

Biomarker variation in patients successfully treated with tocilizumab for severe coronavirus disease 2019 (COVID-19): results of a multidisciplinary collaboration

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Abstract

Objective

Acute respiratory distress syndrome (ARDS) related to SARS-CoV-2 is likely due to a cytokine storm characterised by a major release of pro-inflammatory cytokines, including interleukin-6 (IL-6). Blocking excessive IL-6 production might be the key to the COVID-19-ARDS treatment. Beneficial effects of IL-6 blockade using a humanised anti-IL-6 receptor antibody, tocilizumab (TCZ) were previously reported in patients with COVID-19 related ARDS. The aim of the study was to study the variation over time of several biomarkers, demonstrated to be predictors of poor prognostic, in subjects successfully treated with TCZ for severe COVID-19.

Methods

Retrospective analysis of a case series of patients with COVID-19-ARDS, evidenced by RT-PCR and lung CT-scan. Patients with increasing O₂ requirements, within the window of opportunity for TCZ treatment (Day 7 to Day 17 after onset of symptoms) were treated with TCZ (2 infusions of 8 mg/kg). Demographic, biological and clinical data were collected from the patients' files. Serum levels of CRP, ferritin, fibrinogen, lymphocytes, platelets, creatinine, D-dimer and liver enzymes were assayed at the time of the first TCZ administration, then every two days for 8 days.

Results

40 patients were treated with TCZ. Most of them had several comorbidities, and all had multiple biological abnormalities (lymphopenia, increased CRP, ferritin, fibrinogen, D-dimer, liver enzymes). 30 patients (75%) benefited from TCZ and 10 patients died. In the survivors, following TCZ administration CRP decreased dramatically as early as day 4 (-86.7%, $p < 0.0001$) and returned to normal at day 6. Fibrinogen and lymphocyte count returned to normal values at day 6. Ferritin also decreased significantly. No significant change was observed for D-dimer ($p = 0.68$) and other studied biomarkers (haemoglobin, leucocyte count, AST).

Conclusion

In patients with COVID-19 acute respiratory distress syndrome, treatment with TCZ resulted in favourable evolution in 75% of the cases. Biomarkers of inflammation (CRP, ferritin, fibrinogen) decreased dramatically as early as the 4th day after TCZ injection. Lymphopenia, a predictor of poor prognostic, was reversed 6 days after TCZ injection.

Key words

SARS-CoV-2, COVID-19, C-reactive protein, ferritin, lymphopenia, fibrinogen, inflammation, biomarkers, tocilizumab, IL-6, pneumonia, acute respiratory distress syndrome, cytokines

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Introduction

Only a few months ago, at the beginning of the coronavirus disease 2019 (COVID-19) outbreak (1), no one would have imagined that rheumatologists would be in the very frontline in the management of the disease that has rapidly grown to be a pandemic. In children and young adults COVID-19 is usually mild, even not symptomatic. On the contrary, data from China, the cradle of the pandemic, highlighted that, in older and frail people, SARS-CoV-2 virus can induce very severe clinical features, foremost among which is an acute respiratory distress syndrome (ARDS) (1-5), followed by multi-organ failure, resulting in death in about 4% of the cases (2). Among the wide variety of clinical features related to COVID-19, the most frequently reported are cardiovascular (6), gastro-intestinal (7, 8), neurological (9) and even cutaneous (10). On the other hand, until now, very few musculoskeletal disorders have been reported (11) and nothing seemed to predispose rheumatologists to be major actors in the treatment of COVID-19. The use of hydroxychloroquine for treating COVID-19, based on the antiviral activity of chloroquine (12-14), raised questions within the rheumatologist community that has been using hydroxychloroquine to treat patients with systemic lupus erythematosus and rheumatoid arthritis, for many years. It is mostly the strong rationale to resort to tocilizumab (TCZ) for treating SARS-CoV-2 related ARDS (14-17), and the dramatic effects reported in open-label trials and case series with TCZ (17-21), which put the rheumatologists in the spotlights (22, 23).

Indeed, COVID-19 ARDS is clearly due to a cytokine dysregulation that usually occurs at the second week of the disease. This cytokine "storm" is characterised by a major release of pro-inflammatory cytokines, including interleukin-6 (IL-6), IL-2, IL-7, IL-10, tumor necrosis factor α , interferon γ inducible protein (IP10) and granulocyte-colony-stimulating factor (G-CSF) (14, 18, 24). Elevated IL-6 serum concentration, correlated with clinical severity, was found in patients with COVID-19 (25), and IL-6 blockade therapy using a

humanised anti-IL-6 receptor antibody, tocilizumab (TCZ) showed remarkable beneficial effects in COVID-19-CRS (14, 18, 20).

The Nord Franche-Comté Hospital is located in the North-East of France, an area severely impacted by the COVID-19 outbreak (26). The department of rheumatology was thus changed into a COVID-19 unit, especially dedicated for severe patients needing oxygen therapy. Given the growing number of deaths due to ARDS, and the increasing evidence that TCZ might reduce mortality in patients with COVID-19-CRS, a scientific committee was constituted within the North Franche-Comté Hospital, at the initiative of rheumatologists and infectious diseases specialists, on the very last days of March 2020. The committee, including infectious diseases specialists, rheumatologists, intensivists, biologists and pharmacists, proposed to authorise the off-label use of TCZ to treat the most severe patients, despite the lack of a published controlled randomised trial. Patients were eligible for TCZ therapy in 2 particular situations: first, in patients with severe pneumoniae responsible of ARDS who were not eligible for intensive care unit (ICU) admission, due to older age and/or severe comorbidities, on a compassionate use basis. Second, in patients eligible for ICU admission, who had recently been developing ARDS, as evidenced by increasing O₂ requirements, and the presence of several biological abnormalities that have been demonstrated to be predictors of mortality (2, 5). To be eligible for TCZ therapy each case had to be discussed during a daily multidisciplinary consultation meeting (MCM) including infectious diseases specialists, rheumatologist, pharmacists, biologists and intensivists. One key point was that the disease still had to be within the window of opportunity for TCZ treatment (*i.e.* day 7 to day 17 after onset of symptoms). Contraindications for TCZ were bacterial superinfection, latent tuberculosis infection, macrophage activation syndrome and hypersensitivity to tocilizumab. Between April 1st and May 11th, 2020, 40 severe COVID-19 patients were treated with TCZ in Nord Franche-Comté

Competing interests: none declared.

Hospital. During the same period of time 413 patients were hospitalised for COVID-19 and 94 died. Among the 40 TCZ treated patients, 10 died (25%), with a median survival time of 3.5 days (range 0.5–28 days) following the first TCZ administration. However, this percentage of mortality can be considered as rather low compared to the outcome of patients not treated with TCZ. We have recently published a retrospective case-control study on the first sample of patients treated with TCZ (27). Despite a Charlson comorbidity index higher than in patients from the control group, more severe disease, higher oxygen requirement and poorer biological findings (*i.e.* more severe lymphopenia and higher CRP levels) patients treated with TCZ had a better prognosis than controls ($p=0.002$).

The aim of this work is to report the main biomarker variations the week following the administration of the treatment, in patients successfully treated with TCZ for a potentially fatal COVID-19 ARDS. We also report changes in biomarkers in deceased patients. However, since most of them died within the first 3 days after the first administration of TCZ, the value of the change in biomarkers after day 2 in this subgroup remains questionable.

Patients and methods

Our results come from a retrospective analysis of several relevant serum biomarkers obtained in all patients successfully treated with TCZ for COVID 19-severe pneumonia between April 1st 2020 and May 11th, 2020. All the selected patients required oxygen therapy. Oxygen saturation was measured every 4 hours using a digital saturemeter. The oxygen flow was calculated to obtain oxygen saturation between 90% and 94%.

All patients received 2 intra-venous (IV) infusions of TCZ, ideally at 24 hours interval (in fact 12 to 72 hours), at a dosing regimen of 8 mg per kilogram with a maximum dose of 800mg per infusion. Before treatment administration, patients had to give their informed consent for the off-label use of TCZ. Before TCZ decision of treatment, all patients had to receive, for at

Table I. Characteristics of patients at baseline.

Variables	Alive Mean (range)	Deceased Mean (range)	<i>p</i> -values
Age (years)	73.7 (65-89)	79.7 (73-89)	0.09
Weight (kg)	75.8 (49.5-99.4)	75.8 (53-115)	0.90
BMI (kg/m ²)	27.5 (24-33)	27.7 (17-40)	0.89
Symptom duration (days)	12.2 (4-20)	15.3 (5-21)	0.49
Oxygen (l/min)	9.8 (4-15)	11.1 (5-15)	0.08
O ₂ saturation (%)	91.2 (83-97)	92 (84-98)	0.75
Leucocytes (G/l)	9.9 (2.4-16.0)	8.34 (6.0-12.97)	0.23
Haemoglobin (g/dl)	12.2 (9-17.1)	12.2 (9.7-15.2)	0.99
Platelets (G/l)	303.4 (86-524)	156.2 (54-244)	<0.0001
Lymphocytes (G/l)	1.0 (0.22-2.52)	0.57 (0.17-0.98)	0.04
Prothrombin (%)	84.3 (30-100)	74.3 (49-100)	0.45
Fibrinogen (g/l)	6.6 (2.3-10)	4.65 (0.7-6.9)	0.04
D-Dimer (ng/ml)	8276.6 (542-66,670)	4379 (409-14,669)	0.41
Creatinine (μmol/l)	89.0 (35-170)	99.6 (33-153)	0.56
Bilirubin (μmol/l)	9.65 (3-22.8)	13.7 (5.2-18.7)	0.04
CRP (mg/l)	153.4 (39.4-265)	112.24 (25.3-188.2)	0.16
Ferritin (ng/ml)	1358.6 (156-4490)	3432.9 (707.7-8158)	0.07
AST (U/l)	71.7 (17-196)	95.7 (34-244)	0.19
ALT (U/l)	60.7 (17-216)	63.5 (22-193)	0.84
LDH (U/l)	498.1 (244-1121)	1156.7 (350-4500)	0.11

least 2 days, a standard therapy including IV antibiotics (amoxicillin/clavulanic acid or ceftriaxone or levofloxacin), low molecular weight heparin at anticoagulant dose and paracetamol (1 to 3 g/day).

Data collection

In all patients admitted in the NFC hospital for COVID-19, regardless the severity of the disease, lab tests at entry included blood count, blood electrolytes, glycaemia, creatinine, C-reactive protein (CRP), ferritin, fibrinogen, D-dimer, prothrombin, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatases, gamma glutamyltransferase, triglycerides, lactate dehydrogenase (LDH), creatin kinase (CK) and arterial blood PO₂ and PCO₂. Most of these blood tests were repeated every 2 or 3 days until the patients were discharged from the hospital.

Lung CT-scan was performed in all subjects with respiratory symptoms. The extent of the lung lesions (*i.e.* plaque-like and ground-glass opacities, condensation, crazy paving) was classified <10%, 10–5%, 25–50%, 50–75% or >75%. In all cases, COVID-19 diagnosis was confirmed by real-time RT-PCR on respiratory samples, mainly nasopharyngeal swabs, sputum and bronchial aspirates (28).

Statistics

Qualitative variables were described using frequencies and percentages. Quantitative variables were described using mean, standard deviation and distribution (minimum, maximum and median). Biomarker variations were studied using student *t*-test or Wilcoxon test as appropriate. All statistical tests were carried out two tailed at the 5% level of significance. The statistical analysis was performed using XLstats© statistical and data analysis solution, Paris, France, 2020.

Ethics approval

Due to the retrospective nature of the study, the Ethics & Scientific Committee of Nord Franche-Comté Hospital determined that patient consent was required only for the off-label use of tocilizumab. Due to the retrospective nature of the study, the Ethics Committee determined that patient consent was not required. All patient data are kept confidential and in compliance with the Declaration of Helsinki.

Results

Among the 40 patients treated with TCZ between April 1st and May 11th, 2020, the “survivors” group included 30 subjects (75%). Their main characteristics at baseline are summarised in Table I.

Unsurprisingly there was an overwhelming majority of males (23 males vs. 7 females). The average age was 73, ranging from 51 to 93. Mean body mass index was 28 (range 22–33). At the time of the first TCZ injection, the mean oxygen requirement was 9.5 l/min ranging from 4 to 15 l/min, and average oxygen saturation was 91.9% (range 83–97). Before TCZ treatment 24 patients (80%) received hydroxychloroquine and 16 (53.3%) received IV methylprednisolone (average dose 355 mg, range 80–840 mg). Twenty-five patients suffered from one or more comorbidities, including arterial hypertension (15 patients), other cardio-vascular diseases (11 patients), diabetes (4 patients), cancer (5 cases), chronic obstructive pulmonary disease or emphysema (4 cases), haematological malignancy (2 cases), neurological diseases (2 cases). Unsurprisingly all the patients had multiple biological abnormalities, with a high frequency of lymphopenia (mean 0.99 Giga/l; normal range-NR=1.0 to 4.8 Giga/l), high serum levels of CRP (mean 153.4 mg/l; NR<10), ferritin (mean 1358.6 ng/ml; NR=10 to 291), fibrinogen (6.6 g/l; NR=1.7 to 4.2) and D-dimer (mean 8276.6 ng/l; NR<500). Liver enzymes, AST and ALT, were moderately increased (mean 71.6 and 60.6 UI/l; NR=13 to 40 and 7 to 40 respectively). Details are given in Table I. Survivors differed significantly from deceased patients with a higher platelet count ($p<0.0001$), higher lymphocyte number ($p=0.04$), higher fibrinogen ($p=0.04$) and lower bilirubin concentration ($p=0.04$). There was a trend for a younger age ($p=0.09$), lower oxygen requirement ($p=0.08$), lower ferritin level ($p=0.07$) and higher CRP concentration ($p=0.16$) (Table I).

Following TCZ administration, CRP decreased dramatically as early as day 4 (-86.7% , $p<0.0001$) and returned to normal at day 6 (Fig. 1). Fibrinogen also decreased significantly between day 0 and day 6, returning to normal values 3.5 g/l (Fig. 2). Lymphocytes number increased significantly at day 4 ($p=0.007$), reaching 3.42 G/l at day 10 ($p=0.0008$) (Fig. 3). Ferritin decreased significantly ($p=0.003$) but remained elevated at day 6 compared to normal values.

Fig. 1. C-reactive protein (CRP) serum level variation after tocilizumab treatment in patients with COVID-19 severe pneumonia.

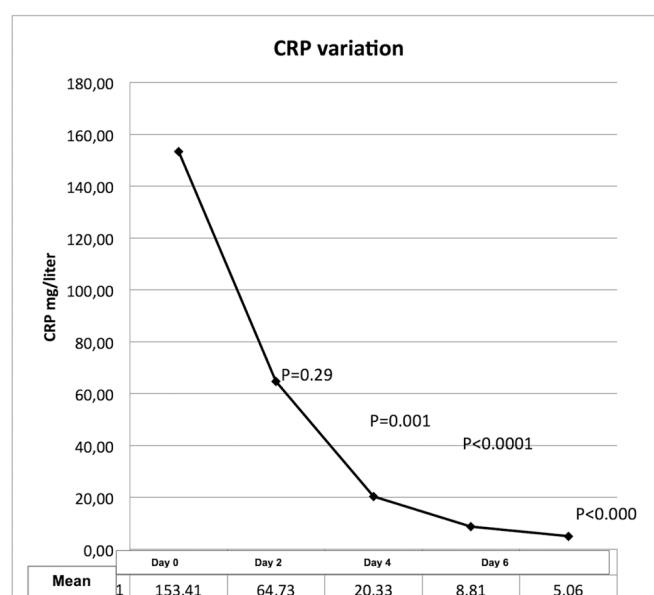


Fig. 2. Fibrinogen serum level variation after tocilizumab treatment in patients with COVID-19 severe pneumonia.

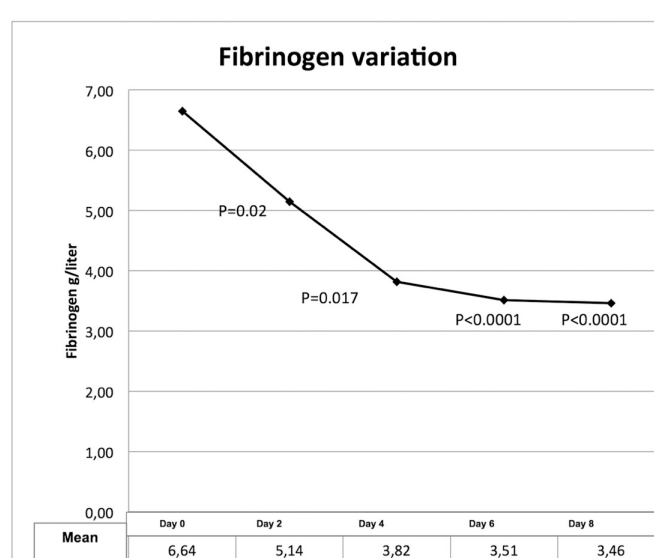


Fig. 3. Lymphocyte serum level variation after tocilizumab treatment in patients with COVID-19 severe pneumonia.

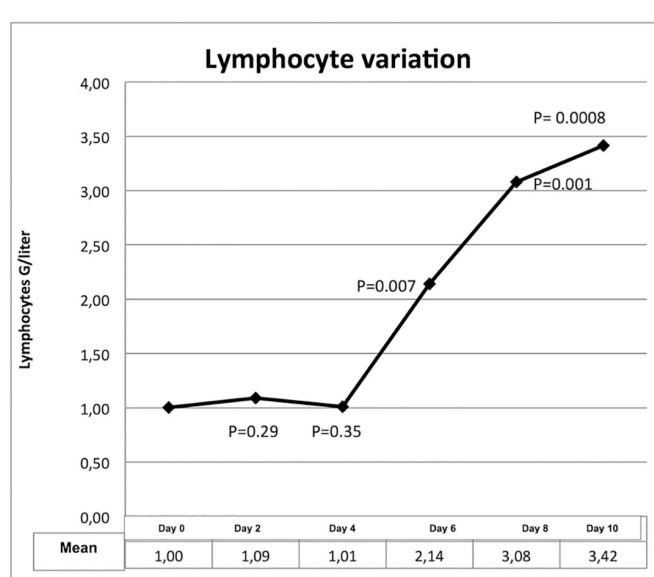


Table II. Mean (range) biomarkers concentration after tocilizumab administration in patients with COVID-19 severe pneumonia.

Biomarkers	Day 0	Day 2	Day 4	Day 6	Day 8
CRP (mg/l)	153.4 (39.4-265)	64.4 (9.5-153.3)	20.3** (5.5-45.5)	8.8**** (1.5-24.5)	5.1**** (0.7-8.7)
Ferritin (ng/ml)	1358.6 (156-4,490)	1316.4 (260.9-4,024.0)	1070.3* (165-2494)	990.7** (519-2,700)	NA
Fibrinogen (g/l)	6.6 (2.3-10)	5.1* (2.4-10)	3.8** (2.5-6.2)	3.5**** (1.8-5.2)	3.5**** (2.4-4.2)
D-dimer (ng/ml)	8,276.6 (542-66,670)	8,307.4 (905-58,190)	9,413.8 (828-18,893)	8,444.0 (563-6,032)	8,098.1 (708-5088)
Leucocytes (G/l)	9.9 (2.4-16.0)	8.6** (3.1-20.4)	8.9 (4.3-16.2)	9.3 (3.2-17.8)	8.7 (4.4-16.4)
Hemoglobin (g/dl)	12.2 (9-17.1)	12.2 (9.5-16.6)	11.6 (8.1-14.7)	11.8 (7.7-16.7)	12.1 (8.9-16.6)
Platelets (G/l)	303.4 (86-524)	343.9** (98-669)	390.3** (144-693)	355.6 (95-760)	326.5 (107-583)
Lymphocytes (G/l)	1.0 (0.22-2.52)	1.1 (0.14-3.74)	1.0 (0.39-2.84)	2.1* (0.2-15.2)	3.1*** (0.6-18-3)
Creatinine (μ mol/l)	89.0 (35-170)	102.8 (27-157)	110.6 (30-329)	114.6 (27-528)	112.9 (30-449)
AST (U/l)	71.7 (17-196)	73.0 (37-197)	79.9 (26-301)	67.7 (29-142)	51 (30-69)
ALT (U/l)	60.7 (17-216)	68.2 (30-165)	93.1* (31-395)	97.8* (25-369)	83.2* (22-151)

p-values: **p*<0.05; ***p*<0.01; ****p*<0.005; *****p*<0.001; NA: data non available.

Creatinine serum levels, platelet count and ALT moderately but significantly increased between day 0 and day 8. No significant change was observed for D dimers (7863.2 ng/l, *p*=0.68) and the other studied biomarkers (haemoglobin, leucocyte count, AST) (Table II). In deceased patients, biomarker changes were analysed only at day 4 because only 4 patients were still alive after day 6. CRP decreased significantly between day 0 and day 4 (23.0 mg/l; *p*=0.004). No significant change was observed for the other biomarkers. However, the sample size was too small (6 patients at day 4, 5 at day 6 and 3 at day 8) to allow a relevant statistical analysis.

Discussion

All the patients of our case series fulfilled the Italian recommendation criteria for the off-label use of TCZ (29): treatment was administered at the end of the initial high viral load phase, in patients with interstitial pneumonia and severe respiratory distress, rapidly worsening respiratory exchanges and high levels of D-dimer/ CRP/ ferritin/ fibrinogen (15). All suffered from a very severe disease, with grim perspectives and a vital prognosis at stake. Among them, 25% died the very next days after TCZ administration, suggesting the treatment was given too late. The 75% who benefited from TCZ were slightly younger, had a less marked lymphopenia, more platelets and higher fibrinogen serum levels. Despite a trend for higher CRP in successfully treated patients, the difference with deceased patients

was not statistically significant. Previous conflicting results were published regarding the predictive value for TCZ effectiveness of serum CRP in patients with RA (30-32). However, in our patients, as usually observed in RA, IL-6 blockade with TCZ was rapidly followed by a dramatic decrease of serum CRP. This phenomenon is well known and attests to the very strong link between IL-6 and CRP (18). However, the dramatic reduction of serum CRP levels were observed both in patients successfully treated with TCZ and in those who died the very next days after TCZ administration. Fibrinogen, another biomarker of inflammation, returned to normal values within one week. This is not surprising since IL-6 is a main regulator of fibrinogen synthesis (33). Ferritin, another biomarker of inflammation, also decreased significantly within 1 week. Hyperferritinemia is frequently observed in rapidly progressive interstitial lung disease with polymyositis and dermatomyositis, and has been shown to be correlated to IL-6 serum levels (34). However, ferritin also depends on IL-18 levels (35). This might explain that ferritin and CRP did not decrease at the same rate after IL-6 blockade. Lymphopenia is one of the major haematologic abnormalities during the course of COVID-19 and has been suggested to be a prognostic factor of mortality (2, 3). After TCZ lymphocyte number increased significantly from day 4 to day 10, reaching rapidly normal values. Despite mild to moderate variations no other significant change

in other biomarker was observed, probably due to the limited number of patients. For example, inversely to what was reported in RA, we did not observe significant decrease in D-dimer after TCZ (36). Unlike biomarkers of acute inflammation, such as CRP and fibrinogen, hepatic enzyme ALT continued to increase, moderately but significantly, the week after TCZ infusion. This may be explained by an increase of the ratio IL-2/IL-6 (due to the diminution of IL-6) since, in drug liver injury, the IL-2/IL-6 ratio was shown to negatively correlate with the pre- vs. post-treatment difference in ALT and AST values and positively correlate with ALT and AST at 1-month post-discharge (37).

Our study suffers from several limitations. All patients were treated within a limited period of time, usually classified as the best “window of opportunity” for TCZ treatment, so we do not have information on the benefits for treating earlier or later. Furthermore, all our patients were suffering from very severe disease with multiple risk factors for mortality (18-20): we thus cannot draw a formal conclusion for the interest for TCZ in more moderate COVID-19 cases. Lastly, due to the very wide between-patient biomarker variation, a larger sample size is mandatory to confirm these results.

In summary the present work, which is the result of a close collaboration between rheumatologists, infectious diseases specialists and biologists, has shown a rapid decrease in inflammation biomarkers and a progressive normali-

sation of the lymphocyte count the very first days after TCZ injection, in three-quarters of patients with very severe and life threatening COVID-19 pneumonia. These results are also consistent with those from the Italian Brescia cohort (38). Further large-scale prospective studies are necessary to better understand these changes and their prognostic values on COVID-19 evolution.

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References

- GORBALENYA AE, BAKER SC, BARIC RS, DE GROOT RJ, DROSTEN C, GULYAEVA AA: The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol* 2020; 5: 536-44.
- WU C, CHEN X, CAI Y *et al.*: Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med* 2020 Mar 13.
- ZHOU F, YU T, DU R *et al.*: Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; 395: 1054-62.
- WEISS P, MURDOCH DR: Clinical course and mortality risk of severe COVID-19. *Lancet* 2020; 395: 1014-5.
- ZHENG Z, PENG F, XU B *et al.*: Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis. *J Infect* 2020 Apr. 23.
- BANSAL M: Cardiovascular disease and COVID-19. *Diabetes Metab Syndr* 2020; 14: 247-50.
- MAO R, QIU Y, HE JS *et al.*: Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2020.
- PAROHAN M, YAGHOUBI S, SERAJ A: Liver injury is associated with severe Coronavirus disease 2019 (COVID-19) infection: a systematic review and meta-analysis of retrospective studies. *Hepatol Res* 2020.
- WU Y, XU X, CHEN Z *et al.*: Nervous system involvement after infection with COVID-19 and other coronaviruses. *Brain Behav Immun* 2020.
- RECALCATI S: Cutaneous manifestations in COVID-19: a first perspective. *J Eur Acad Dermatol Venereol* 2020.
- JOOB B, WIWANITKIT V: Arthralgia as an initial presentation of COVID-19: observation. *Rheumatol Int* 2020; 40: 823.
- FERNER RE, ARONSON JK: Chloroquine and hydroxychloroquine in covid-19. *BMJ* 2020; 369: m1432.
- TOURET F, DE LAMBALLERIE X: Of chloroquine and COVID-19. *Antiviral Res* 2020; 177: 104762.
- ZHANG W, ZHAO Y, ZHANG F *et al.*: The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): the perspectives of clinical immunologists from China. *Clin Immunol* 2020; 214: 108393.
- SARZI-PUTTINI P, GIORGI V, SIROTTI S *et al.*: COVID-19, cytokines and immunosuppression: what can we learn from severe acute respiratory syndrome? *Clin Exp Rheumatol* 2020; 38: 337-42.
- TANAKA T, NARAZAKI M, KISHIMOTO T: Immunotherapeutic implications of IL-6 blockade for cytokine storm. *Immunotherapy* 2016; 8: 959-70.
- LIU B, LI M, ZHOU Z, GUAN X, XIANG Y: Can we use interleukin-6 (IL-6) blockade for coronavirus disease 2019 (COVID-19)-induced cytokine release syndrome (CRS)? *J Autoimmun* 2020: 102452.
- ZHANG C, WU Z, LI JW, ZHAO H, WANG GQ: The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality. *Int J Antimicrob Agents* 2020: 105954.
- XU X, HAN M, LI T *et al.*: Effective treatment of severe COVID-19 patients with tocilizumab. *Proc Natl Acad Sci USA* 2020.
- LUO P, LIU Y, QIU L, LIU X, LIU D, LI J: Tocilizumab treatment in COVID-19: A single center experience. *J Med Virol* 2020 Apr. 6.
- MICHOT J-M, ALBIGES L, CHAPUT N *et al.*: Tocilizumab, an anti-IL6 receptor antibody, to treat Covid-19-related respiratory failure: a case report. *Ann Oncol* 2020 Apr. 2.
- FERRO F, ELEFANTE E, BALDINI C *et al.*: COVID-19: the new challenge for rheumatologists. *Clin Exp Rheumatol* 2020; 38: 175-80.
- GONZÁLEZ-GAY MA, MAYO J, CASTAÑEDA S, CIFRIÁN JM, HERNÁNDEZ-RODRÍGUEZ J: Tocilizumab: from the rheumatology practice to the fight against COVID-19, a virus infection with multiple faces. *Expert Opin Biol Ther* 2020.
- LIU J, LI S, LIU J *et al.*: Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *EBioMedicine* 2020; 55: 102763.
- PEDERSEN SF, HO YC: SARS-CoV-2: a storm is raging. *J Clin Invest* 2020; 130: 2202-5.
- POP R, QUENARDELLE V, HASIU A *et al.*: Impact of the Covid-19 outbreak on acute stroke pathways - Insights from the Alsace region in France. *Eur J Neurol* 2020.
- KLOPFENSTEIN T, ZAYET S, LOHSE A *et al.*: Tocilizumab therapy reduced intensive care unit admissions and/or mortality in COVID-19 patients. *Med Mal Infect* 2020 May 6.
- CORMAN VM, LANDT O, KAISER M *et al.*: Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* 2020; 25: 2000045.
- SIMIT: Vademecum per la cura delle persone con malattia da COVID-19. 2020. <http://www.simit.org/medias/1569-covid19-vademecum-13-03-202.pdf>
- PERS YM, FORTUNET C, CONSTANT E *et al.*: Predictors of response and remission in a large cohort of rheumatoid arthritis patients treated with tocilizumab in clinical practice. *Rheumatology* 2014; 53: 76-84.
- KOJIMA T, YABE Y, KANEKO A *et al.*: Monitoring C-reactive protein levels to predict favourable clinical outcomes from tocilizumab treatment in patients with rheumatoid arthritis. *Mod Rheumatol* 2013 23: 977-85.
- WANG J, DEVENPORT J, LOW JM, YU D, HITRAYA E: Relationship between baseline and early changes in C-reactive protein and interleukin-6 levels and clinical response to tocilizumab in rheumatoid arthritis. *Arthritis Care Res (Hoboken)* 2016; 68: 882-5.
- CARTY CL, HEAGERTY P, HECKBERT SR *et al.*: Interaction between fibrinogen and IL-6 genetic variants and associations with cardiovascular disease risk in the cardiovascular health study. *Ann Hum Genet* 2010; 74: 1-10.
- KAWASUMI H, GONO T, KAWAGUCHI Y *et al.*: IL-6, IL-8, and IL-10 are associated with hyperferritinemia in rapidly progressive interstitial lung disease with polymyositis/dermatomyositis. *Biomed Res Int* 2014; 2014: 815245.
- SLAATS J, TEN OEVER J, VAN DE VEERDONK FL, NETEA MG: IL-1 β /IL-6/CRP and IL-18/ferritin: distinct inflammatory programs in Infections. *PLoS Pathog* 2016; 12: e1005973.
- GUALTIEROTTI R, INGEGNOLI F, GRIFFINI S, GROVETTI E, MERONI PL, CUGNO M: Prothrombotic biomarkers in patients with rheumatoid arthritis: the beneficial effect of IL-6 receptor blockade. *Clin Exp Rheumatol* 2016; 34: 451-8.
- SUN Y, GU J, LIU R *et al.*: IL-2/IL-6 ratio correlates with liver function and recovery in acute liver injury patients. *APMIS* 2019; 127: 468-74.
- TONIATI P, PIVA S, CATTALINI M *et al.*: Tocilizumab for the treatment of severe COVID-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: a single center study of 100 patients in Brescia, Italy. *Autoimmun Rev* 2020 May 3.