Benefits of early aggressive immunomodulatory therapy (tocilizumab and methylprednisolone) in COVID-19: Single center cohort study of 685 patients

Buzon-Martín Luis [a,*], Montero-Baladía Miguel [b], Delgado-López Pedro [c], Iglesias-Posadilla David [b], Astigarraga Itziar [d], Galacho-Harriero Ana [a,c], Iglesias-Julián Enrique [e], López-Veloso María [e], De La Torre-Ferrera Noelia [e], Barraza-Bengoechea Julio César [e], Ubeira- Iglesias Marta [f], San Llorente- Sebastián Rodrigo [g], Colazo-Burlato María [h], Lorenzo-Martín Andrés [h], Minguito de la Iglesia Javier [i], García-Muñoz Juan Pablo [i], Hermida-Fernández Gerardo [j], Navarro-San Francisco Carolina [a], Boado-Lama Jorge [k], Fernández-Regueras María [a], Callejo-Torre Fernando [b], Ossa-Echeverri Sergio [b], Fisac-Cuadrado Lourdes [b], Gero-Escapa María [b], Megías-Lobón Gregoria [l], Simón-Rodríguez Adolfo [i], Fernández-Ratero José Antonio [b], on behalf of HUBU-COVID-19 group

[a] Infectious Diseases Unit, Internal Medicine, Hospital de Burgos, Spain
[b] Intensive Care, Hospital de Burgos, Spain
[c] Neurosurgery, Hospital de Burgos, Spain
[d] Pediatrics, Biocruces Bizkaia Health Research Institute, Hospital Universitario de Cruces, University of the Basque Country UPV/EHU, Baracaldo, Spain
[e] Systemic Autoimmune Diseases, Internal Medicine Department, Hospital de Burgos, Spain
[f] Pharmacy Department, Hospital de Burgos, Spain
[g] Anesthesiology Department, Hospital de Burgos, Spain
[h] Reumatology Department, Hospital de Burgos, Spain
[i] Pneumology Department, Hospital de Burgos, Spain
[j] Hematology Department, Hospital de Burgos, Spain
[k] Internal Medicine, Hospital de Burgos, Spain
[l] Microbiology Department, Hospital de Burgos, Spain

ARTICLE INFO

ABSTRACT

Introduction: A growing evidence suggests that immune dysregulation and thrombotic phenomena are key features in the pathophysiology of COVID-19. Apart from antivirals and respiratory support, anticoagulants, corticoids and immunomodulators are increasingly being prescribed, especially for more severe cases. We describe the clinical outcome of a large cohort of patients preferentially treated with glucocorticoids and interleukin inhibitors.
Methods: Single center and retrospective case series. Adult patients admitted with COVID-19 related respiratory insufficiency were included. Patients who died within 2 days after admission and those testing positive but asymptomatic were excluded. We defined two study periods: from March 3rd to March 31 st, 2020 (beginning of epidemic until peak of incidence) and April 1 st to May 7 th, 2020 (second half of epidemic). The majority of patients received respiratory support, combinations of antimicrobials, anticoagulants, corticoids and interleukin inhibitors. Antivirals were preferentially given in the first period. The clinical outcome (death and ventilator dependency) of both periods was compared.
Results: From March 3 rd to May 7 th, 685 patients were included for analysis (58.4% males, mean age 68.9 years). Patients in the first period (n = 408) were younger (66.6 vs 71.1 years, p = 0.003), presented lower mean P_aO_2/F_iO_2 ratio at admission (256.5 vs 270.4 mm Hg, p = 0.0563), higher ferritin (1520 vs 1221 ng/ml, p = 0.01), higher IL-6 (679 vs 194 pg/ml, p < 0.0001) and similar D-dimer levels (3.59 vs 3.39 μg/mL, p = 0.65) compared

to the second period (n = 277). Lopinavir/ritonavir and interferon were preferentially given in the first period (23.8% and 32% vs 1.8% and 11.9%, p < 0.0001). Use of corticoids (88.2% vs 87.4%, p = 0,74) and tocilizumab (26.29 vs 20.22% p = 0.06) were similarly administered in both periods. Patients in the second period needed less mechanical ventilation (4.9% vs 16.9%, p < 0.0001), fewer ICU admission (6.1% vs 20.1%, p < 0.0001) and showed similar mortality (17.7% vs 15.4%, p = 0.43). Infectious and thrombotic complications were comparable in both periods (both around 8%, with no statistical difference). Patients treated with tocilizumab (n = 163) had lower mortality rate compared to those untreated under the same indication (7.9% vs 24.2%, p < 0.0001).

Conclusions: In this large retrospective COVID-19 in-hospital cohort, lopinavir/ritonavir and interferon showed no significant impact on survival. Extensive use of corticosteroids and tocilizumab resulted in good overall outcome and showed acceptable complication rates.

1. Introduction

COVID-19 pandemic is a worldwide concern affecting all countries with different impact depending on the timing and aggressiveness of implementation of public health measures [1,2]. Overall mortality rate for moderate and severe cases exceed 15%, with some series reporting over 25% [3–7]. Among those admitted to intensive care units (ICU) and requiring mechanical ventilation, mortality can reach 50–70% [3,4,6,7].

A growing evidence suggests that immune dysregulation and thrombotic phenomena are key features in the pathophysiology of moderate and severe SARS-CoV-2 infection [4,8–11]. A certain state of systemic hyperinflammation has been observed in many patients that present rapid respiratory impairment and ventilator dependency [9, 11–13]. A percentage of these may also develop a characteristic massive cytokine release with markedly increased immune inflammatory markers [4,8,11]. In this context, a rationale for immunomodulatory therapy has been proposed [8,10,11,14].

In fact, the use of corticoids and other immunomodulators in COVID-19 has been controversial [14–18]. Initially most guidelines strongly recommended against the use of glucocorticoids and other immuno-modulatory agents outside clinical trials, alluding a theoretical prolonged viral shedding and other complications [19–21]. However, in the last months, substantial evidence favoring the administration of corticoids [7,22–25] and interleukin inhibitors [26–31] has been published.

According to our particular epidemic scenario, we readily observed that patients under corticoid therapy and those treated with interleukin inhibitors seemed to fare better than those under regular antiviral and antibiotic combination schemes. Additionally, we prescribed prophy-lactic anticoagulation [32] as part of the treatment protocol early in the course of the epidemic. We hypothesized that therapy based on the combination of anti-inflammatory (mainly corticoid therapy) and anti-coagulation would result in fewer ventilation dependency and ICU admission, and lower complication and mortality rates.

Therefore, we reviewed the clinical course and outcome of a large cohort of COVID-19 patients admitted to our tertiary center. These pa-tients were preferentially treated with respiratory support and combi-nations of drugs according to actuarial evidence and international recommendations [19–32]. Yet, the great majority received methyl-prednisolone and enoxaparin. Additionally, 163 patients were treated with IL-6 inhibitor tocilizumab. We compared the clinical outcome of patients before and after the peak incidence of the epidemic curve. An-tivirals were used only in the first half of the epidemic. In this case series review we aimed to provide evidence on the effectiveness and safety of immunomodulatory therapy regarding the need for mechanical ventila-tion, requirement of ICU stay, and overall mortality.

2. Patients and methods

2.1. Study design and inclusion criteria

We conducted a retrospective case series at the University Hospital of Burgos, Spain, between March 3 and May 7, 2020. Our center is a tertiary hospital with 700 beds (26 ICU beds) for a catchment area of 350,000 people. The study was approved by the local Ethics Committee (CEIm reference number: 2315) and informed consent was obtained from all participants.

Patients were included if they were 18 years or older and were admitted presenting COVID-19 related respiratory insufficiency upon clinical and blood gas parameters. Patients who died within 48 h of admission and those testing positive but asymptomatic were excluded. Confirmation of SARS-CoV-2 infection was obtained from nasopharyn-geal swabs and PCR analysis A proportion of patients that exhibited clinical and laboratory findings compatible with COVID-19 but failed to test positive, also received treatment and were included in the study.

The primary endpoint was to evaluate the clinical outcome (need for mechanical ventilation or death) of the cohort. Two study periods were defined: from March 3 to March 31, 2020 (beginning of epidemic until peak of incidence) and from April 1 to May 7, 2020 (second half of epidemic). The cutoff was established on April 1st based on the peak incidence and the shift in the treatment protocol. The majority of patients received ventilatory support, combinations of antimicrobials, anticoag-ulants, corticoids and interleukin inhibitors. Antivirals were preferen-tially given in the first period. The study was designed and conducted by a multidisciplinary in-hospital group (including physicians belonging to critical care, infectious diseases, internal medicine, pneumology, hema-tology units and other specialists) specifically created for the COVID-19 epidemic.

2.2. Data and statistical analysis

Quantitative variables are shown as mean (SD) or median (IQR), and qualitative variables as proportions. Fisher's t-test or Mann-Whitney's U test was used to compare the groups based on the distribution of quan-titative variables. Pearson's chi-square ($\chi2$) test or Fisher's exact test was used for the proportion comparison. The primary outcome was defined as a compound of all-cause mortality and the need for invasive mechanical ventilation. Patients received invasive mechanical ventilation according to P_aO_2/F_iO_2 ratio and clinical parameters like tachypnea, altered res-piratory function (tachypnea >30 with increased respiratory effort despite oxygen supplement or non-invasive mechanical ventilation (NIMV), and use of ancillary musculature) and/or hemodynamic insta-bility, with or without impairment in the level of consciousness.

A single-time logistic regression test was performed, setting the combined event death or mechanical ventilation as a dependent variable; subsequently, a multiple logistic regression was performed including all variables showing statistical significance (or a tendency to statistical significance with a value of p < 0.2) in the univariate analysis. Addi-tionally, a survival analysis was performed using the Cox univariate and multivariate regression method and Kaplan-Meier curves, by comparing the time to the need for invasive mechanical ventilation or death in both groups. The results are expressed as hazard ratio (HR) with 95% confi-dence interval. Comparing tocilizumab, the endpoint was only mortality. The statistical analysis was carried out with the statistical package Stata/ IC 16.1 (College Station, TX 77845) and user-written commands. p-value was considered as statistically significant at <0.05.

Table 1
Main clinical characteristics of the COVID-19 cohort.

N (participants)	685
Age (years)	68,89 (±16,12)
Sex (male)	58,39%
History of hypertension	46,71%
Pulmonary disease history	77,81% no pulmonary disease
	9,49% COPD
	2,48% emphysema
	10,22% other respiratory disease
Smoker	13,93%
PaO2/FiO2 at admission	262,22 (±90,22) mm Hg
range	47–470
>300 mm Hg	36,48%
200–300 mm Hg	41,98%
100–200 mm Hg	16,51%
<100 mm Hg	5,03%
PCR SARS-CoV-2	87,01% positive
	2,04% negative
	10,94% negative, but received treatment
Pulmonary CT performed	16,35%
Pulmonary affectation	Unilateral 26,41%
	Bilateral 73,59%
Maximum Ferritin (ng/L)	957 (IQR 1314)
<500	26,60%
500–1000	25,12%
>1000	48,28%
Minimum Lymphocyte count (/μL)	791 (±539)
IL-6 levels (pg/mL)	40 (IQR 155)
<50	53,44%
50–100	14,96%
>100	31,59%
Minimum platellet count (x1000/μL)	194 (±102)
Maximum GPT (UI/L)	51 (IQR 72)
Maximum LDH (UI/L)	333 (IQR 187,5)
<250	20,91%
250–500	60,91%
>500	18,18%
Maximum Troponin (ng/L)	17 (IQR 31)
D-Dimer (μg/mL)	1,4 (IQR 2,4)
D-Dimer (μg/mL) categorized	
<0,5	12,91%
0,5-3,5	62,52%
>3,5	24,57%
Median hospital stay	11 (2–72)
ICU admission	103 (15.0%)
ICU intubation	83 (12.1%)
ICU stay	14 (8–31)

2.3. Treatment protocol

At the beginning of the COVID-19 epidemic (March 2nd, 2020), the standard of care in our institution for patients presenting with hypoxemia included respiratory support, lopinavir/ritonavir (LPV/r), azithromycin, hydroxychloroquine, enoxaparin, interferon 1-β and methylprednisolone. Methylprednisolone dosage varied up to the physician in charge: internists preferentially prescribed a 3-day 250 mg bolus scheme followed by tapering dose, while pneumologists tended to prescribe methylprednisolone at 1 mg/kg of body weight. Ceftriaxone was also prescribed upon physician discretion. By the end of March 2020, LPV/r was abandoned due to the lack of efficacy reported [33] and the presence of troublesome pharmacokinetic interactions, especially among critically ill patients. At that time, IL-6 inhibitor tocilizumab was added to the protocol aimed at patients meeting ARDS criteria [34] and biochemical alterations suggestive of severe systemic inflammation (ferritin levels >1000 ng/ml and/or IL-6 values > 50 pg/ml). IL-1 inhibitor anakinra was also used in patients meeting ARDS criteria and biochemical alterations suggesting severe systemic inflammation but with IL-6 levels less than 50 pg/ml and/or 4-fold blood ALT levels over the normal upper limit.

Etoposide was offered to patients that, despite having received boluses of 250 mg of methylprednisolone for three days plus tocilizumab or anakinra, kept on deteriorating their PaO2/FiO2 ratios and PCO2 values [35]. Orotracheal intubation, mechanical ventilation and prone positioning were applied when necessary according to the course of respiratory function. Given that, since the beginning of the study period, there was evidence that severe COVID-19 infection predisposed to thrombosis, the great majority of patients received prophylactic enoxaparin (40 mg per day for 14 days). Therapeutic anticoagulation was initiated when thrombotic complications appeared and/or at the physician's discretion in patients with high risk of thrombosis according to the clinical and laboratory findings.

3. Results

Within the study period (March 3rd to May 7th, 2020) a total of 1205 patients tested positive for PCR SARS-CoV-2 in our center. After excluding mild and asymptomatic patients and those under 18 years, 685 COVID-19 patients were included in the study (58.4% males, mean age.
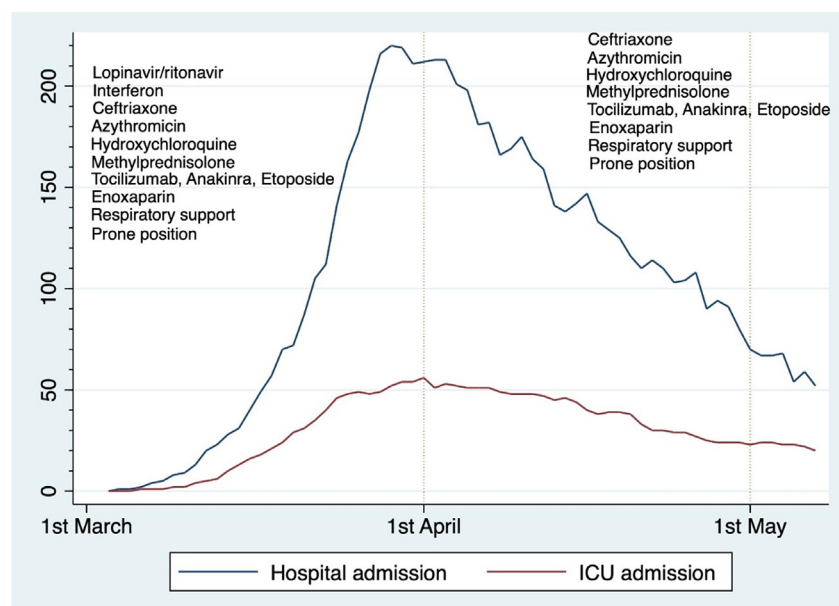

Fig. 1. Evolution of hospital and ICU admission during the pandemic, distinguishing both periods. The list of treatments represents the medications and theraoies used in the first (left)and second (right)periods, respectively.

68.9 ± 16.1 years). The main clinical characteristics of the cohort are detailed in Table 1. More than 45% of the cohort had a history of hypertension, and 63.5% of patients presented with a P_aO_2/F_iO_2 ratio <300. Nearly half of patients showed maximum ferritin levels>1000 ng/ml and IL-6 levels >50 pg/ml. Average D-dimer level was 1.4 µg/mL. For comparison, we subdivided patients in two groups: those admitted before (first period, n = 408) and after (second period, n = 277) the peak of the local epidemic.

Although our treatment protocol varied throughout the study period, according to emerging evidence and international recommendations, the entire cohort was treated with a relatively homogeneous protocol in both periods, with the exception of lopinavir/ritonavir and interferon, which were abandoned in the second period. Fig. 1 depicts the epidemic curve

in our center, and the treatment scheme preferentially used in the two periods.

The similarities and differences of the two periods are shown in Table 2. Patients in the first period were slightly younger (66.5 vs 71.1 years, p = 0.003) and presented with higher ferritin levels (1520 vs 1221 ng/ml, p = 0.017), more profound lymphopenia (711 vs 909, p < 0.0001), and higher IL-6 levels (679 vs 194 pg/ml, p < 0.0001). However, P_aO_2/F_iO_2 ratio at presentation (256 vs 270 mm Hg, p = 0.0563) and D-dimer levels (3.59 vs 3.39 µg/mL, p = 0.65) were similar. Non-invasive ventilation was preferentially used in the first period (16.7% vs 6.49%, p = 0.0002). Noticeably, treatment modalities were equally distributed in the two periods except for lopinavir/ritonavir and interferon that were used almost exclusively in the first period (23.8% and

Table 2
Clinical and demographic variables in the two periods.

	First period (N = 408)	Second period (N = 277)	Difference	P
Age	66.57 (±15.51)	71.09 (±16.65)	−4.52 (−6.96 to −2.08)	0.003
Age categorized				
<50 years	15.20%	11.91%		0.106
50–70 years	38.73%	33.94%		
>70 years	46.08%	54.15%		
Age >70 years	80.03 (±7.13)	84.45 (±7.03)	−4.43 (−5.95 to −2.90)	<0.0001
Female	157 (38.48%)	128 (46.20%)	7.73 (0.2–15%)	0.0441
Duration of symptoms prior to admission (days)	7.01 (±4.99)	7.82 (±7.28)	−0.81 (−1.76 to 0.15)	0.096
Hypertension	43.87%	50.90%	−7.03%	0.07
No prior pulmonary disease	311 (76.23%)	222 (80.14%)		0.278
COPD	37 (9.07%)	28 (10.11%)		
Emphysema	11 (2.70%)	6 (2.17%)		
Other respiratory disease	49 (12.01%)	21 (7.58%)		
Smoker	62 (15.23%)	33 (12%)	3.23% (−2.08 to 8.5)	0.23
P_aO_2/F_iO_2	256.53 (±93.53)	270.41 (±84.72)	−13.88 (−28.12 to 0.37)	0.0563
pCO_2	34.42 (±7.55)	36.27 (±8.20)	−1.85 (−3.10 to −0.61)	0.0036
PCR negative	5 (1.23%)	9 (3.25%)		0.014
PCR positive	367 (89.95%)	229 (82.67%)		
PCR negative but received treatment	36 (8.82%)	39 (14.08%)		
Chest CT performed	56 (13.73%)	56 (20.22%)		0.024
Bilateral pneumoniae	77%	68.61%		0.0153
Ferritin (maximum)	1520 (±93.77)	1221 (±78.55)	300 (53.19–546)	0.0173
Ferritin categorized (ng/mL)				
<500	23.88%	29.93%		0.139
500–1000	24.48%	25.91%		
>1500	51.64%	44.16%		
Lymphocyte count (minimum)	711 (±23)	909 (±38)		<0.0001
IL-6 (maximum)	679 (±126)	194 (±46)	484	<0.0001
IL-6 categorized (pg/mL)				
<50	46.93%	58.26%		0.002
50–100	12.29%	16.94%		
>100	40.78%	24.79%		
Platelet count (maximum)	187 (±5.37)	204(±5.52)	−16.19	0.0421
GPT	123 (±19.69)	68 (±5.45)	54	0.0246
LDH	412 (±14.69)	360 (±17.88)	52.15	0.0243
Troponin	106.63 (±54)	90(±50)	16.32	0.8329
D-Dimer	3.59 (±0.294)	3.39(±0.354)	0.20	0.6564
D-Dimer categorized (µg/mL)				
<0.5	10.03%	17.19%		0.028
0.5–3.5	65.17%	58.59%		
>3.5	24.80%	24.22%		
Ceftriaxone	83.74%	77.97%	5.76%	0.0574
Azythromycin	95.57%	97.11%	1.53%	0.3036
Hydroxychloroquine	89.18%	87.72%	1.46%	0.5551
Lopinavir/ritonavir	23.82%	1.80%	22.02%	<0.0001
Interferon	32.01%	11.91%	20.82%	<0.0001
Corticoids	88.21%	87.36%	0.84%	0.74
No corticoids	11.79%	12.64%		0.026
Methylprednisolone (Bolus)	55.28%	45.13%		
Methylprednisolone (mg/kg)	32.92%	42.24%		
Tocilizumab	26.28%	20.21%		0.0674
Etoposide	1.47%	2.88%		0.2004
Anakinra	0.2457%	1.01%		0.0029
No anticoagulants	7.37%	4.69%		0.322
Prophylactic anticoagulants	61.67%	65.34%		
Therapeutic anticoagulants	30.96%	29.96%		

Table 3
Clinical outcomes of patients in the two periods.

	First period (N = 408)	Second period (N = 277)	Difference	p
Need for non-invasive respiratory support	16.18%	6.49%	9.68%	0.0002
Days under non-invasive support	4.15	6.28	−2.12	0.0152
Mechanical ventilation	16.91%	4.69%	12.22%	<0.0001
ECMO	0.241%	0.361%		0.7826
Nosocomial pneumoniae	1.71%	4.33%		0.0408
Secondary bacteriemia	2.69%	1.44%		0.2725
Bacteriemia associated to catheter	2.69%	0		0.0058
Pneumonia associated to mechanic ventilation	1.47%	0.72%		0.3715
Urinary tract infection	2.45%	1.80%		0.5715
Overall infectious complication rate	8.08%	6.85%		0.5518
Overall thrombotic complication rate				
Pulmonary embolism	3.19%	3.61%		0.946
Arterial embolism	0.98%	0.72%		
Venous embolism	0.74%	0.36%		
ARDS	73.95%	68.59%		0.1264
ICU admission	20.09%	6.13%		<0.0001
Death	15.44%	17.68%		0.4356

32% vs 1.8% and 11.9%, respectively, p < 0.0001. Methylprednisolone, either in bolus or mg/kg dosage, were widely prescribed in both periods (360 patients, 88.1% vs 243 patients, 87.4%, p = 0.026). Tocilizumab was administered in 107 patients, 26.3% and 56 patients, 20.2%, respectively. Anakinra (n = 9) and etoposide (n = 14) were selectively offered to more severe cases, resistant to tocilizumab. Etoposide was used in severely ill patients not responding to interleukin inhibitors that showed laboratory findings similar to that of secondary HLH. The great majority of patients received anticoagulation therapy in both periods (92.7% vs 95.4%, p = 0.322).

Table 3 shows the clinical outcome of patients in both periods. Interestingly, patients in the second period needed less mechanical ventilation (4.69% vs 16.91%, p < 0.0001), and ICU admission (6.1% vs 20.1%, p < 0.0001). Yet, mortality rate was similar in both periods (15.4% vs 17.7%, p = 0.435), as well as overall infectious and thrombotic complication rates.

A total of 163 patients were treated with tocilizumab plus methyl-prednisolone. As shown in Table 4, 66 (40.5%) were admitted to the ICU, 54 required mechanical ventilation (33.13%),and 13 patients died (7.98%).

Overall, there were 83 episodes of infectious complication in 39 patients, 90% of which occurred at the ICU. The survival analysis showed that the hazard risk of death or need for mechanical ventilation was slightly increased in the first period, yet without statistical significance (Fig. 2).

After multiple regression analysis, our model has an AUC = 0.9274. Tocilizumab showed a tendency to be protective for the combined endpoint, with an OR 0.35 without statistical significance (CI95% 0.10–1.20, p = 0.11), but when only mortality was analyzed, tocilizumab shows an OR 0.21 (0.0566–0.7535, p = 0.017). Even in Cox multiple regression showed HR 0.35 (CI95% 0.14–0.90, p = 0.03).

4. Discussion

Since the early reports from China, it was clear that throughout the SARS-CoV-2 pandemic, ICU admission and mechanical ventilation requirements were key issues to acknowledge and deal with [3,4]. Subsequent worldwide expansion of the virus led to overwhelming of ICUs in many countries and the need for ICU beds and ventilators dramatically

Table 4
Clinical characteristics and outcomes of patients treated and not treated with tocilizumab throughout the study period.

	Not treated with tocilizumab (n = 211)	Treated with tocilizumab (n = 163)	P
Age	78.5 (62.5–87.5)	64.5 (57.5–72.5)	<0.0001
<50 years	8.06%	13.61%	<0.0001
50–70 years	29.38%	55.10%	
>70 years	62.56%	31.29%	
Female	35.5%	20.40%	0.002
Duration of symptoms prior to admission (days)	7.35	7.90	<0.0001
Overall stay (days)	10 (range, 7–17)	17 (range, 10–27)	<0.0001
ICU stay (days)	20 (range, 10–50)	13.5 (range, 10–30)	0.944
History of hypertension	54.03%	48.97%	0.343
Prior pulmonary disease			
No	80.57%	85.71%	0.011
COPD	8.53%	2.72%	
Emphysema	0.95%	4.76%	
Others	9.95%	6.80%	
Smoker	13.80%	17.01%	0,4081
P_aO_2/F_iO_2 at admission (mm Hg)	255.14 (±84,05)	208.06 (±86,78)	<0.0001
>300	34.02%	16.20%	<0.0001
200–300	44.33%	41.55%	
100–200	18.04%	28.87%	
<100	3.61%	13.38%	
pCO_2 (mm Hg)	35.38 (±8.73)	33.36 (±7.29)	0.0263
Performance of chest CT	16.58%	32.65%	0.0004
Ferritin, maximum (ng/mL)	1415 (1054–2102)	1936 (1246–2682)	<0.0001
<500	7.66%	3.40%	0.023
500–1000	14.35%	7.48%	
>1000	77.99%	89.12%	
Lymphocyte count, minimum	600 (400–900)	500 (400–700)	0.007
IL-6, maximum (pg/mL)	60.88 (26.1–114.4)	299.25 (68.06–980)	<0.0001
<50	41.61%	20.31%	<0.0001
50–100	30.43%	10.94%	
>100	27.95%	68.75%	
Platelet count, minimum (1000/μL)	176,000	172,000	0.578
GPT (UI/L)	54 (30–98)	113 (64–188)	<0.0001
LDH (UI/L)	380 (302–483)	421 (338–577)	0.046
<250	10.19%	4.83%	0.003
250–500	67.69%	57.93%	
>500	21.84%	37.24%	
Troponin (ng/L)	33.5 (14–79)	15.5 (8–29,5)	<0.0001
D-Dimer (μg/mL)	1.6 (0.9–3.8)	2.4 (1–7.8)	0.027
<0,5	7.35%	6.34%	0.014
0,5-3,5	64.22%	50%	
>3,5	28.43%	43.66%	
Non-invasive mechanical ventilation (NIMV)	5.21%	37.41%	<0.0001
Days under NIMV	4.81 (±2.56)	4.38 (±3.29)	0.6795
Invasive mechanical ventilation (IMV)	11.37%	36.05%	<0.001
Days under IMV	20 (10–36)	12 (8–27)	0.515
Ceftriaxone	83.41%	93.19%	0.006
Azithromycin	96.21%	100%	0.023
Hydroxychloroquine	85.31%	98.64%	<0.0001
Lopinavir/ritonavir	12.80%	12.93%	0.547
Interferon 1-beta	22.27%	31.29%	0.06
Methylprednisolone			
No	10.90%	0.68%	<0.0001
Bolus	47.39%	87.76%	
mg/kg	41.71%	11.56%	
Enoxaparin			
No	3.32%	0%	
Prophylactic	64.93%	40.14%	
Therapeutic	31.75%	59.86%	
Overall infectious complication rate	13.27%	12.93%	0.528
Thrombotic complication rate			

Table 4 (*continued*)

	Not treated with tocilizumab (n = 211)	Treated with tocilizumab (n = 163)	P
No	95.26%	91.16%	0.278
Pulmonary embolism	3.32%	6.12%	
Arterial complications	0.95%	0.68%	
Venous complications	0.47%	2.04%	
ARDS	76.78%	93.20%	<0.0001
ICU requirement	12.80%	40.5%	<0.0001
Mortality rate	24.17%	7.98%	<0.0001

increased in a short period of time [1,2,5,6,36,37]. In our center, some operating rooms and post-surgery recovery units had to be rapidly transformed into new ICU facilities by mid-March 2020, when up to 56 ICU beds and ventilators were required. At that point, SARS-CoV-2 positive patients occupied 80% of the remaining hospital beds.

At the beginning of the epidemic, sound evidence on the effectiveness of the various treatment options lacked. However, pathophysiologic research studies suggested that immune dysregulation was a key feature in the clinical course of moderate and severely ill COVID-19 patients [8–11]. Therefore, we hypothesized that targeting immune dysregulation would result in fewer need for ventilator and lower mortality.

4.1. Hyperinflammatory response in severely ill COVID-19 patients

There is growing evidence showing that COVID-19 infection is a biphasic disease [11]. The initial stage, at which pre-symptomatic or pauci-symptomatic patients exhibit a preliminary and reversible state of immune-suppression associated to the viral load, ideally benefits from antivirals. To date, no specific antiviral drugs, including LPV/r and remdesivir, have proven effective for the treatment of patients with severe COVID-19 in terms of reduction of mortality or requirement of mechanical ventilation [33,38]. The combination of LPV/r, interferon β-1b and ribavirin was safe and superior to lopinavir–ritonavir alone in alleviating symptoms and shortening the duration of viral shedding and hospital stay, only in patients with mild to moderate COVID-19 infection, yet not in severe cases [39].

Ideally, effective antivirals capable of inhibiting SARS-CoV-2 replication should be effective at the first stage of the disease, and likely thereafter, when the dysregulated immune response predisposes to severe multiorgan damage [9–11]. Unfortunately, as of August 30th, 2020, we lack highly efficient antivirals against SARS-CoV-2 [33,38,39]. In our experience, there was no difference in mortality between the two periods of the first wave, in which the only difference in terms of drug treatment was the use of lopinavir/ritonavir plus interferon 1β. Etoposide was used in severely ill patients not responding to interleukin inhibitors that showed laboratory findings similar to that of secondary HLH. In fact,
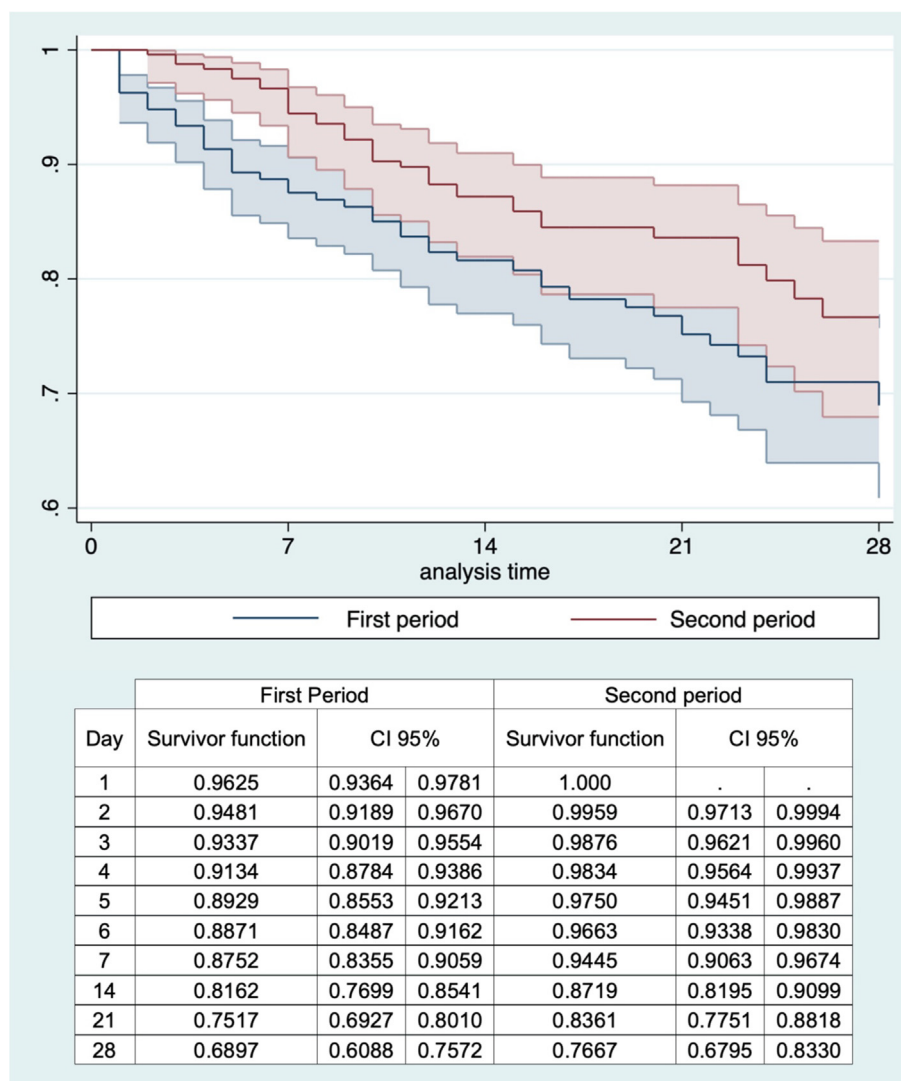

	First Period			Second period		
Day	Survivor function	CI 95%		Survivor function	CI 95%	
1	0.9625	0.9364	0.9781	1.000	.	.
2	0.9481	0.9189	0.9670	0.9959	0.9713	0.9994
3	0.9337	0.9019	0.9554	0.9876	0.9621	0.9960
4	0.9134	0.8784	0.9386	0.9834	0.9564	0.9937
5	0.8929	0.8553	0.9213	0.9750	0.9451	0.9887
6	0.8871	0.8487	0.9162	0.9663	0.9338	0.9830
7	0.8752	0.8355	0.9059	0.9445	0.9063	0.9674
14	0.8162	0.7699	0.8541	0.8719	0.8195	0.9099
21	0.7517	0.6927	0.8010	0.8361	0.7751	0.8818
28	0.6897	0.6088	0.7572	0.7667	0.6795	0.8330

Fig. 2. Survival analysis and kaplan-Meier curves by period. Endpoint: death or need for mechanical ventilation.

when these two agents were abandoned, mortality rate did not change, in line with previous studies reporting unclear in vivo efficacy in severely ill COVID-19 patients [33].

Hydroxychloroquine was the first drug specifically directed to the virus according to the study by Raoult et al. [40]. However, subsequent reports and clinical trials failed to demonstrate a clear benefit, leading to its withdrawal from many ongoing clinical trials [7,41,42]. As we used hydroxychloroquine equally in both periods, no statements can be made regarding its impact in our cohort.

4.2. Corticosteroids and tocilizumab as the cornerstone of aggressive immune downregulation in severely ill COVID-19 patients

Corticosteroid use in COVID-19 patients has been a controversial issue since the beginning of the pandemic. Initially, the World Health Organization (WHO) recommended against the routine use of corticosteroids for treatment of viral pneumonia outside clinical trials [2,19]. Contrarily, the *Corticosteroid Guideline Task Force of the Society of Critical Care Medicine (SCCM)* and the *European Society of Intensive Care Medicine (ESICM)* issued guidelines supporting the use of glucocorticoids in critically ill patients including those with ARDS [20]. They found moderate quality/certainty of evidence for a reduction in the duration of mechanical ventilation and improved overall survival [43]. Thus, a rationale for the prolonged use of corticosteroids in COVID-19 related ARDS was initially proposed [23]. Further evidence supported the use of glucocorticoids in moderate and severely ill COVID-19 patients [7,17,18,25], suggesting that dexamethasone or methylprednisolone would reduce mortality in the more severe cases. The recent publication by Fadel et al. [5], showed that an early short course of methylprednisolone in patients with moderate to severe COVID-19 infection reduced the escalation of

care and reduced mortality compared to late corticoid administration (34.9% vs. 54.3%, p = 0.005). A significant reduction in median hospital stay was also observed in the early corticosteroid group (8 vs. 5 days, p < 0.001). More recently, the preliminary data from the RECOVERY trial demonstrated a statistically significant reduction of mortality at 28 days (21.6% vs 24.6%, p < 0.001) in patients allocated to dexamethasone (6 mg per day) versus those treated with standard of care [7]. In fact, at present, dexamethasone is the only drug showing evidence for a reduction in mortality compared with the standard of care according to randomized clinical trials.

Upon previous research [8,9,11], we realized that immune dysregulation and systemic inflammation played a relevant role in promoting multi-organ damage of severe COVID-19. Therefore, we prescribed glucocorticoids on a regular basis to patients with respiratory failure (P_aO_2 <60 mmHg) and/or bilateral lung infiltrates, rapid imaging progression, and severe inflammatory response. We did not find differences in outcome regarding dosage and both types (bolus and maintenance) resulted beneficial. Further studies are needed to elucidate this point and the impact of other potential confounders. Although we cannot compare the impact of glucocorticoids between the two periods, the mortality rate in our cohort is lower than that of comparable series from other institutions [36,37], supporting the idea that early aggressive glucocorticoid therapy is feasible and effective. In fact, the mortality rate in our cohort (16,35%) is significantly lower than that reported by the conductors of the RECOVERY Trial (22,9%) [7].

Off-label use of tocilizumab was proposed since the beginning of the pandemic upon reports from China and Italy, which suggested a benefit among patients exhibiting severe systemic inflammation (with elevated levels of plasma ferritin and IL-6) and respiratory failure [26,27]. These preliminary reports were further supported by new evidence from case
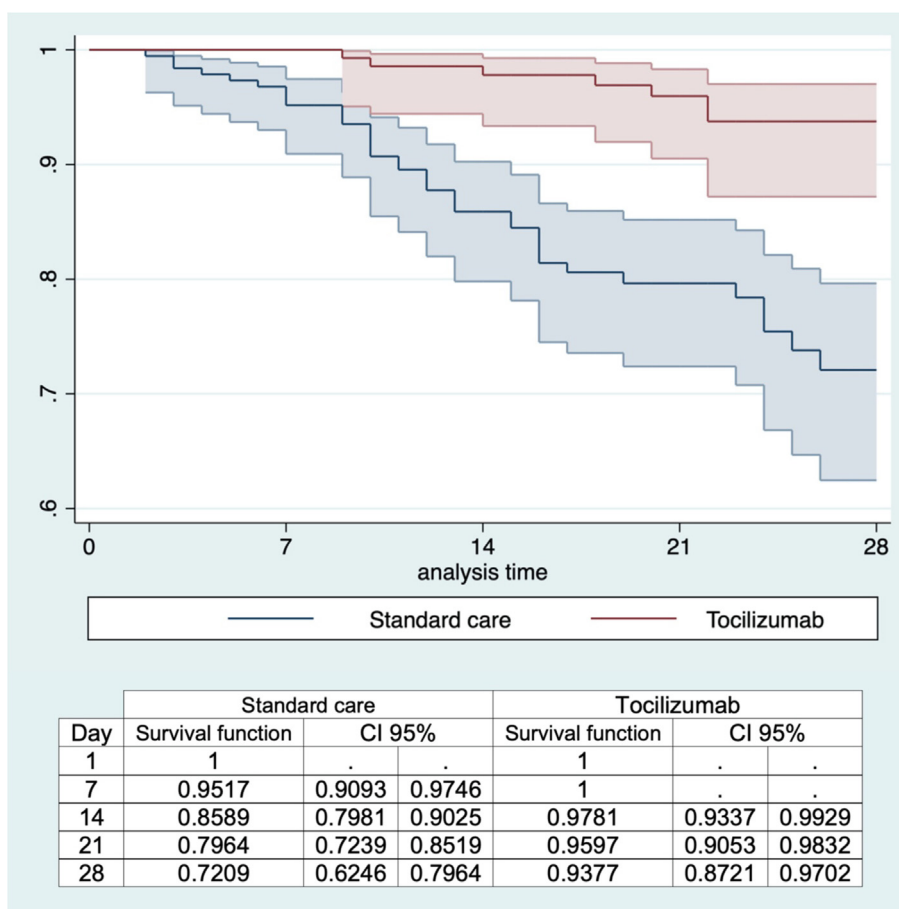

Day	Standard care			Tocilizumab		
	Survival function	CI 95%		Survival function	CI 95%	
1	1	.	.	1	.	.
7	0.9517	0.9093	0.9746	1	.	.
14	0.8589	0.7981	0.9025	0.9781	0.9337	0.9929
21	0.7964	0.7239	0.8519	0.9597	0.9053	0.9832
28	0.7209	0.6246	0.7964	0.9377	0.8721	0.9702

Fig. 3. Tocilizumab survival analysis and Kaplan-Meier curves (death).

reports and observational studies [28,29]. In fact, 64 randomized trials on the efficacy of various tocilizumab schemes are currently ongoing (www.clinicaltrials.gov).

To our knowledge, our series is the largest cohort of patients treated with tocilizumab plus methylprednisolone (n = 163). A similar prior experience from Brescia in Italy resulted in an overall mortality rate of 20% over 100 patients treated with tocilizumab plus dexamethasone. However, the optimal dosing schedule remains unclear [27]. At the beginning of April 2020, and due to national shortage, we were forced to treat patients with only one-two doses of 8 mg/kg, instead of the usual three doses. At our institution, candidates for tocilizumab therapy were selected among severe ill COVID-19 patients upon clinical and laboratory findings. Availability of medication and the presumed potential of benefit for each individual conditioned the indication of treatment, thus not all patients with *a priori* indication for tocilizumab received treatment (Table 4). Interestingly, although patients receiving tocilizumab showed significant lower P_aO_2/F_iO_2 ratio at admission, higher IL-6 and ferritin levels, and required more NIMV and ICU admission, mortality rate was significantly lower compared to those with theoretical indication who remained untreated (7.89% vs 24.17%, p < 0.0001, see Fig. 3). Patients treated with tocilizumab who kept on worsening their respiratory condition and systemic inflammation biochemical parameters, were subsequently treated with anakinra [44] or etoposide [35].

Secondary infections occurred unfrequently among patients hospitalized. Yet, 90% of infections were recorded at the ICU (primarily mechanical ventilation associated pneumonia, catheter related bacteremia, and urinary tract infections). It seems likely that the widespread use of third generation cephalosporins may have decreased the occurrence of nosocomial infection. Additionally, infection rates may have been influenced by the general overwhelming situation of the center, the poor availability of individual protective equipment at some moments, and the need for prone positioning of many patients.

4.3. When should patients with severe COVID-19 be intubated?

The pathophysiological mechanism behind acute respiratory distress syndrome (ARDS) is a pulmonary inflammatory process that induces non-hydrostatic protein-rich pulmonary edema, leading to profound hypoxemia, decreased lung compliance, and increased intrapulmonary shunt and dead space, with subsequent increase in ventilation/perfusion mismatch. ARDS criteria, as well as its severity classifications were used [34]. According to these criteria, ARDS is a common finding among severe COVID-19 pneumonia patients. In our experience, over 58% of COVID-19 patients developed ARDS.

According to international guidelines [45], adult patients presenting with ARDS (P_aO_2/F_iO_2 <300) are immediate candidates for orotracheal intubation and mechanical ventilation. However, unlike usual ARDS, SARS-CoV-2 related ARDS may present with alarming P_aO_2/F_iO_2 ratios (commonly under 150) but relatively preserved ventilatory function (mild dyspnea with or without tachypnea). Additionally, patients commonly show preserved oxygen extraction and adequate organ perfusion without lactic acidosis. Although upon ARDS Berlin criteria, all such patients would be candidates for immediate intubation, this particular ARDS profile allowed avoidance of invasive mechanical ventilation in a significant proportion of our cohort. We hypothesize that SARS-CoV-2 related ARDS distinct pathophysiologic features permit management of many critically ill COVID-19 patients with non-invasive ventilatory support, waiting for the reversal effect of anti-inflammatory therapy.

We observed that severe COVID-19 ARDS patients benefited from adequate oxygenation and correction of ventilation/perfusion mismatch, with alveolar recruitment helped by prone positioning and mild PEEP, which on the one hand, decreases the intrapulmonary shunt, improving arterial oxygenation, and on the other hand, decreases the amount of lung tissue exposed to alveolar opening-closing, thus reducing the risk of ventilator-induced lung injury. Prone positioning is commonly used in patients under mechanical ventilation who persist with severe respiratory impairment despite sedation-analgesia, muscle relaxation and recruitment measures, although it is also described and can be used in patients under non-invasive mechanical ventilation as in COVID-19 [46, 47].

When comparing the need for mechanical ventilation between the both periods, as we gained experience, we readily learned that many COVID-19 ARDS patients tolerated alarmingly low P_aO_2/F_iO_2 ratios (even under 100) without the need for intubation and mechanical ventilation, therefore avoiding invasive measures and ICU stay, yet without a negative impact on mortality.

This is, to our knowledge, the biggest report of patients treated with tocilizumab and glucocorticoids. However, this study has some limitations. It is a single center retrospective case review without a formally defined control group in terms of treatment protocol. This is partly attributable to the lack of standardized treatment guidelines, especially at the beginning of the epidemic. However, our treatment protocol was maintained rather homogeneously throughout the study period in a large sample of hospitalized patients. Our overall mortality rate also compared favorably with previous series. Acknowledging potential sources of bias, we found a significantly reduced mortality rate among patients treated with tocilizumab plus corticoids compared to theoretical candidates who did not received such treatment. In line with previous reports, abandonment of lopinavir/ritonavir plus interferon 1β combination showed no impact on mortality. Finally, infectious complications rate was acceptable and the majority of them occurred in the ICU where predisposing factors may have also contributed.

In summary, we report favorable outcomes from a large single center cohort of hospitalized COVID-19 patients with respiratory impairment, treated with early glucocorticoid therapy and interleukin inhibitors. The combination of methylprednisolone and tocilizumab resulted in improved mortality and acceptable complication rates among severely ill COVID-19 patients. Upcoming results from clinical trials will eventually elucidate the effectiveness of immunomodulatory therapy in this clinical setting.

Author statement

Luis Buzón Martin, Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Supervision, Project administration. Miguel Montero-Baladia, Investigation, Writing - original draft. David Iglesias Posadilla, Investigation, Writing - original draft. Itziar Gastigarraga, Investigation, Writing - original draft. Pedro David Delgado-Lopez, Writing - review & editing. Ana galacho Harriero, Investigation, Conceptualization. Marta Ubeira Iglesias, Investigation, Conceptualization, Resources. Enrique Iglesias Julian, Investigation. Andres Lorenzo Martín, Investigation. Javier Minguito de la Iglesia, Investigation. Juan Pablo García Muñoz, Investigation. Rodrigo Sanllorente Sebastían, Investigation. Maria Lopez-Veloso, Investigation, Resources. Noelia de la Torre Ferrera, Investigation, Julio Cesar Barraza-Bengoechea, Investigation, Conceptualization, Maria Colazo-Burlato Investigation, Gerardo Hermida-Fernández Investigation, Carolina Navarro-San Francisco Investigation, Jorge Boado-Lama Investigation, María Fernandez-Regueras Investigation, Fernando Callejo-Torre Investigation, Sergio Ossa-Echeverri Investigation, Lourdes Fisac-Cuadrado Investigation, María Gero-Escapa Investigation, Gregoria Megías-Lobón Investigation, Conceptualization, Microbiology, Adolfo Simón- Rodriguez Investigation, Jose Antonio Fernández- Ratero Investigation.

Declaration of competing interest

The authors declare no competing financial interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https

://doi.org/10.1016/j.jtauto.2021.100086.

References

[1] J. Bedford, D. Enria, J. Giesecke, D.L. Heymann, C. Ihekweazu, G. Kobinger, et al., COVID-19: towards controlling of a pandemic, Lancet 395 (2020) 1015–1018, https://doi.org/10.1016/S0140-6736(20)30673-5.

[2] WHO coronavirus disease (COVID-19). https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports. (Accessed 21 August 2020).

[3] F. Zhou, T. Yu, R. Du, G. Fan, Y. Liu, Z. Liu, et al., Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study, Lancet 395 (2020) 1054–1062, https://doi.org/10.1016/S0140-6736(20)30566-3.

[4] G. Cheng, D. Wu, W. Guo, Y. Cao, D. Huang, H. Wang, et al., Clinical and immunologic features in severe and moderate Coronavirus Disease 2019, J. Clin. Invest. 130 (5) (2020) 2620–2629.

[5] S. Rubino, N. Kelvin, J.F. Bermejo-Martin, D.J. Kelvin, As COVID-19 cases, deaths and fatality rates surge in Italy, underlying causes require investigation, J Infect Dev Ctries 14 (3) (2020) 265–267, https://doi.org/10.3855/jidc.12734.

[6] Pk Bhratraju, B.J. Ghassemieh, M. Nichols, R. Kim, K.R. Jerome, A.K. Nalla, et al., COVID-19 in critically ill patients in the Seattle region-Case series, N. Engl. J. Med. (2016), https://doi.org/10.1056/NEJMoa2004500.

[7] P. Horby, W.S. Lim, J. Emberson, M. Mafham, J. Bell, L. Linsell, et al., RECOVERY collaborative group, Effect of dexamethasone in hospitalized patients with COVID-19: preliminary report, BMJ (2020), https://doi.org/10.1101/2020.06.22.20137273.

[8] P.M. Metha, D.F. McAuley, M. Brown, E. Sanchez, R.S. Tattersall, J.J. Manson, COVID-19: consider cytokine storm syndromes and immunosupression, Lancet 395 (2020) 1033–1034, https://doi.org/10.1016/S0140-6736(20)30628-0.

[9] S.A. Vardhana, J.D. Wolchok, The many faces of the anti-COVID immune response, J. Exp. Med. 217 (6) (2020), e20200678, https://doi.org/10.1084/jem.20200678.

[10] Q. Ye, B. Wang, J. Mao, The pathogenesis and treatment of the 'Cytokine Storm' in COVID-19, J. Infect. 80 (6) (2020) 607–613, https://doi.org/10.1016/j.jinf.2020.03.037.

[11] Y. Jamilloux, T. Henry, A. Belot, S. Viel, M. Fauter, T. El Jammal, et al., Should we stimulate or suppress immune responses in COVID-19? Cytokine and anti-cytokine interventions, Autoimmun. Rev. (2020 May 4) 102567, https://doi.org/10.1016/j.autrev.2020.102567.

[12] E.J. Giamarellos-Bourboulis, M.G. Netea, N. Rovina, K. Akinosoglou, A. Antoniadou, N. Antonakos, et al., Complex immune dysregulation in COVID-19 patients with severe respiratory failure, Cell Host Microbe (2020), https://doi.org/10.1016/j.chom.2020.04.009.

[13] P. Sarzi-Puttini, V. Giorgi, S. Sirotti, D. Marotto, S. Ardizzone, G. Rizardini, S. Antinori, M. Galli, COVID-19, cytokines and immunosupression: what can we learn from severe acute respiratory syndrome? Clin. Exp. Rheumatol. 38 (2) (2020) 337–342.

[14] W. Zhang, Y. Zhao, F. Zhang, Q. Wang, T. Li, Z. Liu, et al., The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): the perspectives of clinical immunologist from China, Clin. Immunol. (2020) 214, https://doi.org/10.1016/j.clim.2020.108393.

[15] C. Zhang, S. Huang, F. Zheng, Y. Dai, Controversial treatments: an updated understanding of the coronavirus disease 2019, J. Med. Virol. (2020), https://doi.org/10.1002/jmv.25788.

[16] Z. Yang, J. Liu, Y. Zhou, X. Zhao, Q. Zhao, J. Liu, The effect of corticosteroid treatment on patients with coronavirus infection: a systematic review and meta-analysis, J. Infect. 81 (2020) e13–e20, https://doi.org/10.1016/j.jinf.2020.03.062.

[17] N. Veronese, J. Demurtas, L. Yang, et al., Use of corticosteroids in coronavirus disease 2019 pneumonia: a systematic review of the literature, Front. Med. 7 (2020) 170, https://doi.org/10.3389/fmed.2020.00170.

[18] Ye Z, Wang Y, Colunga-Lozano LE, et al. Efficacy and safety of corticosteroids in COVID-19 based on evidence for COVID-19, other coronavirus infections, influenza, community-acquired pneumonia and acute respiratory distress syndrome: a systematic review and meta-analysis [published online ahead of print, 2020 May 14]. CMAJ. 2020;cmaj.200645. https://doi.org/10.1503/cmaj.200645.

[19] World Health Organization, Clinical Management of Severe Acute Respiratory Infection when Novel Coronavirus (nCoV) Infection Is Suspected: Interim Guidance, 2020. Last Access: 21 August, https://www.who.int/publications/i/item/clinical-management-of-covid-19.

[20] W. Alhazzani, M.H. Moller, Y.M. Arabi, M. Loeb, M.N. Gong, E. Fan, et al., Surviving Sepsis Campaign: guidelines on the management of critically ill adults with coronavirus disease 2019 (COVID-19), Crit. Care Med. 48 (6) (2020) e440–e469, https://doi.org/10.1007/s00134-020-06022-5.

[21] Bhimraj A, Morgan RL, Shumaker AH, Lavergne V, Baden L, Cheng VC et al. Infectious Disease Society of America Guidelines on the treatment and management of patients with COVID-19. https://www.idsociety.org/COVID19guidelines. Last Access: 21 August.

[22] J. Villar, C. Ferrando, D. Martinez, et al., Dexamethasone treatment for the acute respiratory distress syndrome: a multicentre, randomised controlled trial, Lancet Respir Med 8 (3) (2020) 267–276, https://doi.org/10.1016/S2213-2600(19)30417-5.

[23] J. Villar, M. Confalonieri, S.M. Pastores, G.U. Meduri, Rationale for prolonged corticosteroid treatment in the acute respiratory distress syndrome caused by

[24] K.Y. Lee, J.W. Rhim, J.H. Kang, Early preemptive immunomodulators (corticosteroids)for severe pneumonia patients infected with SARS-CoV-2, CEP 63 (4) (2020) 117–118, https://doi.org/10.3345/cep.2020.00290.

[25] R. Fadel, A.R. Morrison, A. Vahia, Z.R. Smith, Z. Chaudhry, P. Bhargava, et al., Early short course corticosteroids in hospitalized patients with COVID-19, Clin. Infect. Dis. (2020), https://doi.org/10.1093/cid/ciaa601.

[26] X. Xu, M. Han, T. Li, et al., Effective treatment of severe COVID-19 patients with tocilizumab, Proc. Natl. Acad. Sci. U. S. A 117 (20) (2020) 10970–10975, https://doi.org/10.1073/pnas.2005615117.

[27] P. Toniati, S. Piva, M. Cattalini, et al., Tocilizumab for the Treatment of Severe COVID-19 Pneumonia with Hyperinflammatory Syndrome and Acute Respiratory Failure: A Single Center Study of 100 Patients in Brescia, Italy [published Online Ahead of Print, 2020 May 3], Autoimmun Rev (2020) 102568, https://doi.org/10.1016/j.autrev.2020.102568.

[28] S.K. Alzghari, V.S. Acuña, Supportive treatment with tocilizumab for COVID-19: a systematic review [published online ahead of print, 2020 Apr 21], J. Clin. Virol. 127 (2020) 104380, https://doi.org/10.1016/j.jcv.2020.104380.

[29] C. Campochiaro, E. Della-Torre, G. Cavalli, G. De Luca, M. Ripa, N. Boffini, et al., Efficacy and safety of tocilizumab in severe COVID-19 patients: a single-centre retrospective cohort study, Eur. J. Intern. Med. 76 (2020) 43–49, https://doi.org/10.1016/j.ejim.2020.05.021.

[30] A. Aouba, A. Baldolli, L. Geffray, et al., Targeting the inflammatory cascade with anakinra in moderate to severe COVID-19 pneumonia: case series [published online ahead of print, 2020 May 6], annrheumdis-2020-217706, Ann. Rheum. Dis. (2020), https://doi.org/10.1136/annrheumdis-2020-217706.

[31] L.F. Buckley, G.F. Wohlford, C. Ting, A. Alahmed, B.W. Van Tassell, A. Abbate, et al., Role of anti-cytokine therapies in severe coronavirus disease 2019, Crit Care Expl 2 (2020), e0178, https://doi.org/10.1097/CCE.0000000000000178.

[32] I. Paranjpe, V. Fuster, A. Lala, A. Russak, B.S. Glicksberg, M.A. Levin, et al., Association of treatment dose anticoagulation with in-hospital survival among hospitalized patients with COVID-19, JACC (J. Am. Coll. Cardiol.) 76 (1) (2020) 122–124, https://doi.org/10.1016/j.jacc.2020.05.001.

[33] B. Cao, Y. Wang, D. Wen, et al., A trial of lopinavir-ritonavir in adults hospitalized with severe covid-19, N. Engl. J. Med. 382 (19) (2020) 1787–1799, https://doi.org/10.1056/NEJMoa2001282.

[34] N.D. Ferguson, E. Fan, F.L. Camporota, M. Antonelli, A. Anzueto, R. Beale, et al., The Berlin definition of ARDS: an expanded rationale, justification, and supplementary material, Intensive Care Med. 38 (2012) 1573–1582, https://doi.org/10.1007/s00134-012-2682-1.

[35] M. Montero-Baladia, L. Buzon, I. Astigarraga, P. Delgado, E. Iglesias, M. Lopez-Veloso, et al., Etoposide treatment adjunctive to immunosuppressants for critically ill COVID-19 patients, J. Infect. 81 (3) (2020) 468–470, https://doi.org/10.1016/j.jinf.2020.06.006.

[36] A.M. Borobia, A.J. Carcas, F. Arnalich, R. Alvarez-Sala, J. Montserrat, M. Quintana, et al., A cohort of patients with COVID-19 in a major teaching hospital in Europe, J. Clin. Med. 9 (6) (2020) 1733, https://doi.org/10.3390/jcm9061733.

[37] J. Berenguer, P. Ryan, J. Rodriguez-Baño, M. Yllescas, J.R. Arribas, The COVID-19@Spain Study group. Characteristics and predictors of death among 4,035 consecutively hospitalized patients with COVID-19 in Spain, Clin Microbial Infect (2020), https://doi.org/10.1016/j.cmi.2020.07.024.

[38] Y. Wang, D. Zhang, G. Du, R. Du, J. Zhao, Y. Jin, et al., Remdesivir in adults with severe COVID-19: a randomized, double-bind, placebo-controlled, multicenter trial, Lancet 395 (2020) 1569–1578, https://doi.org/10.1016/s0140-6736(20)31022-9.

[39] I.F. Hung, C. Lung, E.Y. Tso, R. Liu, T.W. Chung, M.Y. Chu, et al., Triple combination of interferon beta-1b, lopinavir-ritonavir and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomized, phase 2 trial, Lancet 395 (2020) 1695–1704, https://doi.org/10.1016/S0140-6736(20)31042-4.

[40] P. Colson, J.M. Rolain, J.C. Lagier, P. Brouqui, D. Raoult, Cloroquine and hydroxicloroquine as available weapons to fight COVID-19, Int. J. Antimicrob. Agents 55 (2020) 105932, https://doi.org/10.1016/j.ijantimicag.2020.105932.

[41] WHO "Solidarity" clinical trial for COVID-19 treatments https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments. Last access 21st August.

[42] NIH halts clinical trial of hydroxychloroquine. Study shows treatment does no harm, but provides no benefit https://www.nih.gov/news-events/news-releases/nih-halts-clinical-trial-hydroxychloroquine. Last access 21st August.

[43] D. Annane, S.M. Pastores, B. Rochwerg, et al., Guidelines for the diagnosis and management of critical illness-related corticosteroid insufficiency (CIRCI) in critically ill patients (Part I): society of critical care medicine (SCCM) and European society of intensive care medicine (ESICM) 2017 [published correction appears in intensive care med. 2018 Feb 23], Intensive Care Med. 43 (12) (2017) 1751–1763, https://doi.org/10.1007/s00134-017-4919-5.

[44] E. Iglesias-Julian, M. Lopez-Veloso, N. De la Torre-Ferrera, J.C. Barraza-Vengoechea, P.D. Delgado-Lopez, M. Colazo Burlato, et al., High dose subcutaneous Anakinra to treat acute respiratory distress syndrome secondary to cytokine storm syndrome among severely ill COVD-19 patients, J. Autoimmun. (2020), https://doi.org/10.1016/j.aut.2020.102537.

[45] E. Fan, L. Del Sorbo, E.C. Goligher, et al., An official American thoracic society/European society of intensive care medicine/society of critical care medicine clinical practice guideline: mechanical ventilation in adult patients with acute

coronavirus disease 2019, e 0111, Crit Care Explor 2 (4) (2020), https://doi.org/10.1097/CCE.0000000000000111.

respiratory distress syndrome [published correction appears in Am J Respir Crit Care Med. 2017 Jun 1; 195(11):1540], Am. J. Respir. Crit. Care Med. 195 (9) (2017) 1253–1263, https://doi.org/10.1164/rccm.201703-0548ST.

[46] L. Ding, L. Wang, W. Ma, H. He, Efficacy and safety of early prone positioning combined with HFNC or NIV in moderate to severe ARDS: a multi-center prospective cohort study, Crit. Care 24 (1) (2020) 28, https://doi.org/10.1186/s13054-020-2738-5. Published 2020 Jan 30.

[47] L. Munshi, L. Del Sorbo, N.K.J. Adhikari, et al., Prone position for acute respiratory distress syndrome. A systematic review and meta-analysis, Ann Am Thorac Soc 14 (Supplement_4) (2017) S280–S288, https://doi.org/10.1513/AnnalsATS.201704-343OT.