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Invited Commentary

Immune Stimulation With Recombinant Human Granulocyte Colony–Stimulating Factor for Coronavirus Disease 2019 (COVID-19)—Beware of Blind Spots

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The sobering mortality of coronavirus disease 2019 (COVID-19), the pandemic triggered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the few proven treatments have stimulated a global effort to rapidly discover and disseminate novel therapies. A noteworthy feature of the disease is the marked degree of lymphopenia in many hospitalized patients with COVID-19 and the association of lymphopenia with adverse outcomes.[1]

Lymphopenia during infection, and particularly during sepsis, is not unique to COVID-19, nor even to viral illness. In studies of hospitalized patients with positive blood cultures, most had lymphopenia, and persistent lymphopenia was associated with mortality.[2] However, during many acute viral respiratory infections, lymphopenia is transient and coincident with peak symptoms but then rapidly resolves as the patient improves.[3] The severity, and in some cases persistence, of lymphopenia in patients with COVID-19 is different.[3] As patients with sepsis have concomitant inflammation and features of immune exhaustion, an attractive strategy might augment specific immune responses, particularly if it could be predicted which patients would be most likely to benefit.

In this issue of *JAMA Internal Medicine*, Cheng et al[4] report an open-label randomized trial of recombinant human granulocyte colony-stimulating factor (rhG-CSF) compared with usual care in 200 hospitalized adults with COVID-19, pneumonia, and lymphopenia (absolute lymphocyte count, ≤ 800/μL [to convert to ×10⁹/μL, multiply by 0.001]).[4] Patients with preexisting conditions, baseline leukocytosis, and those requiring invasive mechanical ventilation at the time of screening were excluded. The patients in the treatment arm received 3 daily doses of subcutaneous rhG-CSF, 5 μg/kg. Usual care included oxygen, assisted ventilation if needed, and antibiotics or adjuvant therapies (corticosteroids, lopinavir-ritonavir, arbidol, or inhaled α-interferon) at the discretion of the treating physician.

The primary outcome was the time to clinical improvement of at least 1 point on a 7-point ordinal scale of clinical and respiratory severity ranging from not hospitalized with normal activities to death.[4] The treatment groups were reasonably well balanced at enrollment, with a slightly more participants in the usual care arm receiving high-flow oxygen and adjuvant therapies before randomization. There was no difference in the primary outcome by treatment group. Treatment with rhG-CSF increased lymphocyte and leukocyte cell counts by day 5. Participants randomized to

receive rhG-CSF were less likely to progress to invasive ventilation or shock and exhibited lower 21-day mortality. Both of these secondary end points were statistically significant.

Cheng et al[4] tested for heterogeneity of rhG-CSF treatment effect by patient factors, including degree of lymphopenia, degree of baseline oxygen support, age, and sex. They detected significant interaction between the effect of rhG-CSF and the degree of lymphopenia, stratifying at a lymphocyte cell count of 400 or less per μL, with the participants with more severe lymphopenia exhibiting greater improvement. In contrast, for the roughly 50% of the study population with a lymphocyte cell count more than 400 per μL, no effect of rhG-CSF was observed. Similarly, Cheng et al[4] report a significant interaction between the level of oxygen support and the rhG-CSF effect. Participants requiring high-flow oxygen or noninvasive ventilation at enrollment demonstrated a significant improvement in the primary outcome. In contrast, those requiring low-flow oxygen or no supplemental oxygen did not manifest a benefit.

Although the subgroup analyses suggesting a heterogeneous effect of rhG-CSF on COVID-19 recovery are provocative, caution should be exercised in interpreting these data, as the findings could be biased. For example, the stratification threshold for lymphopenia (≤400/μL) is not validated. These and other stratified results should be viewed as hypothesis-generating; they require prospective testing before they should influence clinical care. Also, it is not clear whether the administration of adjuvant therapies without a proven benefit for patients with COVID-19 affected the trial results. The more frequent use of these agents in the usual care arm suggests that clinicians viewed patients in this group as having greater illness severity or higher risk, perhaps explaining the higher proportion with worse outcomes.

Conducting a randomized interventional trial during a pandemic is no small feat, and the current trial is a substantial accomplishment. Cheng et al[4] explicitly enriched the study population for patients they hypothesized would benefit in requiring lymphopenia to be eligible. Lymphocyte cell counts are an attractive enrichment marker as an objective measure that is clinically available. Prior trials of rhG-CSF for patients with pneumonia and sepsis showed no benefit, although these trials enrolled predominantly patients with bacterial pneumonia and without leukopenia.[5]

As a treatment choice, rhG-CSF is somewhat unconventional. First, G-CSF has a major effect on the myeloid compartment, significantly increasing neutrophil proliferation, differentiation, mobilization, and survival. Myeloid cells, including neutrophils, have been associated with more severe disease from COVID-19.[1] In a nonrandomized cohort study, blockade of the granulocyte-macrophage–CSF receptor reportedly benefited patients with COVID-19 with systemic hyperinflammation.[6] For these reasons, the authors excluded patients with leukocytosis.

Second, the effect of rhG-CSF on lymphocyte quantity and differentiation is somewhat limited. As reported, rhG-CSF approximately doubled the lymphocyte cell count compared with a 4-fold increase in the total leukocyte cell count.[4] The G-CSF receptor is only moderately expressed in the lymphoid lineage, predominantly on developing thymocytes. One possibility is that G-CSF treatment stimulates the emergence of these T cell precursors from the thymus into the periphery, although the rapidity of the observed effect casts some doubt on whether this is the case. Another possibility is that a small subset of peripheral mature T cells responds to rhG-CSF, or that rhG-CSF–stimulated myeloid lineage cells interact with the T cell compartment to improve its fitness. Beyond stimulation, G-CSF also has regulatory roles and might downregulate neutrophil cytokine production or reset emergency hematopoiesis. Therefore, it is unclear if the effects of rhG-CSF reported by Cheng et al[4] are attributable to an increase in new lymphocytes, changes to activation status of mature lymphocytes, or changes centered on a myeloid to lymphocyte axis of cell survival.

Specifically reversing sepsis-associated lymphopenia has been the focus of immunoadjuvant trials of recombinant human interleukin-7 (rhIL-7), a cytokine favoring lymphocyte survival and proliferation. In a small phase IIb trial of patients with septic shock, rhIL-7 increased lymphocyte cell counts and was well tolerated, although no effect on mortality was seen.[7] RhIL-7 has also been administered to a small series of 12 critically ill patients with COVID-19; the lymphocyte cell count increased, although changes in inflammatory cytokines or survival were not seen.[8]

Recent work with deep immune profiling of peripheral lymphocytes has also highlighted the dramatic heterogeneity of immune responses to COVID-19, even among hospitalized patients with lymphopenia.[9,10] Substantial variation in the degree of T cell and B cell activation and proliferation was evident, with organ failure associating with specific CD8 lymphopenia yet a highly active T cell response, along with a sizable and persistent accumulation of plasmablasts, antibody-secreting cells that are typically low in abundance and only transiently detectable in blood.[9] Innate immune dysregulation was also evident, with decreased expression of the FcγRIII receptor (CD16) on multiple cell types that might indicate either an activated or refractory state.[10] How rhG-CSF treatment, with a likely more dominant impact on myeloid cells, influences lymphocyte responses remains to be determined. Future studies should investigate whether rhG-CSF treatment has distinct effects in patients with different baseline immunological features.

The trial of rhG-CSF conducted by Cheng et al[4] was relatively small, excluded patients with comorbidities, and was conducted early during the COVID-19 pandemic. Moreover, since this trial was conducted, the standard of care for hospitalized patients with COVID-19 has evolved considerably to include the use of remdesivir and dexamethasone. It is unclear how receipt of these medications in a uniform manner might influence the effects of rhG-CSF. Important next steps in determining the role of rhG-CSF or other medications that target lymphopenia in patients with COVID-19 are larger trials that incorporate the evolving standard of care into the control arm and the inclusion of patients with comorbidities.

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