Flipped Inflammatory Time and the Role of Antibodies Against Severe Acute Respiratory Syndrome Coronavirus 2: Optimizing Tocilizumab Against Coronavirus Disease 2019

Pablo Guisado-Vasco,1,2 José Aguirales Gorines,3 María M. Carralón González,1,2 Gabriel Sotres Fernández,1,2 Daniel Carnevali Ruiz1,2

1Internal Medicine Department, Hospital Universitario Quirónsalud Madrid, Madrid, Spain; 2Universidad Europea, Madrid, Spain; and 3Research and Clinical Trials Unit, Hospital Universitario Quirónsalud Madrid, Madrid, Spain

Use of interleukin (IL-6) inhibitors has become one of the most complicated clinical issues in treating coronavirus disease 2019 (COVID-19). Recently, randomized open-label platform trials have found that IL-6 inhibitors have a beneficial effect on mortality in severe COVID-19. However, several questions arise around their mechanism of action in this disease, as well as how, when, and at which dose they should be used. IL-6 has both proinflammatory and anti-inflammatory effects, which may modulate the course of COVID-19, whose immunopathogenesis is driven by the innate immune system, autoantibodies, and interferon. Given that patients with delayed seroconversion against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein would be at the highest risk of complications beyond the second week of disease, we propose that considering patient serostatus at admission could optimize the use of IL-6 inhibitors in COVID-19. We predict that the net treatment benefits could be higher in the subgroup of patients with delayed seroconversion as compared to those who seroconvert more rapidly after SARS-CoV-2 infection.

Keywords. SARS-CoV-2; COVID-19; tocilizumab; IL-6 inhibition; therapy.

Coronavirus disease 2019 (COVID-19) has emerged as a disease with complex pathophysiology with both pulmonary and extrapulmonary manifestations. This novel disease has proven to have a unique immunopathogenesis distinct from influenza, non–COVID-19–related acute respiratory distress syndrome (ARDS), or other coronavirus infections [1]. Since the beginning of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, clinicians and researchers have tested numerous therapies for the most severe manifestations of COVID-19. Most of them have failed to show any positive therapeutic effect in reducing disease severity, hospital length of stay, or mortality rate.

The most notable exceptions are dexamethasone and the interleukin (IL) 6 antagonist tocilizumab. Both drugs have been incorporated into standard of care, together with oxygen support, anticoagulants, and monoclonal antibodies or antiviral drugs against SARS-CoV-2.

However, the interpretation of data supporting these anti-inflammatory agents has not been straightforward when we consider the different modalities of oxygen support and the clinical conditions of patients admitted in hospital wards or intensive care units. That is especially true in the case of interventions that target the IL-6 pathway.

SERUM IL-6 PLASMA ELEVATIONS IN COVID-19: THE CLINICAL PERSPECTIVE

Several preliminary reports have described elevated concentrations of serum IL-6 in patients with severe or critical COVID-19 compared to moderate disease [2]. These preliminary data have led many clinicians to believe that the cytokine release syndrome associated with COVID-19 was the principal cause of lung inflammation in patients with severe disease or with ARDS [3].

The hyperinflammation seen in COVID-19 resembles that of other systemic inflammatory syndromes. In both COVID-19 and hemophagocytic lymphohistiocytosis, there is a similar upregulation of a subset of leukocyte populations, along with similar induction of inflammatory pathways and genes involved in neutrophil activation, cytokine, and chemokine signaling [4, 5].

These observations have prompted both prospective and retrospective observational studies with anti–IL-6 drugs in COVID-19. Most of these studies suggested some benefits of tocilizumab. However, the first randomized clinical trials, which compared tocilizumab with standard of care, did not find a clear benefit when using different primary outcomes, including 28-day mortality,
progression to invasive mechanical ventilation, or C-reactive protein (CRP) or IL-6 plasma level cutoff points [6–8].

More recently, the Randomized, Embedded, Multi-factorial, Adaptive Platform Trial for Community-Acquired Pneumonia (REMAP-CAP) and Randomized Evaluation of COVID-19 Therapy (RECOVERY) trial platforms [9, 10], enrolling larger numbers of patients, did find a benefit of using tocilizumab, particularly in those patients with rapidly developing respiratory insufficiency, those in the first 24 hours of admission to intensive care units, and those on noninvasive ventilation, including patients on high-flow nasal oxygen. The observed benefits were limited to patients who were also being treated with glucocorticoids (dexamethasone), and with a plasma CRP level ≥75 mg/L.

**IMMUNOPATHOGENESIS IN COVID-19: WHAT IS THE ROLE OF IL-6 PLASMA ELEVATION?**

Due to the novelty of the disease and the complexity of its immunopathogenesis, understanding the role of IL-6 in COVID-19 took time to evolve during the pandemic.

Preliminary findings describing IL-6 as a determinant of hyperinflammation were not immediately confirmed as our comprehension of COVID-19 improved. When researchers used control groups with previous sepsis or ARDS, IL-6 did not seem to have a significant role in the severity of COVID-19. First, the median plasma concentration of IL-6 is lower than the median previously reported in ARDS [11]. Second, some studies underscored that COVID-19 is a disease driven mainly by the innate immune system, specifically monocyte dysfunction and suppression of interferon signaling. Few patients exhibited cytokine profiles similar to cytokine storm syndrome. Although upregulation of IL-6, granulocyte colony-stimulating factor, IL-1RA, and MCP1 predicted death among COVID-19 patients, concentrations of these molecules were not statistically higher than those seen in patients with influenza [12]. Third, the magnitude of IL-6 elevations in plasma are several times less than those reported in studies with chimeric antigen receptor T-cell-induced cytokine release syndrome, sepsis, or non–COVID-19 ARDS [13].

In severe COVID-19, cytokine analysis found increased levels of other proinflammatory molecules (including CXCL10 and IL-10), inflammasome-dependent cytokines (IL-18 and IL-1β), and interferons (IFN-α, IFN-γ, and IFN-λ). In addition, severe COVID-19 is characterized by dysregulated immune coordination response, driven mainly by inflammatory monocytes or plasmablast-like neutrophils [14, 15].

Complicating our understanding is that the deficient production or response of type I IFN has proven to be a key factor for development of severe or critical COVID-19. This implicates those inherited gene mutations or the development of auto-antibodies against IFN-α, IFN-ω, or both, which have a negative impact in the clinical progression of the disease, and suggests convergent and singular mechanisms for disease pathogenesis [16–18].

**SEROSTATUS AND IMPROVED USE OF IL-6 INHIBITORS: THE DISMISSED TEST**

At first glance, both perspectives, the clinical and the immunopathogenic mechanism in COVID-19, seem divergent and have led to several questions. Namely, how can we unify the immunopathology of severe COVID-19, the mechanism of IL-6 inhibition, and the potential clinical role of IL-6 inhibitor drugs? To optimize their use, when should we use IL-6 inhibitors? Finally, which patients are expected to benefit most from treatment?

Here, we propose using the serostatus of SARS-CoV-2 for each patient at admission as a day-by-day biomarker to guide tocilizumab scheduling. This hypothesis is grounded on 3 important findings.

First, the timeline of symptoms in COVID-19 is the most relevant factor in disease evolution and is relatively easy to record (apart from individual heterogeneity). Indeed, it is regularly measured in most clinical trials. Most patients with COVID-19 present to hospital around 9 days after their initial symptoms. That was the day of randomization in the RECOVERY trial for the tocilizumab arm (we can assume a similar day of randomization in the REMAP-CAP platform from an exclusion criterion of >14 days of disease symptoms). In both trials, the survival curves of the groups receiving IL-6 inhibitors start to separate from groups receiving standard care 7 days after tocilizumab dose (Figure 1A). That gives us a median time of 16–18 days from symptom onset.

Second, serum antibody dynamics (particularly anti-S1 immunoglobulin G [IgG]) are positively correlated with clinical parameters related to COVID-19 severity and prognosis (Figure 1B, Supplementary Figure 1) [19]. A group of patients with severe or critical cases of COVID-19 showed a delayed anti-S1 IgG response compared with a group containing high levels of those antibodies. Both groups separated in a time-dependent manner around day 10 of the disease. Our first observations seem to suggest that IgG antibodies are discriminatory when determining outcome severity, particularly when compared to immunoglobulin M (IgM). However, further analyses should assess the precise cutoff point for both types of antibodies.

We have also precluded the heterogeneity of antibody titers against SARS-CoV-2, as there is an increasing proportion of individuals with antibodies due to vaccination and/or with previous infection.

Researchers observed the same pattern in the case of neutralizing antibodies (NAb), which exhibited faster kinetics as well as a higher peak in those patients discharged from hospital compared to those who were deceased [19]. We note that the serostatus of SARS-CoV-2 in tocilizumab trials and in daily clinical practice has been largely ignored.

Third, the immune status and trajectories of inflammatory signatures diverge over time and are associated with distinct severe outcomes. Recent data show
Figure 1. A, Mortality of adult patients administered intravenous tocilizumab by body weight (8 mg/kg if weight ≤40 kg; 400 mg if weight >40 to 65 kg; 600 mg if weight >65 to 90 kg; 800 mg if weight >90 kg) plus standard of care compared with standard of care alone. Patients received a single infusion of tocilizumab over 60 minutes (a second dose could be given 12–24 hours later according to attending clinician). Patients with confirmed coronavirus disease 2019 (COVID-19), oxygen saturation <92% on room air or receiving oxygen therapy, and C-reactive protein ≥75 mg/L were randomized. Patients were randomized on day 9 (median) after symptom onset. Blue-shaded area determines the inflammatory "juncture" in the disease course [20]. This figure has been modified from [10]. B, Delayed seroconversion of anti-spike (S1) immunoglobulin G (IgG) in deceased patients compared with recovered patients. Blue-shaded area determines the inflammatory "juncture" in the disease course [20]. Figure represents anti-S1 IgG levels measured by enzyme-linked immunosorbent assay. Lines indicate cross-sectional medians from each group. Discharged, n = 67; deceased, n = 60. Data extracted and figure modified from Lucas et al [19]. C, Divergence around the inflammatory "juncture" (blue-shaded area) in serum interleukin 6 levels comparing deceased and recovered critical COVID-19 patients. Critical patients were determined by disease severity metric as in Liu et al [20]. Discharged, n = 105; deceased, n = 63. Data extracted and figure modified from Liu et al [20]. Abbreviations: IgG, immunoglobulin G; IL-6, interleukin 6; OD, optical density.
that changes in inflammatory chemokine signatures (including IL-6, TNF-α, and IFN-γ) was found to have significantly different kinetics between deceased and recovered groups during days 17–23 of disease, with some inflammatory signatures in severe cases “flipping” from low to high around this inflammatory junction (Figure 1C) [20].

CONCLUSIONS

Based on these observations, we propose that serostatus should guide the use of IL-6 inhibition in cases of severe COVID-19. These drugs must be used together with dexamethasone to prevent a misfiring of cytokines after IL-6 blockade and in accordance with findings in different randomized clinical trials.

A considerable proportion of patients with COVID-19 are admitted to hospital with a median of 9 days of disease. At admission, SARS-CoV-2 serostatus should be evaluated, and patients with low IgG/IgM antibody titers against SARS-CoV-2, or low levels of Nabs (even including those with hematological malignancies or other immunosuppressive conditions), might promptly be considered for IL-6 inhibition. Treatment can be considered independent of oxygen support or CRP values over the next 24–48 hours of admission, providing this subgroup of patients the benefits of IL-6 inhibition.

Effects of tocilizumab take approxi- mately 7 days to manifest. By initiating treatment shortly after admission, we target the inflammatory junction that occurs between days 17 and 23 to prevent an increase in IL-6 in the second/third week of disease and, hence, a severe immunopathologic outcome. Though safety concerns should always be considered when initiating any treatment, we note that studies evaluating tocilizumab in treating COVID-19 did not identify any new safety signal [7–10].

On the other hand, we hypothesize that there is a small or no clinical effect of IL-6 inhibition in patients with high anti-S1 antibody titers after 9 days of COVID-19 symptoms. Nevertheless, physicians might evaluate these cases according to best clinical judgment, need for oxygen support, and plasma CRP levels.

Our hypothesis needs further research to be validated, either using biobank samples of previous studies, including randomized clinical trials, or in prospective specific studies. Future studies would also be needed to find an optimal, easy-to-use cutoff value in serostatus to schedule the IL-6 inhibitor and to optimize the potential benefit of any given patient.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank Timothy Silverstein of Feather Medical Communications for writing and editorial assistance.

Author contributions. P. G.-V. and J. A. G. designed the study, collected and analyzed the data, did the literature search, and drafted the manuscript. M. M. C. G., D. C. R., and G. S. F. took part in the conceptualization and helped with funding acquisition and visualization of the figures. D. C. R. and G. S. F. put forward the hypothesis and revised the drafted manuscript. All authors revised and approved the definitive version of the manuscript.

Financial support. This work was supported by funding associated with the Internal Medicine Research Unit of the Hospital Universitario Quirónsalud Madrid.

Potential conflicts of interest. P. G.-V. has received consultant fees from Angelini Pharma Spain and Pharma Mar, and speaking fees from GlaxoSmithKline (Spain) and Pharma Mar, outside the submitted work. All other authors report no potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Osuchowski MF, Winkler MS, Skirecki T, et al. The COVID-19 puzzle: deciphering pathophysiology and phenotypes of a new disease entity. Lancet Respir Med 2021; 9:622–42.
2. Chen G. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest 2020; 130:2620–9.
3. Moore JB, June CH. Cytokine release syndrome in severe COVID-19. Science 2020; 368:473.
4. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 2020; 395:1033–4.
5. Schimke LF, Marques AHC, Crispim Baocontri G, et al. Multi-layered analyses reveal an immunological overlap between COVID-19 and hemophagocytic lymphohistiocytosis associated with disease severity. bioRxiv [Preprint]. Posted online 14 February 2022. doi:10.1101/2021.07.30.454529.
6. Stone JH, Frigault MJ, Serling-Boyd NJ, et al. Efficacy of tocilizumab in patients hospitalized with Covid-19. N Engl J Med 2020; 383:2333–44.
7. Salama C, Han J, Yau L, et al. Tocilizumab in patients hospitalized with Covid-19 pneumonia. N Engl J Med 2021; 384:20–30.
8. Rosas I, Bräu N, Waters M, et al. Tocilizumab in hospitalized patients with severe COVID-19 pneumonia. N Engl J Med 2021; 384:1503–16.
9. Gordon AC, Mouncey PR, Al-Beidh F, et al; REMAP-CAP Investigators. Interleukin-6 receptor antagonists in critically ill patients with Covid-19. N Engl J Med 2021; 384:1491–502.
10. RECOVERY Collaborative Group. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. Lancet 202; 397:1637–45.
11. Sinha P, Matthay MA, Calfee CS. Is a “cytokine storm” relevant to COVID-19? JAMA Intern Med 2020; 180:1152–4.
12. Mudd PA, Crawford JC, Turner JS, et al. Distinct inflammatory profiles distinguish COVID-19 from influenza with limited contributions from cytokine storm. Sci Adv 2020; 6:eabe3024.
13. Leisman DE, Ronner L, Pinotti R, et al. Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes. Lancet Resp Med 2020; 8:1233–44.
14. Laing AG, Lorenc A, Del Molino Del Barrio I, et al. A dynamic COVID-19 immune signature includes associations with poor prognosis. Nat Med 2020; 26:1623–35.
15. Lucas C, Wong P, Klein J, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature 2020; 584:463–9.
16. Zhang Q, Bastard P, Liu Z, et al. Inborn errors of type I-IFN immunity in patients with life-threatening COVID-19. Science 2020; 370:eabd4570.
17. Bastard P, Bosen LB, Zhang Q, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science 2020; 370:eabd4585.
18. van der Wijst M, Vazquez SE, Hartoularos GC, et al. Type I interferon autoantibodies are associated with systemic immune alterations in patients with COVID-19. Sci Transl Med 2021; 13:eabh2624.
19. Lucas C, Klein J, Sundaram ME, et al. Delayed production of neutralizing antibodies correlates with fatal COVID-19. Nat Med 2021; 27:1178–86.
20. Liu C, Martins AJ, Lau WW, et al. Time-resolved systems immunology reveals a late juncture linked to fatal COVID-19. Cell 2021; 184:1836–57. e22.