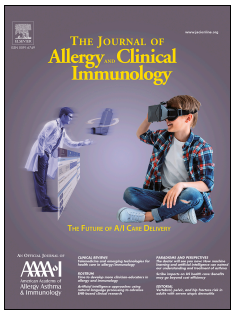
Journal Pre-proof



Anakinra combined with methylprednisolone in patients with severe COVID-19 pneumonia and hyperinflammation: an observational cohort study

Giorgio Bozzi, MD, Davide Mangioni, MD, Francesca Minoia, MD, Stefano Aliberti, MD, Giacomo Grasselli, MD, Laura Barbetta, MD, Valeria Castelli, MD, Emanuele Palomba, MD, Laura Alagna, MD, Andrea Lombardi, MD, Riccardo Ungaro, MD, Carlo Agostoni, MD, Marina Baldini, MD, Francesco Blasi, MD, Matteo Cesari, MD, PhD, Giorgio Costantino, MD, Anna Ludovica Fracanzani, MD, Nicola Montano, MD, Valter Monzani, MD, Antonio Pesenti, MD, Flora Peyvandi, MD, Marcello Sottocorno, PharmD, Antonio Muscatello, MD, Giovanni Filocamo, MD, Andrea Gori, MD, Alessandra Bandera, MD, PhD

PII: S0091-6749(20)31621-3

DOI: https://doi.org/10.1016/j.jaci.2020.11.006

Reference: YMAI 14835

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 7 August 2020

Revised Date: 1 November 2020

Accepted Date: 6 November 2020

Please cite this article as: Bozzi G, Mangioni D, Minoia F, Aliberti S, Grasselli G, Barbetta L, Castelli V, Palomba E, Alagna L, Lombardi A, Ungaro R, Agostoni C, Baldini M, Blasi F, Cesari M, Costantino G, Fracanzani AL, Montano N, Monzani V, Pesenti A, Peyvandi F, Sottocorno M, Muscatello A, Filocamo G, Gori A, Bandera A, Anakinra combined with methylprednisolone in patients with severe COVID-19 pneumonia and hyperinflammation: an observational cohort study, *Journal of Allergy and Clinical Immunology* (2020), doi: https://doi.org/10.1016/j.jaci.2020.11.006.

Anakinra combined with methylprednisolone in patients with severe COVID-19 pneumonia and hyperinflammation: an observational cohort study

Giorgio Bozzi, MD[1§], Davide Mangioni, MD[1,2*§], Francesca Minoia, MD[3], Stefano Aliberti MD[4,5], Giacomo Grasselli, MD[5,6], Laura Barbetta, MD[7], Valeria Castelli, MD[1], Emanuele Palomba, MD[1], Laura Alagna, MD[1], Andrea Lombardi, MD[1], Riccardo Ungaro, MD[1], Carlo Agostoni, MD[3,8], Marina Baldini MD[9], Francesco Blasi MD[4,5], Matteo Cesari MD, PhD[8,10], Giorgio Costantino MD[6,8], Anna Ludovica Fracanzani MD[5,11], Nicola Montano MD[5,12], Valter Monzani MD[7], Antonio Pesenti, MD[5,6], Flora Peyvandi MD[5,13], Marcello Sottocorno, PharmD[14], Antonio Muscatello, MD[1], Giovanni Filocamo MD[3], Andrea Gori, MD[1,5,15], Alessandra Bandera MD, PhD[1,5,15]

1. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Infectious Disease Unit, Milan, Italy
2. University of Milan, Department of Medical Biotechnology and Translational Medicine,
3. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, UOC Pediatria a Media Intensità di Cure, Milan, Italy
4. Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Respiratory Unit and Cystic Fibrosis Adult Center, Milan, Italy
5. University of Milan, Department of Pathophysiology and Transplantation, Milan, Italy
6. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Department of Anesthesia, Critical Care and Emergency, Milan, Italy;
7. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Acute Medical Unit, Milan, Italy;
8. University of Milan, Department of Clinical Sciences and Community Health, Milan, Italy;
9. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Internal Medicine, Milan, Italy

10. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Geriatric Unit, Milan, Italy;
11. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Internal Medicine and Metabolic Diseases, Milan, Italy;
12. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Internal Medicine, Immunology and Allergology, Milan, Italy;
13. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Internal Medicine and Hemostasis and thrombosis Unit, Milano, Italy
14. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Hospital Pharmacy, Milano, Italy
15. Centre for Multidisciplinary Research in Health Science, University of Milan, Milan, Italy.

§ Giorgio Bozzi and Davide Mangioni equally contributed and should both be considered first co-authors

CORRESPONDING AUTHOR

Davide Mangioni, MD

Department of Medical Biotechnology and Translational Medicine, University of Milan

Department of Internal Medicine, Infectious Diseases Unit

IRCCS Ca' Granda Ospedale Maggiore Policlinico Foundation

Via Francesco Sforza 35, 20122, Milan, Italy

Phone. +39 02 5503.4778

Fax. +39 02 5503.4779

@ Mail. davide.mangioni@unimi.it

FUNDING SUPPORT: none

DISCLOSURE STATEMENT: Francesca Minoia reports consultancy fees from SOBI; Giovanni Filocamo reports consultancy fees from SOBI; all the other authors declare no conflict of interest for the submitted work.

STRUCTURED ABSTRACT

Background. Immunomodulants have been proposed to mitigate SARS-Cov-2-induced cytokine storm, which drives acute respiratory distress syndrome in COVID-19.

Objective. To determine efficacy and safety of the association of IL-1 receptor antagonist anakinra plus methylprednisolone in severe COVID-19 pneumonia with hyperinflammation.

Methods. Secondary analysis of prospective observational cohort studies at an Italian tertiary health-care facility. COVID-19 patients consecutively hospitalized (02/25/2020 to 03/30/2020), with hyperinflammation (ferritin ≥1000ng/mL and/or C-reactive protein ≥10mg/dL) and respiratory failure (oxygen therapy from 0.4 FiO2 Venturi mask to invasive mechanical ventilation) were evaluated to investigate the effect of high-dose anakinra plus methylprednisolone on survival. Patients were followed from study inclusion to day 28 or death. Crude and adjusted (sex, age, baseline PaO_2:FiO_2 ratio, Charlson Index, baseline mechanical ventilation, hospitalization to inclusion lapse) risks were calculated (Cox proportional regression model).

Results. 120 COVID-19 patients with hyperinflammation (median age 62 years, 80.0% males, median PaO_2:FiO_2 ratio 151, 32.5% on mechanical ventilation) were evaluated. Of these, 65 were treated with anakinra and methylprednisolone and 55 were untreated historical controls. At 28 days, mortality was 13.9% in treated patients and 35.6% in controls (Kaplan-Meier plots, p=0.005). Unadjusted and adjusted risk of death was significantly lower for treated patients compared to controls (HR 0.33 (95%CI 0.15-0.74), p=0.007 and HR 0.18 (95%CI 0.07-0.50), p=0.001, respectively). No significant differences in bloodstream infections or laboratory alterations were registered.

Conclusions. Treatment with anakinra plus methylprednisolone may be a valid therapeutic option in COVID-19 patients with hyperinflammation and respiratory failure, also on mechanical ventilation. Randomized, controlled trials including use of either agent alone are needed to confirm these results.

CLINICAL IMPLICATIONS:

In the search for an optimal support treatment, combination of high-dose anakinra plus methylprednisolone may be beneficial in COVID-19 severe pneumonia with hyperinflammation. This combined treatment is candidate for further investigation.

CAPSULE SUMMARY:

Treated patients (32% on mechanical ventilation at inclusion) had a significantly higher 28-day survival than controls (86.1% vs 64.3%, respectively). No significant adverse event was registered during treatment. Randomized, controlled trials are needed.

KEYWORDS:

SARS-COV-2; COVID-19; hyperinflammation; anti-interleukin 1; anakinra; corticosteroids; methylprednisolone; immunomodulation; respiratory failure; mechanical ventilation

ABBREVIATIONS:

AE: adverse events

CCI: Charlson Comorbidity Index

COVID-19: coronavirus disease 2019

CRP: C-reactive protein

CSS: cytokine storm syndromes

IL-1: interleukin-1

IQR: interquartile range

MPD: methylprednisolone

MV: mechanical ventilation

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

INTRODUCTION

As of November 2020, the ongoing pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) affected 46 million people world-wide, resulting in over 1.2 million deaths [1].

High levels of pro-inflammatory cytokines, C-reactive protein (CRP) and ferritin correlate with worse outcomes in patients with severe COVID-19 [2-4]. Growing evidence suggests that these patients develop a hyperinflammatory syndrome resembling cytokine storm syndromes (CSS), potentially benefiting from immunomodulatory treatment [5].

IL-1-receptor antagonist anakinra is one of the cytokine-blocking agents employed for COVID-19 treatment [5]. While randomized clinical trials are ongoing [6], single-center experiences have reported encouraging findings [7-10]. The short half-life of anakinra enables to rapidly discontinue its action in case of adverse reactions or secondary infections, making its use suitable also for critically ill patients [7,10]. IL-1 inhibition is also associated with reduction in endothelial dysfunction and microvascular alteration [11], which seem crucial in COVID-19-related thromboembolic events [12].

Corticosteroid treatment is a cornerstone in the management of non-infectious hyperinflammatory conditions, namely CSS [13]. Favorable data have recently emerged in support of the use of corticosteroids in patients with severe COVID-19, especially in those receiving invasive mechanical ventilation [14-17]. In a recent meta-analysis of prospective, randomized clinical trials on critically ill patients with COVID-19, use of corticosteroids compared to placebo or standard of care (SOC) resulted in a significantly lower 28-day mortality [18].

With this study, we aimed at investigating efficacy and safety of combined treatment with anakinra and methylprednisolone (anti-IL-1+MPD) in COVID-19 patients with hyperinflammation and respiratory failure.

RESULTS AND DISCUSSION

Out of 476 COVID-19 patients admitted at our hospital between February 25 and March 30, 2020, a total of 120 (25.2%) patients with hyperinflammation and respiratory failure were included according to inclusion/exclusion criteria (see METHODS in the Online Repository). Of these, 65 were treated with anti-IL-1+MPD and 55 were untreated historical controls.

Median age of study population was 62 years (IQR 54.5-70), 80.0% (96/120) were males and median Charlson Comorbidity Index (CCI) was 0 (IQR 0-1). At inclusion, median PaO2:FiO2 ratio was 151 (105-204.5), 32.5% (39/120) were on MV, median ferritin was 1555 mcg/L (1239-2679), median CRP was 15.2 mg/dl (10.8-23.1). Compared to historical controls, patients treated with anti-IL-1+MPD had less frequently CCI≥1 (25% versus 45.4%, p=0.017), longer duration of hospitalization before inclusion (3 versus 1 median days, p<0.0001), lower baseline PaO2:FiO2 ratio (median of 142 versus 173, p=0.049), reduced proportion of lopinavir/ritonavir treatment (30.8% versus 70.9%, p<0.0001) and higher proportion of anti-coagulant therapy (63.1% versus 38.9%, p=0.009). The two groups did not differ by age, gender, number of patients on MV at inclusion, baseline ferritin, CRP, lymphocyte and platelet counts, hemoglobin and liver enzyme levels, use of remdesivir and hydroxichloroquine during hospitalization (Table 1).

Within the 28-day follow-up, 28/120 (23%) patients died, 9/65 (13.9%) in the anti-IL-1+MPD group compared to 19/55 (35.6%) controls (Kaplan-Meier curves, p=0.004, Figure 1A). Among patients without MV, mortality rate was 6/47 (12.8%) in anti-IL-1+MPD compared to 10/34 (29.4%) in controls (p=0.04, Figure 1B). Among those with MV, it was 3/18 (16.7%) in anti-IL-1+MPD and 9/21 (42.8%) in controls (p=0.076, Figure 1C). Overall cumulative risk of death at 28 days was significantly lower for anti-IL-1+MDP compared to controls (HR 0.33, 95%CI 0.15-0.74, p=0.007). Other factors significantly associated with survival were age <65 years, baseline PaO_2:FiO_2 ratio >100 and CCI 0 compared to ≥1. No association to survival was found for antiviral treatment or for anticoagulant therapy (see Tables E1 and E2 in the Online Repository). At multivariable analysis, treatment with anti-IL-1+MPD was found to be independently associated

with survival when adjusted by gender, age, baseline PaO_2:FiO_2 ratio, CCI, MV at inclusion, and days between hospitalization and inclusion (HR 0.18, 95%CI 0.07-0.50, p=0.001) (see Table E3 in the Online Repository).

Treated patients experienced consistent improvements in respiratory function and a rapid lowering of serum CRP levels during treatment (Figure 2).

Overall, anti-IL-1+MPD treatment was well tolerated. Grade ≥3 gamma-glutamyl transferase increase (27.7%), anemia (24.6%), alanine transaminase increase (6.2%), granulocytopenia (1.5%) were observed in treated patients. However, a comparable proportion of these AEs were observed within controls. No difference in AE were reported between intravenous and subcutaneous route of administration. Nine bloodstream infections (13.8%) were observed in anti-IL-1+MDP and 4 (7.3%) in controls (p=0.23).

To the best of our knowledge, this is the largest observational study evaluating efficacy of anakinra associated with methylprednisolone in COVID-19 patients with hyperinflammation and respiratory failure.

Several clinical trials are currently in progress to evaluate the benefits of anakinra treatment in COVID-19 [6]. In a retrospective study of COVID-19 patients with respiratory failure outside the ICU, Cavalli et al. found a survival benefit in high-dose anakinra (5 mg/kg twice a day intravenously) use compared to SOC (90% vs 56% at day 21) [9]. A significant reduction in a composite outcome of mortality and/or ICU admission was also observed in a French cohort treated with subcutaneous anakinra (100 mg twice a day for 72 hours, then 100 mg daily for 7 days) compared to historical controls (25% vs 73% at day 20) [10]. In contrast to these studies, our analysis encompassed almost one third of patients (32.5%) who were on MV at inclusion. Moreover, combined treatment with high-dose anakinra and methylprednisolone was chosen based on widely approved treatment regimens used in severe cytokine storm syndromes [13]. Of note, corticosteroids such as dexamethasone [14,15] and methylprednisolone [16,17] have recently shown

to be beneficial in COVID-19 patients with respiratory failure. In the RECOVERY trial, the addition of short-course dexamethasone (6mg q24h for 10 days or less) to SOC resulted in lower 28-day mortality compared to SOC alone among hospitalized COVID-19 patients (22.9% vs 25.7%, respectively) [14]. Interestingly, the highest beneficial effect was obtained in patients on invasive mechanical ventilation (29.3% mortality in the dexamethasone group compared to 41.4% mortality in the SOC group at day 28), whereas no difference was seen among those receiving no respiratory support. No treatment with anakinra was reported in any of the study arms. Conversely, in the CoDEX trial, the addition of intravenous dexamethasone (20 mg q24h for 5 days, followed by 10 mg q24h for additional 5 days) to SOC compared to SOC alone in mechanically ventilated COVID-19 patients with moderate to severe ARDS resulted in a significant benefit in the number of ventilator-free days (6.6 vs 4.0 days) but not in all-cause 28-day mortality (56.3% vs 61.5%, respectively) [15]. In their multicenter quasi-experimental study, Fadel et al. compared mortality and/or ICU admission of patients with moderate to severe COVID-19 either on early, short-course methylprednisolone (0.5 to 1 mg/kg/die for 3 days) or SOC [16]. The composite endpoint occurred at lower rate in the methylprednisolone group (34.9% vs 54.3% at day 14). Again, no patient was treated with anakinra. Ramiro et al. prospectively investigated the effect of high-dose intravenous methylprednisolone (250 mg on day 1 followed by 80 mg on days 2–5) on the outcome of patients with severe COVID-19-associated CSS and respiratory failure [17]. In 43% of cases anti-IL6 tocilizumab was added as escalation of immunosuppressive treatment, whereas no patients received anakinra. Compared to matched historical controls, hospital mortality was 65% lower and the need of mechanical ventilation was 71% lower in the treatment group. Table 2 summarizes the major clinical studies that have employed either anakinra alone or steroids alone for the treatment of severe COVID-19 so far.

In our study, patients treated with the combination of anakinra plus methylprednisolone experienced lower mortality than controls (13.9% vs 35.6% at day 28, p=0.004). Notably, mortality in treated patients who were on MV at baseline was as low as 16.7%, yet only a trend towards significance

emerged compared to SOC group, possibly due to limited sample size. The outcomes of this population can be compared to the results of MV patients in the RECOVERY trial (no comparison can be made for non-MV patients due to different disease severity between studies) [14]. While the 28-day mortality is similar between MV patients in control groups (42.8% vs 41.4%), our cohort of patients treated with anti-IL-1+MPD seemed to have experienced a better outcome than patients in the dexamethasone arm of RECOVERY trial (16.7% vs 29.3% mortality at 28 day, respectively). The use of anakinra as add-on therapy to corticosteroids may provide meaningful clinical benefits in this setting and warrants further consideration. The impact of combined treatment was confirmed after adjusting by age, comorbidities, respiratory dysfunction and length of hospitalization before inclusion, with a 18% reduction in mortality. Combined treatment was overall well tolerated, with no significant differences in AE compared to controls. Frequencies of bloodstream infections and laboratory alterations of patients treated with anakinra plus methylprednisolone were similar to those reported in studies investigating anakinra as a single agent [8-10].

Our work has limitations. Firstly, the monocentric nature of the study might affect the generalizability of our results. Secondly, although controls have been recruited in the same setting, their number is lower than the cases, mainly because the association of anti-IL-1+MPD has been implemented relatively early during the pandemic. Thirdly, since no groups treated either with anakinra alone or methylprednisolone alone have been included in the analysis, no definitive conclusions could be drawn on the single or synergistic effect of the two drugs. Moreover, standard-of-care consisted of evolving combinations of antivirals and anti-coagulant therapy which, although not significantly associated to survival, represent a potential bias. Lastly, no primary hard endpoint other than 28-day mortality was considered: intermediate endpoints may help better evaluating treatment efficacy in patients with different severity and length of disease.

In conclusion, combined treatment with anakinra and methylprednisolone may be a valid therapeutic option in COVID-19 patients with hyperinflammation and respiratory failure, also in

mechanically ventilated patients. Randomized, controlled trials that include arms for steroids and anti-IL1 therapy alone are needed to confirm these results.

For detailed methods, please see the Methods section in this article's Online Repository at www.jacionline.org

ACKNOWLEDGMENTS

We thank the health care workers of our Hospital, for their professional and indefatigable commitment to COVID-19 patients' care, in these difficult times.

We would like to thank Dr. Liliane Chatenoud for her endless patience in statistical analysis support.

We are grateful to Chiara Abbruzzese, Nicola Bottino, Paola Tagliabue for their active participation to study conduction; and to Marcello Macchia, Gisella Beatrice Beretta and all the data managers and study coordinators involved in data collection.

"COVID-19 NETWORK" WORKING GROUP: Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico. Scientific Direction: Silvano Bosari, Luigia Scudeller, Giuliana Fusetti, Laura Rusconi, Silvia Dell'Orto; Department of Transfusion Medicine and Biobank: Daniele Prati, Luca Valenti, Giuseppe Lamorte, Maria Manunta, Guido Baselli, Luigi Santoro; Infectious Diseases Unit: Andrea Gori, Alessandra Bandera, Antonio Muscatello, Davide Mangioni, Laura Alagna, Giorgio Bozzi, Andrea Lombardi, Riccardo Ungaro, Teresa Itri, Valentina Ferroni, Valeria Pastore, Roberta Massafra, Ilaria Rondolini; Internal Medicine, Hemophilia and Thrombosis Center and Fondazione Luigi Villa: Flora Peyvandi, Roberta Gualtierotti, Barbara Ferrari, Raffaella Rossio, Elisabetta Corona, Nicolò Rampi, Costanza Massimo; Internal Medicine, Immunology and Allergology: Nicola Montano, Barbara Vigone, Chiara Bellocchi, Elisa Fiorelli, Valerie Melli, Eleonora Tobaldini; Respiratory Unit and Cystic Fibrosis Adult Center: Francesco Blasi, Stefano Aliberti, Maura Spotti, Edoardo Simonetta, Leonardo Terranova, Francesco Amati, Carmen Miele, Sofia Misuraca, Alice D'Adda, Silvia Della Fiore, Marta Di Pasquale, Marco Mantero Martina Contarini, Margherita Ori, Letizia Morlacchi, Valeria Rossetti, Andrea Gramegna, Maria Pappalettera, Mirta Cavallini, Annalisa Vigni; Cardiology Unit: Marco Vicenzi, Irena Rota. Emergency Medicine: Giorgio Costantino, Monica Solbiati, Ludovico Furlan, Marta Mancarella, Giulia Colombo, Giorgio Colombo, Alice Fanin; Acute Internal Medicine: Valter Monzani, Angelo Rovellini, Laura Barbetta, Filippo Billi, Christian Folli; Internal Medicine: Marina Baldini, Irena

Motta, Natalia Scaramellini; Internal Medicine and Metabolic Diseases: Anna Ludovica Fracanzani, Rosa Lombardi, Federica Iuculano; Geriatric Unit: Matteo Cesari, Marco Proietti, Laura Calcaterra. Istituto di Ricerche Farmacologiche Mario Negri IRCCS: Alessandro Nobili, Mauro Tettamanti, Igor Monti.

REFERENCES

1. Johns Hopkins University. The Center for Systems Science and Engineering. https://systems.jhu.edu. Accessed November 1, 2020
2. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. Clinical Infectious Diseases 2020 Jul 28;71(15):762-768
3. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The Lancet 2020 Mar 28;395(10229):1054-1062
4. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. Journal of Clinical Investigation 2020 May 1;130(5):2620-2629
5. Ingraham NE, Lotfi-Emran S, Thielen BK, Techar K, Morris RS, Holtan SG, et al. Immunomodulation in COVID-19. The Lancet Respiratory Medicine 2020 Jun;8(6):544-546
6. King A, Vail A, O'Leary C, Hannan C, Brough D, Patel H, et al. Anakinra in COVID-19: important considerations for clinical trials. Lancet Rheumatology 2020 Jul;2(7):e379-e381
7. Filocamo G, Mangioni D, Tagliabue P, Aliberti S, Costantino G, Minoia F, et al. Use of anakinra in severe COVID-19: A case report. International Journal of Infectious Diseases 2020 Jul;96:607-609
8. Pontali E, Volpi S, Antonucci G, Castellaneta M, Buzzi D, Tricerri F, et al. Safety and efficacy of early high-dose IV anakinra in severe COVID-19 lung disease. Journal of Allergy and Clinical Immunology 2020 Jul;146(1):213-215
9. Cavalli G, De Luca G, Campochiaro C, Della-Torre E, Ripa M, Canetti D, et al. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. The Lancet Rheumatology 2020 Jun;2(6):e325-e331

10. Huet T, Beaussier H, Voisin O, Jouveshomme S, Dauriat G, Lazareth I, et al. Anakinra for severe forms of COVID-19: a cohort study. The Lancet Rheumatology 2020 Jul;2(7):e393-e400
11. Fearon WF, Fearon DT. Inflammation and Cardiovascular Disease. Circulation 2008 May 20;117(20):2577-9
12. Guzik TJ, Mohiddin SA, Dimarco A, Patel V, Savvatis K, Marelli-Berg FM, et al. COVID-19 and the cardiovascular system: implications for risk assessment, diagnosis, and treatment options. Cardiovascular Research 2020 Aug 1;116(10):1666-1687
13. Mehta P, Cron RQ, Hartwell J, Manson JJ, Tattersall RS. Silencing the cytokine storm: the use of intravenous anakinra in haemophagocytic lymphohistiocytosis or macrophage activation syndrome. The Lancet Rheumatology 2020 Jun;2(6):e358-e367
14. RECOVERY Collaborative Group; Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, et al. Dexamethasone in Hospitalized Patients with Covid-19 - Preliminary Report. N Engl J Med. 2020 Jul 17;NEJMoa2021436
15. Tomazini BM, Maia IS, Cavalcanti AB, Berwanger O, Rosa RG, Veiga VC, et al. Effect of Dexamethasone on Days Alive and Ventilator-Free in Patients With Moderate or Severe Acute Respiratory Distress Syndrome and COVID-19. JAMA 2020 Sep 2;324(13):1-11
16. Fadel R, Morrison AR, Vahia A, Smith ZR, Chaudhry Z, Bhargava P, et al. Early Short-Course Corticosteroids in Hospitalized Patients With COVID-19. Clinical Infectious Diseases 2020 May 19;ciaa601
17. Ramiro S, Mostard RLM, Magro-Checa C, van Dongen CMP, Dormans T, Buijs J, et al. Historically controlled comparison of glucocorticoids with or without tocilizumab versus supportive care only in patients with COVID-19-associated cytokine storm syndrome: results of the CHIC study. Ann Rheum Dis Sep;79(9):1143-1151
18. Sterne JAC, Murthy S, Diaz JV, Slutsky AS, Villar J, et al. Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19. JAMA 2020 Sep 2;324(13):1-13

Table 1 – Summarization of the study population characteristics according to treatment with anakinra and methylprednisolone. Continuous variables are presented as median (interquartile range), categorical variables are reported as absolute number (percentage)

	N	Treated	N	Not treated	p value
Demographics					
Age, years	65	60 (54-69)	55	63 (55-76)	0.339
Gender, male	65	52 (80%)	55	44 (80%)	1.000
Charlson Comorbidity Index	65	0 (0-0)	55	0 (0-1)	**0.037**
Charlson Comorbidity Index ≥1	64	16 (25%)	55	25 (45.4%)	**0.017**
Days between hospitalization and inclusion	65	3 (1-6)	55	1 (0-2)	**< 0.0001**
Respiratory function at inclusion					
PaO2:FiO2 ratio					
<100	62	19 (30.7%)	50	7 (14.0%)	**0.049**
100-200	62	32 (51.6%)	50	24 (48.0%)	
200-300	62	9 (14.5%)	50	14 (28.0%)	
300-400	62	2 (3.2%)	50	5 (10.0%)	
Mechanical Ventilation	65	18 (27.7%)	55	21 (37.5%)	0.222
Laboratory markers at inclusion					
Ferritin, ng/mL					
<2000	63	35 (56.45%)	38	27 (71.0%)	0.144
>2000	63	27 (43.55%)	38	11 (29.0%)	
Lymphocyte count, 10^3/l	63	0.7 (0.5-0.9)	55	0.8 (0.5-1.1)	0.458
C-Reactive Protein, mg/dl	65	14.8 (9.0-24.5)	51	15.6 (11.5-21.9)	0.969
Hemoglobin, g/dL	65	12.9 (10.6-14.1)	55	12.5 (10.9-14.1)	0.912
Platelet count, 10^3/l	65	244 (177-326)	55	230 (189-304)	0.436
Alanine transaminase , U/L	62	41 (28-56)	51	38.0 (25.0-73.0)	0.899
Gamma-glutamyl transferase, U/L	41	59.0 (34.4-110.8)	41	53.0 (26.6-95.0)	0.792
d-dimer, mcg/L	56	1220 (855-2906)	47	1271 (1059-1854)	0.944
Concomitant medications					
Remdesivir	65	8 (12.3%)	55	11 (20.0%)	0.250
Hydroxychloroquine	65	65 (100%)	55	52 (94.6%)	0.057
Lopinavir/ritonavir	65	20 (30.8%)	55	39 (70.9%)	**< 0.0001**
Anti-coagulant therapy	65	41 (63.1%)	54	21 (38.9%)	**0.009**

Table 2 – Summarization of major clinical studies that have employed either anakinra alone or steroids alone for the treatment of severe COVID-19

Reference	investigated drug	study design	study population	treatment/intervention	outcomes
Cavalli et al., Lancet Rheumatol 2020 [9]	anakinra	monocentric retrospective case – control study (Italy)	• hyperinflammation (CRP ≥100 mg/L and/or ferritin ≥900 ng/mL) • bilateral pneumonia • PaO2:FiO2 ≤200 mmHg on non-invasive ventilation • No mechanically ventilated patients	IV anakinra 5 mg/kg twice a day (n° 29) *vs* SOT (n° 16, historical controls)	• 21-day survival: 90% in anakinra group *vs* 56% in SOT group (p=0.009) • mechanical ventilation-free survival: 72% in anakinra group *vs* 50% in SOT group (p=0.15)
Huet et al., Lancet Rheumatol 2020 [10]	anakinra	monocentric case-control study (prospective cohort with historical controls) (France)	• bilateral pneumonia • oxygen saturation of ≤93% under oxygen 6 L/min or more, or saturation ≤93% under oxygen 3 L/min with a loss of 3% in 24 hours • No mechanically ventilated patients	SC anakinra 100 mg twice daily for 72 hours followed by 100 mg daily for 7 days (n° 52) *vs* SOT (n° 44, historical controls)	• need for invasive mechanical ventilation or death: 25% in anakinra group *vs* 73% in SOT group (95% CI 0.10-0.49, p=0.00021)
Cauchois et al., PNAS 2020	anakinra	multicentre retrospective case – control study (France)	• hyperinflammation (CRP ≥110 mg/L) • bilateral pneumonia • increase of oxygen requirement of more than 4 L/min in the previous 12 hours • mechanically ventilated patients included (2 in anakinra group *vs* 4 in SOT group)	IV anakinra 300mg daily for 5 days tapered to 200mg daily for 2 days and 100mg for 1 day (n° 12) *vs* SOT (n° 10)	• mortality: 0% in anakinra group *vs* 10% in SOT group (p=0.45) • ventilator-free days during the first 20 days (number of days alive and free from MV): 20 in anakinra group *vs* 17

					in SOT group (p=0.06) • number of days with oxygen requirement less than 3 L/min: 15.5 in anakinra group *vs* 8 in SOT group (p<0.05)
Horby et al., N Engl J Med 2020 [14]	dexamethasone	multicentre randomized open-label trial (United Kingdom)	• Hospitalized patients with SARS-CoV-2 infection • mechanically ventilated patients included	SOT + oral or IV dexamethasone 6 mg once daily for up to 10 days (n° 2104) *vs* SOT (n° 4321)	• overall 28-day mortality: 22.9% in dexamethasone group *vs* 25.7% in SOT group (95% CI 0.75-0.93) [29.3% *vs* 41.4% in mechanically ventilated patients (95% CI 0.51-0.81); 23.3% *vs* 26.2% in patients with oxygen requirement (95% CI 0.72-0.94); 17.8% *vs* 14.0% in patients with no respiratory support (95% CI 0.91-1.55)]
Tomazini et al., JAMA 2020 [15]	dexamethasone	multicentre randomized open-label trial (Brazil)	• mechanically ventilated patients only • MV for less than 48 hours • moderate to severe ARDS (PaO2:FiO2 ≤200 mmHg) • No corticosteroid	SOT + IV dexamethasone 20 mg daily for 5 days followed by 10 mg daily for additional 5 days or until ICU discharge (n° 151) *vs* SOT (n° 148)	• ventilator-free days during the first 28 days: 6.6 in dexamethasone group vs 4.0 in SOT group (p=0.04)

			use in the previous 15 days		• 28-day mortality: 56.3% in dexamethasone group *vs* 61.5% in SOT group (p=0.85)
Fadel et al., Clin Infect Dis 2020 [16]	MPD	multicentre quasi-experimental study (United States)	• bilateral pneumonia • oxygen requirement of 4 L/min or more, or escalating oxygen requirement from baseline • mechanically ventilated patients included	IV MPD 0.5-1 mg/kg/day for 3 days (up to 7 day in ICU patients) (n° 132) vs SOT (n° 81, historical controls)	• mortality: 13.6% in MPD group *vs* 26.3% in SOT group (p=0.024) • need for MV: 21.7% in MPD group *vs* 36.6% in SOT group (p=0.025) • ICU admission during hospitalization: 27.3% in MPD group *vs* 44.3% in SOT group (p=0.017) • composite outcome (all three above): 34.9% in MPD group *vs* 54.3% in SOT group (p=0.005)
Ramiro et al., Ann Rheum Dis 2020 [17]	MPD	monocentric case-control study (prospective cohort with historical controls) (Netherlands)	• hyperinflammation (at least two: CRP ≥100 mg/L, ferritin ≥900 ng/mL, D-dimer >1500 mcg/L) • bilateral pneumonia • oxygen saturation of ≤94% in ambient air or tachypnoea >30/min • mechanically ventilated patients included (1 in MPD group *vs* 13 in SOT	IV MPD 250 mg on day 1 followed by 80 mg daily for 2-7 days with possible escalation with TCZ (single dose 8 mg/kg) at day 2-5 if worsening in clinical or respiratory status (n° 86) vs SOT (n° 86, historical controls)	• clinical improvement (2 points in the WHO 7-point ordinal scale): 74.4% in MPD group *vs* 51.2% in SOT group (p=0.0025) • mortality: 16.3% in MPD group *vs* 47.7% in SOT group (p=0.0004)

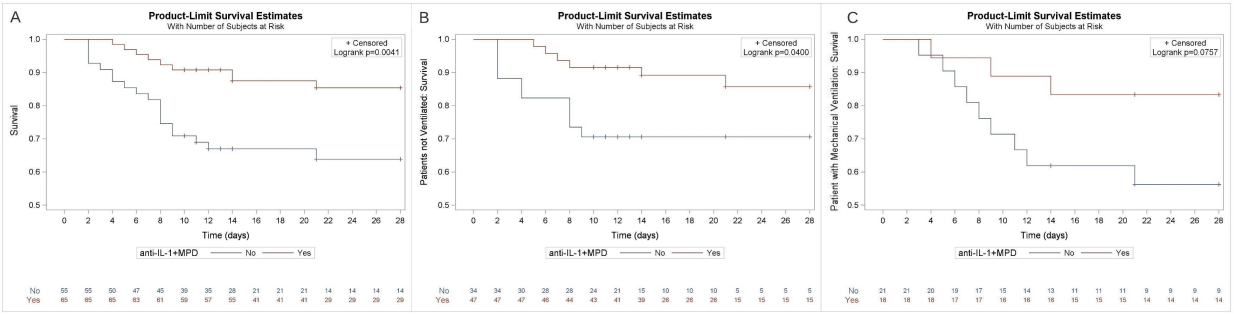
			group)		• need for MV: 11.6% in MPD group *vs* 27.9% in SOT group (p=0.0003)

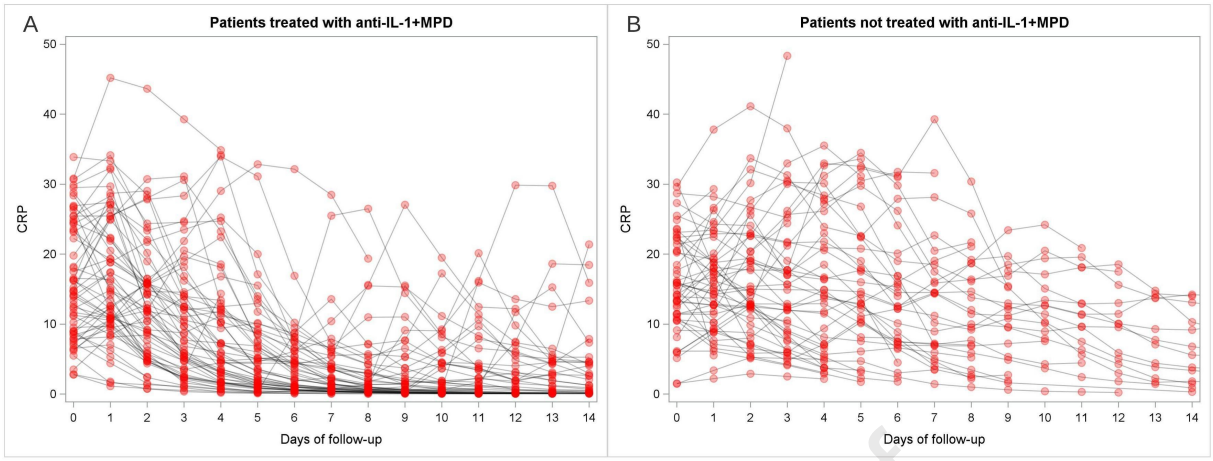
Legend: CRP C-reactive protein, IV intravenous, SOT standard of therapy, SC subcutaneous, CI confidence interval, MV mechanical ventilation, MPD methylprednisolone, ICU intensive care unit, TCZ tocilizumab (humanized monoclonal antibody against the interleukin-6 receptor)

FIGURE LEGENDS

Figure 1 – Survival according to treatment with anakinra and methylprednisolone (anti-IL-1+MPD). Both treated patients and controls were characterized by hyperinflammation and respiratory failure and fulfilled inclusion/exclusion criteria (see METHODS in the Online Repository). Panel A): survival of all individuals exposed to combined treatment is shown in the red color, dotted line; survival of the control group is shown in the blue color, continuous line; Panels B and C): survival of individuals exposed to combined treatment compared to controls in patients without and with mechanical ventilation at inclusion, respectively.

Figure 2 – Daily changes in serum C-reactive protein (CRP) from inclusion to day 14 (overall duration of the treatment with anakinra and methylprednisolone) for treated (Panel A) and untreated (Panel B) patients.





METHODS

A secondary analysis of prospective, observational cohort studies (COVID-19_Network; nCOV-2019_ICU Study) was performed at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy (Institutional Review Board #241_2020; #236_2020). All COVID-19 patients who fulfilled the following inclusion criteria were analyzed: age >18 years; evidence of pneumonia; ferritin ≥1000ng/mL and/or CRP >10mg/dL (see [2-4,6]); respiratory failure with need of supplemental oxygen (oxygen therapy from 0.4 FiO2 Venturi mask to invasive mechanical ventilation-MV). Exclusion criteria were: data available for <48 hours or death within 48 hours from inclusion; symptoms for <7 days; uncontrolled bacterial infections (i.e., sepsis/septic shock); treatment with anti-IL-1 or MPD alone.

From March 5, 2020, patients were treated off-label with anti-IL-1+MPD according to local standard operating procedures. Treatment was implemented at a different time in distinct settings (*i. e.*, COVID-19 intensive care unit, sub-intensive care unit, internal medicine), starting from the intensive care unit. Written informed consent for off-label use was obtained from all patients (except those on MV). The control group included COVID-19 patients admitted and followed from February 25, 2020, to the time of anti-IL-1+MPD introduction. Patients who retrospectively fulfilled all the inclusion and exclusion criteria for treatment were consecutively included in the control group.

Anakinra (Swedish Orphan Biovitrum AB (SOBI), Sweden) was administered subcutaneously at 200mg q8h for 3 days, then 100mg q8h up to day 14 [13]. MV patients were treated with off-label intravenous administration (3-hour infusion time) [8-10,13]. Intravenous route was chosen in view of the pharmacokinetics alterations of critically ill patients in the ICU (*i.e.,* high volume of distribution, massive generalized cutaneous edema consequent to water retention and low albumin). Also, since MV patients were on anti-coagulant therapy, subcutaneous administration could caused to hematomas or infectious complications.

Methylprednisolone was administered at 1mg/kg loading dose, then 1mg/kg/day (fractioned, two doses) for 5 days, then 0.5mg/kg/day (fractioned, two doses) for 5 days, followed by 0.25mg/kg/day (q24h or fractioned) up to day 14.

All subjects received the treatment which was considered standard-of-care at time of the study, which includes hydroxychloroquine in most cases and lopinavir/ritonavir in some. Some patients were also subjected to the use of experimental antiviral remdesivir through compassionate use (Table 1). According to the hospital internal guidelines, all patients received antithrombotic prophylaxis/treatment with enoxaparin sodium during hospitalization. Specifically, until mid March, 2020, hospital guidelines recommended prophylaxis with 100 U/kg q24h for patients <80Kg and 5000 U q12h for >80Kg (if normal renal function), irrespectively of the severity of the disease. From mid March, based on increased observations of thromboembolic events in severe COVID-19 patients, dosage was increased and stratified according to the severity of the disease (and the corresponding risk of thromboembolic events): 100 U/kg q24h in COVID-19 internal medicine (70 U/kg q12h for obese patients), 70 U/kg q12h in COVID-19 sub-intensive care units, 100 U/kg q12h in COVID-19 intensive care unit. This latter scheme was considered in evaluating the percentages of anticoagulant therapy reported in Table 1.

Primary outcome was 28-days survival rate. Adverse events (AE) were graded according to CTCAE_v4.0.

Differences between groups were assessed using two-sample t-test or Wilcoxon rank-sum test for parametric and non-parametric continuous variables and Fisher's exact test for categorical variables. Study inclusion (t0) started at anti-IL-1+MPD initiation (cases) or when ferritin/CRP levels above thresholds were registered (controls). Kaplan-Meier plots were used for survival data. Patients were followed from t0 to day 28 or death. If discharged earlier than day 28, patient status was assessed by post-discharge follow-up phone calls. Unadjusted and adjusted Cox proportional regression models were performed after controlling for proportional hazards assumption. Factors associated with mortality at univariate analysis and hospitalization setting (days elapsed from

hospitalization to t0, MV at inclusion) were considered as covariates. Statistical significance set at $\alpha < 0.05$. Analyses were performed using SAS software v.9.4.

TABLES

Table E1 – Sensitivity analysis of the impact of anti-coagulant therapy on the clinical outcome of treated and control patients. Variables are reported as absolute number (percentage).

Characteristics	**Ranges**	**Number of patients**	**Deaths**	**p value**
anti-IL-1+MPD				
Anti-coagulant therapy	No	24	2 (8.3)	0.466
	Yes	41	7 (17.1)	
No anti-IL-1+MPD				
Anti-coagulant therapy	No	33	10 (30.3)	0.554
	Yes	21	8 (38.1)	

Table E2 – Sensitivity analysis of the impact of antiviral therapy (lopinavir/ritonavir + hydroxychloroquine) on the clinical outcome of treated and control patients. Variables are reported as absolute number (percentage). Three patients in the control group that were treated with lopinavir/ritonavir alone were excluded.

Characteristics	**Ranges**	**Number of patients**	**Deaths**	**p value**
anti-IL-1+MPD				
antiviral therapy	No	45	8 (17.8)	0.169
	Yes	20	1 (5.0)	
No anti-IL-1+MPD				
antiviral therapy	No	13	3 (23.1)	0.488
	Yes	39	13 (33.3)	

Table E3 – Crude and adjusted Cox proportional regression models of the treatment with anakinra and methylprednisolone

	Hazard Ratio	**95% confidence interval**	**p value**
Crude	0.33	0.15-0.77	0.007
Adjusted*	0.18	0.07-0.50	0.001

*Adjusted Cox proportional regression model by: sex, age, PaO2/FiO2 at baseline, Charlson Comorbidity Index, mechanical ventilation at inclusion and days elapsed from hospitalization to inclusion.
The proportional hazards (PH) assumption was checked by using a transform of the Schoenfeld residuals and perform a supremum test of the null hypothesis that the observed pattern of martingale residuals was not different from the expected pattern (https://stats.idre.ucla.edu/sas/seminars/sas-survival/).