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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

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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 lifesaving 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 (104–353) 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.

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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 with 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, immunomodulation 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| Chronic renal disease| Chronic renal disease| Chronic renal disease| Lymphoma| –| Hypertension| –|
| Days from disease onset to ANK treatment | 10| 19| 13| 10| 11| 16| 24| 15| –|
| Days from admission to ANK treatment | 12| 1| 3| 3| 4| 4| 1| 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| HDQ, AZI, MPB|
| Treatment given concomitant to ANK | D1| D3| D7| D1| D3| D7| D1| D7| D7|
| ANK treatment day | PaO2/FiO2 | 0.21| 0.4| 0.28| 0.5| 0.31| 0.21| 0.4| 0.28|
| O2 support | Basal| Basal| Basal| Basal| Basal| Basal| Basal| Basal| Basal|
| Lymphocyte count (/μL) | 800| 1200| 1800| 1094| 809| 800| 1000| 1400| 1700|
| Ferritin (mg/mL) | 4104| 2199| 800| 1094| 809| 800| 1000| 1400| 1700|
| LDH (UI/L) | 389| 314| 273| 246| 233| 246| 253| 256| 261|
| GPT/ALT (UI/L) | 189| 57| 65| 62| 72| 25| 31| 34| 35|
| ANK dose | 100 mg/6 h × 3 days| 100 mg/6 h × 3 days| 100 mg/6 h × 3 days| 100 mg/6 h × 3 days| 100 mg/6 h × 3 days| 100 mg/6 h × 3 days| 100 mg/6 h × 3 days| 100 mg/6 h × 3 days|
| Non-invasive mechanical ventilation | No| No| No| Yes| Yes| No| Yes| Yes| No|
| Intubation | No| No| No| No| No| No| Yes| Yes| No|
| Outcome | No| No| No| Discharge 15 days after initiation of ANK| Discharge at day 10 after initiation of ANK| Discharge 17 days after initiation of ANK| No| Discharge 8 days after initiation of ANK| No|

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 días. 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.

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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 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 U/L (243–598), GOT/AST 36 UI/L (25–189), and GPT/ALT 60.5 U/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 concomitantly. 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, 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 2

| Characteristics                  | Anakinra group (n = 9) | Tocilizumab group (n = 18) | P value |
|----------------------------------|-----------------------|-----------------------------|---------|
| 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   |

### Table 3

| Characteristics                  | Initiation of ANK | Initiation of TCZ | P value* | Day 3 of ANK | Day 3 of TCZ | P value* | Day 7 of ANK | Day 7 of TCZ | P value* | Differences between D1 and D7 of ANK (p value*) |
|----------------------------------|-------------------|-------------------|----------|-------------|-------------|----------|-------------|-------------|----------|-----------------------------------------------|
| 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    | 0.018 (0.004)                               |
| FiO2                              | 0.35 (0.21–1)     | 0.28 (0.21–1)     | 0.219    | 0.4 (0.31–1)| 0.29        | 0.179    | 0.28 (0.21–0.8)| 0.26 (0.21–0.6)| 0.250    | 0.833 (0.574)                               |
| Lymphocyt count (/μL)             | 1000 (200–1400)   | 800 (400–3000)    | 0.765    | 700 (500–1200)| 950        | 0.524    | 1050 (600–1400)| 1150 (500–3800)| 0.569    | 0.750 (0.049)                               |
| Ferritin (mg/mL)                  | 1469 (578–4104)   | 1434 (948–2874)   | 0.790    | 1554.5 (433–2199)| 1512       | 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    | 0.6 (0.3–10.7)| 0.65        | 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 (15.5–90.9)| 0.865    | 29.4 (7–1792)| 67         | 0.475    | 5.2 (1.7–202) | 371.8 (50.7–662.6)| 0.025    | 0.043 (0.285)                               |
| CRP (mg/L)                        | 62 (20–244)       | 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         | 326        | 0.621    | 284.5 (246–435)| 341 (226–670)| 0.281    | 0.345 (0.382)                               |
| GOT/ALT (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          | 68 (11–171)| 0.680    | 90.5 (42–211)| 166       | 0.224    | 0.080 (0.004)                               |

* Student’s T test, Mann-Whitney U test, and Wilcoxon’s test as needed.
without response (cases #2, and 7). Despite developing pneumo-
mediastinum, 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 pa-
rameters 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 signifi-
cance, likely because ANK patients presented poorer oxygenation pa-
rameters 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 re-
ceptor, 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 angio-
tensin II receptors present in type II pneumocytes [1]. The innate im-
une 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 an-
tigen presentation to dendritic cells through TLR-7 promotes the pro-
duction of type I IFN, which limits viral replication and contributes to a
prolonged adaptive immune response. Data from previous studies sug-
gest 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 multiprotein 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 and IL-6 [1] and enhances local T cell cytotoxicity [14]. IL-6 en-
hances 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 amino-
transferases, 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 pre-
senting 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 insti-
tution, 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 upregula-
tion 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 hyper-
transaminasemia 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 labora-
tory, 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 deter-
mined 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-1R1) 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-life 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. Intra-
vavenous ANK has been used in septic patients with peripheral vascon-
striction 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 adminis-
tration 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, hydroxychloroquine, 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 affection 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 size, presence of confounders and lack of placebo control group.

Author statement

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Declaration of competing interest

The authors declare no competing financial interests.

References

[1] Y. Jamilloux, T. Henry, A. Belot, S. Vieil, F. Fauter, T. El Jammal, T. Walzer, B. François, P. Sève, Should we stimulate or suppress immune responses in COVID-19? Cytokine and anti-cytokine interventions, Autoimmun. Rev. (2020) 102567, https://doi.org/10.1016/j.autrev.2020.102567.

[2] C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Z. Cheng, T. Yu, J. Xia, Y. Wei, W. Wu, X. Xie, W. Yin, H. Li, M. Liu, Y. Xiao, H. Gao, L. Guo, J. Xie, G. Wang, R. Jiang, Z. Gao, Q. Jin, J. Wang, B. Cao, Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, Lancet 395 (2020) 497–506, https://doi.org/10.1016/S0140-6736(20)30183-5.

[3] M. Yang, Cell pyroptosis, a potential pathogenic mechanism of 2019-nCoV infection, SSRN Electron. J., 2020, https://doi.org/10.2139/ssrn.3574240.

[4] H.K. Siddiqi, M.R. Mehra, COVID-19 illness in native and immunosuppressed states: a clinical-therapeutic staging proposal, J. Heart Lung Transplant. (2020) 1–18, https://doi.org/10.1016/j.healun.2020.09.012.

[5] N. Kaneko, M. Kurata, T. Yamamoto, S. Morikawa, J. Masumoto, The role of interleukin-1 in general pathology, Inflamm. Regen. 39 (2019) 1–16, https://doi.org/10.1186/s41123-019-0011-0.

[6] A. Ravelli, A.A. Grom, E.M. Behrens, R.Q. Cron, Macrophage activation syndrome as part of systemic juvenile idiopathic arthritis: diagnosis, genetics, pathophysiology and treatment, Gene Immun. 13 (2012) 289–298, https://doi.org/10.1016/j.gene.2012.3.

[7] P.M. Miettunen, A. Navaretd, A. Jayanthan, E.M. Behrens, R.Q. Cron, Successful treatment of severe paediatric rheumatic disease-associated macrophage activation syndrome with interleukin-1 inhibition following conventional immunosuppressive therapy: case series with 12 patients, Rheumatology 50 (2011) 417–419, https://doi.org/10.1093/rheumatology/ker218.
