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High dose subcutaneous Anakinra to treat acute respiratory distress syndrome secondary to cytokine storm syndrome among severely ill COVID-19 patients

Enrique Iglesias-Julián [a,*], María López-Veloso [a], Noelia de-la-Torre-Ferrera [a], Julio Cesar Barraza-Vengoechea [a], Pedro David Delgado-López [b], María Colazo-Burlato [c], Marta Ubeira-Iglesias [d], Miguel Montero-Baladía [e], Andrés Lorenzo-Martín [c], Javier Minguito-de-la-Iglesia [f], Juan Pablo García-Muñoz [f], Rodrigo Sanllorente-Sebastián [g], Blanca Vicente-González [d], Ana Alemán-Alemán [h], Luis Buzón-Martín [i]

[a] Systemic Autoimmune Diseases Unit, Department of Internal Medicine, Burgos University Hospital, Spain
[b] Department of Neurosurgery, Burgos University Hospital, Spain
[c] Department of Rheumatology, Burgos University Hospital, Spain
[d] Department of Pharmacy, Burgos University Hospital, Spain
[e] Department of Intensive Care Medicine, Burgos University Hospital, Spain
[f] Department of Pneumology, Burgos University Hospital, Spain
[g] Department of Anesthesiology, Burgos University Hospital, Spain
[h] Department of Internal Medicine, Burgos University Hospital, Spain
[i] Infectious Diseases Unit, Department of Internal Medicine, Burgos University Hospital, Spain

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ABSTRACT

Objective: Severely ill COVID-19 patients may end in acute respiratory distress syndrome (ARDS) and multi-organ failure. Some of them develop a systemic hyperinflammatory state produced by the massive release of inflammatory agents, known as cytokine storm syndrome (CSS). Inhibition of IL-1 by Anakinra (ANK) is a potential life-saving therapy for severe CSS cases. We propose a rationale for the use of subcutaneous ANK and review our initial experience in a small cohort of severe COVID-19 CSS patients.

Methods: Retrospective cohort study of COVID-19 patients developing ARDS (PaO2/FiO2 <300) and exhibiting signs of hyperinflammation (ferritin >1000 ng/mL and/or d-dimers > 1.5 μg/mL, plus IL-6 < 40 mg/mL) that received ANK. For comparison, a propensity score matched historical cohort of patients treated with IL-6 inhibitor Tocilizumab (TCZ) was used. Patients had previously received combinations of azithromycin, hydroxychloroquine, and methyl-prednisolone. Laboratory findings, respiratory function and adverse effects were monitored. Resolution of ARDS within the first 7 days of treatment was considered a favorable outcome.

Results: Subcutaneous ANK (100 mg every 6 h) was given to 9 COVID-19 ARDS CSS patients (77.8% males). Median age was 62 years (range, 42 to 87). A TCZ cohort of 18 patients was selected by propensity score matching and treated with intravenous single dose of 600 mg for patients weighing >75 Kg, or 400 mg if < 75 Kg. Prior to treatment, median PaO2/FiO2 ratio of the ANK and TCZ cohorts were 193 and 249, respectively ($p = 0.131$). After 7 days of treatment, PaO2/FiO2 ratio improved in both groups to 279 (104–335) and 331 (140–476, $p = 0.099$) respectively. On day 7, there was significant reduction of ferritin ($p = 0.046$), CRP ($p = 0.043$), and IL-6 ($p = 0.043$) levels in the ANK cohort but only of CRP ($p = 0.001$) in the TCZ group. Favorable outcome was achieved in 55.6% and 88.9% of the ANK and TCZ cohorts, respectively ($p = 0.281$). Two patients that failed to respond to TCZ improved after ANK treatment. Aminotransferase levels significantly increased between day 1 and day 7 ($p = 0.004$) in the TCZ group. Mortality was the same in both groups (11%). There were not any opportunistic infection in the groups nor other adverse effects attributable to treatment.

* Corresponding author. Systemic Autoimmune Diseases Unit, Department of Internal Medicine, Burgos University Hospital, Avenida Islas Baleares 3, Burgos, Postal code: 09006, Spain.
E-mail address: eiglesiasjulian@gmail.com (E. Iglesias-Julián).

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Conclusion: Overall, 55.6% of COVID-19 ARDS CSS patients treated with ANK exhibited favorable outcome, not inferior to a TCZ treated matched cohort. ANK may be a potential alternative to TCZ for patients with elevated aminotransferases, and may be useful in non-responders to TCZ.

1. Introduction

RNA virus SARS-CoV-2, the causative agent of the current COVID-19 pandemic, shares 80% and 50% genomic structure with SARS-CoV-1 and MERS-CoV, respectively [1]. Clinical manifestations of COVID-19 range from asymptomatic or pauci-symptomatic to acute respiratory distress syndrome (ARDS) with eventual multi-organ failure associated to a hyper-inflammatory state produced by a *cytokine storm syndrome* (CSS) [1].

Studies have shown that COVID-19 patients can exhibit increased levels of acute phase reactants like C reactive protein (CRP) and ferritin, as well as, TNF-α, IL-1β, IL-1Ra, IL-6, IL-10, IL-18 and IFN-γ among others [2], suggesting a rapid activation of the innate immune response. Although SARS-CoV-2 is able to infect T cells, it cannot replicate inside them, thus cell death occurs as a consequence of apoptosis, necrosis or pyroptosis [3]. These processes induce the production of numerous chemokynes and the recruitment of large amounts of immune cells within the lung, which causes secondary ARDS similar to that occurring in reactive hemophagocytic lymphohistiocytosis (rHLH), also known as macrophagic activation syndrome (MAS). However, in COVID-19 hyperinflammation state, extremely increased ferritin levels and organomegaly, typically found in rHLH, are rarely encountered, likely attributable to immune cell depletion of lymphoid organs [1].

Antivirals and ventilatory support are commonly used in COVID-19 patients. Additionally, anti-inflammatory therapy directed to more severe cases may prevent fatal deterioration. Therefore, immunemodulation therapy has been proposed for the inflammatory stage of the disease (phase III, according to the definition by Siddiqi and Merha [4]) in which cytokine release predominate.

IL-1α and IL-1β are known to be released by macrophages of the lungs, digestive tract and liver among other organs [5]. The exclusive function of the IL-1 antagonist receptor (IL-1Ra) is the inhibition of the biologic response to IL-1. Anakinra (ANK), a human recombinant form of IL-1Ra, acts as a receptor antagonist able to inhibit both IL-1α and IL-1β. ANK has been used in self-inflammatory and autoimmune conditions, like the MAS [6–10] and in severe sepsis [11], showing survival benefit and tolerable adverse effects.

In this paper we propose a rationale for the use of ANK and review our initial experience in a small cohort of severely ill COVID-19 patients exhibiting cytokine release syndrome. Current evidence suggests that IL-6 inhibitor Tocilizumab (TCZ) can be considered a standard therapy for COVID-19-related CSS [12–14]. In fact, in our institution, CSS patients are treated with immunomodulator therapy. Therefore, a historical cohort of patients treated with TCZ were used as comparative control group.

2. Patients and methods

The clinical course and outcome of COVID-19 related ADRS patients receiving ANK, admitted to the University Hospital of Burgos in Spain, within the period April 1st to May 11th, 2020, was retrospectively reviewed. The clinical outcome of this small cohort was compared with a historical cohort of patients treated with TCZ by propensity score matching analysis in a 1:2 ratio. Since this is an off-label indication of ANK and TCZ, informed consent was obtained from all participants or relatives, and the study was approved by the Local Institutional Ethics Committee (CEIm reference number: 2329).

2.1. Confirmation of diagnosis

The diagnosis of COVID-19 infection was established by viral RNA detection with real time PCR technique on nasopharyngeal swabs (Seegene Allplex™ 2019 nCov Assay, South Korea; and SARS-CoV-2 Real Time PCR kit, VIRCELL, Spain). According to the 2012 Berlin criteria [15], ARDS was defined as the presence of bilateral infiltrates in the chest x-ray or CT, along with a ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO2/FiO2 ratio) < 300.

2.2. Inclusion criteria

Patients receiving ANK presented ARDS and hyper-inflammation features, defined as ferritin >1000 ng/mL and/or d-dimers $>$ 1,5 μg/mL, and IL-6 $<$ 40 pg/mL. Patients showing IL-6 $>$ 40 pg/mL were treated with IL-6 inhibitor tocilizumab (TCZ). Patients with IL-6 $>$ 40 pg/mL plus at least a 5-fold increase of the normal value of transaminases were also treated with ANK, given the contraindication for TCZ. All patients were treated after a minimum of seven days from symptom onset.

2.3. Treatment protocol

ANK is administered subcutaneously, 100 mg every 6 h for at least 3 days. Afterwards, dosage can be reduced to every 24 h up to 7 days. Some patients underwent gradual tapering (every 8, 12 and 24 h) according to the clinical course, reversal of organ dysfunction and decreasing inflammatory parameters. Patients in the control group had been treated with intravenous TCZ, a single dose of 600 mg in patients over 75 Kg, and 400 mg in those under 75 Kg.

2.4. Assessment of outcome

Progressive resolution of ARDS was considered a favorable outcome. Patients not improving oxygenation parameters after 7 days of treatment were designated as non-favorable outcome. Laboratory findings and treatment-related adverse events were also monitored and analyzed.

2.5. Data analysis

Clinical and laboratory data were retrieved from the electronic medical history. Continuous variables were described with median and range. Comparison of non-parametric and related parameters over time were analyzed with Wilcoxon's test, and Mann-Whitney U was used for unrelated. Comparison of parametric parameters was analyzed with Student's T test. Statistical analysis was performed with the SPSS v 22 statistical package, considering p value < 0.05 as significative.

3. Results

3.1. Patient characteristics

From April 1st to May 11th, 2020, 238 COVID-19 PCR positive patients were admitted to the center, of which 83 (34.8%) were treated with immunomodulators as adjuvant therapy for ARDS according to the established protocol of the center. Nine out of 83 met the inclusion criteria and were finally treated with ANK (10.8%). A historical comparative cohort of 18 TCZ treated patients was selected by propensity score matching. Previous history and clinical features of the ANK cohort are summarized in Table 1. Two patients were women and 7 were

Table 1
Baseline characteristics and evolution of patients treated with anakinra.

Patient	1	2	3	4	5	6	7	8	9
Age	62	67	84	54	61	42	48	79	87
Sex	Female	Male	Male	Male	Male	Male	Male	Male	Female
Previous history	–	Ex-smoker	Hypertension Chronic renal disease	Dyslipidemia	Hypertension Chronic limphoid leukemia Chronic glomerulonefritis	Lymphoma	–	Hypertension Diabetes Mellitus Neuroendocrine neoplasm	–
Days from disease onset to ANK treatment	10	19	13	10	11	16	24	15	–
Days from admission to ANK treatment	1	12	3	3	4	8	4	1	13
Treatment given prior to ANK	No	HDQ, AZI, MPB, TCZ (2 doses)	HDQ, AZI, MPB	HDQ, AZI, MPB	HDQ, AZI, MPB	HDQ, AZI, MPB	HDQ, AZI, MPB, TCZ	No	HDQ, AZI, MP
Treament given concomitant to ANK	HDQ, AZI, MPB	Ninguno	MP	MP	TCZ, MP	MP	MP	HDQ, AZI, MPB, MP	MP
ANK treatment day	D1 D3 D7	D1 D3 D7	D1 D3 D7	D1 D3 D7	D1 D3 D7	D1 D3 D7	D1 D3 D7	D1 D3 D7	D1 D3 D7
PaO2/FiO2	280 273 279	168 234 286	232 120 237	160 227 108	59* 118* 104*	242 154	286 219 300	65 55	193 155 355
FiO2	0.21 0.4 0.28	0.5 0.31 0.21	0.31 0.5 0.7	0.35 0.5 0.5	0.9* 0.7* 0.8*	0.21 0.31	0.28 0.31 0.21	1 1	0.4 0.4 0.28
O2 support	Basal VMK VMK	VMK VMK Basal	VMK VMK SVNI	VMK VMK VMK	SVNI* SVNI* SVNI*	Basal VMK	GN VMK	SVNI SVNI	VMK VMK GN
Lymphocyte count (/μL)	800 1200 1700	800 1000	700 500 600	1000 500 1100	1400 700 1300	200	1100 1200	1100 600	1100 1400 700
Ferritin (ng/mL)	4104 2199 1800	1094 809	1400 1573 1405	1938 2044 1693	578 433 262	1469 1827	2862 1536	1697 1301	617 568 589
D-dimers (μg/mL)	0.6 0.3 0.3	1.8 0.4	0.4 0.4 0.5	0.3 0.3 0.5	0.6 10.7 13	0.5	0.4 0.4	0.9 1.5	1.6 1.3 0.9
IL-6 (pg/mL)	5.8	624 73	11.7 7 1.7	12.8 7.9 4.6	452.7 1792 202	24.9 39.4	295.6 97.4	31.1	18.9 19.4 2.9
CRP (mg/L)	43 44 4	0 0	62 17 3	5 25 2	141 40 4	63 93	34 4	244 54	83 14 4
LDH (UI/L)	389 314 273	343 246	243 253 296	258 236 261	403 377 435	377 456	373 285	520 350	598 351
GOT/AST (UI/L)	189 57 65	62 72	25 31	34 35	27 16	32 55	187 71	36 27	33
GPT/ALT (UI/L)	168 170 167	98 134	18 42	69 82 211	32 30 44	52 61	228 270	19 60	37 47
ANK dose	100 mg/6 h × 3 days 100 mg/24 h × 4 days	100 mg/6 h × 3 days 100 mg/24 h × 4 days	100 mg/6 h × 3 days 100 mg/24 h × 4 days	100 mg/6 h × 3 days 100 mg/24 h × 4 days	100 mg/6 h × 3 days 100 mg/24 h × 2 days 100 mg/6 h × 10 days 100 mg/8 h × 3 days 100 mg/12 h × 2 days 100 mg/24 h × 2 days	100 mg/6 h × 2 days	100 mg/6 h × 3 days 100 mg/24 h × 4 days	100 mg/6 h × 5 days	100 mg/6 h × 7 days 100 mg/8 h × 2 days 100 mg/12 h × 2 days 100 mg/24 h × 3 days
Non-invasive mechanical ventilation	No	No	Yes	Yes	Yes	No	No	Yes	No
Intubation	No	No	Not candidate	No	No	No	No	Yes	No
Outcome	**Discharge 15 days after initiation of ANK**	ANK given 4 days after second dose of TCZ **Discharge at day 10 after initiation of ANK**	ANK discontinued at day 7 due to inefficacy **Death at day 13 after initiation of ANK.**	**Discharge 17 days after initiation of ANK**	Improved after 100 mg/6 h ANK, worsened during tapered, new improvement at 100 mg/6 h dose. Pneumo-mediastinum at 15 days after initiation of ANK, precluding correct assessment of oxygenation. **Remains hospitalized.**	ANK discontinued after 48 h due to inefficacy. **Treated with another immunomodulator**, according to protocol.	**Discharge 8 days after initiation of ANK**	Needs non-invasive mechanical ventilation at admission (FiO2 1). **Requires intubation** 6 days after initiation of ANK. **Treated with another immunomodulator**, according to protocol	**Discharge 14 days after initiation of ANK**

ANK: Anakinra. HDQ: hydroxy-chloroquine, dose 400 mg every 12 h at day 1, followed by 200 mg every 12 horas for 5 days. AZI: azithromycin, dose 500 mg every 24 h for 7 días, and then every 48 h for 14 days. MPB: intravenous bolus methylprednisolone 250 mg/day, for 3 days. MP: intravenous methylprednisolone, dose by mg/kg/day, with tapering at the physician's criteria. TCZ: tocilizumab, single dose 400 mg if body weight <75 kg, or 600 mg if greater. Repeating dose always 400 mg. VMK: Ventimask® (Venturi-type facial mask for oxygenation). GN: low-flux oxygen nasal cannula.

Table 2
Group characteristics.

Baseline characteristics	Anakinra group (n = 9)	Tocilizumab group (n = 18)	P value[a]
Age (median ± range)	62 (42–87)	62 (34–79)	0.386
Sex (M/F)	7/2	12/6	0.676
Hypertension	3 (33.3%)	8 (44.4%)	0.692
Dyslipidemia	1 (11.1%)	6 (33.3%)	0.363
Diabetes	1 (11.1%)	3 (16.7%)	1.000
Chronic pulmonary disease	0	3 (16.7%)	0.529
Chronic kidney disease	2 (22.2%)	1 (5.6%)	0.250
Neoplasia	1 (11.1%)	2 (11.1%)	1.000
Hematologic disease	2 (22.2%)	0	0.103
Concomitant treatments			
Metilprednisolone	9 (100%)	17 (94.4%)	1.000
Hydroxychloroquine	9 (100%)	18 (100%)	.
Azithromycin	9 (100%)	18 (100%)	.
Beta interferon	0	9 (50%)	0.012
Days of symptoms before immunomodulatory treatment start (median, range)	14 (10–24)	10 (7–18)	0.033
Day of admission before immunomodulatory treatment start (median, range)	4 (1–13)	2 (0–5)	0.014

[a] Student's T test, Fisher's test or Mann-Whitney *U* test as needed.

men, with a mean age of 64.9 ± 31.8 years. Three patients presented hypertension, 1 dyslipidemia, 1 type 2 diabetes, 1 chronic renal disease, 1 an untreated neuro-endocrine tumor, 1 lymphoma undergoing treatment, and 1 untreated chronic lymphoid leukemia. The main characteristics of both cohorts were similar as shown in Table 2.

All patients receiving ANK showed clinical signs of ARDS and impaired blood oxygenation, with a median PaO_2/FiO_2 ratio of 193 (range, 59–286). Oxygen support was delivered by high-flux non-invasive mechanical ventilation device (Optiflow®) in 2 patients, Venturi-type facial mask in 3 patients, low-flux nasal cannula in 1 patient, and 2 additional patients were not under oxygen support at the time initial arterial blood gas was performed, but was initiated as the result of the PaO_2/FiO_2 below 300 was evidenced. Median FiO_2 needed was 0.35 (range, 0.21–1.0). As detailed in Table 3, prior to ANK therapy, the median values for most relevant laboratory findings were: total lymphocyte count 1000/μL (200–1400), ferritin 1469 ng/mL (578–4104), d-dimers 0.6 μg/mL (0.3–1.8), IL-6 28 pg/mL (11.7–624), CRP 62 mg/L (0–244), LDH 377 UI/L (243–598), GOT/AST 36 UI/L (25–189), and GPT/ALT 60.50 UI/L (18–228).

Seven patients (77.8%) had received either intravenous boluses of methylprednisolone (250 mg per day for 3 days) or 1 mg/kg/day methylprednisolone, up to the physician in charge for the patient. Additionally, 2 patients (22.2%) had received TCZ, 600 mg intravenous single dose 48 h prior to ANK in 1 patient, and 600 mg and 400 mg doses 4 days before ANK in another patient. In 1 case ANK and TCZ were given simultaneously. In 2 patients, ANK and methylprednisolone were given concomitantly.

Time until administration of immunomodulator therapy was significantly higher in the ANK (14 days, range 10–24) compared to TCZ group (10 days, range 7–18, p = 0.033). Hospital stay prior to administration of immunomodulator therapy was also higher in the ANK group (median 4 days versus 2 days, p = 0.014), likely attributable to the fact that some patients not responding to TCZ also received ANK as salvage therapy. Patients in the ANK group showed a non-significant trend to worse ventilatory parameters and lower IL-6 levels, the latter reflecting the inclusion criteria.

3.2. Response to treatment

Subcutaneous ANK was given (100 mg every 6 h) for at least 3 days to 8 patients. In 1 patient ANK was discontinued after 48 h due to inefficacy. ANK dosage was reduced to 100 mg every 24 h in 5 patients (62.5%). However, in 2 patients the dosage was kept at 100 mg every 6 h beyond the third day, although in 1 of them it was interrupted at the sixth day because of clinical worsening, receiving etoposide as salvage therapy [16]. The other patient, kept on 100 mg every 6 h for 14 days, presented persistent respiratory failure, finally attributed to the development of spontaneous pneumo-mediastinum. On average, ANK was given at day 14 from the onset of symptoms (range, 10–24), and at day 4 (range, 1–13) from the day of admission.

Outcome was favorable in 5 patients (55.6% of the cohort, patients #1,2,4,7, and 9) according to the improvement of the PaO_2/FiO_2 ratio, need for oxygen requirements, and laboratory inflammation parameters. Median time to hospital discharge was 14 days (range, 8–17) from initiation of ANK treatment. Three out 5 patients had received ANK plus methylprednisolone (cases #1, 4, and 9), and 2 had received prior TCZ

Table 3
Evolution of anakinra and tocilizumab groups.

	Initiation of ANK	Initiation of TCZ	P value[a]	Day 3 of ANK	Day 3 of TCZ	P value[a]	Day 7 of ANK	Day 7 of TCZ	P value[a]	Differences between D1 and D7 of ANK (*p value*)[a]	Differences between D1 and D7 of TCZ (*p value*)[a]
PaO2/FiO2	193 (59–286)	249 (85–387)	0.131	155 (55–273)	238.5 (100–392)	0.070	279 (104–355)	331 (140–476)	0.099	0.189	**0.004**
FiO2	0.35 (0.21–1)	0.28 (0.21–1)	0.219	0.4 (0.31–1)	0.29 (0.24–0.65)	0.179	0.28 (0.21–0.8)	0.26 (0.21–0.6)	0.250	0.833	0.574
Lymphocyte count (/μL)	1000 (200–1400)	800 (400–3000)	0.765	700 (500–1200)	950 (300–2200)	0.524	1050 (600–1400)	1150 (500–3800)	0.569	0.750	**0.049**
Ferritin (ng/mL)	1469 (578–4104)	1434 (948–2874)	0.790	1554.5 (433–2199)	1512 (837–4102)	0.680	1150 (262–1800)	1600 (666–5736)	0.111	**0.046**	0.333
D-dimers (μg/mL)	0.6 (0.3–1.8)	0.75 (0.4–9.9)	0.326	0.4 (0.3–10.7)	0.65 (0.3–8.2)	0.940	0.5 (0.3–13)	0.7 (0.3–20)	0.460	0.753	0.379
IL-6 (pg/mL)	28 (11.7–624)	63.65 (1.5–90.9)	0.865	29.4 (7–1792)	67 (11.3–537.1)	0.475	5.2 (1.7–202)	371.8 (50.7–662.6)	**0.025**	**0.043**	0.285
CRP (mg/L)	62 (0–244)	88.5 (22–362)	0.354	32.5 (4–93)	30.5 (3–110)	0.933	2.5 (0–4)	3 (0–61)	0.723	**0.043**	**0.001**
LDH (UI/L)	377 (243–598)	408 (226–883)	0.698	314 (236–456)	326 (212–536)	0.621	284.5 (246–435)	341 (226–670)	0.281	0.345	0.382
GOT/AST (UI/L)	36 (25–189)	34 (20–101)	0.751	55 (27–71)	40 (17–125)	0.554	34 (16–72)	63 (29–244)	0.087	0.465	0.260
GPT/ALT (UI/L)	60.5 (18–228)	58.5 (12–214)	0.727	61 (30–270)	68 (11–171)	0.680	90.5 (42–211)	166 (37–613)	0.224	0.080	**0.004**

[a] Student's T test, Mann-Whitney *U* test, and Wilcoxon's test as needed.

without response (cases #2, and 7). Despite developing pneumomediastinum, patient #5 improved laboratory parameters. Outcome was not favorable in 3 patients (cases #3, 6, and 8). In 1 patient ANK was discontinued at day 7 and died at day 13. The other 2 patients remain hospitalized both severely affected. Comparison of both groups yielded favorable outcome in 16 patients (88.9%) of the TCZ group ($p = 0.281$); days to discharge after initiation of TCZ were 14 (4–65, $p = 0.920$); 4 (22.2%) needed non-invasive mechanical ventilation ($p = 0.375$), 6 (33.3%) needed intubation ($p = 0.363$), and 2 died in the first 40 days (11.1%, $p = 1.000$).

Table 3 shows the variation of oxygenation and inflammation parameters throughout the hospital stay, in both ANK and TCZ groups. The improvement of PaO2/FiO2 ratio was statistically significant in the TCZ group: 249 (85–387) at day 1 and 331 (140–476) at day 7 ($p = 0.004$). There was also marked improvement in the ANK group: 193 (59–286) at day 1 and 279 (104–335) at day 7, however without statistical significance, likely because ANK patients presented poorer oxygenation parameters at day 1, and also because some of them had been treated with prior TCZ without improvement. At day 7, reduction of IL – 6 was significantly lower in the ANK group, due to TCZ binding to IL – 6 receptor, which increases plasma levels, yet without clinical relevance. Changes in CRP resulted statistically significant in both groups, but ferritin only in the ANK group.

Regarding side effects, ALT was found significantly increased in the TCZ group: 58 (12–214) at day 1, and 166 (37–613) at day 7 ($p = 0.004$), but not in the ANK group: 60.5 (18–228) at day 1, and 90.5 (42–211) at day 7 ($p = 0.080$). No other adverse reactions, including opportunistic infections, were registered in any of the groups during the follow up period (median follow-up 40 days, range 18–49).

4. Discussion

SARS-CoV-2 affects the respiratory tract by attaching to the angiotensin II receptors present in type II pneumocytes [1]. The innate immune response is initiated when pattern-recognition receptors (PRR) recognize pathogen-associated molecular patterns (PAMP), and damage-associated molecular patterns (DAMP), which are released following cellular damage [13]. Both PAMP and DAMP are likely to be generated following initial SARS-CoV-2 cellular infection and lysis [14]. The most relevant variants of such receptors are the so-called *Toll*-type (TLR), expressed by immune cells and some epithelial cells. PAMP antigen presentation to dendritic cells through TLR-7 promotes the production of type I INF, which limits viral replication and contributes to a prolonged adaptive immune response. Data from previous studies suggest that SARS-CoV-2 and MERS-CoV are able to down-regulate or suppress the immune response mediated by type I IFN, and induce T cell apoptosis [1,14]. Another group of intracellular immune mediators are high-molecular weight complex multiproteic signal transducers known as *inflamasomes*. One of them, NLRP3, activates caspase 1 leading to the formation of mature IL-1 β and IL-18. Interestingly, caspase 1 activation can induce cellular *pyroptosis* [3], a specific type of cell death in which large amounts of IL-1 and IL-18 are released to the extracellular matrix. IL-1 β is a powerful inductor of other pro-inflammatory cytokines like TNF-α e IL-6 [1] and enhances local T cell cytotoxicity [14]. IL-6 enhances the immune response by recruiting neutrophils and cytotoxic T cells. In the lung cells, neutrophils release leukotrienes and oxidative products that cause endothelial damage [14]. Within an environment of pro-inflammatory cytokines, it has been reported that IL-6 decreases the cytolytic function of the NK cells, that usually attack the infected antigen-presenting cells, leading to amplification of the inflammatory cytokine cascade. Such cytokine storm activates macrophages that produces newer pro-inflammatory cytokine release [15], in a cascade referred to as CSS [1].

From a clinical and metabolic perspective, CSS closely resembles the inflammatory cascade present in rHLH/MAS, with increase in aminotransferases, LDH, d-dimers, CRP and ferritin [16,17]. The HScore, designed to evaluate and diagnose rHLH [18], has been proposed as a surrogate diagnostic tool to identify CSS in the context of COVID-19 [19]. However, in our experience, many patients do not meet the diagnostic criteria because they do not score high enough in certain items like immune-suppression, organomegaly, or ferritin over 6000 ng/mL. Nevertheless, a marked increase in ferritin level seems to be a good marker of macrophage activation [20], and may help in the early identification of a COVID-19 patient developing CSS [21], in order to initiate immunomodulator therapy before clinical worsening ensues. In line with a previous study [22], at our center, we set the threshold of ferritin >1000 ng/mL for initiating treatment. However, patients presenting ferritin levels between 500 and 1000 ng/mL plus significant increase of d-dimers (>1,5 μg/mL) were also treated, given the potential beneficial effect [23,24]. As in rHLH/MAS, anti-cytokine therapy has been successfully used in COVID-19 patients, especially IL-1 inhibition [25]. Administration of a IL-1β receptor antagonist has been suggested to reduce the severity and mortality linked to SARS-CoV-2 infection [17].

Given the relevant role of IL-6 in COVID-19 infection, at our institution, IL-6 levels are routinely determined by the emergency admission laboratory. Yet, we readily found that a proportion of patients exhibiting typical features of rHLH/MAS showed relatively low levels of IL-6. We hypothesized that such patients, not primarily aimed for IL-6 inhibition, would eventually benefit from IL-1 signaling inhibition, via upregulation mediated by NF-kB (Nuclear Factor Kappa B) involved in the control of cellular response to immune and inflammatory cytokines, including IL-6. Additionally, results from a randomized controlled trial showed that septic patients with impaired liver function including hypertransaminasemia and altered coagulation, a typical phenotype within the COVID-19 spectrum, would benefit from IL-1 inhibition [11]. Although serum levels of IL-1 could not be determined by our laboratory, at present, the *Spanish Society of Immunology* does not recommend routine determination of blood IL-1 to guide treatment, since IL-1 mainly accumulates within the affected tissues, and serum levels do not necessarily reflect the real inflammatory state [19]. As most patients did not show a complete rHLH syndrome, soluble CD25 was not determined either (also unavailable in the emergency setting).

ANK is a non-glycated recombinant homologous of IL-1Ra, which differs from the natural human IL-1Ra in the addition of a single methionine at the amino end. ANK blocks IL-1 activity by competitive inhibition of the type 1 cell surface receptor (IL-1 R1) present in the majority of cell types. ANK subcutaneous bioavailability reaches 95% in adults, and the highest blood concentration is achieved between 3 and 7 h after administration (1–2 mg/kg/day), with a half-life of 4–6 h [20]. This relatively short half-live provides safety in case discontinuation is needed, should adverse effects appear. Local cutaneous reactions at the site of injection are the most common side effects of ANK. According to technical specifications, subcutaneous ANK needs to be administered at a 100 mg daily dose when used in self-inflammatory conditions. Intravenous ANK has been used in septic patients with peripheral vasoconstriction and hypoperfusion, in which subcutaneous absorption may be hindered [8]. However, these findings are not common in severe COVID-19 infection.

Previous studies have shown that high dose ANK (1–2 mg/kg/day) used in septic patients [8] and in MAS [10], was not associated to relevant adverse effects. The recent case series by Cavalli et al. [21] shows that intravenous 10 mg/kg/day ANK can be safely administered until clinical improvement, and can be slowly tapered thereafter. We used subcutaneous 100 mg ANK every 6 h for 3 days, tapered for a minimum duration of 7 more days until clinical improvement. For a typical patient, this dosage corresponds to approximately 5.7 mg/kg/day, a dose considerably higher than that recently reported by others [21,22]. The subcutaneous ANK dose proposed by Cavalli et al. [21] was 100 mg every 12 h.

The high bioavailability and convenience of subcutaneous administration makes this route of administration especially suitable for

hospitalized patients. Preparation of intravenous ANK is subject to instability of the mixture and problems related to drug aggregation due to manipulation. Additionally, intravenous administration requires the infusion of a minimum volume of 400 mL per day, against the usual recommendation of 0.5–1 L negative balance for ARDS [23]. Intravenous ANK is likely to improve bioavailability compared to the subcutaneous route and might be preferable for critically ill patients. However, this route is currently an off-label indication of the drug and studies on the pharmacokinetics of intravenous ANK have shown marked fluctuations precluding constant and adequate bioavailability [24]. Subcutaneous administration has been successfully used in the ICU setting for the treatment of rHLH patients [9].

According to the protocol for moderate-severe COVID-19 established in our center, prior to ANK, patients had received combinations of azithromycin, hydroxy-chloroquine, and methylprednisolone, which introduces a confounding factor and limits the interpretation of results. Unlike our series, in which 77.7% had received methylprednisolone and 22.2% additional TCZ, in the Cavalli et al. [21] study, inclusion criteria precluded prior immunomodulator therapy. Patients in our cohort underwent corticoid therapy, which is a currently-debated issue and a matter of concern. Although, glucocorticoids have been successfully used in respiratory failure linked to COVID-19 within the context of CSS, they have been reported to delay viral clearance and promote secondary infections [30,31]. At present, the use of corticoids remains controversial, with plausible indication in low-dose for moderate-severe COVID-19 related ARDS [32]. The systematic review and metanalysis by Landsbury et al. [31] showed that corticosteroids used in community-acquired pneumonia reduces the need for mechanical ventilation, hospital stay and severity of ARDS, but increases the risk of hyperglycemia. However, those studies included different population subsets, the effect on mortality was unclear, and several drugs and regimens were used, thus precluding generalization to COVID-19. Recently, two studies have shown favorable results with the use of corticoids in severe COVID-19 patients [25,26].

Given the current variability of treatment directed to SARS-CoV-2, it is important to recognize the stage of the disease, according to the scheme proposed by Sidiqqi and Mehra [4]. Early infection (phase I, from inoculation to early onset of the disease) is characterized by mild clinical symptoms and would ideally benefit from antivirals. In phase II (moderate) lung affectation is established and can be subdivided in IIa (non-hypoxemic) and IIb (hypoxemic). In our view, patients in phase IIb might be good candidates for corticoid therapy and other immunomodulators, aimed to ameliorate the deleterious and growing hyperinflammatory response. Phase III (severe) is characterized by pulmonary as well as systemic hyperinflammation with accompanying increased inflammatory markers. Immunomodulator therapy is commonly prescribed at this stage.

We observed favorable outcome in 55.6% of the ANK cohort. Yet, adequate assessment of response in patient #5 was conditioned by the occurrence of spontaneous pneumo-mediastinum, a complication already described in COVID-19 patients [27]. These results are slightly worse than those recently reported from a cohort of 14 patients with similar characteristics to our ANK group [28]. However, patients in this series were treated earlier in the course of the disease, not as salvage therapy as in some of our cases.

We found that CRP, IL-6, and ferritin levels significantly decreased between day 1 and 7. Despite the advanced age of some of the patients in the ANK cohort (3 patients over 75 years) we found no adverse effects including opportunistic infections attributable to ANK administration. It is important to highlight that among TCZ patients there was a significant increase of aminotransferase levels between days 1 and 7 of treatment, reflecting our institutional protocol that favors ANK over TCZ for patients exhibiting >5-fold increase of liver enzymes.

Elderly patients with immune system dysregulation [29], and patients harboring severe comorbidities exhibit higher risk of death [30]. Patients #3, 6 and 8 of the ANK cohort showed non favorable course, without ARDS resolution, partly due to advanced age and comorbidity. One of them (11.1%) died 15 days after ANK was stopped, similar to published case series (between 11 and 15%) [2,31,32].

The limited number of participants, the lack of a comparative placebo group, and the retrospective nature of the study are obvious limitations of this retrospective cohort pilot study. Besides, a relevant proportion of the cohort had received prior immunomodulator therapy, a potential confounder regarding the overall outcome. Therefore, although our results are not readily generalizable, this preliminary experience provides some evidence on the dosage, duration, efficacy and safety of ANK therapy. Yet, in order to elucidate the true effectiveness of ANK, and the hypothetical beneficial association of ANK and TCZ in COVID-19 patients, results from several ongoing prospective controlled studies are needed (NCT04330638, NCT04443881, NCT04341584, NCT04339712, NCT04324021, NCT04364009, NCT04412291, NCT04366232).

In conclusion, 55.6% of this small cohort of severely ill COVID-19 patients may have benefited from high dose subcutaneous ANK therapy, with resolution of ARDS secondary to CSS. In this cohort there was a statistically significant reduction of CRP, IL-6 and ferritin levels following therapy, and found no relevant adverse effects. However, firm conclusions about safety and efficacy are limited by the small sample size, presence of confounders and lack of placebo control group.

Author statement

Enrique Iglesias Julián, Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Supervision, Project administration. María López Veloso, Investigation, Writing - original draft. Noelia de la Torre Ferrera, Investigation, Writing - original draft. Julio Cesar Barraza Vengoechea, Investigation, Writing - original draft. Pedro David Delgado-López, Writing - review & editing. María Colazo Burlato, Investigation, Conceptualization. Marta Ubeira Iglesias, Investigation, Conceptualization, Resources. Miguel Montero Baladía, Investigation. Andrés Lorenzo Martín, Investigation. Javier Minguito de la Iglesia, Investigation. Juan Pablo García Muñoz, Investigation. Rodrigo Sanllorente Sebastián, Investigation. Blanca Vicente González, Investigation, Resources. Ana Alemán Alemán, Investigation, Writing - review & editing. Luis Buzón Martín, Supervision, Project administration.

Declaration of competing interest

The authors declare no competing financial interests.

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