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Autoimmunity Reviews 20 (2021) 102726

Contents lists available at ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

Combined Anakinra and Ruxolitinib treatment to rescue extremely ill COVID-19 patients: A pilot study.

ARTICLE INFO

Keywords
COVID 19
Hyperinflammation
Acute respiratory failure
Invasive mechanical ventilation
Anakinra
Ruxolitinib

Dear Editor,

The SARS Cov-2-induced COVID-19 may evolve in 5% of patients towards an acute respiratory failure (ARF) and multi-system organ failure (MSOF), requiring admission into intensive care unit (ICU) and invasive mechanical ventilation (IMV), with a high mortality rate reported between 30% and 50% [1,2]. ARF is due to an abnormal host immune response with excess circulating inflammatory cytokines, named "cytokine storm" [3]. The cytokine storm constitutes a pathogenic mechanism shared by other well-described syndromes such as primary (p) and secondary (s) hemophagocytic lymphohistiocytosis (HLH) [4]. pHLH mainly depends on IFNγ and an anti-IFNγ mAb (emapalumab) has been recently approved by the US FDA for pHLH treatment [5]. Blocking signaling downstream the IFNγ receptor using ruxolitinib, a Janus Kinase (JAK) 1 and 2 inhibitor, is an alternative effective strategy in pHLH [6]. sHLH appears less dependent on IFNγ, and blocking interleukin (IL)-1, using the IL-1 receptor antagonist anakinra, is protective [7]. Anakinra or tocilizumab have recently been used in the treatment of early COVID-19-associated ARF with a 15–25% reported mortality, but appear insufficient to control critically ill patients in ICU [8–10]. We hypothesized that blocking both IL-1 and IFNγ pathways, using a combination of high-dose anakinra and low-dose ruxolitinib may constitute a rescue treatment in these extremely ill patients.

On April 6th, 2020, we submitted to the French authorities a randomized clinical trial (RCT) evaluating the combination of anakinra and ruxolitinib in extremely severe stages of COVID-19 in ICU, which was accepted by May 15th (EudraCT 2020–001754-21). In the meantime, our institutional ethical committee allowed us to use this treatment on a compassionate basis. After informed telephonic consent from their family (visits in the hospital were not allowed at this time), adults older than 18 years were prospectively included if they presented 1) a COVID-19 pneumonia (positive SARS-CoV-2 RT-PCR), 2) a severe ARF (prone positioning and neuromuscular blocking agents associated with extracorporeal membrane oxygenation (ECMO) and/or inhaled nitric oxide/almitrine) with a PaO2/FiO2 $<$ 100 or a PaO2/FiO2 (from the ventilator) ratio $\leq$ 150 under ECMO (FiO2 on oxygenator being at 100%) and/or a compliance of the respiratory system $\leq$30 ml/cmH2O and 3) a biological inflammatory syndrome with a C-reactive protein $>$100 mg/l and a ferritinemia $>$1000 µg/l. All patients received anticoagulants, targeting an anti-Xa level of 0.5 to 0.7 U/mL. Intravenous anakinra (300 mg/day for 11 days, gradually tapered until day 14, mean total dose: 3800 mg), was associated with oral ruxolitinib (5 mg bid until day 28, mean total dose: 280 mg). The main outcome was mortality at day 28 and secondary objectives included safety.

We initiated this rescue therapy in 11 patients presenting with severe ARF, starting 18 $\pm$ 6 days after the onset of the symptoms, and 13 $\pm$ 5 days after hospitalization. On inclusion, 8 patients (73%) received hydroxychloroquine and/or azithromycine (Table 1) and all had a β-lactam antibiotic. Patients presented with very severe ARF requiring ECMO in 6 of them, with a pronounced organ dysfunction as reflected by the high initial Sequentiel Organ Failure Assessment (SOFA) score (Table 1). We observed a continuous improvement in compliance, reduction of organ dysfunction/failure and a rapid decline in the inflammatory biomarkers (Table 2). All patients were alive on day 28, but one deceased at day 48 (a 77-year-old man, withholding decision). All the 10 survivors (including the 6 who received ECMO) were all successfully weaned from IMV, and discharged from the hospital, 20 $\pm$ 8 days and 32 $\pm$ 7 days following treatment initiation, respectively. The treatment was administered without adverse side effects, except a severe transient liver transaminase elevation at day 15 in one patient, requiring ruxolitinib interruption. Eight nosocomial infections were diagnosed in 4 patients (36%) during the 28-day period following treatment initiation (4 ventilator-associated pneumonia, 2 bacteremia, one cellulitis and one herpetic stomatitis). Six patients had a positive SARS-CoV-2 RT-PCR at the time of treatment initiation, but only four remained positive at day 3 and none at day 7. The total lymphocyte count rapidly increased at day 3 after treatment initiation. Blood serologic tests were found positive for SARS-CoV-2 immunoglobulin G in 8 patients tested.

Recently, dexamethasone has been reported effective in severe COVID-19, but reduces by only 28% the mortality at day 28 in patients under IMV [11]. Similarly, to counterbalance the prolonged cytokine

https://doi.org/10.1016/j.autrev.2020.102726
Received 11 October 2020; Accepted 17 October 2020
Available online 14 December 2020

Table 1
Characteristics of the patients.

	Patients
	N = 11
On admission	
Age	61 ± 13
Male sex	9 (82)
SAPS II score	50 ± 16
Co-morbidities	
Hypertension	2 (18)
Chronic cardiac disease	1 (9)
Hypercholesterolemia	3 (27)
Diabetes	5 (45)
Cancer	0
Chronic obstructive lung disease	1 (9)
Renal chronic insufficiency	0
Chronic liver disease	0
Immunosuppression	0
Previous and ongoing corticotherapy	0
On inclusion	
Time from intubation	10 ± 5
Time from 1st positive COVID 19 PCR	16 ± 11
Hydroxychloroquine and azithromycine	6 (55)
Hydroxychloroquine alone	1 (9)
Azithromycine alone	1 (9)
Mechanical ventilation	
Tidal volume, ml/kg PBW	4.7 ± 1.8
Minute ventilation, l/min	7.7 ± 3.8
Driving pressure, cmH2O	13 ± 5
PEEP, cmH2O	12 ± 3
Adjuvants to mechanical ventilation	
Neuromuscular blocking agents	11 (100)
Prone positioning	10 (91)
Almitrine/Inhaled nitric oxide	8 (73)
Veno-venous ECMO	6 (55)

Results are expressed as the mean ± SD or N (%); SAPS II score, Simplified Acute Physiology Score; PBW, predicted body weight.

Table 2
Evolution under treatment.

	Inclusion	Day 3	Day 7	Day 14	ANOVA
SOFA score	8.3 ± 2.2	7.4 ± 3.1	6.5 ± 2.9	3.2 ± 2.6*	<0.001
Compliance of the respiratory system, ml/cmH2O	24 ± 7	26 ± 12	31 ± 20	41 ± 21†	0.022
C-reactive protein, mg/l	262 ± 75‡	134 ± 98	58 ± 37	63 ± 38	<0.001
Ferritinemia, µg/l	2857 ± 1703#	2036 ± 1378	1374 ± 948	1569 ± 826	<0.001
Lymphocytes, cells/µl	984 ± 549	1560 ± 670	1600 ± 604	1600 ± 560	0.025
Neutrophils/ Lymphocytes ratio	11.8 ± 7.5¶	5.9 ± 3.2	4.5 ± 3.1	4.0 ± 1.9	<0.001

Results are expressed as the mean ± SD;
* Holm-Sidak method: $p < 0.02$ vs. inclusion, day 3 and day 7.
† 8 patients (3 patients had been extubated); Holm-Sidak method: $p < 0.05$ vs. inclusion and day 3.
‡ Holm-Sidak method: $p < 0.001$ vs. day 3, day 7 and day 14.
# Holm-Sidak method: $p < 0.03$ vs. day 3 and $p < 0.001$ vs. day 7 and day 14.
¶ Holm-Sidak method: $p < 0.02$ vs. day 3, day 7 and day 14.

storm observed in extremely severe patients, anakinra or tocilizumab alone may not be sufficient and blocking IFNs may also be necessary [8,10]. Indeed, whereas the first anti-viral defense consisting in IFNα/β production, seems initially inhibited by SARS-CoV-2 in very severe COVID-19 patients, a late IFNα/β signature is present and may play a detrimental role, as suggested in a SARS-CoV animal model [12–14]. In addition, the IL-18/IFN-γ pathway is involved in critically ill COVID-19 patients as suggested by elevated IL-18 concentrations and the strong association with high circulating concentrations of the chemokine interferon-γ-induced protein 10 (IP-10/CXCL10) [3]. Ruxolitinib inhibits JAK1 and 2 kinases downstream the receptors of several cytokines, including IFNα/β/γ and IL-6, but not IL-1β, and has been successfully used at much lower doses than in blood malignancies, in the treatment of pHLH [4,6]. Anakinra is licensed as subcutaneous 100 mg daily injection in rheumatologic disorders, but due to its good safety profile, is commonly used off-label. We used it intravenously, at higher doses, as previously reported in severe sHLH and in COVID-19 ARF [7–9]. This combined treatment raised safety questions, but overall, the safety profile was acceptable. In one patient, severe but transient liver transaminase elevation occurred, requiring ruxolitinib interruption. A nosocomial infection was diagnosed in 36% of the patients, which is not unexpected in severe acute respiratory distress syndrome patients [15]. By comparison, emapalumab induced 10 severe infections among 34 treated pHLH patients [5]. Possible interference with the anti-viral adaptive immune response was another concern. Although 6 patients had a positive SARS-CoV-2 RT-PCR at the time of treatment initiation, none remained positive at day 7, raising the hypothesis that blocking IL-1β-induced harmful NLRP3 activation loop using anakinra, in fact decreases cell pyroptosis and viral spreading out of the cells. Moreover, similarly to observations made in sHLH, total lymphocyte count rapidly increased after treatment initiation, likely reflecting the beneficial effect of blocking the cytokine storm on the lymphocyte exhaustion observed during COVID-19 [16,17]. Finally, serologic tests were positive in all the patients tested to date, showing that the adaptive immune response was not hampered.

This preliminary report strongly suggests that combined anakinra and ruxolitinib treatment may be effective to rescue COVID-19 patients with critical ARF and MSOF, a population with a high mortality rate.

Funding/Support

Treatments were purchased by the Assistance Publique – Hôpitaux de Marseille.

Declaration of Competing Interest

Dr. Papazian reported receiving grants from Sedana and the French Ministry of Health.

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