mean (SD)	63 (12)	61 (11)	65 (7)	.01
Age ≥ 70 (%)	60 (28.6)	32 (21.8)	28 (46.6)	.001
Male sex (%)	148 (70.5)	104 (70.7)	44 (69.8)	.90
Organ transplant (%)				
Kidney	108 (51.4)	74 (50.3)	34 (54.0)	.63
Liver	50 (23.8)	37 (25.2)	13 (20.6)	.48
Heart	33 (15.7)	24 (16.3)	9 (14.3)	.71
Lung	15 (7.1)	9 (6.1)	6 (9.5)	.56
Kidney-pancreas	4 (1.9)	3 (2.0)	1 (1.6)	1.00
Years from transplant to diagnosis, median (IQR)	6.6 (2.8–13.1)	7.1 (3.1–13.8)	5.5 (1.4–11.6)	.048
Comorbidities (%)				
Diabetes mellitus[a]	70 (33.3)	42 (28.6)	28 (44.4)	.03
Chronic lung disease[b]	42 (20.0)	27 (18.4)	15 (23.8)	.37
Chronic cardiopathy[c]	54 (25.7)	31 (21.1)	23 (36.5)	.02
Chronic kidney disease[d]	74 (35.2)	46 (31.3)	28 (44.4)	.07
Chronic liver disease[e]	29 (13.8)	18 (12.2)	11 (17.5)	.32
Cancer[f]	25 (11.9)	15 (10.2)	10 (15.9)	.25
Morbid obesity[g]	10 (4.8)	9 (6.1)	1 (1.6)	.16
Presenting symptoms (%)				
Fever	140 (66.7)	101 (68.7)	39 (61.9)	.34
Rhinorrhea	14 (6.7)	13 (8.8)	1 (1.6)	.10
Odynophagia	16 (7.6)	10 (6.8)	6 (9.5)	.69
Myalgias	54 (25.7)	42 (28.6)	12 (19.0)	.15
Headache	18 (8.6)	16 (10.9)	2 (3.2)	.07
Cough	137 (65.2)	94 (63.9)	43 (68.3)	.55
Expectoration	34 (16.2)	23 (15.6)	11 (17.5)	.74
Pleuritic chest pain	11 (5.2)	10 (6.8)	1 (1.6)	.22
Dyspnea	81 (38.6)	44 (29.9)	37 (58.7)	< .001
Diarrhea	81 (38.6)	59 (40.1)	22 (34.9)	.48
Vomiting	20 (9.5)	14 (9.5)	6 (9.5)	1.00
Impaired consciousness	14 (6.7)	6 (4.1)	8 (12.7)	.046
Days from symptoms onset to diagnosis, median (IQR)	6 (3–10)	6 (3–11)	5 (3–8)	.64
Baseline immunosuppression (%)				
Mofetil mycophenolate	145 (69.0)	101 (68.7)	44 (69.8)	.87
Azathioprine	5 (2.4)	4 (2.7)	1 (1.6)	1.00
Ciclosporin	18 (8.6)	9 (6.1)	9 (14.6)	.05
Tacrolimus	156 (74.3)	110 (74.8)	46 (73.0)	.78
Sirolimus/everolimus	49 (23.3)	38 (25.9)	11 (17.5)	.19
Prednisone	146 (69.5)	97 (66.0)	49 (77.8)	.09

[a]Treated with insulin or antidiabetic oral drugs, or presence of end-organ diabetes-related disease.

[b]Including chronic obstructive pulmonary disease, obstructive sleep apnea, and asthma.

[c]Including cardiac insufficiency, coronary heart disease, aortic aneurysm, and peripheral arterial disease.

[d]Mild (creatinine between 1.5–2 mg/dL) or moderate/severe (creatinine > 3 mg/dL or dialysis) renal impairment.

[e]Mild (without portal hypertension) or moderate/severe (cirrhosis, varices, encephalopathy, ascites) liver disease.

[f]Presence of an active solid or hematologic malignant neoplasm.

[g]Body mass index ≥ 40 kg/m2, or ≥ 35 kg/m2 plus experiencing obesity-related health conditions.

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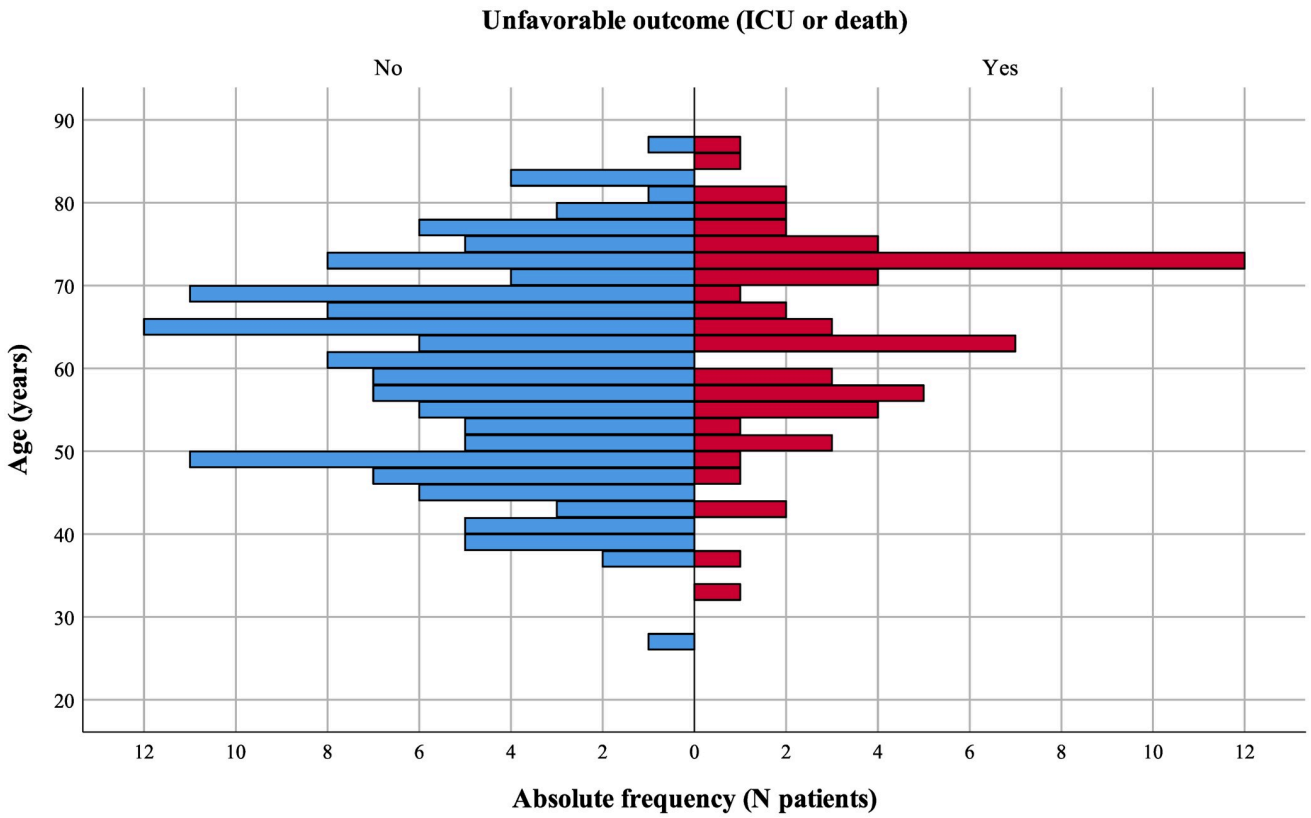

Fig 1. Age distribution of patients stratified by clinical outcome. Twenty-eight (46.6%) out of the 60 patients aged ≥ 70 years experienced an unfavorable outcome *vs*. 35 (23.3%) out of 150 patients aged < 70 years.

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counts, leukocytes were higher and lymphocytes lower in the unfavorable outcome group (*P*-values, respectively, 0.04 and 0.03). By the same token, organ injury and inflammatory biomarkers such as creatinine ($P = 0.002$), lactate dehydrogenase ($P = 0.001$), C-reactive protein ($P = 0.01$), and D-dimer ($P = 0.03$) were higher among patients who later were admitted to the ICU or died. These results and additional clinical details are available in Table 2.

Initial treatment approach, immunosuppression handling, and clinical outcomes

Antiviral or host-targeted therapies were administered to 200 (95.2%) patients, with the most used being hydroxychloroquine (193 (96.5%)), lopinavir/ritonavir (91 (45.5%)), and tocilizumab (49 (24.5%)). Lopinavir/ritonavir ($P = 0.003$) and tocilizumab ($P < 0.001$) during hospitalization, as well as high flow therapy or mechanical ventilation ($P < 0.001$), were more common practices towards severely ill patients (Table 3).

Immunosuppressive therapy was modified in 82.4% of cases, mainly by discontinuing mofetil mycophenolate and reducing tacrolimus, while maintaining prednisone dosages. For each agent, antimetabolite doses were decreased or stopped in 110/150 (73.3%) patients, calcineurin inhibitors in 119/170 (70.0%), and mTOR inhibitors in 35/49 (71.4%) patients. One hundred thirty-three out of 146 (91.1%) patients had steroid doses maintained (Table 3).

Complications were more prevalent in the unfavorable outcome group compared to the non-ICU or alive patients ($P < 0.001$). Twelve (5.7%) patients experienced graft dysfunction at

Table 2. Initial chest x-ray imaging features, hemodynamic, and laboratory values in all patients and by clinical outcome at final follow-up in all patients and by clinical outcome at final follow-up.

	All (n = 210)	Favorable Outcome (n = 147)	Unfavorable Outcome (n = 63)	*P*-value
Infiltrate on chest x-ray (%)	189 (90.0)	126 (85.7)	63 (100)	.002
Signs (%)				
Temperature > 37.5˚C	59 (28.6)	40 (27.6)	19 (31.1)	.61
Systolic blood pressure < 90 mmHg	9 (4.5)	8 (5.7)	1 (1.6)	.19
Diastolic blood pressure < 60 mmHg	20 (9.9)	13 (9.3)	7 (11.3)	.66
Hart rate > 100 bpm	48 (25.1)	32 (24.4)	16 (26.7)	.74
Respiratory rate > 20 bpm	57 (31.1)	27 (21.1)	30 (54.5)	< .001
O_2 sat < 95%	61 (29.2)	36 (24.7)	25 (39.7)	.03
Blood counts, median (IQR)				
White blood cells x 1000/μL	5.6 (4.0–7.8)	5.3 (3.8–7.5)	6.2 (4.4–8.2)	.04
Neutrophils x 1000/μL	4.1 (2.9–5.9)	3.7 (2.8–5.6)	4.7 (3.1–6.8)	.05
Lymphocytes x 1000/μL	.8 (.5–1.0)	.8 (.5–1.1)	.6 (.4-.9)	.03
Platelets x 1000/μL	164 (116–214)	158 (111–215)	173 (123–215)	.26
Blood counts (%)				
White blood cells > 11 x 1000/μL	16 (7.6)	8 (5.4)	8 (12.7)	.13
Neutrophils > 7.5 x 1000/μL	25 (12.3)	14 (9.9)	11 (17.7)	.12
Lymphocytes < 1 x 1000/μL	142 (68.6)	94 (64.4)	48 (78.7)	.04
Platelets < 130 x 1000/μL	67 (33.0)	48 (33.8)	19 (31.1)	.71
Chemistries, median (IQR)				
Creatinine mg/dL	1.6 (1.1–2.3)	1.5 (1.0–2.2)	1.9 (1.3–2.4)	.20
AST U/L	30 (22–44)	29 (21–42)	37 (26–52)	.17
ALT U/L	23 (15–35)	21 (15–32)	27 (17–41)	1.00
Lactate dehydrogenase U/L	270 (223–366)	255 (207–323)	349 (255–484)	.001
Chemistries (%)				
Creatinine > 1.3 mg/dL	133 (63.9)	83 (57.2)	50 (79.4)	.002
AST > 30 U/L	81 (49.7)	50 (45.5)	31 (58.5)	.12
ALT > 40 U/L	37 (18.6)	22 (15.9)	15 (24.6)	.15
Lactate dehydrogenase ≥ 300 U/L	79 (40.9)	42 (31.6)	37 (61.7)	< .001
Additional laboratory values, median (IQR)[a]				
C-reactive protein mg/L	59.6 (26.9–127.2)	44.0 (20.6–112.6)	89.7 (47.3–133.9)	.14
D-dimer ng/mL	612 (367–1399)	574 (340–1060)	799 (476–2315)	.03
Additional laboratory values (%)[a]				
C-reactive protein ≥ 100 mg/L	69 (33.5)	40 (27.8)	29 (46.8)	.01
D-dimer ≥ 600 ng/mL	91 (52.3)	56 (47.9)	35 (61.4)	.09

[a]These values were not available for all patients (C-reactive protein N = 206, D-dimer N = 174).

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the end of follow-up, resulting in transplant loss for five patients (Table 3). Overall, 37 (17.6%) SOTRs required ICU admission, and 45 (21.4%) died. A total of ten (4.8%) patients were discharged and re-admitted during the study period.

Predictors of unfavorable outcomes

Unadjusted baseline predictors of unfavorable outcomes are shown in S2 Table. In the final multivariable analysis, adjusted for gender, comorbidities, type of transplant, and doses of immunosuppressive agents, four baseline risk factors were independently associated with increased odds of ICU admission or death: age ≥70 years ($P = 0.01$), respiratory rate >20 bpm

Table 3. Treatment and complications in all patients and by clinical outcome at final follow-up.

	All (n = 210)	Favorable Outcome (n = 147)	Unfavorable Outcome (n = 63)	*P*-value
Changes in immunosuppression (%)[a]				
Decrease or stop antimetabolite	110/150 (73.3)	77/105 (73.3)	33/45 (73.3)	1.00
Decrease or stop calcineurin inhibitors	119/170 (70.0)	82/118 (69.5)	37/52 (71.2)	.83
Decrease or stop mTOR inhibitors	35/49 (71.4)	26/38 (68.4)	9/11 (81.8)	.63
Decrease or stop steroids	13/146 (8.9)	7/97 (7.2)	6/49 (12.2)	.48
Viral or host-targeted medications (%)[b]				
Hydroxychloroquine	193/200 (96.5)	134/140 (95.7)	59/60 (98.3)	.61
Lopinavir/ritonavir	91/200 (45.5)	54/140 (38.6)	37/60 (61.7)	.003
Darunavir/cobicistat	7/200 (3.5)	4/140 (2.9)	3/60 (5.0)	.74
Interferon	6/200 (3.0)	2/140 (1.4)	4/60 (6.7)	.12
Tocilizumab	49/200 (24.5)	23/140 (16.4)	26/60 (43.3)	< .001
Azithromycin	34/200 (17.0)	28/140 (20.0)	6/60 (10.0)	.09
Methylprednisolone	20/200 (10.0)	14/140 (10.0)	6/60 (10.0)	1.00
Highest level of respiratory support (%)				
High flow/non-invasive mechanical ventilation	22 (10.5)	3 (2.0)	19 (30.2)	< .001
Intubation	24 (11.4)	0 (0)	24 (38.1)	< .001
Complications during hospitalization (%)				
Acute respiratory distress syndrome	54 (26.0)	9 (6.2)	45 (72.6)	< .001
Hospital-acquired coinfections	24 (11.9)	9 (6.3)	15 (25.9)	< .001
Shock	15 (7.3)	0 (0)	15 (25.0)	< .001
Graft dysfunction	12 (5.7)	9 (6.1)	3 (4.8)	.95
Graft lost	5 (2.4)	3 (2.0)	2 (3.2)	1.00

[a]Denominator includes patients on the agent at baseline and known adjustment status.

[b]Denominator includes all patients under viral or host-targeted treatment.

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($P = 0.001$), lymphocytes <1 x 1000/μL ($P = 0.04$), and lactate dehydrogenase ≥300 U/L ($P = 0.04$). A forest plot presenting the respective odds ratio and 95% confidence interval is shown in Fig 2.

Among potential surrogates of immunosuppression intensity, we found a novel association between unfavorable outcomes and the temporal proximity of COVID-19 to transplantation (S3 Table). Through a series of sensitivity analyses, we further demonstrated the negative impact of an earlier post-transplant infection on clinical prognosis (Table 4), as well as the lack of association between the type of graft received and the occurrence of unfavorable outcomes (S4 Table).

Two subgroups of the study population were considered of possible higher risk: patients suffering from graft dysfunction at day 30, and those with COVID-19 acquisition during the first month post-transplant. A detailed description of their main characteristics, outcomes, and management is provided in S5 and S6 Tables.

Discussion

In this large, prospective, nationwide study of SOTRs hospitalized with COVID-19 followed for 30 days, 17.6% required ICU admission, and the mortality rate was 21.4%. Older age, high respiratory rate, lymphopenia, and elevated level of lactate dehydrogenase at presentation were independently associated with ICU admission and/or death. Similarly, an earlier post-transplant SARS-CoV-2 infection was demonstrated as a risk factor for unfavorable outcomes.

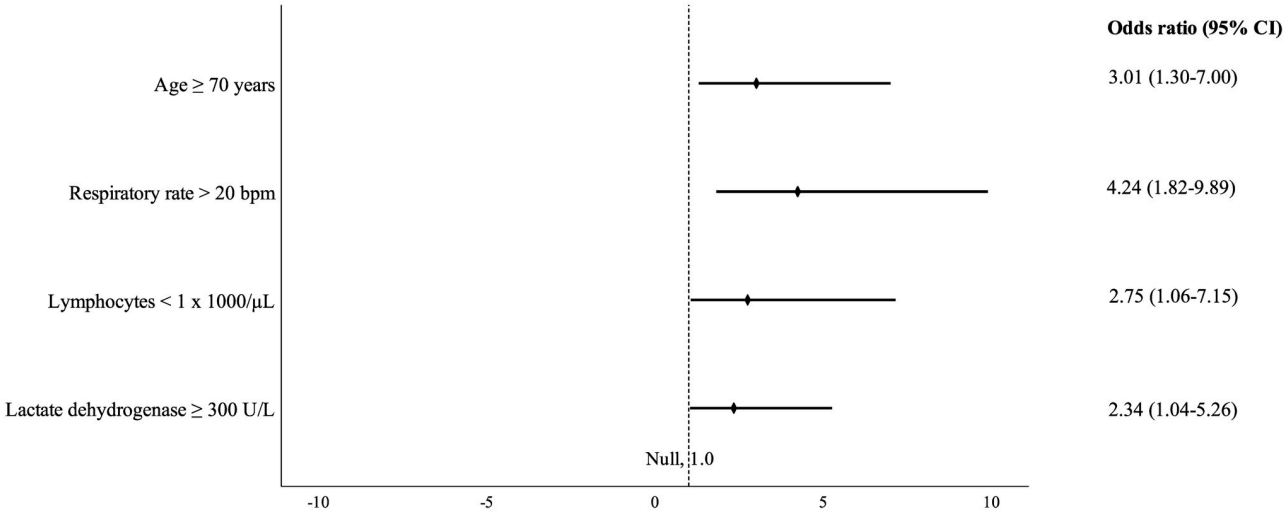

Fig 2. Independent baseline predictors of unfavorable outcome.

https://doi.org/10.1371/journal.pone.0250796.g002

The majority of patients were male with a median age over 60 years, conforming to prior published large nationwide cohorts of the general population hospitalized with COVID-19 [32] and the 2019 Spanish National Transplant Organization Annual Report [33].

The potential negative impact of transplantation on clinical outcomes of COVID-19 has been discussed, and the few authors that directly compared results in SOTRs and general population indicated that ICU admission and death rates were higher among the immunocompromised hosts [34, 35]. However, studies including multivariable analyses of severity risk factors among hospitalized general populations with COVID-19, though with variable durations of follow-up, showed mortality and ICU admission estimates generally comparable to the ones reported for the current SOTR cohort [36–39]. The presented fatality rate in our study was also similar to the average of estimates derived from prior small and heterogeneous studies on hospitalized SOTRs [10–12, 18, 30, 34] and just one percentage point higher than the single previously published multicenter prospective SOTR cohort study (20.5%) [40]. By comparing these incidence rates with those of clinical influenza for high-risk groups, we found close resemblance in the probability of ICU admission (ranging from 11.8 to 28.6%) but less likelihood of dying (between 2.9 and 14.3%) from flu among hospitalized patients [41–43], which may be due to the existence of accessible and effective treatment.

Among the underlying comorbidities assessed, chronic cardiomyopathy, diabetes mellitus, and chronic kidney disease were all present in more than one fourth of the patients included and were associated with increased odds of unfavorable outcomes. This is in accordance with the previously described comorbidities associated with ICU admission or death in the general population [8, 36]. COVID-19 pneumonia at the time of diagnosis (defined by chest X-ray infiltrates) was also associated with unfavorable outcomes, as reported in general population studies [37, 38] and in the US multicenter SOTR cohort [40]. Moreover, no patients without pneumonia in our cohort required ICU admission or died at final follow-up, solidifying pneumonia as a major determinant of unfavorable outcomes in SOTRs.

The most common presenting symptoms in our cohort included fever, cough, and dyspnea, which were significantly associated with a poor clinical outcome. More atypical presentations, such as vomiting or diarrhea, were also reported among a significant proportion of SOTRs. This highlights that immunocompromised hosts often present with unusual or attenuated

Table 4. Baseline risk factors, management, and outcomes *vs.* time from transplantation to COVID-19 diagnosis.

	All (n = 210)	≤ 6 months from transplant to diagnosis (n = 18)	> 6 months from transplant to diagnosis (n = 192)	*P*-value
Baseline risk factors (%)				
Age ≥ 70 years	60 (28.6)	4 (22.2)	56 (29.2)	.53
Diabetes mellitus	70 (33.3)	6 (33.3)	64 (33.3)	1.00
Chronic cardiopathy	54 (25.7)	5 (27.8)	49 (25.5)	1.00
Chronic kidney disease	74 (35.2)	5 (27.8)	69 (35.9)	.49
Dyspnea	81 (38.6)	8 (44.4)	73 (38.0)	.59
Respiratory rate > 20 bpm	57 (31.1)	7 (53.8)	50 (29.4)	.13
O_2 sat < 95%	61 (29.2)	6 (33.3)	55 (28.8)	.69
Lymphocytes < 1 x 1000/μL	142 (68.6)	14 (77.8)	128 (67.7)	.38
Creatinine > 1.3 mg/dL	133 (63.9)	12 (66.7)	121 (63.7)	.80
Lactate dehydrogenase ≥ 300 U/L	79 (40.9)	8 (50.0)	71 (40.1)	.44
C-reactive protein ≥ 100 mg/L	69 (33.5)	7 (41.2)	62 (32.8)	.48
D-dimer ≥ 600 ng/mL	91 (52.3)	12 (85.7)	79 (49.4)	.05
Baseline immunosuppression (%)				
Mofetil mycophenolate	145 (69.0)	15 (83.3)	130 (67.7)	.17
Azathioprine	5 (2.4)	0 (0)	5 (2.6)	1.00
Ciclosporin	18 (8.6)	1 (5.6)	17 (8.9)	1.00
Tacrolimus	156 (74.3)	17 (94.4)	139 (72.4)	.08
Sirolimus/everolimus	49 (23.3)	2 (11.1)	47 (24.5)	.32
Prednisone	146 (69.5)	14 (77.8)	132 (68.8)	.09
Changes in immunosuppression (%)[a]				
Decrease or stop antimetabolite	110/150 (73.3)	8/15 (53.3)	102/135 (75.6)	.12
Decrease or stop calcineurin inhibitors	119/170 (70.0)	9/17 (52.9)	110/153 (71.9)	.11
Decrease or stop mTOR inhibitors	35/49 (71.4)	2/2 (100)	33/47 (70.2)	.91
Decrease or stop steroids	13/146 (8.9)	2/14 (14.3)	11/132 (8.3)	.80
Viral or host-targeted medications (%)[b]				
Hydroxychloroquine	193/200 (96.5)	15/16 (93.8)	178/184 (96.7)	1.00
Lopinavir/ritonavir	91/200 (45.5)	6/16 (37.5)	85/184 (46.2)	.50
Darunavir/cobicistat	7/200 (3.5)	1/16 (6.3)	6/184 (3.3)	1.00
Interferon	6/200 (3.0)	1/16 (6.3)	5/184 (2.7)	.98
Tocilizumab	49/200 (24.5)	6/16 (37.5)	43/184 (23.4)	.34
Azithromycin	34/200 (17.0)	0/16 (0)	34/184 (18.5)	.11
Methylprednisolone	20/200 (10.0)	1/16 (6.3)	19/184 (10.3)	.86
Highest level of respiratory support (%)				
High flow/non-invasive mechanical ventilation	22 (10.5)	3 (16.7)	19 (9.9)	.62
Intubation	24 (11.4)	5 (27.8)	19 (9.9)	.06
Complications during hospitalization (%)				
Acute respiratory distress syndrome	54 (26.0)	7 (41.2)	47 (24.6)	.23
Hospital-acquired coinfections	24 (11.9)	4 (25.0)	20 (10.8)	.20
Graft dysfunction	12 (5.7)	2 (11.1)	10 (5.2)	.62
Graft lost	5 (2.4)	1 (5.6)	4 (2.1)	.91
Final outcome (%)				
Intensive care unit admission	37 (17.6)	8 (44.4)	29 (15.1)	.01

(*Continued*)

Table 4. (Continued)

	All (n = 210)	≤ 6 months from transplant to diagnosis (n = 18)	> 6 months from transplant to diagnosis (n = 192)	*P*-value
Death	45 (21.4)	6 (33.3)	39 (20.3)	.32
Unfavorable*	63 (30.0)	10 (55.6)	53 (27.6)	.01

[a]Denominator includes patients on the agent at baseline and known adjustment status.

[b]Denominator includes all patients under viral or host-targeted treatment.

*Clinical outcome is categorized into favorable (full recovery and discharged or stable clinical condition) and unfavorable (admission to ICU or death).

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signs and symptoms of infection, leading to late presentations or missed diagnosis, and potentially worse results.

Among the inflammatory parameters measured at hospital admission, creatinine, lactate dehydrogenase, C-reactive protein, and D-dimer levels were higher within the unfavorable outcome group. However, the overall variation in these biomarkers was less pronounced than that observed in the general population of hospitalized patients with COVID-19 [31, 44–46], which is biologically plausible. This being the case, further investigation is required to address whether the lower inflammatory response and greater immunosuppression characterizing SOTRs have impacts on COVID-19 clinical outcomes.

The fundamental implication of our study is the identification of specific and independent predictors (age ≥70 years, respiratory rate >20 bpm, lymphocytes <1 x 1000/μL, and lactate dehydrogenase ≥300 U/L) for unfavorable outcomes in hospitalized SOTRs with COVID-19, which could ease the development of future research and guidelines targeted at high-risk transplanted populations. Furthermore, we showed that an interval shorter than six months between transplantation and COVID-19 diagnosis has a negative impact on mortality and ICU admission rates, which is a risk that should be considered when deciding which patients should proceed with transplantation. Finally, although analogous to the general population, mortality in SOTRs hospitalized with SARS-CoV-2 infection is dramatically high, and the promotion of preventive strategies and treatments will be crucial to mitigate the adverse impacts of the COVID-19 pandemic in these patients.

The strengths of the present study are the strong design, the multicenter participation approach to make the results generalizable and comparable, the standardized and anonymous collection of data using an electronic Case Report Form, and the 30-day duration of follow-up. In parallel, we have faced some limitations. First, our study is centered on hospitalized patients, and thus the conclusions reached may not be applicable to those SOTRs attended in the outpatient setting. Second, testing limitations probably led to undercounting of mild or asymptomatic cases, and the ensuing selection bias towards more severely ill patients. Finally, the cases included only represent the early COVID-19 epidemic. Therefore, the potential benefit of therapies that are now implemented more widely, such as remdesivir and convalescent plasma, have not been addressed.

In summary, among hospitalized SOTR with COVID-19, ICU admission and death rates were high, and they were similar to those reported in the general population. Unfavorable outcomes were mainly driven by respiratory pathology (represented by a high breathing rate), older age, and two laboratory features at presentation, namely lymphopenia and elevated level of lactate dehydrogenase. An earlier post-transplant SARS-CoV-2 infection was established as a novel risk factor for ICU need and mortality. While this study provides preliminary indicators available upon hospital admission for identifying patients at risk of critical disease or death, it is an urgent priority to find efficacious antiviral treatments and to investigate the role

of the immune response in COVID-19, especially in the population of SOTRs, where it is vital to guide suitable and prompt immunomodulatory management.

Supporting information

S1 File. The COVIDSOT working team.
(DOCX)

S2 File. Institutional review board approval number of each participating center.
(DOCX)

S1 Table. STROBE checklist.
(DOCX)

S2 Table. Univariable models of baseline risk factors associated with unfavorable outcome.
(DOCX)

S3 Table. Univariable models of potential surrogates of immunosuppression intensity *vs.* unfavorable outcome.
(DOCX)

S4 Table. Clinical outcomes according to the type of transplant received.
(DOCX)

S5 Table. Description of patients suffering from graft dysfunction at day 30 (n = 12).
(DOCX)

S6 Table. Description of patients with COVID-19 acquisition during the first month post-transplant (n = 6).
(DOCX)

S1 Dataset. Minimal anonymized data set necessary to replicate the study findings.
(XLSX)

Acknowledgments

COVIDSOT working team

Lead author: Elisa Cordero (elisacorderom@gmail.com).

Virgen del Rocío University Hospital-IBiS, *University of Seville*, *Seville*, *Spain*: Elisa Cordero, Jerónimo Pachón, Manuela Aguilar-Guisado, Judith Berastegui-Cabrera, Gabriel Bernal-Blanco, Pedro Camacho, Marta Carretero, José Miguel Cisneros, Juan Carlos Crespo, Miguel Angel Gómez-Bravo, Carmen Infante-Domínguez, Silvia Jiménez-Jorge, Laura Merino, Clara Rosso, Sonsoles Salto-Alejandre, Javier Sánchez-Céspedes, José Manuel Sobrino-Márquez. *Bellvitge University Hospital*, *Bellvitge Biomedical Research Institute-IDIBELL*, *University of Barcelona*, *Barcelona*, *Spain*: Jordi Carratalá, Nuria Sabé, Carme Baliellas, Oriol Bestard, Carles Diez, José Gonzàlez-Costello, Laura Lladó, Eduardo Melilli. *Puerta de Hierro University Hospital*, *Madrid*, *Spain*: Antonio Ramos-Martínez, Jorge Calderón-Parra, Ana Arias-Milla, Gustavo Centeno-Soto, Manuel Gómez-Bueno, Rosalía Laporta-Hernández, Alejandro Muñoz-Serrano, Beatriz Sánchez-Sobrino. *Clinic University Hospital-IDIBAPS*, *University of Barcelona*, *Barcelona*, *Spain*: Asunción Moreno, María Ángeles Marcos, Laura Linares, Marta Bodro, María Ángeles Castel, Frederic Cofán, Jordi Colmenero, Fritz Dieckmann, Javier Fernández, Dra. Marta Farrero, Miquel Navasa, Félix Pérez-Villa, Pedro Ventura. *Gregorio Marañón*

University Hospital, *CIBERES*, *Madrid*, *Spain*: Maricela Valerio, Patricia Muñoz, Víctor Fernández-Alonso, Maria Olmedo-Samperio, Carlos Ortíz, Sara Rodríguez-Fernández, Maria Luisa Rodríguez-Ferrero, Magdalena Salcedo, Eduardo Zataraín. *Ramón y Cajal University Hospital*, *Madrid*, *Spain*: Pilar Martín-Dávila, Jesús Fortún-Abete, Juan Carlos Galán, Cristina Galeano-Álvarez, Francesca Gioia, Javier Graus, Sara Jiménez, Mario J. Rodríguez. *12 de Octubre University Hospital/i+12*, *CIBERCV*, *Madrid*, *Spain*: José María Aguado, Mario Fernández-Ruiz, Amado Andrés, Juan F. Delgado, Carmelo Loinaz, Francisco López-Medrano, Rafael San Juan. *Marqués de Valdecilla University Hospital-IDIVAL*, *University of Cantabria*, *Santander*, *Spain*: Carmen Fariñas, Francisco Arnaiz de las Revillas, Marta Fernández-Martínez, Ignacio Fortea-Ormaechea, Aritz Gil-Ongay, Mónica Gozalo-Marguello, Claudia González-Rico, Milagros Heras-Vicario, Víctor Mora-Cuesta. *La Fe University Hospital*, *Valencia*, *Spain*: Marino Blanes-Julia, Rosa Blanes-Hernández, Luis Almener-Bonet, María Isabel Beneyto-Castelló, Victoria Miguel Salavert-Lletí, Aguilera-Sancho-Tello, Amparo Solé-Jover. *Reina Sofía University Hospital-IMIBIC*, *Córdoba*, *Spain*: Elisa Vidal, Julián de la Torre-Cisneros, Rafael León, Álvaro Torres de Rueda, José Manuel Vaquero. *Virgen Macarena University Hospital-IBiS*, *Seville*, *Spain*: Zaira R. Palacios-Baena, Luis E. López-Cortés, David Gutiérrez-Campos, Marie-Alix Clement, Marta Fernández-Regaña, Inmaculada López-Hernández, Natalia Maldonado-Lizarazo, Ana Belén Martín-Gutiérrez, Rocío Valverde. *Badajoz University Hospital*, *Extremadura*, *Spain*: Román Hernández-Gallego, Elena García de Vinuesa-Calvo.

Author Contributions

Conceptualization: Jerónimo Pachón, Javier Sánchez-Céspedes, Elisa Cordero.

Data curation: Silvia Jiménez-Jorge, Nuria Sabé, Antonio Ramos-Martínez, Laura Linares, Maricela Valerio, Pilar Martín-Dávila, Mario Fernández-Ruiz, María Carmen Fariñas, Marino Blanes-Juliá, Elisa Vidal, Zaira R. Palacios-Baena, Román Hernández-Gallego, Jordi Carratalá, Jorge Calderón-Parra, María Ángeles Marcos, Patricia Muñoz, Jesús Fortún-Abete, José María Aguado, Francisco Arnaiz-Revillas, Rosa Blanes-Hernández, Julián de la Torre-Cisneros, Luis E. López-Cortés, Elena García de Vinuesa-Calvo, Clara M. Rosso.

Formal analysis: Sonsoles Salto-Alejandre.

Funding acquisition: Elisa Cordero.

Methodology: Jerónimo Pachón, Javier Sánchez-Céspedes, Elisa Cordero.

Project administration: Silvia Jiménez-Jorge, Jerónimo Pachón, Elisa Cordero.

Supervision: Jerónimo Pachón, Javier Sánchez-Céspedes, Elisa Cordero.

Validation: Jerónimo Pachón, Javier Sánchez-Céspedes, Elisa Cordero.

Visualization: Silvia Jiménez-Jorge, Nuria Sabé, Antonio Ramos-Martínez, Laura Linares, Maricela Valerio, Pilar Martín-Dávila, Mario Fernández-Ruiz, María Carmen Fariñas, Marino Blanes-Juliá, Elisa Vidal, Zaira R. Palacios-Baena, Román Hernández-Gallego, Jordi Carratalá, Jorge Calderón-Parra, María Ángeles Marcos, Patricia Muñoz, Jesús Fortún-Abete, José María Aguado, Francisco Arnaiz-Revillas, Rosa Blanes-Hernández, Julián de la Torre-Cisneros, Luis E. López-Cortés, Elena García de Vinuesa-Calvo, Clara M. Rosso, Jerónimo Pachón, Elisa Cordero.

Writing – original draft: Sonsoles Salto-Alejandre.

Writing – review & editing: Nuria Sabé, Antonio Ramos-Martínez, Laura Linares, Maricela Valerio, Pilar Martín-Dávila, Mario Fernández-Ruiz, María Carmen Fariñas, Marino Blanes-Juliá, Elisa Vidal, Zaira R. Palacios-Baena, Román Hernández-Gallego, Jordi Carratalá, Jorge Calderón-Parra, María Ángeles Marcos, Patricia Muñoz, Jesús Fortún-Abete, José María Aguado, Francisco Arnaiz-Revillas, Rosa Blanes-Hernández, Julián de la Torre-Cisneros, Luis E. López-Cortés, Elena García de Vinuesa-Calvo, Clara M. Rosso, Jerónimo Pachón, Javier Sánchez-Céspedes, Elisa Cordero.

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S1 File. The COVIDSOT Working Team.

Lead author: Elisa Cordero (elisacorderom@gmail.com)

Virgen del Rocío University Hospital-IBiS, University of Seville, Seville, Spain: Elisa Cordero, Jerónimo Pachón, Manuela Aguilar-Guisado, Judith Berastegui-Cabrera, Gabriel Bernal-Blanco, Pedro Camacho, Marta Carretero, José Miguel Cisneros, Juan Carlos Crespo, Miguel Angel Gómez-Bravo, Carmen Infante-Domínguez, Silvia Jiménez-Jorge, Laura Merino, Clara Rosso, Sonsoles Salto-Alejandre, Javier Sánchez-Céspedes, José Manuel Sobrino-Márquez. *Bellvitge University Hospital, Bellvitge Biomedical Research Institute-IDIBELL, University of Barcelona, Barcelona, Spain*: Jordi Carratalá, Nuria Sabé, Carme Baliellas, Oriol Bestard, Carles Diez, José Gonzàlez-Costello, Laura Lladó, Eduardo Melilli. *Puerta de Hierro University Hospital, Madrid, Spain*: Antonio Ramos-Martínez, Jorge Calderón-Parra, Ana Arias-Milla, Gustavo Centeno-Soto, Manuel Gómez-Bueno, Rosalía Laporta-Hernández, Alejandro Muñoz-Serrano, Beatriz Sánchez-Sobrino. *Clinic University Hospital-IDIBAPS, University of Barcelona, Barcelona, Spain*: Asunción Moreno, María Ángeles Marcos, Laura Linares, Marta Bodro, María Ángeles Castel, Frederic Cofán, Jordi Colmenero, Fritz Dieckmann, Javier Fernández, Dra. Marta Farrero, Miquel Navasa, Félix Pérez-Villa, Pedro Ventura. *Gregorio Marañón University Hospital, CIBERES, Madrid, Spain*: Maricela Valerio, Patricia Muñoz, Víctor Fernández-Alonso, Maria Olmedo-Samperio, Carlos Ortíz, Sara Rodríguez-Fernández, Maria Luisa Rodríguez-Ferrero, Magdalena Salcedo, Eduardo Zataraín. *Ramón y Cajal University Hospital, Madrid, Spain*: Pilar Martín-Dávila, Jesús Fortún-Abete, Juan Carlos Galán, Cristina Galeano-Álvarez, Francesca Gioia, Javier Graus, Sara Jiménez, Mario J. Rodríguez. *12 de Octubre University Hospital/i+12, CIBERCV, Madrid, Spain*: José María Aguado, Mario Fernández-Ruiz, Amado Andrés, Juan F. Delgado, Carmelo Loinaz, Francisco López-Medrano, Rafael San Juan. *Marqués*

de Valdecilla University Hospital-IDIVAL, University of Cantabria, Santander, Spain: Carmen Fariñas, Francisco Arnaiz de las Revillas, Marta Fernández-Martínez, Ignacio Fortea-Ormaechea, Aritz Gil-Ongay, Mónica Gozalo-Marguello, Claudia González-Rico, Milagros Heras-Vicario, Víctor Mora-Cuesta. *La Fe University Hospital, Valencia, Spain*: Marino Blanes-Julia, Rosa Blanes-Hernández, Luis Almener-Bonet, María Isabel Beneyto-Castelló, Victoria Miguel Salavert-Lletí, Aguilera-Sancho-Tello, Amparo Solé-Jover. *Reina Sofía University Hospital-IMIBIC, Córdoba, Spain*: Elisa Vidal, Julián de la Torre-Cisneros, Rafael León, Álvaro Torres de Rueda, José Manuel Vaquero. *Virgen Macarena University Hospital-IBiS, Seville, Spain*: Zaira R. Palacios-Baena, Luis E. López-Cortés, David Gutiérrez-Campos, Marie-Alix Clement, Marta Fernández-Regaña, Inmaculada López-Hernández, Natalia Maldonado-Lizarazo, Ana Belén Martín-Gutiérrez, Rocío Valverde. *Badajoz University Hospital, Extremadura, Spain*: Román Hernández-Gallego, Elena García de Vinuesa-Calvo.

S2 File. Institutional review board approval number of each participating center.

Bellvitge University Hospital (C.I. PR116/20)

Puerta de Hierro University Hospital (C.I. 0683-N-20)

Clinic University Hospital (C.I. HCB/2020/0383)

Gregorio Marañón University Hospital (C.I. 142.20)

Ramón y Cajal University Hospital (C.I. 0683-N-20)

12 de Octubre University Hospital (C.I. 20/154)

Marqués de Valdecilla University Hospital (C.I. 2020.146)

La Fe University Hospital (C.I. 0683-N-20)

Reina Sofía University Hospital (C.I. 4638)

Badajoz University Hospital (C.I. 0683-N-20)

S1 Table. STROBE checklist.

	Item No	Recommendation	Manuscript location
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Title and abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Abstract
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Introduction
Objectives	3	State specific objectives, including any prespecified hypotheses	Introduction, last paragraph
Methods			
Study design	4	Present key elements of study design early in the paper	Methods, first paragraph
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Methods, first to third paragraph
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Methods, first to third paragraph
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Methods, third and fourth paragraph
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Results, Tables 1-3
Bias	9	Describe any efforts to address potential sources of bias	Discussion, eighth paragraph
Study size	10	Explain how the study size was arrived at	Methods, first paragraph
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Methods, Statistical analysis
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Methods, Statistical analysis

		(*b*) Describe any methods used to examine subgroups and interactions	Methods, Statistical analysis
		(*c*) Explain how missing data were addressed	Not applicable
		(*d*) If applicable, explain how loss to follow-up was addressed	Not applicable
		(*e*) Describe any sensitivity analyses	Supplementary Table S4 and S5
Results			
Participants	13	(a) Report numbers of individuals at each stage of Study (eg, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed)	Results, Tables 1-3
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14	(a) Give characteristics of study participants (eg, demographic, clinical, social) and information on exposures and potential confounders	Results, Tables 1-3
		(b) Indicate number of participants with missing data for each variable of interest	Results, Tables 1-3
		(c) Summarize follow-up time (eg, average and total amount)	Methods, first paragraph
Outcome data	15	Report numbers of outcome events or summary measures over time	Results, first and third paragraph
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table S2 and S3, and Figure 2
		(*b*) Report category boundaries when continuous variables were categorized	Tables 1-3
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done (eg, analyses of subgroups and interactions, and sensitivity analyses	Table S3, S4, S5, and S6
Discussion			
Key results	18	Summarize key results with reference to study objectives	Discussion, first paragraph

Limitations	19	Discuss limitations of the study, considering sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Discussion, eighth paragraph
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Discussion
Generalizability	21	Discuss the generalizability (external validity) of the study results	Discussion, last paragraph
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Funding

S2 Table. Univariable models of baseline risk factors associated with unfavorable outcome.

	Crude Odds ratio (95% CI)	***P*-value**
Age ≥ 70 years	2.88 (1.53-5.41)	.001
Diabetes mellitus	2.00 (1.08-3.69)	.03
Chronic cardiopathy	2.15 (1.13-4.11)	.02
Chronic kidney disease	1.76 (.96-3.22)	.07
Dyspnea	3.33 (1.80-6.15)	< .001
Respiratory rate > 20 bpm	4.49 (2.28-8.86)	< .001
O_2 sat < 95%	2.01 (1.07-3.77)	.03
Lymphocytes < 1 x 1000/μL	2.04 (1.01-4.11)	.046
Creatinine > 1.3 mg/dL	2.87 (1.44-5.75)	.003
Lactate dehydrogenase ≥ 300 U/L	3.49 (1.85-6.58)	< .001
C-reactive protein ≥ 100 mg/L	2.29 (1.23-4.24)	.01
D-dimer ≥ 600 ng/mL	1.73 (.91-3.30)	.098

S3 Table. Univariable models of potential surrogates of immunosuppression intensity *vs.* unfavorable outcome.

	Crude Odds ratio (95% CI)	*P*-value
Time from transplant to COVID-19 diagnosis		
Within ≤ 6 months from transplant (N = 18)	3.28 (1.53-5.41)	**.001**
Organ transplanted		
Thoracic graft (N = 48)	1.08 (.54-2.17)	.83
Immunosuppressant dosages		
Mofetil mycophenolate ≥ 1080 mg/day (N = 35)	.65 (.28-1.51)	.32
Prednisone ≥ 20 mg/day (N = 8)	2.42 (.59-10.01)	.22
Baseline immunosuppressive regimen		
Triple therapy (N = 128)	.94 (.52-1.73)	.85

S4 Table. Clinical outcomes according to the type of transplant received.

	All patients 210	Type of transplant		Statistic (df)	Estimated Risk (95% CI)	*P*-value
		Kidney 108 (51.4)	Others 102 (48.6)			
Renal complications[a]	83 (40.3)	53 (49.5)	30 (30.3)	7.90 (1)	2.26 (1.27-4.00)	.01
Intensive care unit admission	37 (17.6)	21 (19.4)	16 (15.7)	.51 (1)	1.30 (.63-2.65)	.48
Mortality	45 (21.4)	26 (24.1)	19 (18.6)	.92 (1)	1.39 (.71-2.70)	.34
Graft dysfunction, at day 30	12 (8.3)	11 (16.4)	1 (1.3)	10.72 (1)	14.93 (1.87-119.02)	.001
Graft lost, at day 30	5 (3.8)	5 (7.8)	0 (0)	3.65 (1)	..	.06

	All patients 210	Type of transplant		Statistic (df)	Estimated Risk (95% CI)	*P*-value
		Liver 50 (23.8)	Others 160 (76.2)			
Intensive care unit admission	37 (17.6)	7 (14.0)	30 (18.8)	.59 (1)	.71 (0.29-1.72)	.44
Mortality	45 (21.4)	8 (16.0)	37 (23.1)	1.15 (1)	.63 (0.27-1.47)	.28
Graft dysfunction, at day 30	12 (8.3)	0 (0)	12 (11.4)	3.48 (1)	..	.06
Graft lost, at day 30	5 (3.8)	0 (0)	5 (5.2)	.77 (1)	..	.38

	All patients 210	Type of transplant		Statistic (df)	Estimated Risk (95% CI)	*P*-value
		Heart 33 (15.7)	Others 177 (84.3)			
Cardiac complications[b]	16 (7.6)	3 (9.1)	13 (7.3)	.00 (1)	1.26 (.34-6.70)	1.00
Intensive care unit admission	37 (17.6)	5 (15.2)	32 (18.1)	.16 (1)	.81 (.29-2.26)	.69
Mortality	45 (21.4)	6 (18.2)	39 (22.0)	.25 (1)	.79 (.30-2.04)	.62
Graft dysfunction, at day 30	12 (8.3)	1 (4.3)	11 (9.1)	.12 (1)	.46 (.06-3.70)	.73
Graft lost, at day 30	5 (3.8)	0 (0)	5 (4.5)	.13 (1)	..	.72

	All patients 210	Type of transplant		Statistic (df)	Estimated Risk (95% CI)	*P*-value
		Lung 15 (7.1)	Others 195 (92.9)			
Respiratory complications[c]	68 (32.4)	5 (33.3)	63 (32.3)	.00 (1)	1.05 (.34-3.19)	1.00
Intensive care unit admission	37 (17.6)	3 (20.0)	34 (17.4)	.00 (1)	1.18 (.32-4.42)	1.00
Mortality	45 (21.4)	5 (33.3)	40 (20.5)	.71 (1)	1.94 (.63-5.99)	.40
Graft dysfunction, at day 30	12 (8.3)	0 (0)	12 (9.2)	.46 (1)	..	.50
Graft lost, at day 30	5 (3.8)	0 (0)	5 (4.1)	.00 (1)	..	1.00

	All patients 210	Type of transplant		Statistic (df)	Estimated Risk (95% CI)	*P*-value
		Combined 4 (1.9)	Others 206 (98.1)			
Intensive care unit admission	37 (17.6)	1 (25.0)	36 (17.5)	.00 (1)	1.57 (.16-15.57)	1.00
Mortality	45 (21.4)	0 (0)	45 (21.8)	.19 (1)	..	.66
Graft dysfunction, at day 30	12 (8.3)	0 (0)	12 (8.4)	.00 (1)	..	1.00
Graft lost, at day 30	5 (3.8)	0 (0)	5 (3.8)	.00 (1)	..	1.00

[a]New onset or exacerbation of renal insufficiency.

[b]New onset arrhythmia, heart failure, or acute coronary event.

[c]Need for mechanical ventilation, acute distress respiratory syndrome, empyema, or pleural effusion.

S5 Table. Description of patients suffering from graft dysfunction at day 30 (n=12).

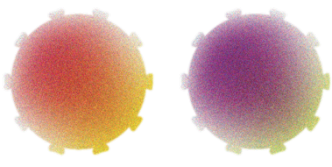
Identification, demographics, type of transplant (dd.mm.yy), and presence of pneumonia	Previous rejection history	Hospital admission	Intensive care unit admission	Graft loss	Death	Specific treatment for COVID-19	Baseline regimen	Handle of immunosuppression
1. 59-year-old male, kidney transplant (01.01.94), pneumonic	No	**Yes**	No	**Yes**	No	LPV/r + HCQ + TCZ + IFN + MPS	Pred + Tac + MMF	End of Tac and MMF. Reduction of Pred
2. 79-year-old male, kidney transplant (24.02.08), pneumonic	No	**Yes**	No	No	No	HCQ + AZM	Pred + Tac + SRL/EVR	End of Tac and SRL/EVR. Pred kept.
3. 57-year-old male, kidney transplant (13.06.10), pneumonic	No	**Yes**	**Yes**	**Yes**	**Yes**	LPV/r + HCQ + AZM	Pred + Tac + SRL/EVR	End of Tac and SRL/EVR. Pred kept.
4. 38-year-old male, **heart** transplant (09.07.11), pneumonic	**Yes, chronic**	**Yes**	No	No	No	HCQ	Pred + Tac + MMF	End of MMF. Tac and Pred kept.
5. 48-year-old male, kidney transplant (30.07.11), pneumonic	No	**Yes**	No	No	No	HCQ + AZM	Pred + Tac + SRL/EVR	End of Tac and SRL/EVR. Pred kept.
6. 73-year-old male, kidney transplant (12.03.14), pneumonic	No	**Yes**	**Yes**	**Yes**	**Yes**	LPV/r + HCQ + TCZ	Pred + Tac + MMF	End of Tac and MMF. Pred kept.
7. 56-year-old male, kidney transplant (16.09.14), pneumonic	No	**Yes**	**Yes**	No	**Yes**	LPV/r + HCQ + TCZ	Pred + Tac + SRL/EVR	End of Tac and SRL/EVR. Pred kept.
8. 50-year-old male, kidney transplant (08.05.16), pneumonic	No	**Yes**	No	No	No	HCQ + TCZ + AZM	Pred + Tac + SRL/EVR	End of Tac and SRL/EVR. Pred kept.
9. 45-year-old female, kidney transplant (20.12.16), pneumonic	No	**Yes**	No	**Yes**	No	LPV/r + HCQ	Pred + Tac + MMF	End of Tac and MMF. Pred kept.
10. 39-year-old male, kidney transplant (24.03.17), pneumonic	No	**Yes**	No	No	No	HCQ	Pred + Tac + SRL/EVR	End of Tac and SRL/EVR. Pred kept.
11. 61-year-old female, kidney transplant (18.02.20), **non-pneumonic**	**Yes, acute**	**Yes**	No	**Yes**	No	HCQ	Pred + Tac + MMF	**No changes**
12. 40-year-old female, kidney transplant (08.03.20), pneumonic	No	**Yes**	No	No	No	**None**	Pred + Tac + MMF	End of MMF. Tac and Pred kept.

Abbreviations: LPV/r, lopinavir/ritonavir; HCQ, hydroxychloroquine; TCZ, tocilizumab; IFN, interferon; MPS, methylprednisolone; AZM, azithromycin; Pred, prednisone; Tac, tacrolimus; MMF, mofetil mycophenolate; SRL/EVR, sirolimus/everolimus.

S6 Table. Description of patients with COVID-19 acquisition during the first month posttransplant (n=6).

Identification, demographics, type of transplant (dd.mm.yy), and presence of pneumonia	Previous rejection history	Hospital admission	Intensive care unit admission	Graft dysfunction at day 30	Death	Specific treatment for COVID-19	Baseline regimen	Handle of immunosuppression
1. 40-year-old female, kidney transplant (08.03.20), pneumonic	No	**Yes**	No	**Yes (but no graft loss)**	No	**None**	Pred + Tac + MMF	End of MMF. Tac and Pred kept.
2. 61-year-old female, kidney transplant (18.02.20), **non-pneumonic**	**Yes, acute**	**Yes**	No	**Yes (with graft loss)**	No	HCQ	Pred + Tac + MMF	**No changes**
3. 70-year-old male, kidney transplant (20.03.20), **non-pneumonic**	No	**Yes**	No	No	No	HCQ	Pred + Tac	Reduction of Tac. Pred kept.
4. 43-year-old male, **heart** transplant (02.03.20), pneumonic	No	**Yes**	**Yes**	No	No	LPV/r + HCQ	Pred + Tac + MMF	**No changes**
5. 59-year-old female, **first week** post **heart** transplant (30.03.20), pneumonic	No	**Yes**	**Yes**	No	**Yes**	LPV/r + HCQ + TCZ	Pred + Tac + MMF	End of Tac and MMF. Pred kept.
6. 72-year-old female, **first week** post kidney transplant (27.03.20), pneumonic	No	**Yes**	**Yes**	No	**Yes**	HCQ	Pred + MMF	Reduction of MMF. Pred kept.

Abbreviations: LPV/r, lopinavir/ritonavir; HCQ, hydroxychloroquine; TCZ, tocilizumab; IFN, interferon; MPS, methylprednisolone; AZM, azithromycin; Pred, prednisone; Tac, tacrolimus; MMF, mofetil mycophenolate; SRL/EVR, sirolimus/everolimus.



ARTICLE 2

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.

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Letter to the Editor

Serum IFN-γ and RNAemia temporal profiles as biomarkers of severe COVID-19 in solid organ transplant and immunocompetent patients

Dear editor,

Currently, the availability of SARS-CoV-2 vaccines, despite the great worldwide differences in vaccination rates,[1] have focused the impact of the pandemic in immunodepressed patients. Therefore, we read with interest the recent meta-analysis about COVID-19 vaccine in patients with solid malignancies[2] which found that 42% and 86% of patients achieved serological response after one and two doses, respectively. Other authors have found that the pooled odds ratio for developing anti-SARS-CoV-2 spike protein IgG was higher in the control group than in solid organ transplant (SOT) recipients.[3] The infection by SARS-CoV-2 elicits an innate and specific cellular and humoral immune response. Interferons (IFN) are key in the innate immune response during the acute phase of the viral infection, as seen with plasmacytoid dendritic cells expressing high concentrations of types I and III IFNs in COVID-19 patients.[4,5] In acute COVID-19 and convalescent patients, intracellular cytokine staining after stimulation with SARS-CoV-2 peptide pools, has showed significant IFN-γ increases in CD8 + T-cells, which is associated with viral elimination, and without differences in both phases of the disease.[6,7]

In this context, to gain insight in the innate immune response in SOT recipients with COVID-19, compared with no SOT patients, we have assessed the IFN-α and IFN-γ serum levels and RNAemia at hospital admission and by days from the symptom's onset (DfSO; $\leq$1 to $\geq$15 days), as well as their association with unfavorable clinical outcomes (death and/or invasive mechanical ventilation [IMV]). With this aim, we conducted a multicentre prospective observational cohort study, including consecutive adult inpatients with confirmed COVID-19 (RT-PCR in nasopharyngeal swabs) and available samples for IFN-α/IFN-γ serum levels and RNAemia determinations (Methods, Supplementary material), from January 6th 2020 to August 13th 2021, followed until hospital discharge, death, or 30 days, whichever occurred first. The study was approved by The Ethics Committee of University Hospitals Virgen Macarena and Virgen del Rocío (C.I. 0771-N-20 and 0842-N-20).

Data were separately analyzed for SOT recipients and no SOT patients. In addition, we performed a matched cohort analysis in which patients undergoing SOT were paired with those from the no SOT cohort (1:2) according to their propensity score (PS), using callipers of a 0.01 standard deviation, to control for residual confounders. Mortality in the matched pairs was compared using Cox regression. IFN-α and IFN-γ levels were analyzed as discrete (undetectable and detectable) and continuous (pg/mL) variables. Multivariate Cox regression and logistic regression analysis were performed to identify factors independently associated with 30-day all-cause mortality and unfavorable clinical outcomes (Methods, Supplementary material).

Forty-seven (10.3%) SOT recipients and 408 (89.7%) no SOT patients (Supplementary Table 2) were recruited. The mean DfSO to hospital admission was 7.1 $\pm$ 4.3, without differences between groups. Undetectable IFN-α occurred in 8.5% and 13.5% ($p = 0.36$) of both groups, respectively (Fig. 1A), independently of the DfSO. In SOT recipients, IFN-α levels were higher with <7 DfSO than with $\geq$7 DfSO ($p = 0.015$), with a decrease from 19.5 pg/mL to 1.4 pg/mL. In no SOT, IFN-α levels were higher than in SOT recipients with $\geq$7 DfSO (Supplementary Table 3). Undetectable IFN-γ was more frequent in SOT recipients than in no SOT patients (42.6% and 19.4%, $p < 0.001$) and this difference was higher with $\geq$7 DfSO (Fig. 1A). IFN-γ levels were similar over the different time-periods, both in SOT and no SOT, and without differences between groups (Supplementary Table 3), which is consistent with other studies.[6,7] RNAemia was more frequent in SOT recipients (57.4%) than in no SOT patients (18.9%, $p < 0.001$) (Fig. 1A, Supplementary Table 2). In SOT recipients, RNAemia detection was independent of the DfSO, and in no SOT patients decreased with $\geq$11 DfSO ($p = 0.014$) (Supplementary Table 3). Mortality was higher in SOT recipients than in no SOT patients with $\geq$4 DfSO (Fig. 1A). In SOT recipients, mortality was not associated to the DfSO at admission; however, in no SOT patients, mortality was much higher in patients with $\leq$3 DfSO, decreasing to 0% in patients with $\geq$11 DfSO ($p < 0.001$) (Fig. 1A).

In the PS matched cohorts (Table 1), SOT recipients showed higher prevalence of undetectable IFN-γ than no SOT patients (39.4% *vs.* 10.6%, respectively; $p = 0.001$), lower plasma IFN-α and IFN-γ levels in those with RNAemia ($p = 0.013$ and $p = 0.001$, respectively; Supplementary Fig. 1B), higher RNAemia detection (57.6% *vs.* 13.6%; $p < 0.001$) and mortality (27.3% *vs.* 4.5%; $p = 0.003$).

In SOT recipients, the multivariate logistic regression model selected RNAemia as predictor of unfavorable clinical outcome (Supplementary Table 6). Regarding no SOT patients, in the Cox regression multivariate analysis, 30-day all-cause mortality was associated with RNAemia and undetectable IFN-γ levels (Supplementary Table 7). In the Kaplan-Meier analysis, patients with RNAemia had lower survival, both in SOT ($p < 0.0133$) and no SOT ($p = 0.001$) groups (Fig. 1B). RNAemia has been associated with COVID-19 mortality.[8] The present data confirm it, with a higher sample size and including SOT recipients, in which the RNAemia impact had not yet been analyzed. The Kaplan-Meier analysis also showed an association of undetectable IFN-γ with lower survival in SOT recipients ($p = 0.048$) (Fig. 1B). Our results, showing an association of undetectable IFN-γ in serum with mortality, support the protective role of the specific T-cells response.

The Kaplan-Meier analysis did not show association of undetectable IFN-α levels with the survival at 30 days, both in SOT and no SOT groups. It has been reported that inborn errors of type I

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Table 1
Comparison of solid organ transplant (SOT) recipients matched (1:2) with no SOT patients according to propensity score.[a]

Variable	SOT recipients (n = 33)	No SOT patients (n = 66)	P value
Male sex	20 (60.6)	37 (56.1)	0.666
Age >70 years	4 (12.1)	21 (31.8)	0.033
Dyspnoea	15 (45.5)	36 (54.5)	0.394
SpO_2 <95%	17 (51.5)	26 (39.4)	0.251
Neutrophil count >7500/μL	6 (18.2)	6 (9.1)	0.327
Lymphocyte count <1000/µL	18 (54.5)	30 (45.5)	0.394
C-reactive protein >100 mg/L	9 (27.3)	20 (30.3)	0.755
Ferritin >1000 ng/mL	6 (18.2)	10 (15.2)	0.699
D-dimer >600 ng/mL	29 (87.9)	48 (72.7)	0.087
LDH >300 IU/L	14 (42.4)	33 (50.0)	0.477
IFN-α undetectable	3 (9.1)	8 (12.1)	0.747
IFN-α (pg/mL)[b]	1.43 (0.60–22.01)	11.98 (3.24–23.11)	0.163
IFN-γ undetectable	13 (39.4)	7 (10.6)	0.001
IFN-γ (pg/mL)[b]	26.14 (0.00–240.96)	145.35 (40.00–330.96)	0.347
RNAemia positive	19 (57.6)	9 (13.6)	<0.001
RNAemia (log_{10} copies/mL)[b]	2.38 (2.12–3.19)	2.36 (1.92–2.98)	0.921
CCI ≥3	25 (75.8)	37 (56.1)	0.056
CURB-65 ≥2	12 (36.4)	12 (18.8)	0.057
WHO basal score 6–9[c]	3 (9.1)	4 (6.1)	0.683
IMV	10 (30.3)	5 (7.6)	0.003
Mortality at day 30	9 (27.3)	3 (4.5)	0.003
WHO final score 7-10[c]	13 (39.4)	7 (10.6)	0.001

Data are presented as No. (%). P values are calculated by Cox regression.
Abbreviations (in order of appearance): SpO_2, peripheral capillary oxygen saturation; LDH, lactate dehydrogenase; IFN, interferon; CCI, Charlson Comorbidity Index (30); CURB-65 (31), Severity Score for Community-Acquired Pneumonia; IMV, invasive mechanical ventilation.
[a] Variables included in the propensity score were sex, dyspnea, SpO_2, neutrophil and lymphocyte counts, C-reactive protein, ferritin, D-dimer, and LDH.
[b] Median (IQR). P values are calculated by the Mann-Whitney U test.
[c] Severity rating according to the WHO Clinical Progression Scale (doi: 10.1016/S1473-3099(20)30483-7), ranged from 0 (not infected) to 10 (dead), of which scores 6–9 represent severe disease.

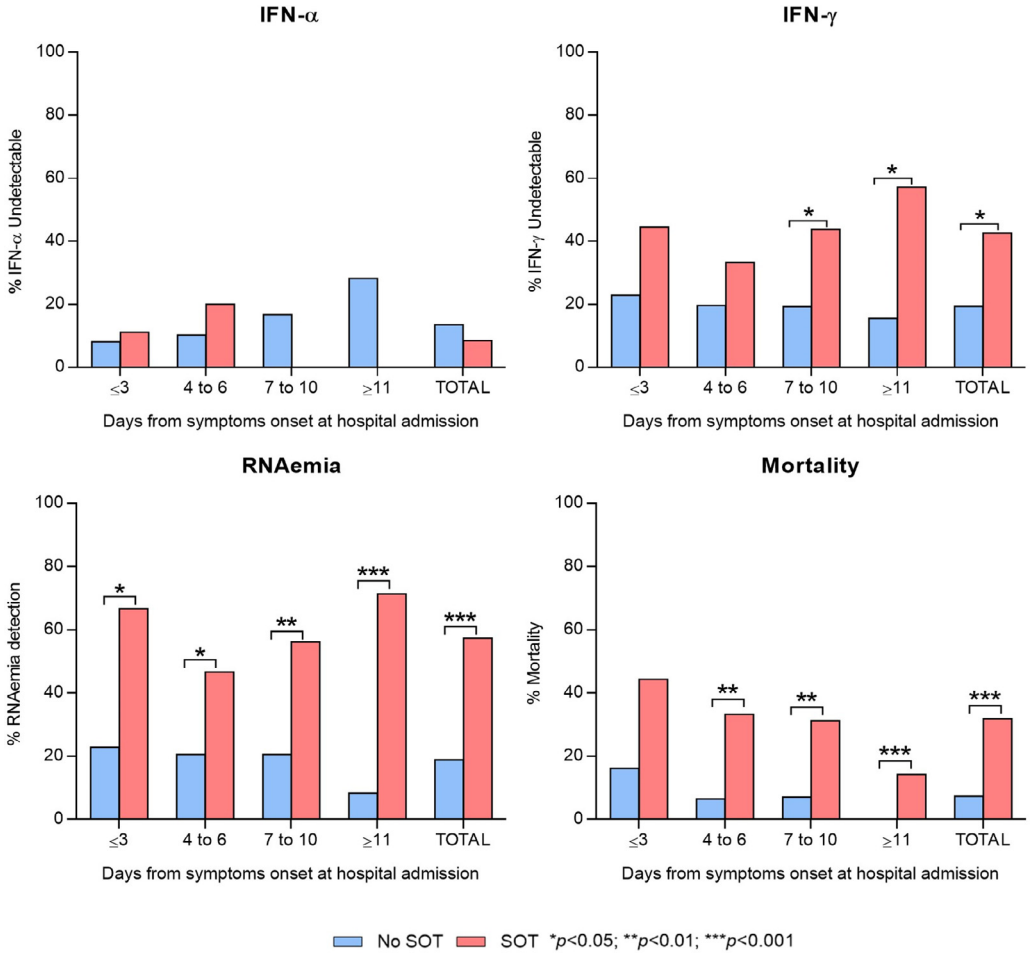

Fig. 1. SOT recipients (n = 47) comparison with no SOT patients (n=408) regarding (A) undetectable IFN-α and IFN-γ serum levels, RNAemia, and mortality, by days from symptoms onset at hospital admission, and (B) Survival Kaplan Meier analysis of patients with and without undetectable IFN-γ serum levels and RNAemia detection.

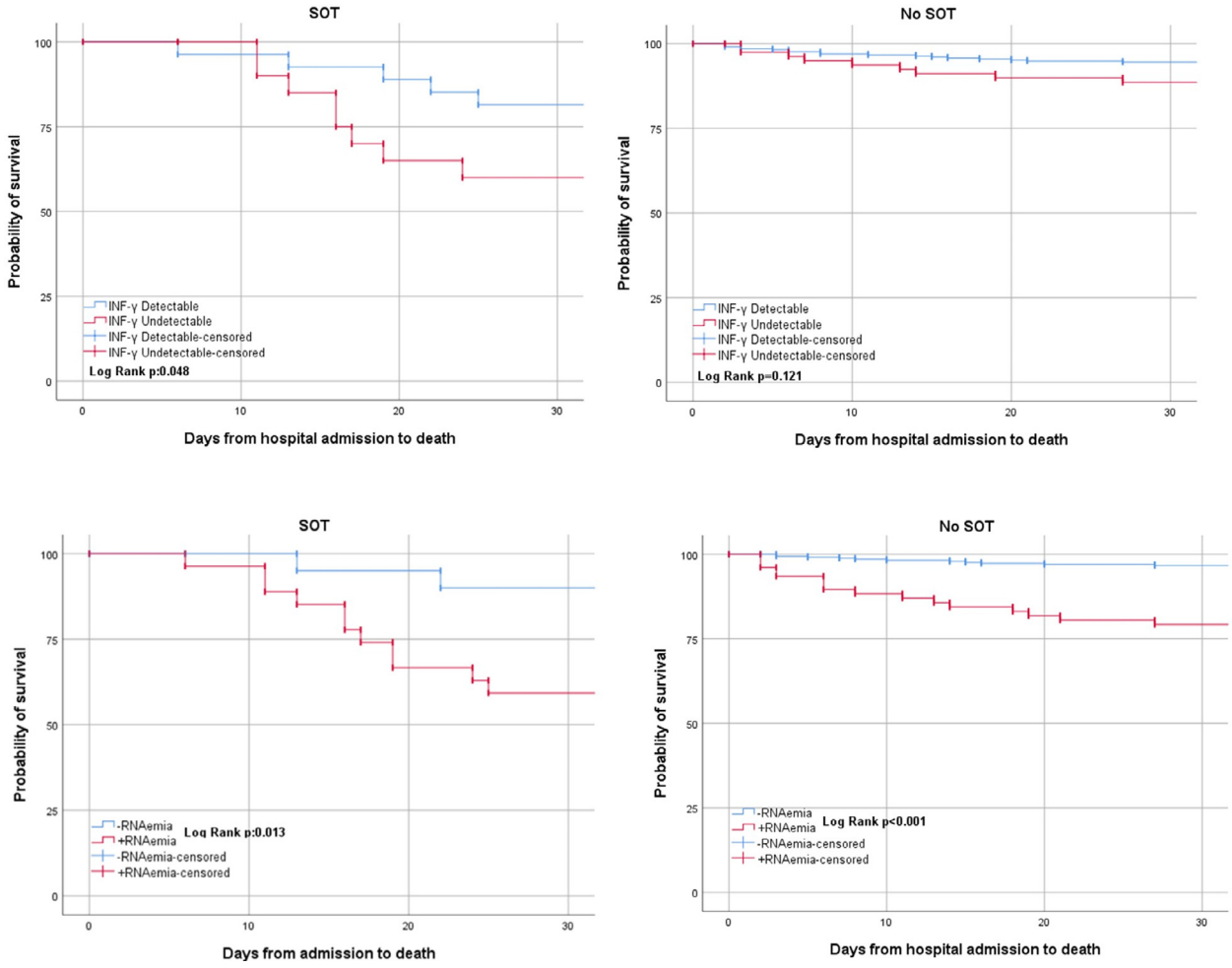

Fig. 1. Continued

IFN immunity accounts for life-threatening COVID-19 pneumonia[9] and that autoantibodies against type I IFNs increased the infection fatality rate.[10] However, we have not found association of serum undetectable IFN-α with unfavorable outcome, including immunosuppressed patients as the SOT recipients. A limitation is that we did not analyse IFN-stimulated genes to define a type I IFN signature nor interferon autoantibodies, because of our purpose was to identify easy-to-measure variables in the clinical setting.

In summary, the present results support the RNAemia and IFN-γ serum levels determinations, at hospital admission, in all adult COVID-19 patients, to guide their management and to assess the antiviral therapy efficacy in the case of RNAemia.

Declaration of Competing Interest

The authors have declared that no competing interests exist.

Author contributions

JS-C, EC, JMC, and JP conceived and designed the study; obtained public funding from the Spanish Ministry of Economy, Industry, and Competitiveness; and took responsibility for the integrity of the data and the accuracy of its analysis. JP and SS-A did the scientific literature search. PC-M, JB-C, CI, RR-A, JA, PP-P, EG-D, CR, JPR, MJB-V, SS, RV-O, JNA, CG-G, MJB-V, DG-C, NM, GB, MAG-B, JMS, MA-G, RA-M, JG-A, JAO, ZRP-B, AP, JAL and JRB supported the inclusion of patients and the acquisition of data. JS-C, MC-L and JB-C processed the research clinical samples and obtained the data. SS-A, MC-L and JB-C processed the data, SS-A, MC-L and JP did the statistical analysis. SS-A, MC-L, JP, MA-G, JS-C and EC did the interpretation of data and wrote the draft of the manuscript. All authors critically revised the manuscript for important intellectual content and gave final approval for the version published.

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dalucía, Spain. The funders have not role nor any influence on the design, data analysis or interpretation.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2023.01.019.

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Sonsoles Salto-Alejandre[1], Marta Carretero-Ledesma[1], Pedro Camacho-Martínez, Judith Berastegui-Cabrera, Carmen Infante
Division of Infectious Diseases, Microbiology and Parasitology, Virgen del Rocío University Hospital, Seville, Spain
Institute of Biomedicine of Seville (IBiS), Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.

Regino Rodríguez-Álvarez
Department of Infectious Diseases, Hospital Universitario Cruces, Bizkaia, Spain

Jorge Alba
Department of Infectious Diseases, San Pedro-CIBIR University Hospital, La Rioja, Logroño, Spain

Patricia Pérez-Palacios
Institute of Biomedicine of Seville (IBiS), Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.
Unit of Infectious Diseases and Microbiology, Virgen Macarena University Hospital, Seville, Spain

Emilio García-Díaz
Medical Surgical Respiratory Unit, Virgen del Rocío University Hospital, Seville, Spain.

Cristina Roca, Julia Praena
Division of Infectious Diseases, Microbiology and Parasitology, Virgen del Rocío University Hospital, Seville, Spain
Institute of Biomedicine of Seville (IBiS), Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.

María José Blanco-Vidal
Department of Infectious Diseases, Hospital Universitario Cruces, Bizkaia, Spain

Sonia Santibáñez
Department of Infectious Diseases, San Pedro-CIBIR University Hospital, La Rioja, Logroño, Spain

Rocío Valverde-Ortiz
Unit of Nephrology, Virgen Macarena University Hospital, Seville, Spain

Javier Nieto-Arana
Department of Infectious Diseases, Hospital Universitario Cruces, Bizkaia, Spain

Concepción García-García
Department of Infectious Diseases, San Pedro-CIBIR University Hospital, La Rioja, Logroño, Spain

David Gutiérrez-Campos
Institute of Biomedicine of Seville (IBiS), Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.
Unit of Infectious Diseases and Microbiology, Virgen Macarena University Hospital, Seville, Spain

Natalia Maldonado
Institute of Biomedicine of Seville (IBiS), Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.
Unit of Infectious Diseases and Microbiology, Virgen Macarena University Hospital, Seville, Spain
CIBERINFEC, ISCIII - CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain

Gabriel Bernal
Unit of Urology and Nephrology, Virgen del Rocío University Hospital, Seville, Spain.

Miguel Ángel Gómez-Bravo
General and Digestive Service, Virgen del Rocío University Hospital, Seville, Spain.

José Manuel Sobrino
Unit of Cardiology and Cardiovascular Surgery, Virgen del Rocío University Hospital, Seville, Spain.

Manuela Aguilar-Guisado, Rocío Álvarez-Marín
Division of Infectious Diseases, Microbiology and Parasitology, Virgen del Rocío University Hospital, Seville, Spain
Institute of Biomedicine of Seville (IBiS), Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.
CIBERINFEC, ISCIII - CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain

Josune Goikoetxea-Aguirre
Department of Infectious Diseases, Hospital Universitario Cruces, Bizkaia, Spain

José Antonio Oteo
Department of Infectious Diseases, San Pedro-CIBIR University Hospital, La Rioja, Logroño, Spain

Zaira R. Palacios-Baena
Institute of Biomedicine of Seville (IBiS), Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.
Unit of Infectious Diseases and Microbiology, Virgen Macarena University Hospital, Seville, Spain
CIBERINFEC, ISCIII - CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain

Álvaro Pascual
Institute of Biomedicine of Seville (IBiS), Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.
Unit of Infectious Diseases and Microbiology, Virgen Macarena University Hospital, Seville, Spain
CIBERINFEC, ISCIII - CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain
Department of Microbiology, University of Seville, Seville, Spain

José Antonio Lepe
Division of Infectious Diseases, Microbiology and Parasitology, Virgen del Rocío University Hospital, Seville, Spain
Institute of Biomedicine of Seville (IBiS), Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.
CIBERINFEC, ISCIII - CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain
Department of Microbiology, University of Seville, Seville, Spain

Jesús Rodríguez-Baño
Institute of Biomedicine of Seville (IBiS), Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.
Unit of Infectious Diseases and Microbiology, Virgen Macarena University Hospital, Seville, Spain
CIBERINFEC, ISCIII - CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain
Department of Medicine, School of Medicine, University of Seville, Seville, Spain

José Miguel Cisneros
Division of Infectious Diseases, Microbiology and Parasitology, Virgen del Rocío University Hospital, Seville, Spain
Institute of Biomedicine of Seville (IBiS), Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.
CIBERINFEC, ISCIII - CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain
Department of Medicine, School of Medicine, University of Seville, Seville, Spain

Jerónimo Pachón
Institute of Biomedicine of Seville (IBiS), Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.
Department of Medicine, School of Medicine, University of Seville, Seville, Spain

Javier Sánchez-Céspedes*
Division of Infectious Diseases, Microbiology and Parasitology, Virgen del Rocío University Hospital, Seville, Spain
Institute of Biomedicine of Seville (IBiS), Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.
CIBERINFEC, ISCIII - CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain

Elisa Cordero
Division of Infectious Diseases, Microbiology and Parasitology, Virgen del Rocío University Hospital, Seville, Spain
Institute of Biomedicine of Seville (IBiS), Virgen del Rocío and Virgen Macarena University Hospitals/CSIC/University of Seville, Seville, Spain.
CIBERINFEC, ISCIII - CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain
Department of Medicine, School of Medicine, University of Seville, Seville, Spain

*Corresponding author at: Division of Infectious Diseases, Microbiology and Parasitology, Virgen del Rocío University Hospital, Av. Manuel Siurot s/n, Seville 41013, Spain.
E-mail address: jsanchez-ibis@us.es (J. Sánchez-Céspedes)
[1] These authors contributed equally to this work.

SUPPLEMENTARY MATERIAL

Serum IFN-γ and RNAemia temporal profiles as biomarkers of severe COVID-19 in solid organ transplant and immunocompetent patients

1. SUPPLMENTARY METHODS

Interferon-α and interferon-γ plasma levels

Serum samples collected from patients were stored at −80 ºC. The IFN-α (USCN Life Science & Technology Company, Missouri, TX, USA) and IFN-γ (RayBiotech, Norcross, GA, USA) were quantified by ELISA according to the manufacturer's instructions. Briefly, serum samples for IFN-α quantification were diluted 1:2 in assay diluent and incubated 1 h at 37 °C. After that, working reagent A was added, plate was incubated 1 h at 37 °C and washed 3 times before adding reagent B and let for 30 min at 37°C. Finally, plate was washed 5 times, and revealed with TMB and stop solution. For IFN-γ quantification, serum samples were also diluted 1:2 in assay diluent and incubated 2.5 h at room temperature (RT). Then, biotin antibodies were added, and plates were incubated for one hour at RT. Afterwards, streptavidin solution was incubated in the plate 45 min at RT and, at the end, the assay was revealed with TMB and stop solution. Plates were washed 4 times between each step of incubation. Optical density was measured at 450 nm using Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

The relative levels of IFN were analysed using a log/log fit curve using the statistical package GraphPad Prism 6. This assay was performed in duplicate for each sample. The lower limits of detection were 3 pg/ml y 2 pg/ml for IFN-α and IFN-γ, respectively. As reference values, IFN-α and IFN-γ levels were determined in 32 healthy uninfected adults, 12 males and 20 females, with a median age of 38 (IQR, 28-49; range 22-81) years, without SARS-CoV-2 infection, primary or secondary immunodeficiency, chronic underlying diseases, and any acute disease in the previous month.

SARS-CoV-2 RT-PCR in nasopharyngeal swabs, RNAemia detection and blood viral load

SARS-CoV-2 RNA was extracted from plasma samples and NP swabs using the MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics GmbH, Mannheim, Germany) following manufacturer recommendations. RT-PCR was conducted using the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel and the GoTaq® Probe 1-Step RT-qPCR System (Wisconsin, USA) in a LightCycler 96 Instrument (Roche, Germany) following the CDC's instructions. SARS-CoV-2 viral load quantification was calculated by the interpolation of the Ct values obtained using the Quantitative Synthetic SARS-CoV-2 RNA: ORF, E, N kit (ATCC, VA, USA) and expressed in copies/mL and $\log_{10}$ copies/mL.

Statistical analysis

A descriptive analysis of all obtained data was performed, and results were presented as crude number (%) or means ± SEM. The $\chi 2$, Fisher's exact test, Mann-Whitney U test, and Kruskal-Wallis test were used to compare between-group differences. When appropriate, continuous variables were dichotomized using data classification analysis, in accordance with their association with mortality[1,2]. Being this a contemporary and accessible cohort, we were able to recover all the necessary data for the agreed aims achievement. Hence, the small number of missing values (Supplementary Table 1) and the fact that they were missing completely at random, enabled the implementation of a complete-case analysis.

To identify bivariate correlation among IFN-α and IFN-γ levels, SARS-CoV-2 RNAemia, quantitative baseline variables, and the final WHO Clinical Progression Scale[3] at 30 days, Spearman's rank correlation coefficient ρ was calculated using Origin 2021b

(OriginLab, Northampton, MA, USA) and visualized in a heat map using the app Correlation Plot 1.30.

The outcome variables were 30-day all-cause mortality and need for invasive mechanical ventilation (IMV). The main exposures of interest, recorded at hospital admission, were IFN-α and IFN-γ serum levels and SARS-CoV-2 RNAemia at hospital admission (Methods in Supplementary material). Additional exposure variables were demographics, chronic underlying conditions, Charlson Comorbidity Index (CCI), DfSO, symptoms and signs, hemogram, liver and renal biochemistry, inflammatory biomarkers, pneumonia, CURB-65 score, quick Sequential Organ Failure Assessment (qSOFA) score, respiratory support, and COVID-19 severity according to the WHO Clinical Progression Scale[3].

Cox regression was used to analyse the impact of undetectable IFN-α/IFN-γ levels and RNAemia detection on 30-day all-cause mortality. Variables with a p value <0.10 in univariate comparisons and those considered clinically relevant were included in the multivariate models. Interaction, confusion, and collinearity were thoroughly explored. A propensity score (PS) for patients with *vs.* without RNAemia was calculated, and its predictive ability for the observed data was assessed using the area under the receiver operating characteristic curve (AUROC) with a 95% confidence interval (CI). Statistical analyses were carried out using the statistical package SPSS (SPSS 26.0, IBM Corp, Armonk, New York, USA). GraphPad Prism 9.0.0 (GraphPad Software, San Diego, CA, USA) was used for graphing and analysis of survival curves using the Kaplan-Meier method, and significance was determined using the Mantel-Cox test.

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2. SUPPLEMENTARY RESULTS, TABLES AND FIGURES

Supplementary Results

INF-α levels were higher in no SOT patients *vs.* healthy controls (p=0.042) and SOT recipients (p=0.047). IFN-γ levels were higher in no SOT patients (p<0.001) and SOT recipients (p=0.02) *vs.* healthy controls (Supplementary Fig. 1A).

The 75 patients with a WHO final score of 7-10, had more frequent RNAemia (58.7% vs. 15.8%, p<0.001) as well as higher plasma RNAemia levels (2.53 [2.18-3.17] vs. 2.05 [1.75-2.72] log10 copies/mL; p=0.0065), compared with the 380 patients with a WHO final score of 4-6 (Supplementary Fig. 2A and 2B).

The pairwise correlations heatmaps between IFN-α and IFN-γ plasma levels, SARS-CoV-2 RNAemia viral load, baseline variables, and unfavourable clinical outcome (WHO final score 7-10) for the SOT recipients and no SOT patients are detailed in Supplementary Fig. 3). As for the IFN-α, it showed positive correlation with the age, CCI, plasma creatinine, and WHO basal score, and negative correlation with lymphocytes and platelets counts in the no SOT patients. In the SOT recipients, IFN-α only showed negative correlation with the platelets count. IFN-γ had positive correlation with the platelets count in the no SOT patients; in the SOT recipients it showed positive correlation with lymphocytes count, and negative correlation with the age, CCI, and WHO basal score. SARS-CoV-2 RNAemia showed positive correlation with neutrophils, C-reactive protein, and the WHO final score in no SOT patients, and positive correlation with the heart rate, LDH, neutrophils, and platelets, and negative correlation with lymphocytes count in the SOT recipients.

Supplementary Tables

Supplementary Table 1. Missing data for the variables collected in the cohort.	
Variable	**% Missing data**
Demographics and underlying conditions	
Sex	0
Age	0
Chronic kidney disease	0.2
Chronic liver disease	0.2
SOT	0
Admission symptoms and signs	
Dyspnoea	0.7
Temperature	0.7
SpO_2	4.4
HR	13.8
Admission image and laboratory findings	
Infiltrates on chest X-rays	2.2
Neutrophil count	5.3
Lymphocyte count	2.2
Platelets	1.5
Creatinine	3.1
C-reactive protein	4.8
Ferritin	11.2
D-dimer	16.7
LDH	11.2
IFN alpha	0
IFN gamma	0
RNAemia	0
Admission scores	
CCI	0.4
CURB-65	3.1
qSOFA	2.9
WHO basal score [a]	0.2
Outcomes	
IMV	0
Mortality at day 30	0
WHO final score [a]	0

Abbreviations (in order of appearance): SOT, solid organ transplant; SpO_2, peripheral capillary oxygen saturation; HR, heart rate; LDH, lactate dehydrogenase; IFN, interferon; CCI, Charlson Comorbidity Index; CURB-65, Severity Score for Community-Acquired Pneumonia; qSOFA, quick Sequential Organ Failure Assessment score; IMV, invasive mechanical ventilation.

[a] WHO Clinical Progression Scale, doi: 10.1016/S1473-3099(20)30483-7.

Supplementary Table 2. Demographics, underlying chronic diseases, clinical features, IFN-α, IFN-γ, RNAemia, and outcomes in all, SOT recipients, and no SOT patients.

Variable	All cohort (n = 455)	SOT recipients (n = 47)	No SOT patients (n = 408)	*P* value [e]
Demographics and underlying conditions				
Male sex	261 (57.4)	28 (59.6)	233 (57.1)	0.746
Age >70 years	157 (34.5)	11 (23.4)	146 (35.8)	0.091
Chronic kidney disease [a]	42 (9.3)	7 (15.2)	35 (8.6)	0.173
Chronic liver disease [b]	13 (2.9)	1 (2.2)	12 (2.9)	1.000
Admission symptoms and signs				
Dyspnoea	231 (51.1)	20 (44.4)	211 (51.8)	0.346
Temperature ≥37.5ºC	129 (28.5)	8 (17.8)	121 (29.7)	0.092
DBP <60 mmHg	21 (5.9)	3 (8.6)	18 (5.6)	0.449
SpO_2 <95%	203 (46.6)	19 (43.2)	184 (46.9)	0.636
HR >100 bpm	117 (29.8)	12 (31.6)	105 (29.7)	0.806
RR >30 bpm	7 (5.3)	2 (15.4)	5 (4.2)	0.140
Admission image and laboratory findings				
Infiltrates on chest X-rays	403 (88.6)	35 (74.5)	368 (90.2)	0.001
Neutrophil count >7500/μL	85 (19.7)	7 (16.7)	78 (20.1)	0.600
Lymphocyte count <1000/μL	224 (50.3)	26 (61.9)	198 (49.1)	0.115
Platelets <130 000/μL	62 (13.8)	12 (27.9)	50 (12.3)	0.005
Creatinine >1.3 mg/dL	105 (23.8)	30 (71.4)	75 (18.8)	<0.001
C-reactive protein >100 mg/L	151 (34.9)	14 (35.0)	137 (34.9)	0.986
Ferritin >1000 ng/mL	88 (21.8)	7 (19.4)	81 (22.0)	0.722
D-dimer >600 ng/mL	224 (59.1)	28 (84.8)	196 (56.6)	0.002
LDH >300 IU/L	189 (46.8)	16 (41.0)	173 (47.4)	0.448
IFN-α undetectable	59 (13.0)	4 (8.5)	55 (13.5)	0.337
IFN-α (pg/mL) [c]	15.52 (3.45–29.48)	3.74 (0.60–22.99)	16.79 (5.29–32.72)	0.231
IFN-γ undetectable	99 (21.8)	20 (42.6)	79 (19.4)	<0.001
IFN-γ (pg/mL) [c]	111.90 (7.20–305.51)	26.14 (0.00–180.00)	120.71 (12.33–350.70)	0.247
RNAemia positive	104 (22.9)	27 (57.4)	77 (18.9)	<0.001
RNAemia (log_{10} copies/mL) [c]	2.39 (1.88–3.08)	2.43 (2.17–3.23)	2.37 (1.87–3.03)	0.282
Admission scores				
CCI ≥3	258 (57.0)	35 (77.8)	223 (54.7)	0.003
CURB-65 ≥2	108 (24.5)	16 (35.6)	92 (23.2)	0.069
qSOFA ≥2	13 (2.9)	2 (5.9)	11 (2.7)	0.264
WHO basal score 6–9 [d]	32 (7.0)	4 (8.5)	28 (6.9)	0.761
Outcomes				
IMV	35 (7.7)	13 (27.7)	22 (5.4)	<0.001
Mortality at day 30	45 (9.9)	15 (31.9)	30 (7.4)	<0.001
WHO final score 7-10 [d]	75 (16.5)	21 (44.7)	54 (13.2)	<0.001

Data are presented as No. (%). P values are calculated by χ^2 or Fisher's test, as appropriate.

Abbreviations (in order of appearance): SOT, solid organ transplant; DBP, diastolic blood pressure; SpO_2, peripheral capillary oxygen saturation; HR, heart rate; RR, respiratory rate; LDH, lactate dehydrogenase; IFN, interferon; CCI, Charlson Comorbidity Index; CURB-65, Severity Score for Community-Acquired Pneumonia; qSOFA, quick Sequential Organ Failure Assessment score; IMV, invasive mechanical ventilation.

[a] Kidney transplant recipients are excluded from this category.

[b] Liver transplant recipients are excluded from this category.

[c] Median (IQR). P values are calculated by the Mann-Whitney U test.

[d] Severity rating according to the WHO Clinical Progression Scale (doi: 10.1016/S1473-3099(20)30483-7), ranged from 0 (not infected) to 10 (dead), of which scores 6–9 represent severe diseases.

[e] Comparisons between SOT recipients and no SOT patients.

Supplementary Table 3. IFN-α, IFN-γ, and RNAemia detection and plasma levels, and mortality rates by days from symptoms onset (DfSO), in SOT recipients and no SOT patients.				
DfSO at hospital admission	**Total cohort (N = 455)**	**SOT recipients (N =47)**	**No SOT patients (N = 408)**	**p value**
IFN-α				
≤ 3	76/83 (91.5) *	8/9 (88.9)	68/74 (91.9)	0.567
		14.2 (2.1-24.1) **, #	23.5 (13.8-52.7)	0.054
4 to 6	108/122 (88.5)	12/15 (80)	96/107 (89.7)	0.236
		19.5 (3.9-40.4)	20.7 (9.7-39.4)	0.358
7 to 10	146/172 (84.8)	16/16 (100)	130/156 (83.3)	0.064
		1.4 (0.5-20.4)	18.9 (9.6-31.4)	0.001
≥ 11	66/78 (84.6)	7/7 (100)	59/71 (71.8)	0.295
		1.03 (0.7-5.8)	15.2 (6.05-30.0)	0.004
TOTAL	396/455 (86.8)	43/47 (91.48)	353/408 (86.5)	0.360
		4.9 (0.75-23.4)	19.98 (10.74-37.98)	**0.000**
IFN-γ				
≤ 3	62/83 (74.7) *	5/9 (55.5)	57/74 (77.1)	0.159
		55.9 (36.34-197.1) **	134.4 (71.1-399.8)	0.194
4 to 6	96/122 (78.7)	10/15 (66.7)	86/107 (80.4)	0.187
		187.4 (77.5-375.8)	179.8 (70.6-377.4)	0.792
7 to 10	135/172 (78.5)	9/16 (56.2)	126/156 (80.7)	0.031
		158.3 (35.9-543.4)	155.18 (61.8-369.7)	0.853
≥ 11	63/78 (80.7)	3/7 (42.8)	60/71 (84.5)	0.023
		187.9 (144-2-188)	265.96 (70.29-506.98)	0.570
TOTAL	356/455 (78.02)	27/47 (57.4)	329/408 (80.6)	0.000
		172.27 (52.3-303.8)	164.83 (69.06-410.74)	0.834
RNAemia				
≤ 3	23/83 (27.7) *	6/9 (66.7)	17/74 (22.9) ##	0.012
		3.3 (2.11-4.04) ***	2.7 (2.3-3.4)	0.609
4 to 6	29/122 (23.7)	7/15 (46.7)	22/107 (20.5)	0.047
		2.8 (2.7-3.2)	2.6 (2.2-3.3)	0.263
7 to 10	41/172 (23.8)	9/16 (56.2)	32/156 (20.5)	0.003
		3.3 (2.5-3.9)	2.5 (3.14-3.7)	0.841
≥ 11	11/78 (14.1)	5/7 (71.4)	6/71 (8.4)	0.000
		2.9 (2.9-4.25)	2.3 (1.8-2.9)	0.052
TOTAL	104/455 (22.8)	27/47 (57.4)	77/408 (18.9)	0.000
	2,89 (2,41-3,61)	2,95 (2,71-3,71)	2,87 (2,39-3,57)	0.154
Mortality				
≤ 3	15/83 (18.1) *	4/9 (44.4) ###	12/74 (16.2) ####	0.064
4 to 6	12/122 (9.8)	5/15 (33.3)	7/107 (6.5)	0.006
7 to 10	15/172 (8.7)	5/16 (31.3)	11/156 (7.1)	0.008
≥ 11	1/78 (1.3)	1/7 (14.3)	0/71 (0.0)	0.089
TOTAL	43/455 (9.5)	15/47 (31.9)	30/408 (7.4)	<0.001

SOT: solid organ transplantation; * n/N (%); ** pg/mL (median [IQR]); *** log_{10} copies/mL (median [IQR]); # IFN-α levels alongside the four time-periods (p=0.015, Kruskal-Wallis test); ## RNAemia rates alongside the four time-periods (p=0.014); ### Mortality rates alongside the four time-periods in SOT (p=0.346); #### Mortality rates alongside the four time-periods in no SOT (p=0.001).

Supplementary Table 4. Demographics, underlying chronic diseases, clinical features, and outcomes of patients with COVID-19 according to the presence of RNAemia.

Variable	RNAemia (n = 104)	No RNAemia (n = 351)	*P* value
Demographics and underlying conditions			
Male sex	63 (60.6)	198 (56.1)	0.45
Age >70 years	33 (31.7)	124 (35.1)	0.50
Chronic kidney disease [a]	11 (10.7)	31 (8.8)	0.57
Chronic liver disease [b]	5 (4.9)	8 (2.3)	0.18
SOT	29 (27.9)	20 (5.7)	<0.001
Admission symptoms and signs			
Dyspnoea	62 (61.4)	169 (48)	0.01
Temperature ≥37.5 ºC	30 (29.4)	100 (28.4)	0.83
DBP <60 mmHg	7 (9.3)	14 (5)	0.17
SpO_2 <95%	54 (54.5)	149 (44)	0.07
HR >100 bpm	30 (34.1)	87 (28.4)	0.32
RR >30 bpm	5 (15.6)	2 (2)	0.01
Admission image and laboratory findings			
Infiltrates on chest X-rays	92 (91.1)	282 (81.3)	0.02
Neutrophil count >7500/μL	22 (22.2)	63 (18.9)	0.48
Lymphocyte count <1000/μL	68 (67.3)	157 (45.4)	<0.001
Platelets <130 000/μL	20 (19.6)	42 (12.1)	0.06
Creatinine >1.3 mg/dL	36 (36.4)	69 (20.1)	<0.001
C-reactive protein >100 mg/L	50 (51.5)	102 (30.2)	<0.001
Ferritin >1000 ng/mL	26 (29.2)	62 (19.6)	0.05
D-dimer >600 ng/mL	51 (64.6)	174 (57.6)	0.27
LDH >300 IU/L	61 (67)	128 (40.6)	<0.001
IFN-α undetectable	17 (16.3)	42 (12.0)	0.24
IFN-α (pg/mL) [c]	15.73 (0.75-45.42)	15.52 (5.06-25.48)	0.15
IFN-γ undetectable	28 (26.9)	71 (20.2)	0.15
IFN-γ (pg/mL) [c]	140.34 (0.00-222.85)	103.29 (11.00-360.47)	0.94
Admission scores			
CCI ≥3	66 (64.1)	193 (54.8)	0.10
CURB-65 ≥2	34 (34)	74 (21.6)	0.01
qSOFA ≥2	5 (5.2)	8 (2.3)	0.17
WHO basal score 6–9 [d]	17 (16.5)	14 (4)	<0.001
Outcome			
IMV	25 (24.0)	10 (2.8)	<0.001
Mortality at day 30	32 (30.8)	13 (3.7)	<0.001
WHO final score 7-10 [d]	44 (42.3)	31 (8.8)	<0.001

Data are presented as No. (%). *P* values are calculated by χ^2 or Fisher's test, as appropriate.

Abbreviations (in order of appearance): SOT, solid organ transplant; DBP, diastolic blood pressure; SpO_2, peripheral capillary oxygen saturation; HR, heart rate; RR, respiratory rate; LDH, lactate dehydrogenase; IFN, interferon; CCI, Charlson Comorbidity Index; CURB-65, Severity Score for Community-Acquired Pneumonia; qSOFA, quick Sequential Organ Failure Assessment score; IMV, invasive mechanical ventilation.

[a] Kidney transplant patients are excluded from this category.

[b] Liver transplant patients are excluded from this category.

[c] Median (IQR). P values are calculated by the Mann-Whitney U test.

[d] Severity rating according to the WHO Clinical Progression Scale (doi: 10.1016/S1473-3099(20)30483-7), ranged from 0 (not infected) to 10 (dead), of which scores 6–9 represent severe disease.

Supplementary Table 5. Analytical data of patients with COVID-19 according to the presence of RNAemia.				
Variable	**Total cohort (n = 455)**	**RNAemia (n = 104)**	**No RNAemia (n = 351)**	***P* value**
Neutrophil count, $x10^3/\mu L$	5.43 (3.32)	5.44 (3.25)	5.43 (3.35)	0.98
Lymphocyte count, $x10^3/\mu L$	1.17 (0.73)	0.98 (0.65)	1.22 (0.74)	0.004
Platelets, $x10^3/\mu L$	216.9 (99.8)	178.1 (64.9)	228.4 (105.3)	<0.001
Creatinine, mg/dL	1.21 (1.02)	1.41 (1.15)	1.15 (0.97)	0.03
C-reactive protein, mg/L	92.3 (92.2)	116.0 (83.6)	85.5 (93.6)	0.004
Ferritin, ng/mL	705.7 (775.9)	914.0 (857.4)	646.8 (742.2)	0.004
D-dimer, ng/mL	610.0 (1390.1)	892.0 (907.9)	1110.0 (1489.3)	0.22
LDH, IU/L	318.5 (130.1)	366.8 (145.8)	304.4 (121.8)	<0.001

Data are presented as Mean (SD) or Median (IQR). *P* values are calculated by Student t-test or Mann-Whitney test, as appropriate. LDH: lactate dehydrogenase; IFN: interferon.

Supplementary Table 6. Multivariate logistic regression analysis of risk factors associated with unfavourable outcome [a] in SOT recipients (n = 47)

Variable	B coefficient	Standard error	Wald	Degree of freedom	Adjusted analysis [b]	
					OR (95% CI)	*P* value
IFN alpha undetectable	-0.621	1.503	0.171	1	0.537 (0.028–10.232)	0.680
IFN gamma undetectable	1.678	0.985	2.901	1	5.353 (0.777–36.899)	0.089
Days from symptoms onset ≤10	2.197	1.284	2.927	1	8.995 (0.726–111.412)	0.087
Presence of RNAemia	1.816	0.858	4.479	1	6.147 (1.144–33.046)	0.034
CCI ≥3	0.545	1.058	0.265	1	1.724 (0.217–13.720)	0.607
CURB-65 ≥2	0.846	0.897	0.891	1	2.331 (0.402–13.524)	0.345
Constant	-4.791	1.844	6.749	1	0.008	0.009

Abbreviations (in order of appearance): SOT, solid organ transplant; OR, odds ratio; IFN, interferon; CCI, Charlson Comorbidity Index; CURB-65, Severity Score for Community-Acquired Pneumonia.

[a] Invasive mechanical ventilation and/or death (Final WHO score 7-10).

[b] The area under the receiver operating characteristic (AUROC) curve of the model was 0.84 (95% CI, 0.72–0.97), p<0.001, and no interactions were identified.

Supplementary Table 7. Multivariate analyses of risk factors associated with 30-day all-cause mortality using Cox regression in the no SOT patients (n = 408)

Variable	Adjusted analysis [a]		Adjusted by PS [b]	
	HR (95% CI)	***P* value**	**HR (95% CI)**	***P* value**
IFN alpha undetectable	1.773 (0.662–4.750)	0.255	0.981 (0.176–5.478)	0.983
IFN gamma undetectable	1.073 (0.441–2.607)	0.877	3.860 (1.046–14.238)	0.043
Days from symptoms onset ≤3	2.048 (0.931–4.506)	0.075	3.805 (0.906–15.983)	0.068
Presence of RNAemia	5.331 (2.381–11.937)	<0.001	8.457 (2.009–35.598)	0.004
CCI ≥3	3.378 (0.880–12.968)	0.076	2.715 (0.419–17.595)	0.295
CURB-65 ≥2	3.352 (1.320–8.515)	0.011	2.703 (0.676–10.803)	0.160
Propensity score [b]	…	…	0.137 (0.001–16.421)	0.415

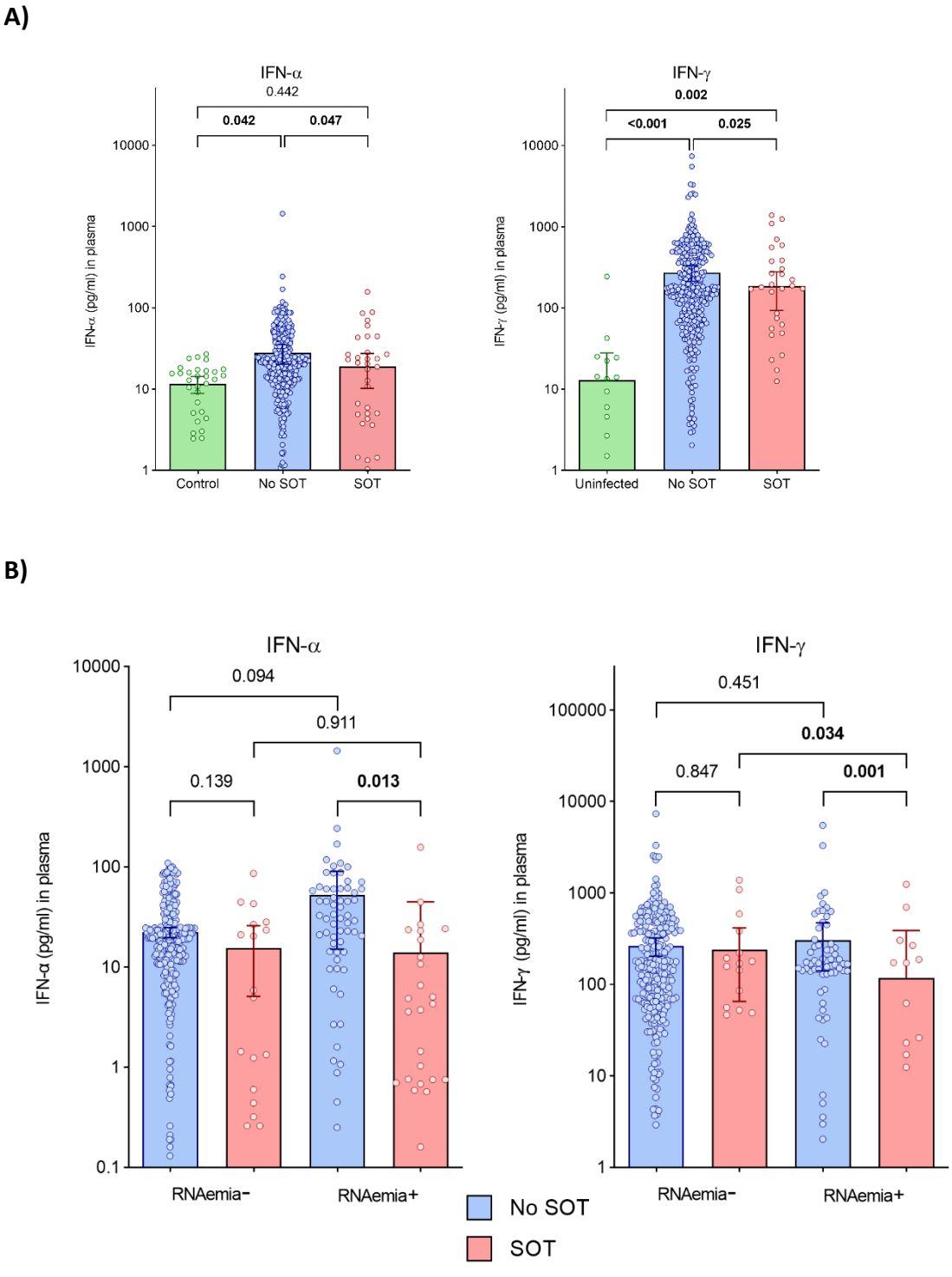
Abbreviations (in order of appearance): SOT, solid organ transplant; PS, propensity score; HR; hazard ratio; CI, confidence interval; IFN, interferon; CCI, Charlson Comorbidity Index; CURB-65, Severity Score for Community-Acquired Pneumonia.

[a] The area under the receiver operating characteristic (AUROC) curve of the model was 0.84 (95% CI, 0.80–0.91), p<0.001, and no interactions were identified.

[b] The variables included in the propensity score were sex, dyspnoea, peripheral capillary oxygen saturation, neutrophil and lymphocyte count, C-reactive protein, ferritin, D-dimer, and lactate dehydrogenase. The AUROC curve of the PS model was 0.84 (95% CI, 0.75–0.93), p<0.001.

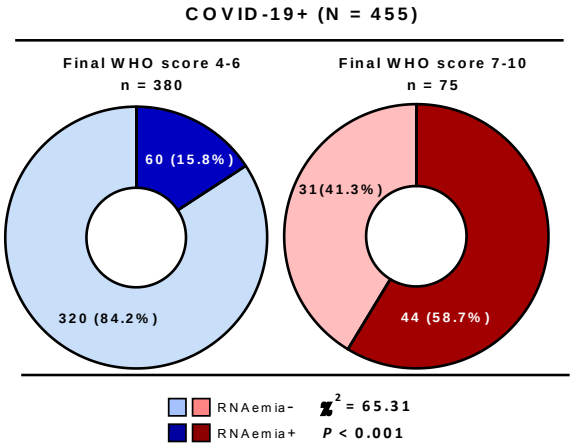
Supplementary Figures

Supplementary Figure 1. IFN-α and IFN-γ plasma levels (means ± SEM) in (A) healthy uninfected controls *vs.* no SOT and SOT recipients and (B) patients without *vs.* with RNAemia in no SOT and SOT recipients.

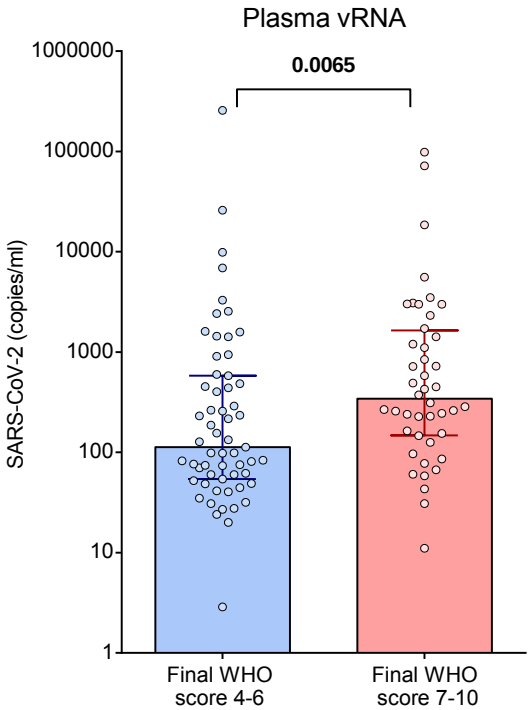


Supplementary Figure 2. SARS-CoV-2 RNAemia rates (A) and plasma viral load (median, IQR) (B) regarding the Final WHO Clinical Progression Scale (33) in all cohort (n=455).

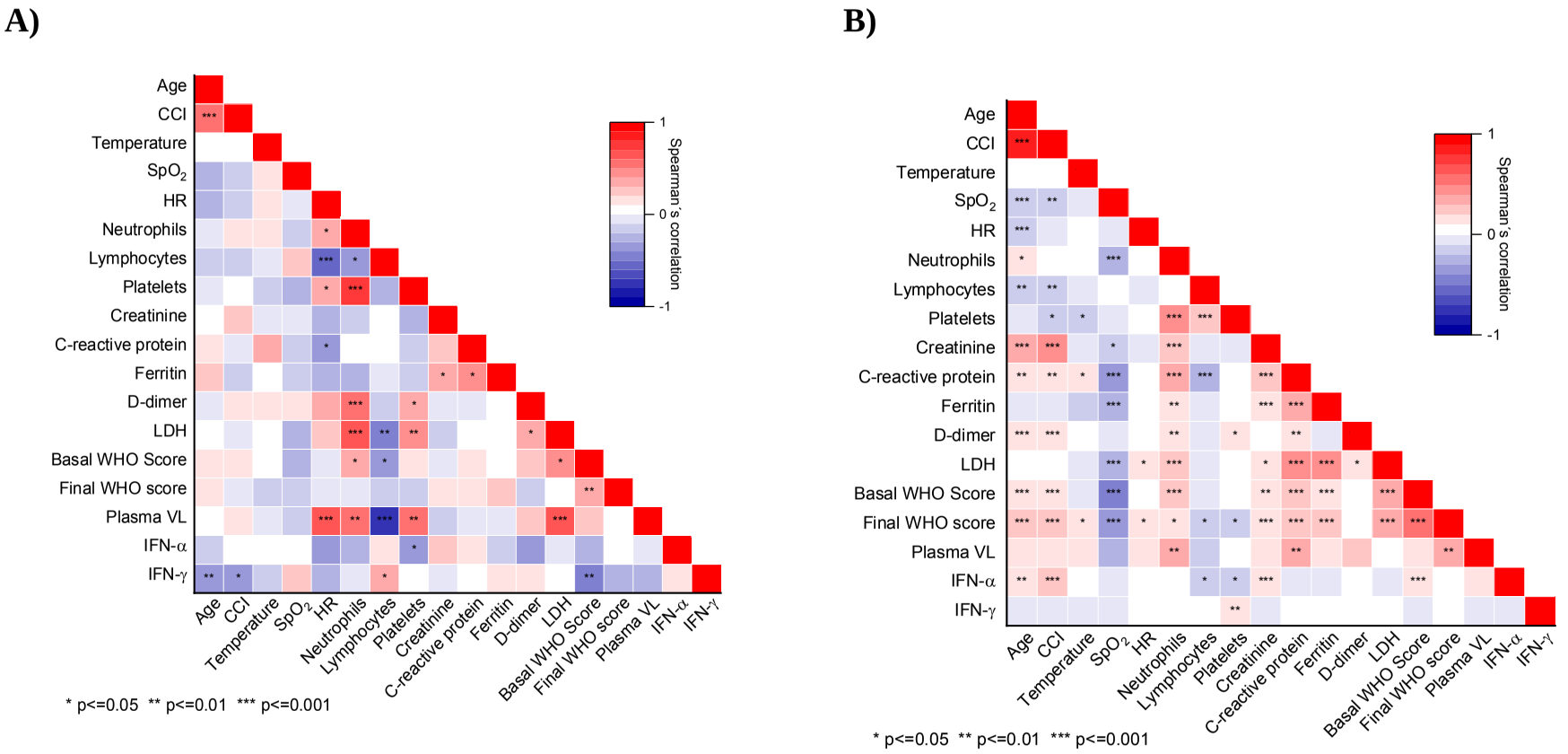
A)



B)



Supplementary Figure 3. Pairwise correlation heatmaps between IFN-α and IFN-γ plasma levels and SARS-CoV-2 RNAemia at hospital admission and baseline factors (Spearman's rank correlation coefficient) in (A) SOT recipients (n=47) and (B) no SOT patients (n=408). CCI, Charlson Comorbidity Index; SpO_2, capillary oxygen saturation; HR: heart rate; LDH, lactate dehydrogenase; Plasma VL: RNAemia (copies/mL); IFN: interferon.



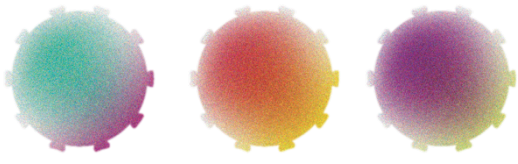
3. MEMBERS OF THE COVIDSOT WORKING TEAM

***Hospital Universitario Cruces*:**
Elena Bereciartua
Mikel Del Álamo Martínez
Gorane Euba
Mikel Gallego
Laura Guio
Marta Ibarrola
Leire López
Oscar Martínez-Expósito

***Virgen del Rocío University Hospital*:**
José María Álamo-Martínez
María Balsera-Manzanero
Carmen Bernal-Bellido
Carmen Cepeda-Franco
Nuria Espinosa
Manuel García-Gutiérrez
María Victoria Gil-Navarro
Luis Giménez
María José Gómez-Gómez
Ana Belén Guisado-Gil
Albin Hermansson
Silvia Jiménez-Jorge
José Manuel Lomas
Laura Merino-Díaz
José Molina
María Dolores Navarro
María Paniagua
Carmen Pérez-López
Manuel Poyato-Borrego
Esther Quintana
Marta Rey-Rodríguez
César Sotomayor
Alejandro Suárez
Marta Sunyer

***Virgen Macarena University Hospital*:**
Marta Fernández-Regaña
Ana Belén Martín-Gutiérrez
Ana Silva-Campos
Adoración Valiente

***San Pedro-CIBIR University Hospital*:**
María Iñiguez
Sonia Omatos
Patricia Pérez-Matute
Aránzazu Portillo
Pablo Villoslada



ARTICLE 3

scientific reports

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SARS-CoV-2 viral load in nasopharyngeal swabs is not an independent predictor of unfavorable outcome

Sonsoles Salto-Alejandre[1,2], Judith Berastegui-Cabrera[1,2], Pedro Camacho-Martínez[1,2], Carmen Infante-Domínguez[1,2], Marta Carretero-Ledesma[1,2], Juan Carlos Crespo-Rivas[1,2], Eduardo Márquez[3], José Manuel Lomas[1,2], Claudio Bueno[4], Rosario Amaya[5], José Antonio Lepe[1,2], José Miguel Cisneros[1,2], Jerónimo Pachón[2,6]✉, Elisa Cordero[1,2,6], Javier Sánchez-Céspedes[1,2] & The Virgen del Rocío Hospital COVID-19 Working Team*

The aim was to assess the ability of nasopharyngeal SARS-CoV-2 viral load at first patient's hospital evaluation to predict unfavorable outcomes. We conducted a prospective cohort study including 321 adult patients with confirmed COVID-19 through RT-PCR in nasopharyngeal swabs. Quantitative Synthetic SARS-CoV-2 RNA cycle threshold values were used to calculate the viral load in log_{10} copies/mL. Disease severity at the end of follow up was categorized into mild, moderate, and severe. Primary endpoint was a composite of intensive care unit (ICU) admission and/or death (n = 85, 26.4%). Univariable and multivariable logistic regression analyses were performed. Nasopharyngeal SARS-CoV-2 viral load over the second quartile (≥ 7.35 log_{10} copies/mL, $p = 0.003$) and second tertile (≥ 8.27 log_{10} copies/mL, $p = 0.01$) were associated to unfavorable outcome in the unadjusted logistic regression analysis. However, in the final multivariable analysis, viral load was not independently associated with an unfavorable outcome. Five predictors were independently associated with increased odds of ICU admission and/or death: age ≥ 70 years, SpO_2, neutrophils > 7.5×10^3/μL, lactate dehydrogenase ≥ 300 U/L, and C-reactive protein ≥ 100 mg/L. In summary, nasopharyngeal SARS-CoV-2 viral load on admission is generally high in patients with COVID-19, regardless of illness severity, but it cannot be used as an independent predictor of unfavorable clinical outcome.

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causative agent of coronavirus disease 2019 (COVID-19), has spread worldwide, becoming a pandemic of historic dimensions[1]. The clinical spectrum of COVID-19 ranges from asymptomatic disease to pneumonia, life-threatening complications, and, ultimately, death[2,3]. Despite most infected individuals develop solely a mild illness, the mortality rate for severe cases is as high as that caused by other etiologies of severe community-acquired pneumonia[4].

For coping with the best clinical attention to COVID-19 patients it is crucial to perform prognosis estimations at the first clinical evaluation, offering personalized attention based on early and easily detectable predictors that support decision making, guide level of care, and optimize the allocation of health resources. Different studies have already addressed this issue, identifying clinical signs and several biomarkers as predictors of unfavorable outcome[5–7].

In this regard, different studies have addressed the possible association between the viral load in nasopharyngeal (NP) swabs and the clinical outcomes. Some studies have reported that a high number of virus copies in NP swabs, mainly defined as a cycle threshold (Ct) < 25 or < 22 in the real-time polymerase chain reaction (RT-PCR), was an independent risk factor for intubation and/or death[8–11]. However, other studies have not found independent association between low Ct values and critical care admission or death[12,13]. In short, the real impact

[1]Unit of Infectious Diseases, Microbiology, and Preventive Medicine, Virgen del Rocío University Hospital, Seville, Spain. [2]Institute of Biomedicine of Seville (IBiS), Virgen del Rocío University Hospital/CSIC/University of Seville, Seville, Spain. [3]Medico-Surgical Unit of Respiratory Diseases, Virgen del Rocío University Hospital, Seville, Spain. [4]Unit of Emergencies, Virgen del Rocío University Hospital, Seville, Spain. [5]Intensive Care Unit, Virgen del Rocío University Hospital, Seville, Spain. [6]Department of Medicine, University of Seville, Seville, Spain. *A list of authors and their affiliations appears at the end of the paper ✉email: pachon@us.es

	Total cohort (n = 321)	Mild disease (n = 56)	Moderate disease (n = 180)	Severe disease (n = 85)	*p* value[c]
Age in years, median (IQR)	63 (52–77)	48 (40–60)	62 (52–75)	75 (63–84)	< 0.001
Age ≥ 70 (%)	118 (36.8)	8 (14.3)	58 (32.2)	52 (61.2)	< 0.001
Male sex (%)	169 (52.6)	29 (51.8)	90 (50.0)	50 (58.8)	0.40
Chronic underlying diseases (%)					
Arterial hypertension	150 (46.7)	16 (28.6)	80 (44.4)	54 (63.5)	< 0.001
Diabetes mellitus	57 (17.8)	6 (10.7)	35 (19.4)	16 (18.8)	0.31
Chronic lung disease[a]	38 (11.8)	3 (5.4)	26 (14.4)	9 (10.6)	0.17
Cardiovascular disease	64 (19.9)	5 (8.9)	32 (17.8)	27 (31.8)	0.002
Chronic kidney disease	22 (6.9)	2 (3.6)	13 (7.2)	7 (8.2)	0.54
Chronic liver disease	10 (3.1)	1 (1.8)	6 (3.3)	3 (3.5)	0.82
Cancer[b]	25 (7.8)	5 (8.9)	9 (5.0)	11 (12.9)	0.08
Symptoms (%)					
Fever	237 (73.8)	38 (67.9)	142 (78.9)	57 (67.1)	0.07
Rhinorrhea	19 (5.9)	6 (10.7)	10 (5.6)	3 (3.5)	0.20
Odynophagia	22 (6.9)	6 (10.7)	11 (6.1)	5 (5.9)	0.45
Myalgias	70 (21.8)	13 (23.2)	37 (20.6)	20 (23.5)	0.83
Headache	57 (17.8)	11 (19.6)	30 (16.7)	16 (18.8)	0.84
Cough	216 (67.3)	40(71.4)	128 (71.1)	48 (56.5)	0.04
Expectoration	33 (10.3)	7 (12.5)	16 (8.9)	10 (11.8)	0.64
Pleuritic chest pain	14 (4.4)	3 (5.4)	9 (5.0)	2 (2.4)	0.57
Dyspnea	147 (45.8)	19 (33.9)	75 (41.7)	53 (62.4)	0.001
Diarrhea	52 (16.2)	12 (21.4)	33 (18.3)	7 (8.2)	0.06
Vomiting	20 (6.2)	1 (1.8)	16 (8.9)	3 (3.5)	0.08
Impaired consciousness	12 (3.7)	1 (1.8)	6 (3.3)	5 (5.9)	0.42
Days from symptom onset to diagnosis, median (IQR)	7 (3–10)	7 (5–12)	6 (3–10)	6 (2–10)	0.35
Infiltrate on chest X-ray (%)	224 (69.8)	9 (16.1)	140 (77.8)	75 (88.2)	< 0.001
Signs (categorized, %)					
Temperature > 37.5 °C	82 (26.9)	5 (11.1)	48 (27.0)	29 (35.4)	0.01
SBP < 90 mmHg	7 (2.5)	1 (2.4)	4 (2.4)	2 (2.8)	0.99
DBP < 60 mmHg	24 (8.6)	1 (2.4)	9 (5.5)	14 (19.4)	0.001
Hart rate > 100 bpm	70 (21.8)	5 (8.9)	41 (22.8)	24 (28.2)	0.02
Respiratory rate > 20 bpm	17 (20.0)	0 (0)	5 (10.6)	12 (35.3)	0.01
SpO_2 < 95%	127 (39.6)	4 (7.1)	52 (28.9)	71 (83.5)	< 0.001

Table 1. Demographics, comorbidities, and clinical data of 321 patients with COVID-19 stratified according to disease severity. [a]Chronic obstructive pulmonary disease, obstructive sleep apnea, or asthma. [b]Active solid or hematologic malignant neoplasms. [c]Across all three groups.

of initial SARS-CoV-2 viral load in NP swabs on COVID-19 patients' outcomes is not been fully elucidated, and this issue remains controversial[14].

In the present prospective study on adult COVID-19 patients, stratified into mild disease (attended as outpatients) and hospital admitted with moderate or severe disease, we analyzed if the viral load of SARS-CoV-2 in NP swabs was associated with the disease severity, and the ability of NP SARS-CoV-2 viral load at the first hospital evaluation to predict unfavorable outcomes.

Results

Demographics, clinical characteristics, and outcome. The cohort included 321 adult patients, with the first evaluation at the Emergency room. Fifty-six (17.4%) patients had a mild disease and were discharged after the first evaluation, and subsequently attended as outpatients until the end of follow-up; 180 (56.1%) had a moderate course, being hospitalized in general wards, and with full recovery and hospital discharged; and 85 (26.5%) patients were categorized as severe COVID-19 because of required admission to the ICU (32 patients [10.0%]), in-hospital death (40 [12.5%]), or both (13 [4.0%]).

Demographics, symptoms, and signs of the total cohort and the three categories of disease severity are shown in Table 1. In the total cohort, males accounted for 169 (52.6%), median age was 63 (IQR 52–77) years, and 36.8% were ≥ 70 years old. The most common symptoms were fever (73.8%), cough (67.3%), and dyspnea (45.8%). Two

	Total cohort (n = 321)	Mild disease (n = 56)	Moderate disease (n = 180)	Severe disease (n = 85)	*p* value[b]
Blood counts, median (IQR)					
WBC × 10^3/μL	6.5 (4.7–9.0)	5.0 (4.0–7.0)	6.5 (4.8–8.7)	8.1 (5.3–11.7)	< 0.001
Neutrophils × 10^3/μL	4.7 (3.2–7.1)	3.4 (2.4–4.7)	4.6 (3.2–6.7)	6.9 (4.0–9.9)	0.64
Lymphocytes × 10^3/μL	1.1 (.8–1.6)	1.3 (1.1–1.6)	1.1 (.8–1.6)	.9 (.6–1.4)	< 0.001
Platelets × 10^3/μL	198 (163–257)	202 (164–243)	197 (165–253)	200 (161–268)	0.70
Blood counts (categorized, %)					
WBC > 11 × 10^3/μL	42 (13.1)	1 (1.8)	16 (8.9)	25 (29.4)	< 0.001
Neutrophils > 7.5 × 10^3/μL	65 (20.2)	0 (0)	31 (17.2)	34 (40.0)	< 0.001
Lymphocytes < 1 × 10^3/μL	125 (38.9)	7 (12.5)	68 (37.8)	50 (58.8)	< 0.001
Platelets < 130 × 10^3/μL	26 (8.1)	5 (8.9)	11 (6.1)	10 (11.8)	0.28
Biochemistry and inflammatory biomarkers, median (IQR)					
Creatinine mg/dL	.9 (.7–1.2)	.8 (.7–1.0)	.9 (.7–1.1)	1.1 (.8–1.6)	0.42
AST U/L	29 (22–49)	24 (18–32)	27 (21–46)	39 (28–64)	0.01
LDH U/L	309 (231–415)	222 (185–280)	293 (229–376)	400 (319–502)	< 0.001
CRP mg/L[a]	57.0 (20.8–136.6)	16.0 (5.8–33.9)	53.0 (20.0–113.8)	142.5 (67.2–252.0)	< 0.001
D-dimer ng/mL[a]	770 (463–1608)	515 (345–755)	730 (428–1578)	1145 (708–2453)	0.07
Biochemistry and inflammatory biomarkers (categorized, %)					
Creatinine > 1.3 mg/dL	60 (18.7)	4 (7.1)	28 (15.6)	28 (32.9)	< 0.001
AST > 30 U/L	124 (38.6)	11 (19.6)	62 (34.4)	51 (60.0)	< 0.001
LDH ≥ 300 U/L	145 (45.2)	4 (7.1)	79 (43.9)	62 (72.9)	< 0.001
CRP ≥ 100 mg/L[a]	102 (31.8)	1 (1.8)	52 (28.9)	49 (57.6)	< 0.001
D-dimer ≥ 600 ng/mL[a]	171 (53.3)	13 (23.2)	97 (53.9)	61 (71.8)	< 0.001
Nasopharyngeal viral load (log_{10} copies/mL, median [IQR])					
Viral load (VL)	7.35 (5.85–8.80)	6.44 (4.70–8.32)	7.10 (5.92–8.66)	8.18 (6.31–8.90)	0.88
VL ≥ 6.33 (1st tertile, %)	215 (67.0)	29 (51.8)	122 (67.8)	64 (75.3)	0.01
VL ≥ 7.35 (50th percentile, %)	163 (50.8)	24 (42.9)	84 (46.7)	55 (64.7)	0.01
VL ≥ 8.27 (2nd tertile, %)	107 (33.3)	16 (28.6)	52 (28.9)	39 (45.9)	0.02

Table 2. Laboratory values and nasopharyngeal SARS-CoV-2 viral load of 321 patients with COVID-19 stratified according to disease severity. [a]Values were available in 293 and 276 patients for CRP and D-dimer, respectively. [b]Across all three groups.

hundred twenty-four (69.8%) patients had chest X-ray infiltrates at first hospital evaluation: 16.1% within the mild group, 77.8% in the moderate one, and 88.2% in the severe group ($p < 0.001$). During the follow-up, 100% of the patients in the moderate and severe groups showed pulmonary infiltrates in the evolutive chest X-ray after hospital admission. Between-group differences regarding baseline laboratory values were also identified and are detailed in Table 2.

Seventy-eight (24.3%) patients required respiratory support with high flow therapy or non-invasive mechanical ventilation, which was more frequent in patients with severe than with moderate disease (55 [64.7%] vs. 23 [12.8%], respectively, being $p = 0.001$). Twenty-eight (32.9%) patients, all admitted to ICU, required invasive mechanical ventilation.

Median NP viral load at first hospital evaluation was not different among the mild, moderate, or severe groups according to their clinical outcomes (Table 2). However, we found higher frequencies of NP viral load above the first tertile, the 50th percentile, and the second tertile in the severe group when comparing the three groups ($p = 0.01$).

We also analyzed the possible differences among demographics, chronic underlying diseases, and the days from symptoms onset to diagnosis according to the SARS-CoV-2 viral load (Table 3). Although the median days from symptoms onset to diagnosis was lower in the group with higher SARS-CoV-2 viral load (2nd vs. 1st tertile) this difference was not significant. Additionally, we performed a linear regression analysis which did not show association between both variables ($p = 0.389$). The only significant differences ($p < 0.05$) were found for the frequency of cardiovascular diseases and age ≥ 70 years. Finally, we made a linear regression analysis between the SARS-CoV-2 viral load and age, finding a significant correlation between both variables ($p < 0.001$) though not clinically relevant (adjusted R^2 0.036).

Predictors of unfavorable outcome. Twenty-three categorical variables at first hospital evaluation were identified as baseline risk factors for unfavorable outcome (admission to ICU or death) in the unadjusted logistic regression analysis: advanced age, arterial hypertension, cardiovascular disease, cancer, dyspnea, higher temperature and respiratory rate, lower diastolic blood pressure and capillary oxygen saturation, leukocytes > 11 × 10^3/

	VL ≤ 6.33 (1st tertile) (n = 107)	VL 6.34–8.26 (1st to 2nd tertile) (n = 107)	VL ≥ 8.27 (2nd tertile) (n = 107)	*p* value
Age ≥ 70 years	27 (25.2)	40 (37.4)	51 (47.7)	0.003
Male sex	57 (53.3)	64 (59.8)	48 (44.9)	0.09
Arterial hypertension	40 (37.4)	57 (53.3)	53 (49.5)	0.05
Diabetes mellitus	22 (20.6)	14 (13.1)	21 (19.6)	0.30
Chronic lung disease[a]	12 (11.2)	11 (10.3)	15 (14.0)	0.68
Cardiovascular disease	19 (17.8)	15 (14.0)	30 (28.0)	0.03
Chronic kidney disease	5 (4.7)	7 (6.5)	10 (9.3)	0.40
Chronic liver disease	4 (3.7)	4 (3.7)	2 (1.9)	0.66
Cancer[b]	9 (8.4)	8 (7.5)	8 (7.5)	0.96
Days from symptom onset to diagnosis, median (IQR)	7 (5–12)	7 (4–10)	4 (1–7)	0.27[c]

Table 3. Demographics, comorbidities, and days from symptoms onset to diagnosis of 321 patients with COVID-19 stratified according to nasopharyngeal viral load (VL, log_{10} copies/mL). Data are presented as n (%) unless otherwise indicated. [a]Chronic obstructive pulmonary disease, obstructive sleep apnea, or asthma. [b]Active solid or hematologic malignant neoplasms. [c]Viral load ≤ 6.33 (1st tertile) vs. viral load ≥ 8.27 (2nd tertile).

	Crude odds ratio (95% CI)	*p* value
Age ≥ 70 years	4.06 (2.41–6.83)	< 0.001
Arterial hypertension	2.54 (1.52–4.24)	< 0.001
Cardiovascular disease	2.50 (1.41–4.45)	0.002
Cancer	2.36 (1.03–5.42)	0.043
Cough	.53 (.31-.88)	0.01
Dyspnea	2.50 (1.50–4.17)	< 0.001
Diarrhea	.38 (.17-.88)	0.02
Infiltrate on chest X-ray	4.38 (2.15–8.92)	< 0.001
Temperature > 37.5 °C	1.76 (1.02–3.04)	0.04
DBP < 60 mmHg	4.73 (2.00–11.21)	< 0.001
Respiratory rate > 20 bpm	5.02 (1.57–16.01)	0.006
SpO_2 < 95%	16.30 (8.54–31.13)	< 0.001
WBC > 11×10^3/μL	5.37 (2.72–10.59)	< 0.001
Neutrophils > 7.5×10^3/μL	4.41 (2.48–7.84)	< 0.001
Lymphocytes < 1×10^3/μL	3.07 (1.84–5.12)	< 0.001
Creatinine > 1.3 mg/dL	3.13 (1.74–5.63)	< 0.001
AST > 30 U/L	3.35 (2.00–5.60)	< 0.001
LDH ≥ 300 U/L	4.97 (2.87–8.60)	< 0.001
CRP ≥ 100 mg/L	4.70 (2.77–7.97)	< 0.001
D-dimer ≥ 600 ng/mL	2.91 (1.70–4.98)	< 0.001
Viral load ≥ 7.35 log_{10} copies/mL (50th percentile)	2.17 (1.30–3.63)	0.003
Viral load ≥ 8.27 log_{10} copies/mL (2nd tertile)	2.10 (1.26–3.49)	0.01

Table 4. Baseline risk factors for unfavorable outcome (intensive care unit admission and/or death): Univariable logistic regression analysis.

μL, neutrophils > 7.5×10^3/μL, lymphocytes < 1×10^3/μL, and higher levels of creatinine, aspartate aminotransferase, lactate dehydrogenase (LDH), C-reactive protein (CRP), and D-dimer, among others (Table 4). Regarding the NP viral load, values over the second quartile and second tertile were also associated with unfavorable outcome in the unadjusted logistic regression analysis (Table 4).

In the final multivariable analysis, despite the previous link between a higher viral load and the occurrence of unfavorable outcome, the number of virus copies in the NP swabs was not independently associated with an unfavorable clinical result (Table 5). Five of the previous predictors were independently associated with increased odds of ICU admission and/or death: age ≥ 70 years (odds ratio [OR] 3.58, $p < 0.001$), SpO_2 < 95% (OR 11.07, $p < 0.001$), neutrophils > 7.5×10^3/μL (OR 3.67, $p = 0.001$), LDH ≥ 300 U/L (OR 2.11, $p = 0.04$), and CRP ≥ 100 mg/L (OR 2.61, $p = 0.01$). Information on the overall apparent performance of the model is presented in Table 5 and Fig. 1.

	Adjusted odds ratio (95% CI)	*p* value
Age ≥ 70 years	3.58 (1.83–6.99)	< 0.001
SpO_2 < 95%	11.07 (5.34–22.97)	< 0.001
Neutrophils > 7.5×10^3/μL	3.67 (1.74–7.74)	0.001
LDH ≥ 300 U/L	2.11 (1.04–4.31)	0.04
CRP ≥ 100 mg/L	2.61 (1.32–5.14)	0.01
Viral load ≥ 7.35 $\log_{10}$ copies/mL (50th percentile)	1.49 (.75–2.96)	0.25
Viral load ≥ 8.27 $\log_{10}$ copies/mL (2nd tertile)	1.84 (.92–3.68)	0.09

Table 5. Independent predictors of unfavorable outcome (ICU admission and/or death): Multivariable logistic regression model. The final multivariable model was composed of five variables (therefore 17 events per variable) demonstrated as independent predictors of unfavorable outcome: Age ≥ 70 years, SpO_2 < 95%, neutrophils > 7.5×10^3/μL, LDH ≥ 300 U/L, and CRP ≥ 100 mg/L). Such model reported a Beta Coefficient of -4.08 (standard error = 0.46), a Wald statistic of 78.72 (degrees of freedom = 1), and an overall apparent performance of 84.2% (sensitivity = 70.6%, specificity = 89.4%, PPV = 70.3%, NPV = 89.1%). The variables included were explanatory and contributed to giving the model an ability to explain roughly 52.1% of the variation of the outcome (Nagelkerke R^2 value = 0.521). A higher nasopharyngeal viral load (above the second quartile or the second tertile) was not independently linked to an increased risk of ICU admission or death.

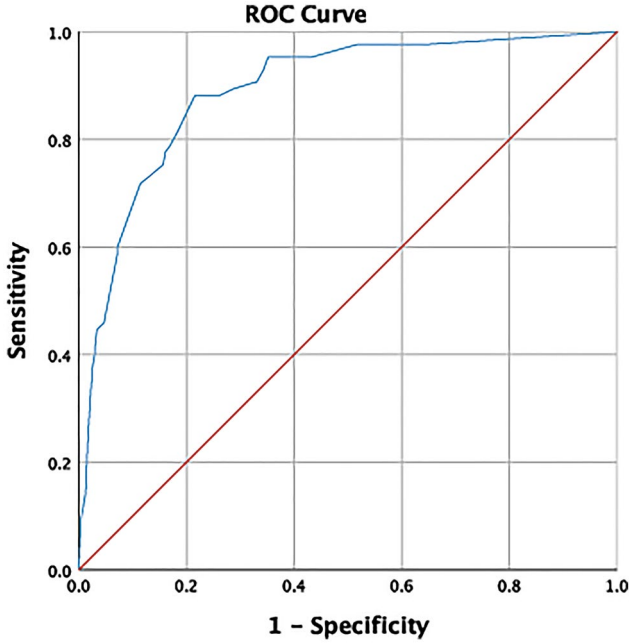

Figure 1. Discrimination power of the final multivariable model: ROC Curve plot. Discrimination power of the model (including Age ≥ 70 years, SpO_2 < 95%, neutrophils > 7.5×10^3/μL, LDH ≥ 300 U/L, and CRP ≥ 100 mg/L) expressed by an area under the ROC Curve of 0.89 (95% CI 0.85–0.93), standard error of 0.02 (under the non-parametric assumption), and $p < 0.001$ (being the null hypothesis a true area = 0.50).

Discussion

The main finding of the present study is that patients with SARS-CoV-2 infection, regardless of their illness severity, generally have a high rate of viral replication in the upper respiratory airways. Consequently, this parameter cannot be used as a predictor of COVID-19 unfavorable outcome, defined as admission to ICU and/or death. Moreover, this prospective cohort confirms that the independent risk factors for ICU admission or death are those previously identified by Salto-Alejandre S. *et al*[7]. Thus, at first hospital evaluation, advanced age, hypoxemia, neutrophilia, and increased levels of LDH and CRP have high sensitivity and specificity to accurately discriminate patients that would potentially develop a critical disease from those with a favorable course.

The evidence to date reveals that the relationship between SARS-CoV-2 viral load and the pathogenicity and virulence of this microorganism is not fully understood. Furthermore, as there are many methods to perform the molecular detection of SARS-CoV-2 genome, the interpretation and comparison of results in literature is highly controversial. As an example, droplet digital PCR (ddPCR) has shown slightly higher sensitivity than standard RT-PCR[15].

Several previous studies have demonstrated that a high value of SARS-CoV-2 viral load in the upper respiratory tract (URT), defined as a Ct < 25 or < 22 in the RT-PCR, is an independent risk factor for respiratory failure[8], intubation, or death[9], using multivariate logistic regression and time-based analyses[10]. Pujadas et al., using a Cox proportional hazards model, also showed an independent association between viral load in the URT and mortality[11].

Such results have often led to the thought that viral load could be used along with other features to decide upon the need for hospital admission, and even that a stratification for baseline NP viral load would benefit the design of clinical trials. Nevertheless, other studies show contradictory results. Maltezou et al., using a Ct < 25 to define high URT viral load, reported an association between higher viral load and the development of COVID-19 disease, while no association was found with ICU admission, mechanical ventilation or death[12]. Amodio et al. demonstrated that the median PCR Ct was significantly lower in patients who died or needed critical care than in those who were hospitalized and discharged alive, or exclusively attended at home, but after adjusting for age and sex, there was not and independent association with critical care need or death[13].

Similarly, in our study, despite the patients with higher viral load (above the first tertile, the 50th percentile, and the second tertile) often belonged to the severe disease group, the adjusted multivariable model did not find an association between the copies per mL and the need for critical care or mortality. Argyropoulos et al., on the other hand, showed that viral load was inversely correlated with disease severity, being higher in patients with mild COVID-19[16]. The reason for this conflictive result was, however, that NP sampling in patients with severe or critical symptoms was obtained at a later time point in the disease course. Lastly, Lee et al. found that viral load quantification was similar among symptomatic and asymptomatic patients[17], and our results support this conclusion. Certainly, most patients in the present cohort who suffered an unfavorable outcome had a high SARS-CoV-2 viral load quantification (above the 50th percentile) at hospital admission, but half of the patients with mild COVID-19 also exceeded said limit. This corroborates that the number of virus copies is not strongly related to COVID-19 prognosis.

To further test our hypothesis, we stratified the patients according to disease severity at the end of follow up, and having confirmed that the medium time from symptoms onset to diagnosis was similar among groups, we performed multiple comparations for each viral load cut-off value. Mild patients could only be distinguished through the first tertile, above which a small increment of moderate and severe cases was found. For higher viral load cut-off points, the probability of belonging to the mild or moderate group was similar. The percentage of patients having NP viral load over the 50th percentile and second tertile was significantly higher for those with severe COVID-19, and the univariable analysis showed that a viral load quantification over the mentioned levels could be a risk factor for ICU admission or death. However, through the multivariable model, we concluded that a high viral load could not be used as an independent predictor of such outcomes.

Our study highlights several substantial issues. First, there is not a clear viral load cut-off point capable of discriminating between the various levels of COVID-19 severity, as the ROC Curve analysis demonstrated. Secondly and contrary to expectations, a higher number of SARS-CoV-2 copies in NP swabs at first patient's evaluation is not predictive of whether ICU admission or death might occur. Nevertheless, according to the Spanish nationwide seroepidemiological study, this finding should not be surprising: a third of the population with positive PCR was asymptomatic, and 20% of the seropositive symptomatic participants did not have previous SARS-CoV-2 genome detection[18]. Finally, through the external cohort validation of hypoxemia, neutrophilia, and increased levels of LDH and CRP as independent predictors of unfavorable outcome[7], we contribute to the identification of higher-risk patients with COVID-19 in whom suitable and prompt management is vital.

The main strength of the present study is that a wide spectrum of COVID-19 severity, from mild symptomatic to critically ill patients, is represented in the analyzed cohort, allowing novel conclusions to be drawn about the efficacy and predictive reliability of previously studied clinical factors. The study has also some limitations. The viral load quantification in the URT samples, through NP swabs, was only performed at a single time point, and we have not data on the dynamics according to the clinical outcomes. Additional synchronous and longitudinal sampling from other sources, such as blood or stools, would have been important comparators. Regarding the quantification of SARS-CoV-2 viral load, further studies are required to refine the use of the standard and novel techniques, which is especially important due to variabilities in specimen collection, the lack of systematic quantification assays, and inconsistencies in protocols between different laboratories. Also, the lack of association of NP viral load with unfavorable outcome, should be confirmed when COVID-19 be caused by the new SARS-CoV-2 variants.

In summary, we found that higher values of SARS-CoV-2 viral load in NP samples at first hospital evaluation are more frequent in patients with unfavorable in-hospital outcome, but that a high viral load is not an independent risk factor for ICU admission or death among adult patients with COVID-19.

Methods

Design, patients, and data collection. We conducted a prospective observational cohort study in Virgen del Rocío University Hospital, a Spanish care-teaching center with 1177 beds (including 72 adult ICU beds). The study protocol was approved by the Ethics Committee of Virgen Macarena and Virgen del Rocío University Hospitals (C.I. 0771-N-20), and complied the Declaration of Helsinki. An informed consent was established as a mandatory requirement for all patients. Consecutive patients with confirmed COVID-19 by RT-PCR assay for SARS-CoV-2 in NP samples were enrolled, from February 29th to May 1st, 2020. Baseline was the date of first hospital evaluation. Follow-up censoring date was May 29th, 2020, for a minimum observation period in each patient of 28 days.

The clinical data source was the electronic medical record system. Variables registered included demographics, comorbidities, symptoms and signs at admission, baseline laboratory tests and chest X-ray findings, complications during hospitalization, and clinical outcome.

SARS-CoV-2 infection diagnosis and viral load. SARS-CoV-2 total RNA was extracted from NP swabs using EZ1 Virus Mini Kit v2.0 (Qiagen Inc., Valencia, CA, USA) following manufacturer's instruction. SARS-CoV-2 genomic RNA was amplified by LightCycler 96 Instrument (Roche, Germany) using CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel and the GoTaq® Probe 1-Step RT-qPCR System (Wisconsin, USA) following the CDC's instructions. The Quantitative Synthetic SARS-CoV-2 RNA: ORF, E, N kit (ATCC, VA, USA) for each NP sample was run and Ct values were interpolated into the curve obtained to calculate the viral load in $\log_{10}$ copies/mL. The lower and upper limits of quantification for our RT-PCR were 3.9 and 8.9 $\log_{10}$ copies/mL, respectively.

Statistical analysis. Primary endpoint was the occurrence of unfavorable outcome at the end of follow-up, defined as a composite of ICU admission and/or death. For analyzing the ability of NP SARS-CoV-2 viral load at first patient's evaluation to predict an unfavorable outcome, the severity of COVID-19 at the end of the 28 days follow-up was categorized into (1) mild, patients exclusively attended as outpatients after the first hospital evaluation; (2) moderate, hospitalized with full recovery and discharged; and (3) severe, hospitalized and admitted to the ICU or dead.

A descriptive analysis of all data obtained was performed. Categorical variables were presented as n (%) and continuous as median (interquartile range [IQR]). We used the χ^2-test, Fisher's Exact Test, One-Way Analysis of Variance (ANOVA), Kruskal Wallis Test, Student's *t*-test, or Welch's *t*-test to compare between-group differences, and linear regression analysis to assess association between variables, as appropriate.

To examine the factors associated with unfavorable outcome, a univariable logistic regression analysis was performed. Additionally, bivariate correlations were thoroughly explored to account for potential confusion and interaction effects. To increase the applicability of our results within the scope of clinical practice, continuous variables were dichotomized based on normal ranges and cut-off values previously identified as predictors of unfavorable outcome[7].

Regarding SARS-CoV-2 viral load, for which there is no prior clinical consensus for categorization, we tried to determine the optimal cut-off value through a ROC curve plot (figure not shown). However, all the points in the curve approached the diagonal segment, with an area underneath of 0.57 (close to the null value) that called into question the usefulness of this parameter. Finally, we decided to analyze viral load based on three prespecified cut-off points: the first tertile, the second quartile or 50th percentile, and the second tertile.

For identifying which of the predictors obtained from the univariable analysis were to be considered independent, a multivariable logistic regression model was built using three criteria to achieve the highest accuracy: relevance to clinical situation, statistical significance ($p < 0.10$), and adequate number of events to allow meaningful analysis. An automated backward stepwise selection was used for exclusion of variables, utilizing a probability threshold of 5%. The model was first assessed for sensitivity, specificity, positive and negative predictive values, and overall apparent performance. Secondly, the fraction of variance explained by said model was estimated through the Nagelkerke R^2 value. The internal validity was finally evaluated using the area under the ROC curve, where ≥ 0.70 (being the null hypothesis a true area of 0.50) is considered as evidence of good discrimination ability.

Ethics approval. The study protocol was approved by the Ethics Committee of Virgen Macarena and Virgen del Rocío University Hospitals (C.I. 0771-N-20) and complied the Declaration of Helsinki.

Data availability

All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).

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Author contributions

J.S.-C., E.C., J.M.C., and J.P. conceived and designed the study; obtained public funding from the Spanish Ministry of Economy, Industry, and Competitiveness; and took responsibility for the integrity of the data and the accuracy of its analysis. J.P., J.B.-C., and S.S.-A. did the scientific literature search. P.C.-M., C.I.-D., M.C.-L., J.C.C.-R., E.M., J.M.L., C.B., R.A., and J.A.L. provided the data. JB-C and SS-A processed the data, did the statistical analysis, and wrote the draft of the manuscript. All authors critically revised the manuscript for important intellectual content and gave final approval for the version published.

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Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to J.P.

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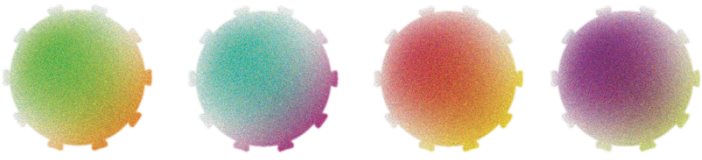
The Virgen del Rocío Hospital COVID-19 Working Team

José Miguel Cisneros[1,2], Manuela Aguilar-Guisado[1,2], Almudena Aguilera[7], Clara Aguilera[8], Teresa Aldabo-Pallas[5], Verónica Alfaro-Lara[9], Cristina Amodeo[4], Javier Ampuero[10], María Dolores Avilés[4], Maribel Asensio[3], Bosco Barón-Franco[9], Lydia Barrera-Pulido[1], Rafael Bellido-Alba[5], Máximo Bernabeu-Wittel[9], Candela Caballero-Eraso[3], Macarena Cabrera[11], Enrique Calderón[4], Jesús Carbajal-Guerrero[5], Manuela Cid-Cumplido[5], Yael Corcia-Palomo[5], Juan Delgado[9], Antonio Domínguez-Petit[4], Alejandro Deniz[12], Reginal Dusseck-Brutus[5], Ana Escoresca-Ortega[5], Fátima Espinosa[9], Nuria Espinosa[1,2], Michelle Espinoza[3], Carmen Ferrándiz-Millón[5], Marta Ferrer[3], Teresa Ferrer[10], Ignacio Gallego-Texeira[5], Rosa Gámez-Mancera[9], Emilio García[4], Horacio García-Delgado[5], Manuel García-Gutiérrez[1], María Luisa Gascón-Castillo[5], Aurora González-Estrada[9], Demetrio

González[3], Carmen Gómez-González[5], Rocío González-León[9], Carmen Grande-Cabrerizo[13], Sonia Gutiérrez[9], Carlos Hernández-Quiles[9], Inmaculada Concepción Herrera-Melero[5], Marta Herrero-Romero[1], Luis Jara[3], Carlos Jiménez-Juan[9], Silvia Jiménez-Jorge[1,2], Mercedes Jiménez-Sánchez[5], Julia Lanseros-Tenllado[9], Carmina López[9], Isabel López[4], Álvaro López-Barrios[4], Luis F. López-Cortés[1,2], Rafael Luque-Márquez[1,2], Daniel Macías-García[11], Guillermo Martín-Gutiérrez[1,2], Luis Martín-Villén[5], José Molina[1,2], Aurora Morillo[4], María Dolores Navarro-Amuedo[1,2], Dolores Nieto-Martín[9], Francisco Ortega[3], María Paniagua-García[1], Amelia Peña-Rodríguez[7], Esther Pérez[4], Manuel Poyato[1], Julia Praena-Segovia[1,2], Rafaela Ríos[4], Cristina Roca-Oporto[1,2], Jesús F. Rodríguez[4], María Jesús Rodríguez-Hernández[1], Santiago Rodríguez-Suárez[9], Ángel Rodríguez-Villodres[1,2], Nieves Romero-Rodríguez[7], Ricardo Ruiz[14], Zaida Ruiz de Azua[5], Celia Salamanca[1], Sonia Sánchez[4], Víctor Manuel Sánchez-Montagut[9], César Sotomayor[1,2], Alejandro Suárez Benjumea[13] & Javier Toral[4]

[7]Cardiology and Cardiovascular Surgery Unit, Virgen del Rocío University Hospital, Seville, Spain. [8]Rheumatology Unit, Virgen del Rocío University Hospital, Seville, Spain. [9]Internal Medicine Unit, Virgen del Rocío University Hospital, Seville, Spain. [10]Digestive and Hepatobiliary Unit, Virgen del Rocío University Hospital, Seville, Spain. [11]Neurology and Clinical Neurophysiology Unit, Virgen del Rocío University Hospital, Seville, Spain. [12]Endocrinology and Nutrition Unit, Virgen del Rocío University Hospital, Seville, Spain. [13]Urology and Nephrology Unit, Virgen del Rocío University Hospital, Seville, Spain. [14]Unit of Clinical Investigation and Clinical Trials, Virgen del Rocío University Hospital, Seville, Spain.



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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,l], Pablo Ryan [h,m,n], Marta de Miguel-Montero [o], Jesús Rodríguez-Baño [b,c,d,p,*,2], Jerónimo Pachón [a,b,p,*], 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 (IiSGM), 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
[o] Fundación SEIMC/GeSIDA, Madrid, Spain
[p] Department of Medicine, Universidad de Sevilla, Seville, Spain

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ABSTRACT

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, and the main exposure of interest was subcutaneous administration of interferon-β, defined as early if started ≤ 3 days from admission. Multivariate logistic and Cox regression analyses were conducted to identify the associations of different variables with receiving early interferon-β therapy and to assess its impact on 30-day 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, 0.82–1.30) 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.
E-mail addresses: jesusrb@us.es (J. Rodríguez-Baño), pachon@us.es (J. Pachón).
[1] Join first author.
[2] Join last author.

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Conclusions: In this multicenter cohort of admitted COVID-19 patients, receiving early interferon-β therapy after hospital admission did not show an association with lower mortality. Whether interferon-β might be useful in the earlier stages of the disease or specific subgroups of patients requires further research.

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, ivermectin, and remdesivir based on their empirically observed in vitro activity against coronaviruses. Most of these drugs are not used today because they did not demonstrate clinical efficacy in clinical trials, and there is currently no antiviral agent that can unequivocally reduce mortality. In this context, knowing the role of the inflammatory response in the development of severe complications, it is likely that developing a compound with both antiviral and immunomodulatory effects would be the most powerful approach to combat COVID-19.

Interferons (IFNs) are a group of cytokines that are crucial not only for antiviral immunity but also to dampen the innate response, preventing damage from pathogen-induced inflammation. 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 Universitario 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 $\leq$ 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 χ^2 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

Table 1
Features of Patients with COVID-19 According to Interferon Group.

Variable	EIT (n = 683)	LIT (n = 440)	NIT (n = 2685)	*P* Value (Early vs NIT)	*P* Value (Late vs NIT)
Male sex	451 (67)	297 (68.1)	1559 (58.8)	< .001	< .001
Age > 75 years	193 (28.3)	140 (31.9)	968 (36.1)	< .001	.09
Comorbidities					
Hypertension	337 (49.8)	240 (54.9)	1337 (50.1)	.90	.06
Diabetes	150 (22)	103 (23.7)	553 (20.8)	.49	.16
Obesity (BMI >30)	101 (16.3)	73 (18.3)	283 (11.9)	.003	< .001
Chronic heart disease	138 (20.3)	100 (23.3)	632 (23.8)	.05	.81
Chronic pulmonary disease (not asthma)	132 (19.4)	92 (21.3)	456 (17.1)	.16	.04
Asthma	52 (7.6)	33 (7.7)	197 (7.4)	.83	.85
Liver cirrhosis	5 (.7)	10 (2.3)	33 (1.2)	.17	.08
Chronic kidney disease stage 4 (eGFR <30 mL/min/1.73 m^2)	24 (3.5)	17 (3.9)	149 (5.6)	.03	.15
Chronic neurologic disorder	36 (5.3)	24 (5.6)	278 (10.4)	< .001	.002
Solid/hematologic neoplasm (active)	28 (4.1)	38 (8.8)	267 (10)	< .001	.42
Admission symptoms and signs					
Headache	65 (10)	47 (11.3)	292 (11.6)	.23	.85
Myalgia/arthralgia	178 (27.2)	119 (29)	611 (24.1)	.10	.03
Cough	547 (81)	320 (74.1)	1850 (69.7)	< .001	.07
Dyspnea	411 (60.8)	213 (49.1)	1191 (45)	< .001	.12
Vomiting/nausea	76 (11.4)	54 (12.7)	329 (12.6)	.43	.92
Diarrhea	92 (13.8)	62 (14.6)	290 (11.1)	.05	.04
Low SpO_2 (age-adjusted)[a]	261 (43.8)	101 (26.6)	498 (20.9)	< .001	.01
Heart rate ≥ 100 bpm	175 (27)	90 (21.5)	565 (22)	.007	.83
SBP < 90 or DPB ≤ 60 mmHg	122 (19.1)	73 (17.8)	468 (18.3)	.64	.84
Temperature ≥ 38.5 ºC	82 (12.5)	70 (16.5)	257 (9.9)	.06	< .001
More than 7 days from symptoms onset to admission	142 (20.8)	66 (15.0)	415 (15.5)	.001	.81
Admission laboratory findings					
Neutrophil count > 7500/μL	122 (17.9)	51 (11.6)	388 (14.8)	.047	.08
Lymphocyte count < 1000/μL	406 (59.9)	254 (58.1)	1357 (51.7)	< .001	.01
Platelets < 150,000/μL	239 (35.3)	163 (37.4)	783 (29.9)	.007	.002
D-dimer levels > 500 ng/mL	192 (62.7)	95 (55.9)	557 (56.2)	.04	.94
Lactate dehydrogenase > 250 U/L	369 (83.1)	197 (68.2)	1008 (58.8)	< .001	.003
C-reactive protein > 100 mg/L	295 (46.3)	112 (26.7)	603 (25.1)	< .001	.47
Treatment during hospitalization					
Remdesivir	30 (4.5)	10 (2.3)	8 (.3)	< .001	< .001
Lopinavir/ritonavir	635 (93.1)	413 (94.1)	1660 (62.4)	< .001	< .001
Tocilizumab	150 (22.4)	97 (22.5)	117 (4.5)	< .001	< .001
Corticosteroids	260 (38.4)	175 (40.1)	615 (23.3)	< .001	< .001
NIV or high flow (score of 6)[b]	178 (26.4)	116 (26.9)	214 (8.1)	< .001	< .001
Intubation and mechanical ventilation (score of 7)[b]	283 (41.4)	142 (32.3)	169 (6.3)	< .001	< .001
Vasopressors (score of 8)[b]	226 (33.4)	114 (26.5)	118 (4.5)	< .001	< .001
Dialysis or ECMO (score of 9)[b]	62 (9.2)	33 (7.6)	42 (1.7)	< .001	< .001
Outcome					
Alive currently hospitalized	110 (16.1)	56 (12.7)	132 (4.9)	< .001	< .001
Discharged alive	346 (50.7)	215 (48.9)	1930 (71.9)	< .001	< .001
Mortality at day 30	227 (33.2)	169 (38.4)	623 (23.2)	< .001	< .001
Center with high mortality	239 (35)	196 (44.5)	1042 (38.8)	.07	.02
Center with high interferon-β prescription	420 (61.5)	168 (38.2)	522 (19.4)	< .001	< .001
COVID-19 SEIMC Score (Median [IQR])[c]	8 (5–13)	8 (5–13)	8 (4–16)	.89	.92
COVID-19 SEIMC Score Risk category[c]					
Low (0–2 points)	34 (5.9)	22 (5.9)	307 (13.8)	< .001	< .001
Moderate (3–5 points)	122 (21.3)	75 (20.3)	473 (21.2)	.99	.89
High (6–8 points)	140 (24.4)	92 (24.9)	410 (18.4)	.046	.04
Very high (9–30 points)	277 (48.3)	181 (48.9)	1040 (46.6)	.72	.65
Days from hospital admission to intubation (Median [IQR])	2 (1–4)	5 (3–7)	4 (1–7)	.01	.05

Data are presented as No. (%). P values are calculated by $\chi 2$, Fisher's test or Mann-Whitney's *U* test.
Abbreviations: EIT, early interferon-β treatment; LIT, late interferon-β treatment; NIT, no interferon-β treatment; BMI, body mass index; eGFR, estimated glomerular filtration rate; HIV, human immunodeficiency virus infection; AIDS, acquired immunodeficiency syndrome; SpO_2, peripheral capillary oxygen saturation; SBP, systolic blood pressure; DBP, diastolic blood pressure; NIV, non-invasive ventilation; ECMO, extracorporeal membrane oxygenation; IQR, interquartile range.
[a]Age-adjusted low SpO_2 ≤ 90% for patients aged > 50 years and ≤ 93% for patients aged ≤ 50 years.
[b]Severity rating according to the WHO Clinical Progression Scale, ranged from 0 (not infected) to 10 (dead).
[c]Simple scoring system to predict 30-day mortality on presentation in hospitalized patients with COVID-19 based on age (years), low SpO_2 (age-adjusted), neutrophil-to-lymphocyte ratio, estimated glomerular filtration rate (CKD-EPI), dyspnea and sex (14).

(CI). The PS was used in two ways: as a covariate to control for residual confounders in multivariate models and to perform a matched cohort analysis in which patients undergoing EIT and NIT were paired (1:1) according to their PS using calipers with 0.01 standard deviation. Mortality in the matched pairs was compared by Cox regression. Regarding missing data, Little's MCAR test was used to verify a random pattern, and imputation was performed using the Markov chain Monte Carlo method. All statistical analyses were carried out using SPSS software (SPSS 26.0, IBM Corp., Armonk, NY, USA).

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, 5 (4−8)), and 2685 (70.5%) received no IFN-β treatment. 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 and high values of inflammatory biomarkers, and received treatment with tocilizumab, corticosteroids, and respiratory and hemodynamic support in higher proportions.

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,

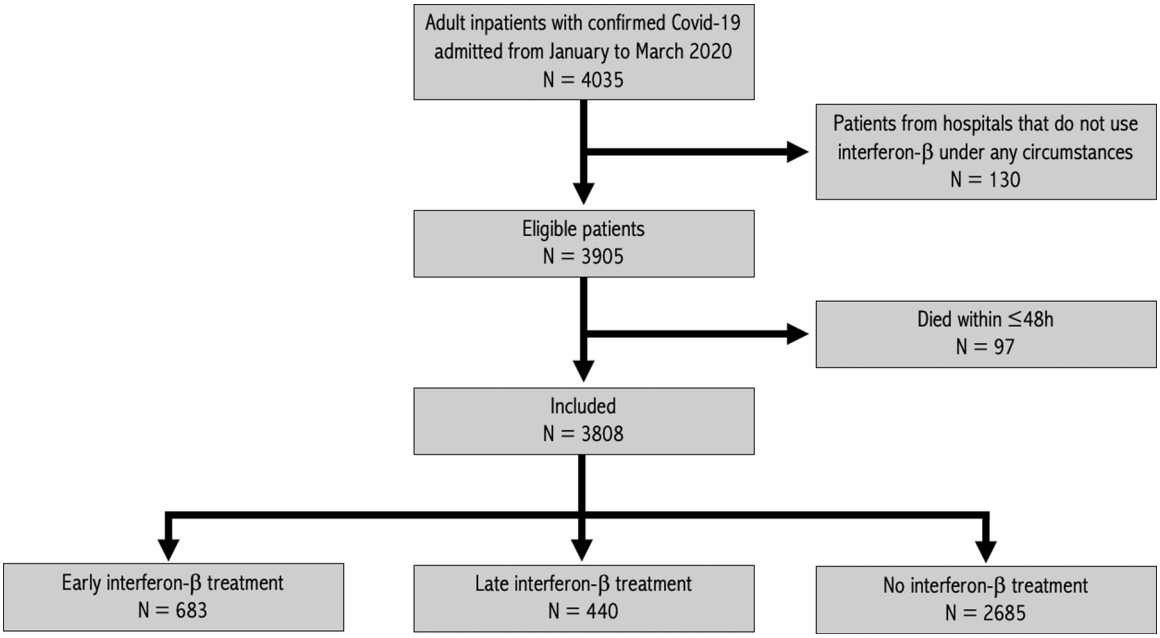

Fig. 1. Study flowchart showing the initial patients from the COVID-19@Spain cohort and the reasons for exclusion, for being treated at centers where IFN-β was not used and because they died ≤ 48 h after hospital admission. Finally, 3808 patients were included for analysis of the impact of early interferon-β treatment.

Table 2
Analysis of the Association of Different Variables with Early Interferon-β Treatment.

Variable	EIT (n = 683)	LIT or NIT (n = 3125)	Crude OR (95% CI)	*P* Value
Male sex	451 (67)	1856 (60.1)	1.34 (1.25–1.44)	< .001
Age > 75 years	193 (28.3)	1108 (35.5)	.72 (.67–.77)	< .001
Obesity (BMI >30)	101 (16.3)	356 (12.8)	1.31 (1.19–1.43)	< .001
Chronic heart disease	138 (20.3)	732 (23.7)	.81 (.75–.89)	< .001
Dyspnea	411 (60.8)	1404 (45.6)	1.84 (1.72–1.98)	< .001
Low SpO_2 (age-adjusted)[a]	261 (43.8)	599 (21.7)	2.69 (2.51–2.89)	< .001
Heart rate ≥ 100 bpm	175 (27)	655 (21.9)	1.31 (1.21–1.42)	< .001
More than 7 days from symptoms onset to admission	142 (20.8)	481 (15.4)	1.44 (1.33–1.57)	< .001
Neutrophil count > 7500/μL	122 (17.9)	439 (14.4)	1.30 (1.19–1.42)	< .001
Lymphocyte count < 1000/μL	406 (59.9)	1611 (52.6)	1.34 (1.26–1.44)	< .001
Platelets < 150,000/μL	239 (35.3)	946 (30.9)	1.22 (1.13–1.31)	< .001
D-dimer levels > 500 ng/mL	192 (62.7)	652 (56.2)	1.32 (1.18–1.46)	< .001
Lactate dehydrogenase > 250 U/L	369 (83.1)	1205 (60.2)	3.26 (2.93–3.63)	< .001
C-reactive protein > 100 mg/L	295 (46.3)	715 (25.3)	2.55 (2.37–2.74)	< .001
Lopinavir/ritonavir	635 (93.1)	2073 (66.9)	6.70 (5.91–7.59)	< .001
Tocilizumab	150 (22.4)	214 (7)	3.83 (3.49–4.20)	< .001
Corticosteroids	260 (38.4)	790 (25.7)	1.80 (1.68–1.94)	< .001
NIV or high flow (score of 6)[b]	178 (26.4)	330 (10.7)	2.96 (2.73–3.22)	< .001
Intubation and mechanical ventilation (score of 7)[b]	283 (41.4)	311 (10)	5.94 (5.51–6.41)	< .001
Vasopressors (score of 8)[b]	226 (33.4)	232 (7.6)	6.00 (5.52–6.53)	< .001
Center with high interferon-β prescription[c]	420 (61.5)	690 (22.1)	5.64 (5.25–6.06)	< .001

Data are presented as No. (%) unless otherwise indicated.
Abbreviations: EIT, early interferon-β treatment; LIT, late interferon-β treatment; NIT, no interferon-β treatment; OR, odds ratio; CI, confidence interval; BMI, body mass index; SpO_2, peripheral capillary oxygen saturation; NIV, non-invasive ventilation.
[a]Age-adjusted low SpO_2 ≤ 90% for patients aged > 50 years and ≤ 93% for patients aged ≤ 50 years.
[b]Severity rating according to the WHO Clinical Progression Scale, ranged from 0 (not infected) to 10 (dead).
[c]The centers were dichotomized into low (<40%) and high (≥40%) proportion of IFN-β prescription.

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 (Clinical Trials.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 Kalil 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
Univariate and Multivariate Analyses of Risk Factors Associated with All-cause 30-Day Mortality Using Cox Regression.

Variable	Deceased (n = 1019)	Alive (n = 2789)	Crude Analysis		Adjusted Analysis[a]		EIT vs NIT, Adjusted by PS[b]	
			HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Male sex	700 (69.5)	1607 (58.4)	1.31 (1.15–1.50)	< .001	…	…	…	…
Age > 75 years	621 (61)	680 (24.4)	2.66 (2.34–3.01)	< .001	2.37 (2.00–2.81)	< .001	2.51 (2.06–3.05)	< .001
Obesity (BMI > 30)	163 (18.3)	294 (11.7)	1.29 (1.09–1.52)	.004	…	…	…	…
Chronic heart disease	399 (39.6)	471 (17.1)	1.87 (1.65–2.13)	< .001	…	…	…	…
Dyspnea	613 (61.2)	1202 (43.7)	1.74 (1.54–1.98)	< .001	1.49 (1.24–1.78)	< .001	1.39 (1.12–1.71)	.003
Low SpO_2 (age-adjusted)[c]	366 (42.9)	494 (19.8)	2.05 (1.75–2.41)	< .001	1.55 (1.26–1.90)	< .001	1.67 (1.30–2.14)	< .001
Heart rate ≥ 100 bpm	239 (24.5)	591 (22.4)	1.15 (.99–1.33)	.06	…	…	…	…
More than 7 days from symptoms onset to admission	99 (9.7)	524 (18.8)	.67 (.54–.83)	< .001	…	…	…	…
Neutrophil count > 7500/μL	244 (24.3)	317 (11.6)	1.60 (1.38–1.84)	< .001	…	…	…	…
Lymphocyte count < 1000/μL	650 (64.9)	1367 (49.9)	1.55 (1.36–1.77)	< .001	1.28 (1.08–1.53)	.01	1.25 (1.03–1.51)	.03
Platelets < 150,000/μL	382 (37.9)	803 (29.4)	1.30 (1.14–1.48)	< .001	1.29 (1.08–1.53)	.004	1.28 (1.05–1.56)	.01
D-dimer levels > 500 ng/mL	233 (67.1)	611 (54.6)	1.27 (1.01–1.59)	.04	…	…	…	…
Lactate dehydrogenase > 250 U/L	458 (73.4)	1116 (61.2)	1.49 (1.25–1.78)	< .001	1.44 (1.19–1.76)	< .001	1.50 (1.20–1.88)	< .001
C-reactive protein > 100 mg/L	407 (44.1)	603 (23.7)	1.87 (1.65–2.14)	< .001	1.42 (1.19–1.69)	< .001	1.47 (1.21–1.79)	< .001
Lopinavir/ritonavir	743 (72.9)	1982 (71.1)	.93 (.81–1.07)	.29	.92 (.75–1.13)	.42	.88 (.64–1.20)	.41
Tocilizumab	122 (12.2)	242 (8.9)	.90 (.75–1.09)	.27	.80 (.63–1.03)	.08	.76 (.46–1.26)	.28
Corticosteroids	439 (43.6)	611 (22.3)	1.52 (1.34–1.72)	< .001	1.32 (1.11–1.56)	.002	1.33 (1.08–1.63)	.01
Interferon-β treatment								
No interferon-β treatment	623 (61.1)	2062 (73.9)	Reference	.01	Reference	.34	Reference	…
Early interferon-β treatment	227 (26.7)	456 (18.1)	1.28 (1.10–1.49)	.001	1.01 (.80–1.26)	.97	1.03 (.82–1.30)	.78
Late interferon-β treatment	169 (21.3)	271 (11.6)	1.08 (.91–1.28)	.37	1.19 (.95–1.49)	.14	Excluded	…
Center with high mortality	543 (53.3)	934 (33.5)	1.72 (1.52–1.95)	< .001	1.69 (1.43–2.00)	< .001	1.68 (1.39–2.03)	< .001
Propensity score[d]	…	…	…	…	…	…	.98 (.27–3.62)	.97

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; SpO_2, peripheral capillary oxygen saturation.
[a]The area under the receiver operating characteristic (AUROC) curve of the model was.86 (95% CI,.84–.91), $P = .004$.
[b]Patients in the late interferon-β treatment group were excluded from this analysis.
[c]Age-adjusted low $SpO_2 \leq 90\%$ for patients aged > 50 years and ≤ 93% for patients aged ≤ 50 years.
[d]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 SpO_2, 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), $P < .001$.

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

Table 4
Comparison of Matched Patients According to Propensity Score.

	Overall Cohort (N = 3368)[a]			Propensity Score-Matched Cohort (N = 288)[b]		
Variable	EIT (n = 683)	NIT (n = 2685)	*P* Value	EIT (n = 144)	NIT (n = 144)	*P* Value
Male sex	451 (67)	1559 (58.8)	< .001	97 (67.4)	98 (68.1)	.90
Age > 75 years	193 (28.3)	968 (36.1)	< .001	30 (20.8)	38 (26.4)	.27
Obesity (BMI >30)	101 (16.3)	283 (11.9)	.003	23 (16)	19 (13.2)	.50
Chronic heart disease	138 (20.3)	632 (23.8)	.05	23 (16)	24 (16.7)	.87
Dyspnea	411 (60.8)	1191 (45)	< .001	93 (64.6)	86 (59.7)	.40
Low SpO_2 (age-adjusted)[c]	261 (43.8)	498 (20.9)	< .001	59 (41)	53 (36.8)	.47
Heart rate ≥ 100 bpm	175 (27)	565 (22)	.01	40 (27.8)	39 (27.1)	.90
> 7 days from onset to admission	142 (20.8)	415 (15.5)	.001	29 (20.1)	31 (21.5)	.77
Neutrophil count > 7500/μL	122 (17.9)	388 (14.8)	.047	21 (14.6)	23 (16)	.74
Lymphocyte count < 1000/μL	406 (59.9)	1357 (51.7)	< .001	91 (63.2)	83 (57.6)	.34
Platelets < 150,000/μL	239 (35.3)	783 (29.9)	.01	48 (33.3)	54 (37.5)	.46
D-dimer levels > 500 ng/mL	192 (62.7)	557 (56.2)	.04	96 (66.7)	91 (63.2)	.54
Lactate dehydrogenase > 250 U/L	369 (83.1)	1008 (58.8)	< .001	115 (79.9)	117 (81.3)	.77
C-reactive protein > 100 mg/L	295 (46.3)	603 (25.1)	< .001	66 (45.8)	68 (47.2)	.81
Lopinavir/ritonavir	635 (93.1)	1660 (62.4)	< .001	142 (98.6)	142 (98.6)	.99
Tocilizumab	150 (22.4)	117 (4.5)	< .001	32 (22.2)	36 (25)	.56
Corticosteroids	260 (38.4)	615 (23.3)	< .001	63 (43.8)	64 (44.4)	.91
Deceased	227 (33.2)	623 (23.2)	< .001	38 (26.4)	38 (26.4)	1.00
Center with high mortality	239 (35)	1042 (38.8)	.07	50 (34.7)	47 (32.6)	.71

Data are presented as No. (%). P values are calculated by Cox regression.
Abbreviations: EIT, early interferon-β treatment; NIT, no interferon-β treatment; BMI, body mass index; SpO_2, peripheral capillary oxygen saturation.
[a]Patients in the late interferon-β treatment group were excluded from this analysis.
[b]The 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 SpO_2, 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.
[c]Age-adjusted low SpO_2 ≤ 90% for patients aged > 50 years and ≤ 93% for patients aged ≤ 50 years.
[d]Severity rating according to the WHO Clinical Progression Scale, ranged from 0 (not infected) to 10 (dead).

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 statistical analyses, including the imputation of missing data using the Markov chain Monte Carlo method.

In conclusion, our findings did not find an association between early IFN-β therapy after hospital admission and any mortality benefit in patients admitted because of COVID-19. Additional data are needed for IFN-β administration at even earlier stages of the disease and in association with other drugs such as tocilizumab or corticosteroids. Finally, whether the drug would be useful specifically in patients with low IFN production needs to be investigated.

Collaborators

The COVID-19@Spain Study Group members.

Fundación SEIMC-GESIDA: 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, Gandullo-Moro María, Ruíz-Hueso Rocío, Moya-González Natalia, Peral Enrique, Valido-Morales Agustín, Pavón-Masa María. **Hospital Universitario La Paz:** Díaz Menéndez Marta, De la Calle Prieto Fernando, Arsuaga Vicente Marta, Ramos Ramos Juan Carlos, De Miguel Buckley Rosa, Cadiñanos Loidi Julen, Marcelo Calvo Cristina, Vasquez Manau Julia, Mora Rillo Marta, Loeches Yagüe Belén, Ramos Ruperto Luis, García-Rodríguez Julio, Montejano Sánchez Rocío, Diaz Pollan Beatriz. **Hospital Universitario Gregorio Marañón:** López Juan Carlos, Ramírez-Schacke Margarita, Gutiérrez Isabel, Tejerina Francisco, Aldámiz-Echevarría Teresa, Díez Cristina, Fanciulli Chiara, Pérez-Latorre Leire, Parras Francisco, Catalán Pilar, García-Leoni María E., Pérez-Tamayo Isabel, Puente Luis, Cedeño Jamil. **Hospital Infanta Leonor:** Such-Diaz Ana, Álvaro-Alonso 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 Josefa, Brañas Fátima, Valencia Jorge, Pérez-Butragueño Mario, Muñoz-Rivas Nuria. **Hospital Universitari de Bellvitge:** Abelenda-Alonso Gabriela, Ardanuy Carmen, Bergas Alba, Cuervo Guillermo, Domínguez María Ángeles, Fernández-Huerta Miguel, Gudiol Carlota, Lorenzo-Esteller Laia, Niubó Jordi, Pérez-Recio Sandra, Podzamczer Daniel, Pujol Miquel, Rombauts Alexander, Trullen Núria. **Hospital Universitario Virgen del Rocío:** Molina José, Álvarez-Marín Rocío, García-Gutiérrez Manuel, Paniagua María, Alarcón Arístides, Gil-Navarro María Victoria, Giménez Luis, Camacho-Martínez Pedro, Merino Laura, Caballero-Eraso Candela, Paradas Carmen, Valencia-Martín José, Fernández-Delgado Esperanza. **Complejo Hospitalario Virgen de la Salud:** Sepúlveda Berrocal Mª Antonia, Yera Bergua Carmen, Toledano Sierra Pilar, Cano Llorente Verónica, Zafar Iqubal-Mirza Sadaf, Muñiz Gema, Martín Pérez Inmaculada, Mozas Moriñigo Helena, Alguacil Ana, García Butenegro María Paz. **Hospital Universitario Rafael Méndez:** Peláez Ballesta Ana Isabel, Morcillo Rodríguez Elena. **Hospital Universitario de Cruces:** Goikoetxea Agirre Josune, Bereciartua Bastarrica Elena, Guio Carrion Laura, Euba Ugarte Gorane. **Hospital de Melilla:** Pérez Hernández Isabel A., Román Soto Sergio. **Hospital San Eloy de Barakaldo:** Silvariño Fernández Rafael, Ugalde Espiñeira Jon. **Hospital Universitario Central de Asturias:** Asensi Victor, 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-Lora Javier, Velasco Garrido José Luis, López-Jodar María, Santos González Jesús. **Hospital Universitario Puerto Real:** Ruiz Aragón Jesús, Virto Peña Ianire. **EOXI Pontevedra e Salnés:** Alende Castro Vanessa, Fernandez Morales Marta. **Hospital de Figueres:** Vega Molpeceres Sonia, Pons Viñas Estel. **Hospital Sant Jaume de Calella:** del Río Pérez Oscar, Valero Rovira Silvia. **Hospital del Mar:** Gómez-Junyent Joan, Castañeda Espinosa Silvia, Cánepa María Cecilia, Villar-García Judit, Gimenez Argente Carmen, Soldado Folgado Jade, Nogués Solán Xavier, de Pablo Miró Mar, Cazador Labat Miriam. **Hospital Clínico Universitario Virgen de**

la Arrixaca-IMIB: García Vázquez Elisa, Marín Real Sonia, Roura Piloto Aychel Elena. **Hospital de Can Misses:** García Almodóvar Esther. **Hospital de Sagunto:** Sáez Barberá Carmen, Karroud Zineb. **Hospital Clínico San Cecilio:** Vinuesa García David, Hernández Quero José, Faro-Míguez Naya, Benavente-Fernandez Alberto. **Hospital Universitario Príncipe de Asturias:** Novella Mena María, Hernández Gutiérrez Cristina, Sanz Moreno José, Pérez Tanoira Ramón, Barbero Allende José María, Culebras López Ana María, García Sánchez Marta, Arranz Caso Alberto, Cuadros González Juan, Álvarez de Mon Soto Melchor. **Parc Sanitari Sant Joan de Déu:** Díaz-Brito Fernández Vicens, Sanmarti Vilamala Montserrat, Gabarrell Pascuet Aina, Esteve-Palau Erika, España Cueto Sergio, Álvarez Moya Maria Carmen, Medina Salas Francisco, García Aranda Geneva. **Hospital Nuestra Señora de Gracia:** Sáez Escolano Paula, Solsona Fernández Sofía. **HC Marbella Internacional Hospital:** Sempere Alcocer Marco Antonio, Martin Nicole. **Hospital Universitario La Princesa**: De los Santos Gil Ignacio, García-Fraile Lucio, Sampedro Núñez Miguel, Barrios Blandino Ana, Rodríguez Franco Carlos, Useros Brañas Daniel, Villa Martí Almudena, Oliver Ortega Javier, Costanza Espiño Álvarez Alexia, Sanz Sanz Jesús. **Hospital Josep Trueta:** Rexach Fumaña María, Policarpo Torres Guillem, Ortega Montoliu Meritxell. **Hospital Dos de Maig-Consorcio Sanitari Integral:** Sala Jofre Clara, Casas Rodríguez Susana. **Hospital Arnau de Vilanova-Lliria:** Tortajada Alamilla Cecilia, Oltra Carmina. **Hospital General Universitario de Elche:** Masiá Canuto Mar, Gutiérrez Rodero Félix. **Hospital Clínico Universitario de Valencia:** Oltra Sempere Mª Rosa, Vela Berna Sara. **Complejo Asistencial de Ávila:** Pedromingo Kus Miguel, Garcinuño María Ángeles, Fiorante Silvana, Pérez Pinto Sergio. **Hospital Comarcal de Alcañiz:** Hernández Machín Pilar, Alastrué Violeta Alba. **Hospital Universitario Marqués de Valdecilla:** Fariñas Álvarez María Carmen, González Rico Claudia, Arnaiz de las Revillas Francisco, Calvo Jorge, Gozalo Mónica. **Hospital Quirón-Salud de Torrevieja:** Mora Gómez Francisco. **Hospital Universitario Miguel Servet:** Latorre-Millán Miriam, Milagro Beamonte Ana, Rezusta López Antonio, Roc Lourdes. **Hospital de Barcelona SCIAS:** Meije Yolanda, Ribera Puig Alba, Duarte-Borjes Alejandra, Sanz Salvador Xavier. **Fundación Hospital Universitario Alcorcón:** Losa García Juan Emilio, Martín-Segarra Oriol. **Hospital Álvaro Cunqueiro:** Pérez-Rodríguez M. Teresa, Pérez González Alexandre. **Complejo Asistencial Universitario de Salamanca:** Belhassen-García Moncef, Tejera Pérez Rosa, López-Bernus Amparo, Carbonell Cristina. **Hospital Universitario Severo Ochoa:** Cantón De Seoane Juan, Torres Perea Rafael, Cervero Jiménez Miguel, Avilés Parra Juan Pablo, Cayuela Rodríguez Lucia, Kamel Rey Sara Lidia, Roa Alonso David, Martín Rojo Lidia, García Escudero Laura, Orejas Gallego Alberto. **Hospital CIMA-Sanitas:** Pelegrín Iván, Rouco Esteves Marques Rosana. **Hospital HLA Inmaculada:** Parra Ruiz Jorge, Ramos Sesma Violeta. **Hospital Universitario Rio Hortega:** Abadia Otero Jésica. **Hospital de Guadalajara:** Salillas Hernando Juan, Torres Sánchez del Arco Robert, Torralba González de Suso Miguel, Serrano Martínez Alberto, Gilaberte Reyzábal Sergio, Pacheco Martínez-Atienza Marina, Liébana Gómez Mónica, Fernández Rodríguez Sara, Varela Plaza Álvaro, Calvo Sánchez Henar. **Hospital Universitario Infanta Sofía:** Martínez Martín Patricia, González-Ruano Patricia, Malmierca Corral Eduardo, Rábago Lorite Isabel, Pérez-Monte Mínguez Beatriz. **Hospital Comarcal de Blanes:** García Flores Ángeles, Comas Casanova Pere. **Hospital Universitari de Tarragona Joan XXIII:** Sirisi Escoda Merce, Peraire Forner Joaquim. **Hospital Universitario Basurto:** Ibarra Ugarte Sofía, Muñoz Sanchez Pepa, López Azkarreta Iñigo. **Hospital Universitario de Canarias:** Alemán Valls Remedios, Alonso Socas María del Mar. **Hospital Universitario de Gran Canaria Dr. Negrín:** Sanz Peláez Oscar, Robaina Bordon Jose Maria. **Hospital Son Espases:** Riera Jaume Melchor, Vilchez Helem Haydee, Albertí Francesc, Cañabate Ana Isabel. **Hospital Universitario de Móstoles:** Moreno Cuerda Víctor J., Álvarez Kaelis Silvia, Álvarez Zapatero Beatriz, García García Alejandro, Isaba Ares Elena, Morcate Fernández Covadonga, Pérez Rodríguez Andrea. **Complejo Hospitalario Universitario A Coruña:** Ramos Merino Lucía, Castelo Corral Laura, Rodríguez Mahía María, González Bardanca Mónica, Sánchez Vidal Efrén, Míguez Rey Enrique. **Hospital Costa del Sol:** Correa Ruiz Ana, García de Lomas Guerrero José Mª. **Hospital Clínico Universitario Lozano Blesa:** Cano Alberto, Alda Alicia, Merino Izarbe. **Hospital Mutua de Terrassa:** Gómez García Lucía, Boix Palop Lucia, Dietl Gómez-Luengo Beatriz. **Hospital de la Plana:** Pedrola Gorrea Iris, Blasco Claramunt Amparo. **Hospital Virgen de la Concha-Complejo Asistencial de Zamora:** López Mestanza Cristina, Fraile Villarejo Esther. **Complejo Hospitalario Universitario Insular Materno-Infantil:** Carmona Tello Maria Nieves, Suárez Hormiga Laura. **Hospital de la Marina Baixa:** Algado Rabasa José Tomas, Garijo Saiz Ana María, Amador Prous Concepción. **Hospital Universitario y Politécnico La Fe:** Tasias Pitarch Mariona. **Hospital Universitario del Vinalopó:** Hernández Belmonte Adriana, Pérez Soto María Isabel. **Hospital Parc Taulí de Sabadell:** Navarro Vilasaró Marta, Calzado Isbert Sonia, Cervantes García Manuel, Gomila Grange Aina, Gasch Blasi Oriol, Machado Sicilia María Luisa, Van den Eynde Otero Eva, Falgueras López Luis, Navarro Sáez María del Carmen. **Hospital Clinic de Barcelona:** Martínez Esteban, Marcos Mª Ángeles, Mosquera Mar, Blanco José Luis, Laguno Montserrat, Rojas Jhon, González-Cordón Ana, Inciarte Alexy, Torres Berta, De la Mora Lorena, Soriano Alex. **Hospital Universitario de la Ribera:** Martínez Macias Olalla, Borrás Máñez María. **Fundación Jiménez Díaz:** Cabello Úbeda Alfonso, Carrasco Antón Nerea, Álvarez Álvarez Beatriz, Petkova Saiz Elizabet, Górgolas Hernández-Mora Miguel, Prieto Pérez Laura, Carrillo Acosta Irene, Heili Frades Sara, Villar Álvarez Felipe, Fernández Roblas Ricardo, Milicua José María. **Hospital Clínico Universitario de Valladolid:** Fernández Espinilla Virginia, Castrodeza Sanz José Javier, Dueñas Gutiérrez Carlos Jesús. **Hospital Clínico San Carlos:** González-Romo Fernando, Merino Amador Paloma, Rueda López Alba, 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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. **José Ramón Arribas: Juan Berenguer:** Investigation, Resources, Data curation. **Jordi Carratalà:** Investigation, Resources, Data curation. **Inmaculada Jarrín:** Investigation, Resources, Data curation. **Pablo Ryan:** Investigation, Resources, Data curation. **Marta de Miguel-Montero:** Investigation, Resources, Data curation. **Jesús Rodríguez-Baño:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Jerónimo Pachón:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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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.biopha.2021.112572.

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SUPPLEMENTARY INFORMATION

Table S1. STROBE checklist

	Item	Recommendation	Manuscript location
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	Title and abstract
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Abstract
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Introduction
Objectives	3	State specific objectives, including any prespecified hypotheses	Introduction, last paragraph
Methods			
Study design	4	Present key elements of study design early in the paper	Methods, first paragraph
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Methods, first paragraph
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Methods, first paragraph
		(b) For matched studies, give matching criteria and number of exposed and unexposed	Table 4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Methods, second and third paragraph
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Results, Tables 1-4
Bias	9	Describe any efforts to address potential sources of bias	Methods, Statistical analysis
Study size	10	Explain how the study size was arrived at	Methods, first paragraph
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Methods, Statistical analysis
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Methods, Statistical analysis

		(b) Describe any methods used to examine subgroups and interactions	Methods, Statistical analysis
		(c) Explain how missing data were addressed	Methods, Statistical analysis
		(d) If applicable, explain how loss to follow-up was addressed	Not applicable
		(e) Describe any sensitivity analyses	Supplementary Table S1, S2 and S3
Results			
Participants	13	(a) Report numbers of individuals at each stage of Study (eg, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed)	Results, Figure 1
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14	(a) Give characteristics of study participants (eg, demographic, clinical, social) and information on exposures and potential confounders	Results, Tables 1-3
		(b) Indicate number of participants with missing data for each variable of interest	Results, Table 1
		(c) Summarise follow-up time (eg, average and total amount)	Methods, first paragraph
Outcome data	15	Report numbers of outcome events or summary measures over time	Results, first to fourth paragraph
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Results, Tables 1-4
		(b) Report category boundaries when continuous variables were categorised	Tables 1-3
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done (eg, analyses of subgroups and interactions, and sensitivity analyses	Table 4, S1, S2 and S3
Discussion			
Key results	18	Summarise key results with reference to study objectives	Discussion, first paragraph

Limitations	19	Discuss limitations of the study, considering sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Discussion, eighth paragraph
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Discussion
Generalisability	21	Discuss the generalisability (external validity) of the study results	Discussion, last paragraph
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Funding

Table S2. Multivariate Analysis of Risk Factors Associated with All-cause 30-Day Mortality Using Cox Regression for EIT vs NIT (excluding LIT), with COVID-19 SEIMC Score as continuous variable (instead of age, dyspnea, SpO2 and lymphocyte count)

					Adjusted Analysis	
Variable	B Coefficient	Standard Error	Wald	Degree of Freedom	HR (95% CI)	*P* Value
COVID-19 SEIMC Score	.091	.005	380.893	1	1.095 (1.085–1.105)	<.001
Platelets <150 000/μL	.192	.065	8.746	1	1.212 (1.067–1.376)	.003
Lactate dehydrogenase >250 U/L	.325	.083	15.473	1	1.384 (1.177–1.628)	<.001
C-reactive protein >100 mg/L	.384	.075	26.454	1	1.468 (1.268–1.699)	<.001
Lopinavir/ritonavir	-.146	.104	1.976	1	.864 (.706–1.259)	.160
Tocilizumab	-.510	.107	22.599	1	.601 (.487–.741)	<.001
Corticosteroids	.335	.070	23.077	1	1.398 (1.220–1.603)	<.001
Center with high mortality	.506	.063	64.056	1	1.658 (1.465–1.876)	<.001
Early interferon-β treatment	.075	.076	.981	1	1.078 (.929–1.252)	.322
Propensity score[a]	1.326	.262	25.608	1	3.766 (2.253–6.294)	<.001

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

Abbreviations: EIT, early interferon-β treatment; NIT, no interferon-β treatment; LIT, late interferon-β treatment HR, hazard ratio; CI, confidence interval.

[a]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 SpO_2, 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.

Table S3. Multivariate Analysis of Risk Factors Associated with All-cause 30-Day Mortality Using Cox Regression for EIT vs NIT (excluding LIT), without lopinavir/ritonavir, tocilizumab, and corticosteroids treatment

					Adjusted Analysis	
Variable	B Coefficient	Standard Error	Wald	Degree of Freedom	HR (95% CI)	*P* Value
Age >75 years	.788	.060	172.429	1	2.200 (1.956–2.474)	<.001
Dyspnea	.351	.064	30.176	1	1.420 (1.253–1.609)	<.001
Low SpO_2 (age-adjusted)[a]	.627	.066	91.070	1	1.872 (1.646–2.129)	<.001
Lymphocyte count <1000/μL	.169	.062	7.340	1	1.184 (1.048–1.338)	.007
Platelets <150 000/μL	.218	.061	12.710	1	1.244 (1.103–1.403)	<.001
Lactate dehydrogenase >250 U/L	.509	.077	43.522	1	1.664 (1.431–1.936)	<.001
C-reactive protein >100 mg/L	.406	.064	40.399	1	1.501 (1.324–1.701)	<.001
Center with high mortality	.422	.058	52.224	1	1.525 (1.360–1.710)	<.001
Early interferon-β treatment	.099	.070	1.973	1	1.104 (.962–1.267)	.160
Propensity score[b]	-.373	.181	4.235	1	.688 (.482–.982)	.040

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

Abbreviations: EIT, early interferon-β treatment; NIT, no interferon-β treatment; LIT, late interferon-β treatment HR, hazard ratio; CI, confidence interval.

[a]Age-adjusted low SpO_2 ≤90% for patients aged >50 years and ≤93% for patients aged ≤50 years.

[b]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 SpO_2, 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.

Table S4. Multivariate Analysis of Risk Factors Associated with All-cause 30-Day Mortality Using Cox Regression for the overall cohort (including LIT population), studying interferon treatment as a time-dependent variable

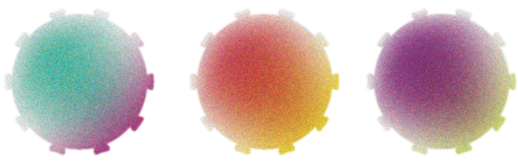
					Adjusted Analysis	
Variable	B Coefficient	Standard Error	Wald	Degree of Freedom	HR (95% CI)	*P* Value
Age >75 years	.666	.175	14.459	1	1.947 (1.381–2.746)	<.001
Dyspnea	.419	.185	5.131	1	1.520 (1.058–2.185)	.024
Low SpO_2 (age-adjusted)[a]	.709	.224	10.017	1	2.031 (1.310–3.150)	.002
Lymphocyte count <1000/μL	-.011	.177	.004	1	.989 (.699–1.401)	.952
Platelets <150 000/μL	.318	.176	3.275	1	1.375 (.974–1.941)	.070
Lactate dehydrogenase >250 U/L	.606	.277	7.100	1	1.832 (1.174–2.861)	.008
C-reactive protein >100 mg/L	.650	.207	9.819	1	1.916 (1.276–2.877)	.002
Lopinavir/ritonavir	.222	.348	.408	1	1.249 (.632–2.469)	.523
Tocilizumab	-.173	.287	.362	1	.841 (.479–1.477)	.548
Corticosteroids	.625	.198	9.925	1	1.868 (1.266–2.755)	.002
Center with high mortality	-.120	.202	.356	1	.887 (.597–1.317)	.551
Early interferon-β treatment (Time-dependent)	.351	.177	3.905	1	1.420 (1.003–2.011)	.048
Propensity score[b]	-1.047	.994	1.110	1	.351 (.050–2.463)	.292

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

Abbreviations: LIT, late interferon-β treatment HR, hazard ratio; CI, confidence interval.

[a]Age-adjusted low SpO_2 ≤90% for patients aged >50 years and ≤93% for patients aged ≤50 years.

[b]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 SpO_2, 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.



GLOBAL SUMMARY OF THE RESULTS

5. GLOBAL SUMMARY OF THE RESULTS

Article 1 results

In this large, prospective, nationwide study of SOTRs hospitalized with COVID-19 followed for 30 days, 17.6% required ICU admission, and the mortality rate was 21.4%. Older age, high respiratory rate, lymphopenia, and elevated level of lactate dehydrogenase at presentation were independently associated with ICU admission and/or death. Similarly, an earlier post-transplant SARS-CoV-2 infection was demonstrated as a risk factor for unfavorable outcomes. Most patients were male with a median age over 60 years. Among the underlying comorbidities assessed, chronic cardiomyopathy, diabetes mellitus, and chronic kidney disease were all present in more than one fourth of the patients included and were associated with increased odds of unfavorable outcomes. COVID-19 pneumonia at the time of diagnosis (defined by chest X-ray infiltrates) was also associated with unfavorable outcomes. The most common presenting symptoms in our cohort included fever, cough, and dyspnea, which were significantly associated with a poor clinical outcome. More atypical presentations, such as vomiting or diarrhea, were also reported among a significant proportion of SOTRs. This highlights that immunocompromised hosts often present with unusual or attenuated signs and symptoms of infection, leading to late presentations or missed diagnosis, and potentially worse results. Among the inflammatory parameters measured at hospital admission, creatinine, lactate dehydrogenase, C-reactive protein, and D-dimer levels were higher within the unfavorable outcome group.

Article 2 results

Forty-seven (10.3%) SOTRs and 408 (89.7%) non SOTRs were recruited. The mean days from symptoms onset (DfSO) to hospital admission was 7.1 ± 4.3, without differences between groups. Undetectable IFN-α occurred in 8.5% and 13.5% (p = 0.36) of both groups, respectively, independently of the DfSO. Undetectable IFN- γ was more frequent in SOTRs (42.6% *vs*. 19.4%; p < 0.001), as well as RNAemia (57.4% *vs*. 18.9%; p < 0.001). In the PS matched cohorts, SOTRs showed higher prevalence of undetectable IFN-γ (39.4% *vs*. 10.6%, p = 0.001), higher RNAemia detection (57.6% *vs*. 13.6%; p < 0.001), and mortality (27.3% *vs*. 4.5%; p = 0.003). In SOTRs, the multivariable logistic regression model selected RNAemia as an independent predictor of unfavorable clinical outcome. Regarding non SOTRs, 30-day all-cause mortality was associated with RNAemia and undetectable IFN-γ levels. All patients with RNAemia and SOTRs with undetectable IFN-γ showed lower survival. We did not find an association between serum undetectable IFN-α with unfavorable outcome.

Article 3 results

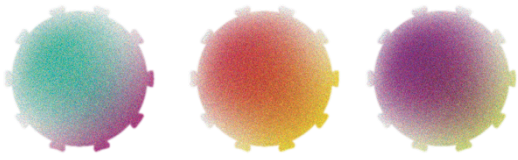
The cohort included 321 adult patients, with the first evaluation at the Emergency room. Fifty-six (17.4%) patients had a mild disease and were discharged after the first evaluation, and subsequently attended as outpatients until the end of follow-up; 180 (56.1%) had a moderate course, being hospitalized in general wards, and with full recovery and hospital discharged; and 85 (26.5%) patients were categorized as severe COVID-19 because of required admission to the ICU (32 patients [10.0%]), in-hospital death (40 [12.5%]), or both (13 [4.0%]). In the total

cohort, males accounted for 169 (52.6%), median age was 63 (IQR 52–77) years, and 36.8% were ≥ 70 years old. The most common symptoms were fever (73.8%), cough (67.3%), and dyspnea (45.8%). Two hundred twenty-four (69.8%) patients had chest X-ray infiltrates at first hospital evaluation: 16.1% within the mild group, 77.8% in the moderate one, and 88.2% in the severe group ($p < 0.001$). During the follow-up, 100% of the patients in the moderate and severe groups showed pulmonary infiltrates in the evolutive chest X-ray after hospital admission. Seventy-eight (24.3%) patients required respiratory support with high flow therapy or non-invasive mechanical ventilation, which was more frequent in patients with severe than with moderate disease (55 [64.7%] *vs*. 23 [12.8%], respectively, $p = 0.001$). Twenty-eight (32.9%) patients, all admitted to ICU, required IMV. Median NP viral load at first hospital evaluation was not different among the mild, moderate, or severe groups according to their clinical outcomes. However, we found higher frequencies of NP viral load above the first tercile, the 50th percentile, and the second tercile in the severe group when comparing the three groups ($p = 0.01$). Although the median days from symptoms onset to diagnosis was lower in the group with higher SARS-CoV-2 viral load (2nd *vs*. 1st tercile) this difference was not significant. Additionally, we performed a linear regression analysis which did not show association between both variables ($p = 0.389$). Nasopharyngeal viral load values over the second quartile and second tercile were associated with unfavorable outcomes in the unadjusted logistic regression analysis. In the final multivariable analysis, however, the number of virus copies in NP swabs was not linked to an unfavorable clinical result.

Article 4 results

In all, 3808 patients were included in this study: 683 (17.9%) received early IFN-β treatment (EIT), 440 (11.6%) received late IFN-β treatment (LIT) and 2685 (70.5%) received no IFN-β treatment (NIT). 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) and did not receive as broad therapy (including remdesivir, tocilizumab, and corticosteroids) as patients in the EIT group. Patients receiving EIT more frequently had severe signs and symptoms and high values of inflammatory biomarkers, and received treatment with tocilizumab, corticosteroids, and respiratory and hemodynamic support in higher proportions. 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). Early IFN-β treatment did not show an association with mortality in the multivariable analysis. We then investigated the impact of EIT *vs*. NIT, including the PS for EIT. Similarly, no difference was observed among the patients

undergoing EIT (adjusted HR, 1.03; 95% CI, 0.82–1.30; p = 0.78). Finally, we matched 144 pairs of patients receiving EIT or NIT based on PS. 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).



GENERAL DISCUSSION

6. GENERAL DISCUSSION

The fundamental implication of our first article is the identification of specific and independent predictors (age ≥ 70 years, respiratory rate > 20 bpm, lymphocytes < 1 x 1000/μL, and lactate dehydrogenase ≥ 300 U/L) for unfavorable outcomes in hospitalized SOTRs with COVID-19, which could ease the development of future research and guidelines targeted at high-risk transplanted populations. Furthermore, we showed that an interval shorter than six months between transplantation and COVID-19 diagnosis has a negative impact on mortality and ICU admission rates, which is a risk that should be considered when deciding which patients should proceed with transplantation. Finally, although analogous to the general population, mortality in SOTRs hospitalized with SARS-CoV-2 infection is dramatically high, and the promotion of preventive strategies and treatments will be crucial to mitigate the adverse impacts of the COVID-19 pandemic in these patients.

Our second article is the first analyzing the interplay among the SARS-CoV-2 RNAemia, IFN-α, and IFN-γ serum levels at the first clinical evaluation of COVID-19 in a cohort of 455 adult patients. We showed that undetectable IFN-γ in serum at hospital admission was independently associated with 30-day all-cause mortality in COVID-19, specifically in non SOTRs. However, undetectable IFN-α in serum was not associated with mortality neither in SOTRs or non SOTRs. RNAemia at hospital admission was also as a robust predictor of 30-day all-cause mortality in all adult COVID-19 inpatients, SOTRs and non SOTRs.

The main finding of our third article was that patients with SARS-CoV-2 infection, regardless of their illness severity, have a high rate of viral replication in the upper

respiratory airways. Consequently, this parameter cannot be used as a predictor of COVID-19 unfavorable outcome, defined as admission to ICU and/or death. There is not a clear viral load cut-off point capable of discriminating between the various levels of COVID-19 severity and, contrary to expectations, a higher number of SARS-CoV-2 copies in NP swabs at first patient's evaluation is not predictive of whether ICU admission or death might occur.

Our fourth article is, to the best of our knowledge, 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. We did not find an association between early IFN-β therapy after hospital admission and any mortality benefit in patients admitted because of COVID-19.

In the large, prospective, and nationwide study of SOTRs hospitalized with COVID-19 followed for 30 days, we found that 17.6% required ICU admission, and the mortality rate was 21.4%. Older age, high respiratory rate, lymphopenia, and elevated level of lactate dehydrogenase at presentation were independently associated with ICU admission and/or death. Similarly, an earlier post-transplant SARS-CoV-2 infection was demonstrated as a risk factor for unfavorable outcomes.

Most patients were male with a median age over 60 years, conforming to prior published large nationwide cohorts of the general population hospitalized with COVID-19[118] and the 2019 Spanish National Transplant Organization Annual Report[119].

The potential negative impact of transplantation on clinical outcomes of COVID-19 has been discussed, and the few authors that directly compared results in SOTRs and general population indicated that ICU admission and death rates were higher among the immunocompromised hosts[120,121]. However, studies including multivariable analyses of severity risk factors among hospitalized general populations with COVID-19, though with variable durations of follow-up, showed mortality and ICU admission estimates generally comparable to the ones reported for the current SOTR cohort[122–125]. The presented fatality rate in our study was also similar to the average of estimates derived from prior small and heterogeneous studies on hospitalized SOTRs[126–129] and just one percentage point higher than the single previously published multicenter prospective SOTR cohort study (20.5%)[130]. By comparing these incidence rates with those of clinical influenza for high-risk groups, we found close resemblance in the probability of ICU admission (ranging from 11.8 to 28.6%) but less likelihood of dying (between 2.9 and 14.3%) from flu among hospitalized patients[131–133], which may be due to the existence of accessible and effective treatment.

Among the underlying comorbidities assessed, chronic cardiomyopathy, diabetes mellitus, and chronic kidney disease were all present in more than one fourth of the patients included and were associated with increased odds of unfavorable outcomes. This is in accordance with the previously described comorbidities

associated with ICU admission or death in the general population[134,135]. COVID-19 pneumonia at the time of diagnosis (defined by chest X-ray infiltrates) was also associated with unfavorable outcomes, as reported in general population studies[136,137] and in the US multicenter SOTR cohort[138]. Moreover, no patients without pneumonia in our cohort required ICU admission or died at final follow-up, solidifying pneumonia as a major determinant of unfavorable outcomes in SOTRs.

The most common presenting symptoms in our cohort included fever, cough, and dyspnea, which were significantly associated with a poor clinical outcome. More atypical presentations, such as vomiting or diarrhea, were also reported among a significant proportion of SOTRs. This highlights that immunocompromised hosts often present with unusual or attenuated signs and symptoms of infection, leading to late presentations or missed diagnosis, and potentially worse results.

Among the inflammatory parameters measured at hospital admission, creatinine, lactate dehydrogenase, C-reactive protein, and D-dimer levels were higher within the unfavorable outcome group. However, the overall variation in these biomarkers was less pronounced than that observed in the general population of hospitalized patients with COVID-19[2,14,118], which is biologically plausible. This being the case, further investigation is required to address whether the lower inflammatory response and greater immunosuppression characterizing SOTRs have impacts on COVID-19 clinical outcomes.

The fundamental implication of our study is the identification of specific and independent predictors (age ≥70 years, respiratory rate >20 bpm, lymphocytes <1 x 1000/μL, and lactate dehydrogenase ≥300 U/L) for unfavorable outcomes in

hospitalized SOTRs with COVID-19, which could ease the development of future research and guidelines targeted at high-risk transplanted populations. Furthermore, we showed that an interval shorter than six months between transplantation and COVID-19 diagnosis has a negative impact on mortality and ICU admission rates, which is a risk that should be considered when deciding which patients should proceed with transplantation. Finally, although analogous to the general population, mortality in SOTRs hospitalized with SARS-CoV-2 infection is dramatically high, and the promotion of preventive strategies and treatments will be crucial to mitigate the adverse impacts of the COVID-19 pandemic in these patients.

The strengths of the present study are the strong design, the multicenter participation approach to make the results generalizable and comparable, the standardized and anonymous collection of data using an electronic Case Report Form, and the 30-day duration of follow-up. In parallel, we have faced some limitations. First, our study is centered on hospitalized patients, and thus the conclusions reached may not be applicable to those SOTRs attended in the outpatient setting. Second, testing limitations probably led to undercounting of mild or asymptomatic cases, and the ensuing selection bias towards more severely ill patients. Finally, the cases included only represent the early COVID-19 epidemic. Therefore, the potential benefit of therapies that are now implemented more widely, such as remdesivir and convalescent plasma, have not been addressed.

In summary, among hospitalized SOTR with COVID-19, ICU admission and death rates were high, and they were similar to those reported in the general population.

Unfavorable outcomes were mainly driven by respiratory pathology (represented by a high breathing rate), older age, and two laboratory features at presentation, namely lymphopenia and elevated level of lactate dehydrogenase. An earlier post-transplant SARS-CoV-2 infection was established as a novel risk factor for ICU need and mortality. While this study provides preliminary indicators available upon hospital admission for identifying patients at risk of critical disease or death, it is an urgent priority to find efficacious antiviral treatments and to investigate the role of the immune response in COVID-19, especially in the population of SOTRs, where it is vital to guide suitable and prompt immunomodulatory management.

The analysis of the SARS-CoV-2 RNAemia and interferon types I and II, at the first clinical evaluation, shows that undetectable IFN-γ in serum, at hospital admission, is independently associated with 30-day all-cause mortality in COVID-19, specifically in the no SOT patients. In the SOT recipients there is a non-significant trend to higher mortality in the adjusted analysis, and the Kaplan-Meier survival analysis shows an association of undetectable IFN-γ with mortality. However, undetectable IFN-α in serum was not associated with mortality both in no SOT patients and SOT recipients. RNAemia at hospital admission is also a robust predictor of 30-day all-cause mortality in adult COVID-19 inpatients, and it´s

predictive ability of unfavourable clinical outcome persists when SOT recipients and no SOT patients are analyzed separately. Although CURB-65 score ≥2 predicts mortality in no SOT patients, this association does not persist after adjusting by PS. Finally, lesser than three and ten days from symptoms onset show non-significant trends to association with mortality and unfavourable outcome in no SOT patients and SOT recipients, respectively.

We found high IFN-α and IFN-γ serum levels at hospital admission in COVID-19 patients, when compared with healthy uninfected controls, as other authors[63,139]. Regarding IFN-γ serum levels, we did not observe differences depending on the DfSO, both in SOT and no SOT groups, which is consistent with other studies[59,71], which did not find differences in the expression of IFN-γ by specific CD8+ T-cells in acute disease and convalescent COVID-19 patients. At hospital admission, we also found more frequent RNAemia in SOT recipients with undetectable IFN-γ in serum, both factors associated with unfavourable clinical outcome in the adjusted analysis. In COVID-19 patients, a significant increase of intracellular IFN-γ expression by specific CD8+ T-cells, after stimulation with SARS-CoV-2 peptide pools, have been associated with viral elimination. A recent study[140], using an ELISpot technology to detect IFN-γ release from T-cells after exposure to four SARS-CoV-2 peptides, found an association between higher T-SPOT responses (especially with the Spike protein S1 domain) and increasing disease severity at the time of sampling, but without concluding whether these T-cell responses were protective or deleterious. The results of the present study, after adjusted analysis by PS, showing an association of undetectable IFN-γ in serum with mortality, strongly suggest the protective role of the specific T-cells response.

The relevance of impaired type I IFN activity has been proposed as a hallmark of severe COVID-19 through an extensive study of the inflammatory response and associated with persistent plasma SARS-CoV-2 RNAemia[61]. However, this study was carried out in only 11 and 21 patients with mild-to-moderate and severe/critical disease, respectively, and did not report information on survival. Other study in 50 patients, using a univariate analysis, found higher IFN-α plasma levels in patients with more severe disease[67]. It has been also reported that inborn errors of type I IFN immunity accounts for life-threatening COVID-19 pneumonia in 2.6% of women and 12.5% of men, after an analysis of differences in proportions[141]. The same authors, after analyzing 1,261 unvaccinated deceased patients and 34,159 individuals of the general population sampled before the pandemic, found that autoantibodies against type I IFNs increased the infection fatality rate, especially when neutralizing both IFN-α2 and IFN-ω[142]. In the present study, we have also included a cohort of SOT recipients, to know the IFN-α role in immunosuppressed patients. As it may be expected, SOT recipients had lower IFN-α serum levels than no SOT patients, as well as occurred among the SOT recipients when the disease was longer than six days. Also, at hospital admission we found higher disease severity and RNAemia frequency in SOT recipients and no SOT patients, respectively, with undetectable IFN-α in serum. However, after adjusting by demographics, chronic underlying diseases, symptoms and signs, and inflammatory variables, using a PS, we showed that undetectable IFN-α in serum was not associated with mortality in adult COVID-19 inpatients.

Patients with RNAemia more frequently have a critical rate score in the WHO score scale, lower lymphocyte count, and higher values of CRP and LDH, which have been previously identified as independent predictors of mortality in the general

population and in SOT recipients [8,34]. In the pairwise analysis, we did not find correlation between RNAemia viral load and IFN-α and IFN-γ serum levels, also confirming the data from other studies[139,143]. The RNAemia frequency in SOT recipients quadruplicated that in no SOT patients. Previously, only case reports of SOT[144] and hematopoietic stem cells transplant[145] recipients with RNAemia have been reported.

Other studies have showed that COVID-19 adult patients with RNAemia had higher mortality[46,48,139] and severe disease[47,146,147] than patients without RNAemia at hospital admission, after adjusting by different confounding variables. The samples sizes ranged from 61 to 199 patients, with RNAemia detection between 23% and 50.8%[46–48,139,147]. The present study analyzed a higher sample size, 455 patients, and includes 47 (10.3%) SOT recipients, a population in which the RNAemia impact had not yet been analyzed. The RNAemia rate in the present study was 22.9%, in the lower frequency of the reported range[47], and we proved that RNAemia was higher in SOT recipients than in the no SOT patients. Previous studies have found a relatively higher proportion of RNAemia in patients with active neoplasia, SOT, immunosuppression, and chronic liver disease in small cohorts of 41 to 72 patients and using non-adjusted analysis[53,148,149]. The mortality rate of 9.4% in our cohort is in the range of previous studies[46,139,147]. The high mortality (23%) found by Brunet-Ratnasingham[139], with RNAemia rate of 50.8%, may be biased by the inclusion of 47.5% critical patients in the small size derivation cohort. Because of our objective was to test the prognosis value of RNAemia at hospital admission, we did not address if the persistence of RNAemia during the admission was associated with higher mortality[50].

The main strength of the present study is that mortality, a robust endpoint, was chosen to test the main hypothesis, its association with SARS-CoV-2 RNAemia and interferon I/II mediated innate immune response at hospital admission. The multicenter design permitted to include a sample size of 455 participants and, for the first-time, including SOT recipients. The general population is well represented, according to age and sex, comorbidities, moderate to severe/critical COVID-19, need of IMV, and 30-day mortality. Finally, the multivariate analysis, adjusting for well-known variables associated with unfavourable outcomes, the PS-matched and other sensitivity analysis, allowed dragging robust conclusions. The study also presents limitations. The RNAemia viral load and the IFN-α and IFN-γ serum levels quantification was only performed at hospital admission, which precludes longitudinal analysis of variables such as SARSCoV-2 RNAemia and interferons, although we have introduced the days from symptoms onset as covariable. We did not analyze IFN-stimulated genes to define a I IFN signature[150] nor interferon autoantibodies, because of our purpose was to identify easy-to measure variables in the clinical setting.

In summary, the results of the present study prove that undetectable IFN-γ in serum, at hospital admission, is a predictor of 30-day all-cause mortality in adult COVID-19 inpatients. In the SOT recipient's cohort, there is a trend to unfavourable clinical outcome in patients with undetectable IFN-γ, and the survival analysis shows its association with mortality. However, undetectable IFN-α in serum was not associated with mortality both in no SOT patients and SOT recipients.

RNAemia at hospital admission is also a robust predictor of 30-day all-cause mortality in adult COVID-19 inpatients, and it´s predictive ability of unfavourable clinical outcome persists when SOT recipients and no SOT patients are analyzed separately. These data strongly support the inclusion of RNAemia and IFN-γ serum levels determinations, at hospital admission, in all adult COVID-19 patients, to guide their management and to assess the antiviral therapy efficacy. Finally, lesser than three and ten days from symptoms onset show non-significant trends to association with mortality and unfavourable outcome in no SOT patients and SOT recipients, respectively.

The main finding of the SARS-CoV-2 NP viral load study is that patients with SARS-CoV-2 infection, regardless of their illness severity, generally have a high rate of viral replication in the upper respiratory airways. Consequently, this parameter cannot be used as a predictor of COVID-19 unfavorable outcome, defined as admission to ICU and/or death. Moreover, this prospective cohort confirms that the independent risk factors for ICU admission or death are those previously identified by Salto Alejandre S. *et al.*[151] Thus, at first hospital evaluation, advanced age, hypoxemia, neutrophilia, and increased levels of LDH and CRP have high sensitivity and specificity to accurately discriminate patients that would potentially develop a critical disease from those with a favorable course.

The evidence to date reveals that the relationship between SARS-CoV-2 viral load and the pathogenicity and virulence of this microorganism is not fully understood. Furthermore, as there are many methods to perform the molecular detection of SARS-CoV-2 genome, the interpretation and comparison of results in literature is highly controversial. As an example, droplet digital PCR (ddPCR) has shown slightly higher sensitivity than standard RT-PCR[152].

Several previous studies have demonstrated that a high value of SARS-CoV-2 viral load in the upper respiratory tract (URT), defined as a Ct < 25 or < 22 in the RT-PCR, is an independent risk factor for respiratory failure[42], intubation, or death[40], using multivariate logistic regression and time-based analyses[41]. Pujadas et al., using a Cox proportional hazards model, also showed an independent association between viral load in the URT and mortality[39].

Such results have often led to the thought that viral load could be used along with other features to decide upon the need for hospital admission, and even that a stratification for baseline NP viral load would benefit the design of clinical trials. Nevertheless, other studies show contradictory results. Maltezou et al., using a Ct < 25 to define high URT viral load, reported an association between higher viral load and the development of COVID-19 disease, while no association was found with ICU admission, mechanical ventilation or death[43]. Amodio et al. demonstrated that the median PCR Ct was significantly lower in patients who died or needed critical care than in those who were hospitalized and discharged alive, or exclusively attended at home, but after adjusting for age and sex, there was not and independent association with critical care need or death[44].

Similarly, in our study, despite the patients with higher viral load (above the first tertile, the 50th percentile, and the second tertile) often belonged to the severe disease group, the adjusted multivariable model did not find an association between the copies per mL and the need for critical care or mortality. Argyropoulos et al., on the other hand, showed that viral load was inversely correlated with disease severity, being higher in patients with mild COVID-19[153]. The reason for this conflictive result was, however, that NP sampling in patients with severe or critical symptoms was obtained at a later time point in the disease course. Lastly, Lee *et al*. found that viral load quantification was similar among symptomatic and asymptomatic patients[154], and our results support this conclusion. Certainly, most patients in the present cohort who suffered an unfavorable outcome had a high SARS-CoV-2 viral load quantification (above the 50th percentile) at hospital admission, but half of the patients with mild COVID-19 also exceeded said limit. This corroborates that the number of virus copies is not strongly related to COVID-19 prognosis.

To further test our hypothesis, we stratified the patients according to disease severity at the end of follow up and having confirmed that the medium time from symptoms onset to diagnosis was similar among groups, we performed multiple comparations for each viral load cut-off value. Mild patients could only be distinguished through the first tertile, above which a small increment of moderate and severe cases was found. For higher viral load cut-off points, the probability of belonging to the mild or moderate group was similar. The percentage of patients having NP viral load over the 50th percentile and second tertile was significantly higher for those with severe COVID-19, and the univariable analysis showed that a viral load quantification over the mentioned levels could be a risk factor for ICU

admission or death. However, through the multivariable model, we concluded that a high viral load could not be used as an independent predictor of such outcomes.

Our study highlights several substantial issues. First, there is not a clear viral load cut-off point capable of discriminating between the various levels of COVID-19 severity, as the ROC Curve analysis demonstrated. Secondly and contrary to expectations, a higher number of SARS-CoV-2 copies in NP swabs at first patient's evaluation is not predictive of whether ICU admission or death might occur. Nevertheless, according to the Spanish nationwide seroepidemiological study, this finding should not be surprising: a third of the population with positive PCR was asymptomatic, and 20% of the seropositive symptomatic participants did not have previous SARS-CoV-2 genome detection[155]. Finally, through the external cohort validation of hypoxemia, neutrophilia, and increased levels of LDH and CRP as independent predictors of unfavorable outcome[151], we contribute to the identification of higher-risk patients with COVID-19 in whom suitable and prompt management is vital.

The main strength of the present study is that a wide spectrum of COVID-19 severity, from mild symptomatic to critically ill patients, is represented in the analyzed cohort, allowing novel conclusions to be drawn about the efficacy and predictive reliability of previously studied clinical factors. The study has also some limitations. The viral load quantification in the URT samples, through NP swabs, was only performed at a single time point, and we have not data on the dynamics according to the clinical outcomes. Additional synchronous and longitudinal sampling from other sources, such as blood or stools, would have been important comparators. Regarding the quantification of SARS-CoV-2 viral load, further

studies are required to refine the use of the standard and novel techniques, which is especially important due to variabilities in specimen collection, the lack of systematic quantification assays, and inconsistencies in protocols between different laboratories. Also, the lack of association of NP viral load with unfavorable outcome, should be confirmed when COVID-19 be caused by the new SARS-CoV-2 variants.

In summary, we found that higher values of SARS-CoV-2 viral load in NP samples at first hospital evaluation are more frequent in patients with unfavorable in hospital outcome, but that a high viral load is not an independent risk factor for ICU admission or death among adult patients with COVID-19.

The post hoc analysis of a multicenter cohort from the first wave of the COVID-19 pandemic was aimed to analyze 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. 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 (Clinical Trials.gov identifier: NCT04449380)[156].

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[157]; (ii) previous encouraging experience in mice and nonhuman primate models of MERS[158,159]; (iii) promising results in reducing mortality when combined with lopinavir–ritonavir and started within seven days after symptom onset[160]; and (iv) safety in patients with ARDS, in addition to long-term consolidated evidence of tolerability as an established treatment for multiple sclerosis[161,162].

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[163]. 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[164]. 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[165].

The WHO Solidarity Trial[166], 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 Kalil et al. did not show efficacy of INF-β combined with remdesivir compared to remdesivir alone concerning time to recovery[167]. 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, based on the WHO Ordinal Scale for Clinical Improvement, during the dosing period in the intention-to-treat population[168].

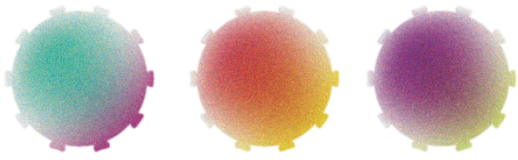
With this as background, we conducted a post hoc propensity score adjusted study of 3808 consecutive patients with moderate-to-severe COVID-19, investigating the effectiveness of subcutaneous INF-β treatment. In this observational study, we mimicked the assignment of patients to treatment arms and the 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 to avoid immortal time bias. We used a single robust primary outcome, mortality, because some 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[19,114,151], 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[109] or with neutralizing auto-Abs against type I IFNs[141] 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[169]. Other issues also must 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[170,171]. 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 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 statistical analyses, including the imputation of missing data using the Markov chain Monte Carlo method.

In conclusion, our findings did not find an association between early IFN-β therapy after hospital admission and any mortality benefit in patients admitted because of COVID-19. Additional data are needed for IFN-β administration at even earlier stages of the disease and in association with other drugs such as tocilizumab or corticosteroids. Finally, whether the drug would be useful specifically in patients with low IFN production needs to be investigated.

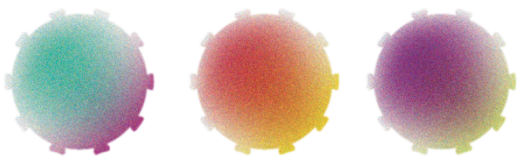


CONCLUSIONS

7. CONCLUSIONS

1. Among hospitalized SOTR with COVID-19, ICU admission and death rates were high, and they were similar to those reported in the general population.
2. Unfavorable outcomes were mainly driven by respiratory failure (represented by a high breathing rate), older age, and two laboratory features at presentation, namely lymphopenia and elevated level of lactate dehydrogenase, as inflammatory biomarker.
3. An earlier post-transplant SARS-CoV-2 infection was established as a novel risk factor for ICU need and mortality.
4. Undetectable IFN-γ in serum, at hospital admission, is a predictor of 30-day all-cause mortality in adult COVID-19 inpatients.
5. In SOTRs, there is an association between undetectable IFN-γ and unfavorable clinical outcomes.
6. RNAemia at hospital admission is the most robust predictor of 30-day all-cause mortality in all adult COVID-19 inpatients.
7. RNAemia and IFN-γ serum levels determinations at hospital admission should be used to guide the management of patients and to assess the antiviral therapy efficacy.
8. Higher values of SARS-CoV-2 viral load in NP samples at first hospital evaluation are more frequent in patients with unfavorable in hospital outcome, but they are not an independent predictor of ICU admission or death among adult patients with COVID-19.

9. The administration of early IFN-β therapy after hospital admission does not have a survival benefit in patients with moderate-to-severe COVID-19. Whether the drug would be useful specifically in patients with low IFN production needs to be investigated.



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