

Prognostic and Predictive Biomarkers in Patients With Coronavirus Disease 2019 Treated With Tocilizumab in a Randomized Controlled Trial*

OBJECTIVES: To explore candidate prognostic and predictive biomarkers identified in retrospective observational studies (interleukin-6, C-reactive protein, lactate dehydrogenase, ferritin, lymphocytes, monocytes, neutrophils, D-dimer, and platelets) in patients with coronavirus disease 2019 pneumonia after treatment with tocilizumab, an anti-interleukin-6 receptor antibody, using data from the COVACTA trial in patients hospitalized with severe coronavirus disease 2019 pneumonia.

DESIGN: Exploratory analysis from a multicenter, randomized, double-blind, placebo-controlled, phase 3 trial.

SETTING: Hospitals in North America and Europe.

PATIENTS: Adults hospitalized with severe coronavirus disease 2019 pneumonia receiving standard care.

INTERVENTION: Randomly assigned 2:1 to IV tocilizumab 8 mg/kg or placebo.

MEASUREMENTS AND MAIN RESULTS: Candidate biomarkers were measured in 295 patients in the tocilizumab arm and 142 patients in the placebo arm. Efficacy outcomes assessed were clinical status on a seven-category ordinal scale (1, discharge; 7, death), mortality, time to hospital discharge, and mechanical ventilation (if not receiving it at randomization) through day 28. Prognostic and predictive biomarkers were evaluated continuously with proportional odds, binomial or Fine-Gray models, and additional sensitivity analyses. Modeling in the placebo arm showed all candidate biomarkers except lactate dehydrogenase and D-dimer were strongly prognostic for day 28 clinical outcomes of mortality, mechanical ventilation, clinical status, and time to hospital discharge. Modeling in the tocilizumab arm showed a predictive value of ferritin for day 28 clinical outcomes of mortality (predictive interaction, $p = 0.03$), mechanical ventilation (predictive interaction, $p = 0.01$), and clinical status (predictive interaction, $p = 0.02$) compared with placebo.

CONCLUSIONS: Multiple biomarkers prognostic for clinical outcomes were confirmed in COVACTA. Ferritin was identified as a predictive biomarker for the effects of tocilizumab in the COVACTA patient population; high ferritin levels were associated with better clinical outcomes for tocilizumab compared with placebo at day 28.

KEY WORDS: biomarkers; coronavirus disease 2019; ferritin; interleukin-6; severe acute respiratory syndrome coronavirus 2; tocilizumab

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Proinflammatory cytokines, such as interleukin (IL)-6, may be involved in the immune dysregulation reported in coronavirus disease 2019 (COVID-19), possibly resulting in more severe clinical

manifestations (1–3). Elevated IL-6 levels are associated with severe COVID-19 (4), and preliminary findings from case-control and retrospective cohort studies (5, 6) supported investigation of tocilizumab, an anti-IL-6 receptor α antibody, in severe COVID-19 pneumonia.

COVACTA was a global, double-blind, randomized, placebo-controlled, phase 3 trial of tocilizumab in patients hospitalized with severe COVID-19 pneumonia. The primary endpoint of improved clinical status on a seven-category ordinal scale at day 28 was not met. No difference was observed in mortality at day 28; however, time to hospital discharge was shorter in the tocilizumab group. Subgroup analysis suggested reduced risk for ICU transfer among patients not in the ICU at randomization and potential reduction in clinical failure (death, mechanical ventilation, ICU transfer, or study withdrawal) among patients not receiving mechanical ventilation at randomization. Additional studies are investigating these findings further (7).

Heterogeneity in clinical presentation and pathologic manifestations of COVID-19 (8) present challenges in investigating effective treatments and identifying biomarkers to prognosticate or predict treatment response. Laboratory measures of hyperinflammation (IL-6, C-reactive protein [CRP]), macrophage activation (ferritin), dysregulated immune cells (lymphocytes, monocytes, neutrophils), tissue damage (lactate dehydrogenase [LDH]), and coagulopathy (D-dimer, platelets) have been reported as risk factors for poor prognosis in COVID-19 (4, 9–13), and most of them are mechanistically linked to IL-6 activity (14–16). These biomarkers have been investigated only in retrospective observational studies of COVID-19.

We aimed to determine the prognostic and predictive value of previously identified candidate biomarkers regarding clinical outcomes in a prespecified exploratory analysis of the COVACTA trial.

METHODS

Patients and Study Design

COVACTA (ClinicalTrials.gov; NCT04320615) was a randomized, placebo-controlled, double-blind, global, multicenter, phase 3 trial investigating the efficacy and safety of tocilizumab versus placebo in patients hospitalized with severe COVID-19 pneumonia receiving standard care according to local practice (7).

Tocilizumab is investigational for the treatment of severe COVID-19 pneumonia and is not currently indicated for use in this patient population. Eligible adult patients—stratified by geographic region (North America, Europe) and mechanical ventilation (yes, no)—received IV tocilizumab or placebo (2:1). Key inclusion criteria were hospitalization with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection confirmed by polymerase chain reaction of any specimen and blood oxygen saturation less than or equal to 93% or $\text{PaO}_2/\text{FiO}_2$ less than 300 mm Hg despite local standard care (could include antivirals and low-dose steroids). Key exclusion criteria were active or suspected infection (other than SARS-CoV-2), alanine aminotransferase or aspartate aminotransferase greater than 10 \times upper limit of normal, absolute neutrophil count less than 1,000/mL, and platelet count less than 50,000/mL. The primary endpoint was clinical status assessed using a seven-category ordinal scale. Secondary endpoints included mortality at day 28, incidence of mechanical ventilation (in patients not receiving it at randomization), and time to hospital discharge. Informed consent was obtained for all enrolled patients. The study was conducted in accordance with the International Council for Harmonization E6 guideline for good clinical practice and the Declaration of Helsinki or local regulations, whichever afforded greater patient protection. The protocol was reviewed and approved by all appropriate institutional review boards and ethics committees (**Appendix 1**, <http://links.lww.com/CCM/G718>).

Biomarker Analysis

Biomarkers in peripheral blood were assessed at baseline to examine their potential prognostic value and potential predictive value for tocilizumab efficacy. Efficacy was determined by clinical status assessed on the seven-category ordinal scale at day 28 (primary), mortality at day 28, time to hospital discharge, and requirement for mechanical ventilation by day 28 (in patients not receiving it at randomization). These endpoints were selected to ensure a robust and consistent signal across different, clinically meaningful outcomes. Imputation rules were assigned as published (7). Prespecified candidate biomarkers included IL-6 and CRP as markers of hyperinflammation, ferritin as an acute-phase protein and as a marker of macrophage

activation, LDH as a marker of tissue damage, lymphocytes as markers of dysregulated immune response, and D-dimer as a marker of coagulopathy. The potential for neutrophil, monocyte, platelet, and WBC counts as biomarkers was also explored. IL-6 was measured using an immunoassay validated at QPS (Quantikine ELISA; R&D Systems, Minneapolis, MN). CRP was measured using an in vitro diagnostic method validated at PPD (Roche Cobas; Roche Diagnostics, Rotkreuz, Switzerland). All other biomarkers were assessed using standard clinical chemistry and hematology methods at local clinical laboratories.

Statistical Analysis

Biomarkers were assessed in the modified intention-to-treat population (any randomly assigned patients who received study medication). Subgroup analysis was conducted in patients in ordinal scale categories 4 (ICU or non-ICU hospital ward, requiring noninvasive ventilation or high-flow oxygen) and 5 (ICU, requiring intubation and mechanical ventilation) at baseline. Histograms, scatterplots, and tables were generated to assess the completeness of data by treatment arm at baseline, determine the balance of biomarker levels at baseline, and identify outliers. Biomarkers not normally distributed (D-dimer, ferritin, IL-6, lymphocytes [absolute], neutrophil-to-lymphocyte ratio, and WBCs) were log-transformed before analysis. Pearson correlation coefficient was calculated between outcomes and biomarkers, between baseline covariates and biomarkers, and between individual biomarkers. Six candidate biomarkers assessed (IL-6, CRP, ferritin, LDH, lymphocytes, D-dimer) were prespecified in a biomarker analysis plan. No adjustments were made for multiple comparisons.

Prognostic modeling was assessed in the placebo arm only, controlling for the following covariates: mechanical ventilation status at randomization, antiviral use, steroid use, age, sex, and region (Europe/North America). No endpoints were considered primary because the motivation was to identify a robust, consistent signal across endpoints. Sensitivity analyses were performed on the placebo arm only using unadjusted analysis, on the tocilizumab and placebo arms adjusting for the same covariates plus treatment arm, and on the tocilizumab and placebo arms controlling only for treatment arm. Candidate prognostic biomarkers were

assessed using a proportional odds model with the ordinal scale score at day 28 as a dependent variable and biomarker and covariates (depending on the model) as independent variables. Odds ratios, CIs, and *p* values were reported, and proportional odds assumptions were assessed graphically. A Fine-Gray model was fit for time to discharge, with death as a competing risk. A Cox proportional hazards model was fit as a sensitivity analysis. A binomial model with outcome as a dependent variable, biomarkers as independent variables, and covariates (depending on the model) was used for binary outcomes (death, discharge, mechanical ventilation).

Candidate predictive biomarkers were modeled as for candidate prognostic biomarkers, with addition of an interaction term between continuous biomarker levels and treatment. All reported predictive *p* values rely on the interaction *p* value term except those generated in the multivariate model assessing multiple biomarkers. Tertile analysis of predictive biomarkers was performed by creating vectors for each tertile (low, medium, high) and fitting a single model with interaction terms for medium and high tertiles with treatment. Treatment effects within each tertile were calculated from the estimates. No cut point optimization was performed, but analysis and visualization were performed using tertiles and quartiles. Combined predictive biomarkers were assessed by dichotomizing the biomarkers using median values as cutoffs.

Supportive analysis was conducted using data from the placebo arm of COVACTA and data from a phase 2 trial of tocilizumab in moderate-to-severe COVID-19 pneumonia (ClinicalTrials.gov: NCT04363736; MARIPOSA) (**Appendix 2** and **Table S1**, <http://links.lww.com/CCM/G718>).

RESULTS

Biomarkers at Baseline

Baseline biomarker levels (**Fig. S1A**, <http://links.lww.com/CCM/G718>), except IL-6 and CRP, were generally balanced between treatment arms in COVACTA (**Table 1**; and **Fig. S1B**, <http://links.lww.com/CCM/G718>). Median levels were higher in the tocilizumab than the placebo arm for IL-6 (88.1 vs 70.3 ng/L) and CRP (169.3 vs 151.9). Median levels of IL-6, CRP, ferritin, D-dimer, LDH, and neutrophils (absolute level and percentage) were above normal, and lymphocytes

TABLE 1.
Biomarker Levels at Baseline (Modified Intention-to-Treat Population)

| Biomarker | Normal Range | Placebo | Tocilizumab |
|---|---------------------|---------------------|--------------------|
| Interleukin-6, ng/mL ^a | ≤ 0.007 | <i>n</i> = 99 | <i>n</i> = 233 |
| Mean (sd) | | 192.2 (368.7) | 201.9 (418.4) |
| Median (range) | | 70.3 (3.1–2,810) | 88.1 (3.1–4,020) |
| C-reactive protein, mg/L | ≤ 5 | <i>n</i> = 126 | <i>n</i> = 256 |
| Mean (sd) | | 177.8 (117.1) | 187.8 (119.8) |
| Median (range) | | 151.9 (1.6–500) | 169.3 (1.1–500) |
| Ferritin, pmol/L ^a | 27 to ≤ 337 (women) | <i>n</i> = 124 | <i>n</i> = 240 |
| Mean (sd) | 27 to ≤ 674 (men) | 3,792 (7,463) | 3,069 (3,113) |
| Median (range) | | 2,168 (96.9–75,300) | 2,250 (3.6–24,045) |
| D-dimer, µg/mL fibrinogen equivalent units ^a | ≤ 0.5 | <i>n</i> = 66 | <i>n</i> = 131 |
| Mean (sd) | | 4.2 (7.6) | 4.6 (8.4) |
| Median (range) | | 1.2 (0.3–46.7) | 1.3 (0.2–58.1) |
| Lactate dehydrogenase, IU/L | 105–333 | <i>n</i> = 121 | <i>n</i> = 243 |
| Mean (sd) | | 469.7 (291.7) | 479.4 (303.5) |
| Median (range) | | 422 (1.3–2,323) | 430 (0.7–3,282) |
| Leukocytes, 10 ⁹ /L ^a | 4.5–11 | <i>n</i> = 140 | <i>n</i> = 280 |
| Mean (sd) | | 9.2 (4.1) | 9.3 (4.5) |
| Median (range) | | 8.5 (2.4–22.4) | 8.3 (2.7–28.2) |
| Lymphocytes, 10 ⁹ /L ^a | 0.9–2.9 | <i>n</i> = 139 | <i>n</i> = 288 |
| Mean (sd) | | 0.96 (0.83) | 0.98 (0.57) |
| Median (range) | | 0.9 (0–8.9) | 0.9 (0–5.4) |
| Lymphocytes, % | 20–40 | <i>n</i> = 133 | <i>n</i> = 268 |
| Mean (sd) | | 13 (10) | 12 (7) |
| Median (range) | | 11 (0–55) | 11 (0–48) |
| Neutrophils, 10 ⁹ /L | 1.7–7 | <i>n</i> = 139 | <i>n</i> = 291 |
| Mean (sd) | | 7.5 (3.9) | 7.6 (4.1) |
| Median (range) | | 7.2 (0.9–23.1) | 6.8 (1.0–24.6) |
| Neutrophils, % | 40–60 | <i>n</i> = 134 | <i>n</i> = 268 |
| Mean (sd) | | 79 (12) | 79 (9) |
| Median (range) | | 82 (24–98) | 81 (44–99) |
| Neutrophils/lymphocytes, ratio ^a | 1–3 | <i>n</i> = 132 | <i>n</i> = 265 |
| Mean (sd) | | 65.0 (566.5) | 64.8 (683.6) |
| Median (range) | | 7.1 (0.4–6,509) | 7.5 (1.2–10,463) |
| Monocytes, % | 2–8 | <i>n</i> = 131 | <i>n</i> = 269 |
| Mean (sd) | | 6 (4) | 6 (4) |
| Median (range) | | 5 (0–19) | 5 (0–32) |
| Platelets, 10 ⁹ /L | 150–400 | <i>n</i> = 142 | <i>n</i> = 295 |
| Mean (sd) | | 262.0 (117.7) | 265.7 (113.3) |
| Median (range) | | 240 (53–814) | 253 (10.2–825) |

^aLog-transformed for all additional analyses.

(absolute level and percentage) were below normal (neutrophil-to-lymphocyte ratio was elevated). Monocytes (percentages), platelet counts, and WBC counts were within normal ranges. Limited correlation was observed between prespecified biomarkers (Fig. S1C, <http://links.lww.com/CCM/G718>).

Evaluation of Prognostic Biomarkers

All biomarkers—except LDH, D-dimer, and neutrophil-to-lymphocyte ratio—correlated with clinical outcomes, including mortality, mechanical ventilation, ordinal scale score, and time to hospital discharge, at day 28 (Fig. 1A). Some biomarkers correlated with baseline covariates; IL-6, CRP, neutrophils (absolute level and percentage), and WBC count were positively correlated with higher baseline ordinal scale category (Fig. 1B). Adjusting for covariates, IL-6, CRP, ferritin, lymphocytes, monocytes, neutrophils, platelets, and WBCs showed robust evidence across sensitivity analyses, supporting their prognostic value, but LDH and D-dimer did not show prognostic value (Fig. S2, <http://links.lww.com/CCM/G718>). Prognostic modeling showed a consistent direction of effect across all clinical outcomes at day 28 for mortality, mechanical ventilation, ordinal scale score, and time to hospital discharge (Fig. 2A; and Fig. S2, <http://links.lww.com/CCM/G718>). Analysis of the ordinal scale at day 28 according to baseline

continuous ferritin revealed a potential prognostic effect of ferritin in the placebo arm ($p = 0.01$; $n = 124$).

Evaluation of Predictive Biomarkers

Predictive modeling identified ferritin as a consistent and significant predictive biomarker for the effects of tocilizumab in COVACTA, whereas other biomarkers, including IL-6, CRP, D-dimer, LDH, lymphocytes, monocytes, neutrophils, platelets, and WBC, did not show consistent or significant predictive value for the effects of tocilizumab across endpoints (Fig. 2B; and Fig. S3, <http://links.lww.com/CCM/G718>). Analysis of the ordinal scale at day 28 according to continuous baseline ferritin values showed that placebo patients with higher baseline levels had worse clinical status, whereas clinical outcomes at day 28 were similar across baseline ferritin tertiles in tocilizumab patients ($n = 364$; predictive effect interaction, $p = 0.02$; visualized using tertiles in Fig. 3A). Modeled data showed a predictive effect for ferritin as a biomarker for tocilizumab effects on mortality by day 28 (predictive interaction, $p = 0.03$) (Fig. 3B) and mechanical ventilation by day 28 (predictive interaction, $p = 0.01$) (Fig. 3C). Tocilizumab treatment decreased the probability of mechanical ventilation and mortality by day 28 compared with placebo in patients with elevated ferritin levels based on continuous assessment across a range of ferritin levels. Although time to hospital discharge was nominally shorter with tocilizumab than

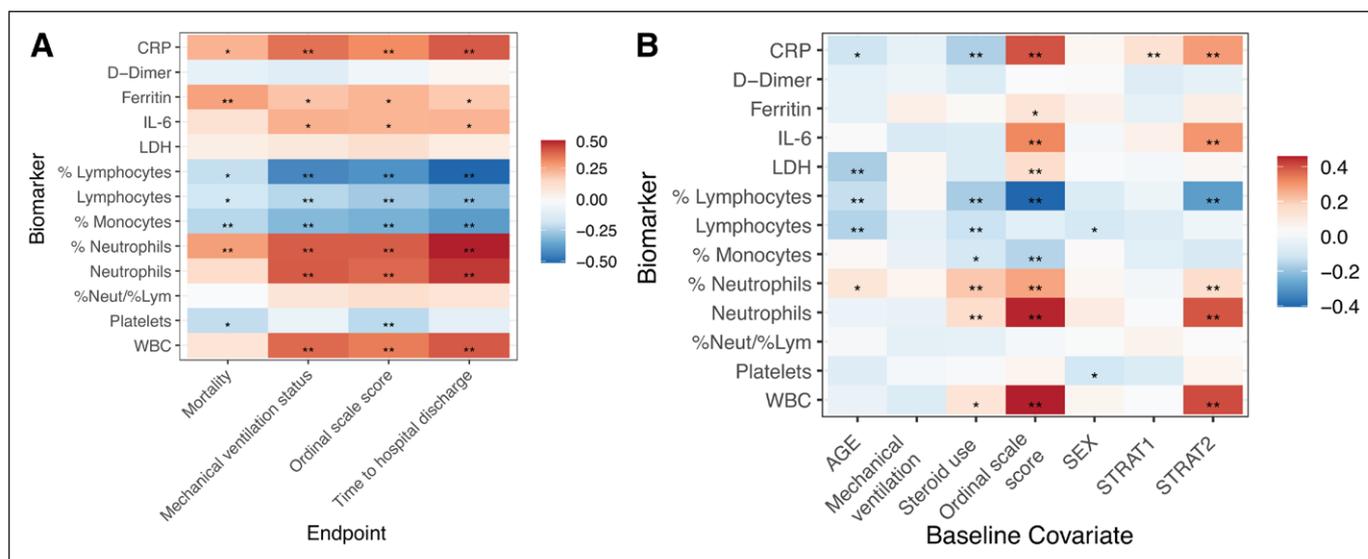


Figure 1. Correlation between baseline biomarkers and clinical outcomes (A) and biomarkers and baseline covariates (B). p values are based on Pearson correlation unadjusted for covariates, placebo arm only for A and for placebo and tocilizumab arms for B. * $p \leq 0.05$; ** $p \leq 0.01$. CRP = C-reactive protein, IL-6 = interleukin-6, LDH = lactate dehydrogenase, %Lym = % lymphocytes, %Neut = % neutrophils, STRAT = stratification factor.

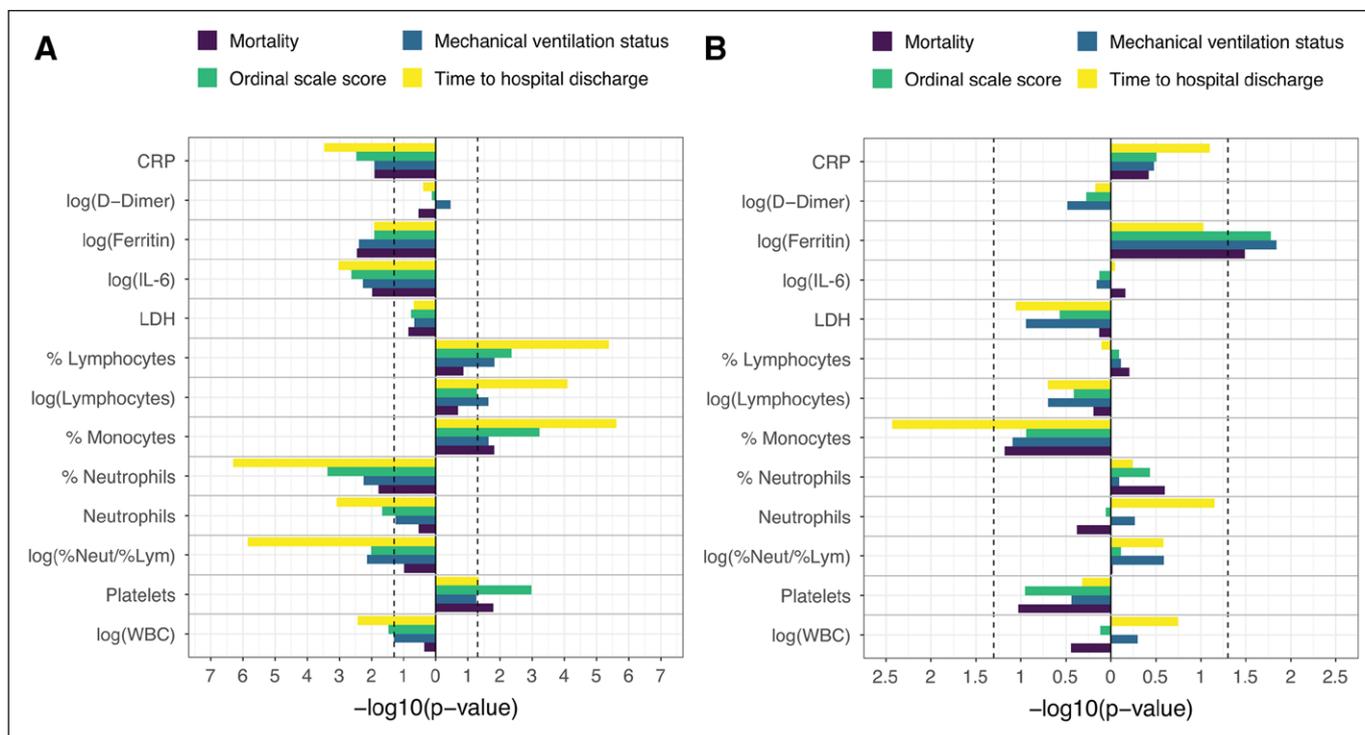


Figure 2. Efficacy outcomes. Placebo-adjusted, scaled prognostic model (A) and predictive model (B). Model adjusted for the stratification factors of region (Europe, North America) and mechanical ventilation (yes, no) as well as age, sex, baseline antiviral use (yes, no), and baseline steroid use (yes, no). Vertical dashed lines indicate $p = 0.05$. Outcomes are at day 28 and have been aligned, so that bars pointing in the same direction have the same direction of effect. Last observation carried forward was used for ordinal scale score at day 28. CRP = C-reactive protein, IL-6 = interleukin 6, LDH = lactate dehydrogenase, %Lym = % lymphocytes, %Neut = % neutrophils

placebo in COVACTA (7), ferritin was not a significant predictive biomarker for the effect of tocilizumab on time to hospital discharge ($p = 0.09$) (Fig. 3D); however, the direction of effect was consistent with that observed for ordinal scale and mortality outcomes.

Analysis comparing data from patients with severe disease in the tocilizumab 8 mg/kg arm from MARIPOSA with data from the placebo arm of COVACTA supported the predictive value of ferritin as a biomarker for mortality (ATT estimand based, $p = 0.044$) and mechanical ventilation status (Fig. S4, <http://links.lww.com/CCM/G718>).

Additional sensitivity analyses were performed on the negative signals observed for the biomarkers of interest: IL-6 and CRP. Analysis of the ordinal scale and mortality at day 28 confirmed that baseline IL-6 is not a predictive marker for the effects of tocilizumab (Fig. S5, <http://links.lww.com/CCM/G718>). Predictive analysis of CRP at a cutoff of 75 mg/L (17) was not significant for time to hospital discharge ($p = 0.46$), mortality ($p = 0.92$), or ordinal scale ($p = 0.78$) (data not shown), which is consistent with the tertiles analysis (Fig. S3, <http://links.lww.com/CCM/G718>). Analysis of combinations of the biomarkers such as ferritin, IL-6, and

CRP ($n = 257$) as well as ferritin, LDH, and D-dimer ($n = 165$) did not show any synergistic effect between biomarkers (Table S2, <http://links.lww.com/CCM/G718>), further suggesting that ferritin alone was the strongest predictive biomarker for the effects of tocilizumab in COVACTA.

Subgroup Analyses

Patients who required high-flow oxygen or noninvasive or invasive mechanical ventilation without other advanced life support (ordinal scale categories 4 and 5) at baseline had higher baseline ferritin levels than patients in all other ordinal scale categories (Fig. S6 and Table S3, <http://links.lww.com/CCM/G718>), and those in the tocilizumab arm had better clinical outcomes than those in the placebo arm. Analysis of this subgroup using a continuous scale for baseline ferritin levels also demonstrated a predictive association of baseline ferritin levels and the effects of tocilizumab for mortality (interaction, $p = 0.02$). Tertile analysis was also performed (Fig. 4A; and Fig. S7, <http://links.lww.com/CCM/G718>). The predictive signal of ferritin as a continuous biomarker was significant for clinical status by ordinal scale at day 28 (interaction,

$p = 0.01$) and incidence of mechanical ventilation by day 28 in patients who were not on mechanical ventilation at randomization (interaction, $p = 0.01$).

Baseline IL-6 levels were predictive for tocilizumab effects in this subgroup for time to hospital discharge only (Fig. 4B; and Fig. S8, <http://links.lww.com/CCM/G718>) (adjusted Fine-Gray, $p = 0.03$), but this was not statistically significant in sensitivity analysis (Cox proportional hazards adjusted, $p = 0.09$).

Furthermore, baseline IL-6 levels were not predictive for tocilizumab effects on hospital discharge (yes vs no; $p = 0.13$) or any other outcome in this subgroup. There was no evidence that baseline CRP levels were significantly predictive of tocilizumab effects based on the continuous or tertiles analysis (Fig. S7, <http://links.lww.com/CCM/G718>). Baseline CRP based on a cutoff of 75 mg/L was predictive for time to hospital discharge (Fine-Gray model, $p = 0.03$; Cox proportional hazards sensitivity analysis, $p = 0.05$) (data not shown) but not for the other outcomes.

DISCUSSION

Trials of tocilizumab in COVID-19 have produced mixed results. The COVACTA study did not show a mortality benefit of tocilizumab over standard care, but potential clinical benefits including shortened hospital stay and time to clinical improvements were observed (7). Tocilizumab did not demonstrate any mortality benefit above standard care in another randomized controlled

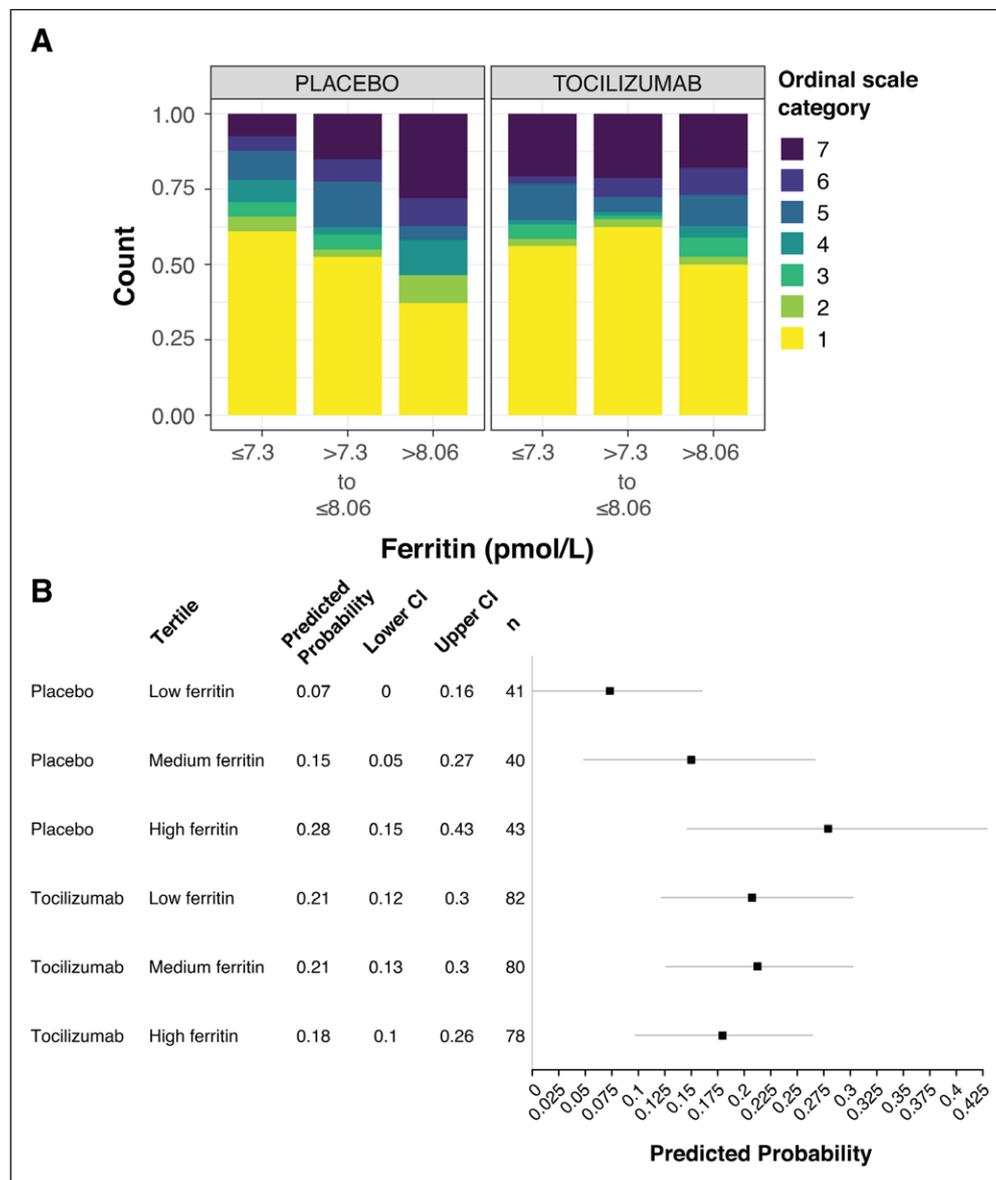


Figure 3. Ferritin as a predictor for efficacy outcomes. **A**, Day 28 all-comers, **B**, unadjusted tertiles model-predicted probability of death to day 28, **C**, unadjusted tertiles model-predicted probability of mechanical ventilation to day 28 (in patients who did not receive mechanical ventilation at randomization), and **D**, cumulative incidence function plot of hospital discharge and death to day 28. Seven-category ordinal scale: 1, discharged or ready for discharge; 2, non-ICU hospital ward, not requiring supplemental oxygen; 3, non-ICU hospital ward, requiring supplemental oxygen; 4, ICU or non-ICU hospital ward, requiring noninvasive ventilation or high-flow oxygen; 5, ICU, requiring intubation and mechanical ventilation; 6, ICU, requiring extracorporeal membrane oxygenation or mechanical ventilation and additional organ support; and 7, death. Ferritin values were log-transformed (sample size, $n = 364$). Ferritin tertile cutoff values were 3.63 pmol/L (minimum), 1,480.77 pmol/L, 3,150.29 pmol/L, and 75,299.67 pmol/L (maximum) in an unadjusted model showing predicted probability and 95% CI (**B**, **C**). Cumulative incidence was determined by Aalen-Johansen estimator, with an arbitrary median cut point of 7.7 on logarithmic scale (**D**). (Continued)

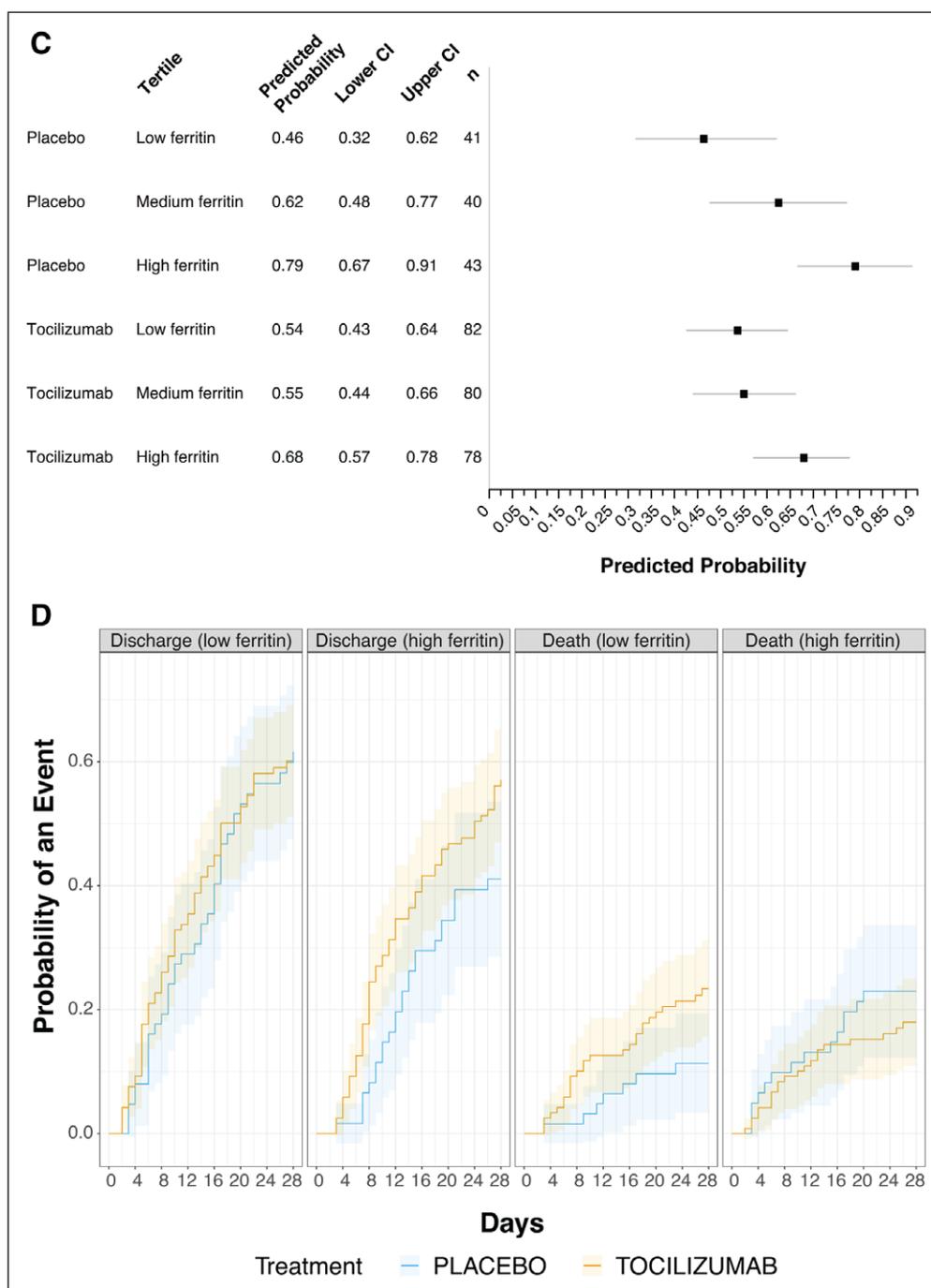


Figure 3. (Continued)

trial (18), but the Evaluating Minority Patients with Actemra study showed tocilizumab reduced the likelihood of progression to mechanical ventilation or death (19). The open-label Randomized, Embedded, Multifactorial Adaptive Platform Trial for Community-Acquired Pneumonia study showed improved outcomes, including survival, above standard care in critically ill patients treated with tocilizumab (20), and the open-label Randomized Evaluation of Covid-19 Therapy

study demonstrated that tocilizumab was associated with reduced all-cause mortality across all levels of respiratory support, higher probability of hospital discharge alive, and reduced need for mechanical ventilation (17).

Our exploratory analysis of the COVACTA trial is the first, to our knowledge, to report on prognostic and predictive biomarkers from a global, multicenter, randomized controlled trial in patients hospitalized with COVID-19 pneumonia. The candidate biomarkers we assessed—IL-6, CRP, ferritin, LDH, lymphocytes, neutrophils, monocytes, D-dimer, and platelets—are among the biomarkers previously identified in case series and observational studies as potential biomarkers for disease outcomes in COVID-19 (9, 12, 21).

IL-6, CRP, ferritin, LDH, lymphocyte, neutrophil, and D-dimer levels were abnormal at baseline in COVACTA, consistent with manifestations of hyperinflammation, macrophage activation, tissue

damage, dysregulated immune cells, and coagulopathy in patients with severe COVID-19 (4, 9–13). Only a modest correlation was observed between biomarkers representing different manifestations, potentially reflecting the overlapping yet differentiated pathogenic pathways in COVID-19. Elevated levels of IL-6, CRP, ferritin, and neutrophils and decreased levels of lymphocytes demonstrated robust and consistent prognostic value as biomarkers of poor disease outcomes across all clinical outcomes assessed. Monocytes

(percentage) and platelet counts also demonstrated prognostic value, although at baseline they were within normal ranges. Elevated LDH levels appeared to have prognostic value, but this was not robust across sensitivity analyses. Our findings support the hypothesis that systemic hyperinflammation, tissue damage, and dysregulated immune responses are associated with poor disease outcomes in patients with severe COVID-19 (4, 9, 10). Elevated D-dimer levels as a marker of coagulopathy showed weak and inconsistent prognostic signals in COVACTA. However, there were potential confounding factors for D-dimer assessment in COVACTA, including higher levels among survivors compared with other studies (10) (Table S4, <http://links.lww.com/CCM/G718>), disproportionate missing data by region (59.2%, Europe; 40.8%, North America), an association with the stratification factor region that was not evident for any other biomarkers tested, and an unadjusted *p* value of 0.023 for mortality in prognostic modeling compared with an adjusted *p* value of 0.54 for region. Thus, the prognostic value of D-dimer levels in COVID-19 remains controversial (12, 22, 23).

Ferritin was the only biomarker in this exploratory analysis that predicted the effects of tocilizumab treatment in COVACTA across multiple clinical outcomes. During infection and inflammation, ferritin, as a marker of myeloid cell activation in blood and inflamed tissue, is released

from activated macrophages and hepatocytes to protect cells from oxidative damage and sequester iron from pathogens (24, 25). Once released, it can further activate macrophages to produce cytokines such as IL-6 and IL-1 β , driving a positive feedback loop of ferritin-inducing proinflammatory cytokine release (24, 25). Altered myeloid cell activation, elevated IL-6 levels, and elevated ferritin levels were reported in

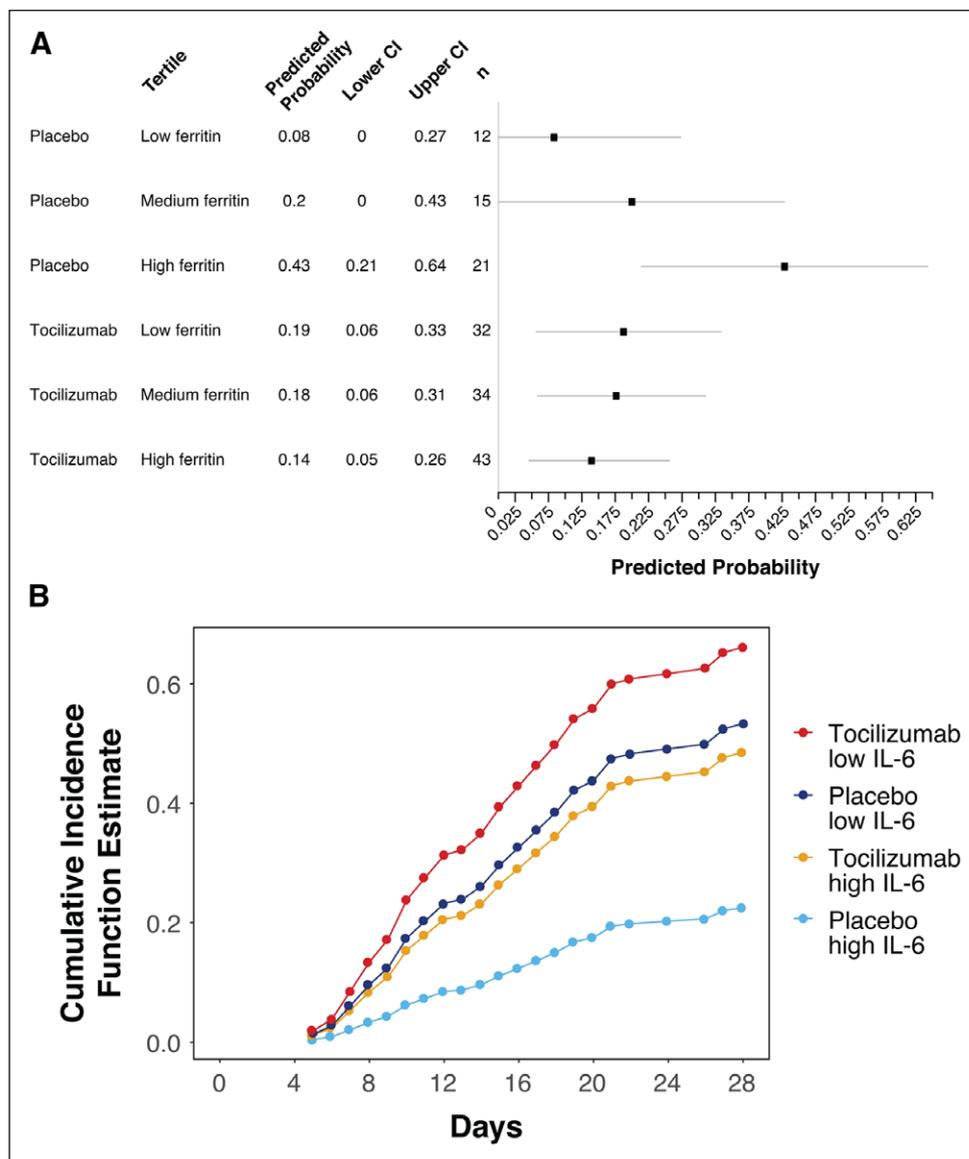


Figure 4. Predictive biomarker assessment for the subset of patients in ordinal scale categories 4 and 5 at baseline in COVACTA (*n* = 157). **A**, Death by day 28 according to baseline ferritin levels. Ferritin values were log-transformed. Shown are predicted probabilities for death within each tertile of ferritin (tertile cutoff values were 3.63 pmol/L [minimum], 1,480.77 pmol/L, 3,150.29 pmol/L, and 75,299.67 pmol/L [maximum]) showing predicted probability and 95% CI based on an unadjusted model (calculated based on all-comers) fit using samples restricted to those for patients in ordinal scale categories 4 and 5 at baseline. **B**, Time to hospital discharge by baseline interleukin (IL)-6 levels. Figure shows the cumulative incidence function for time to hospital discharge based on the median IL-6 cutoff value.

COVID-19 and correlated with increased severity of COVID-19 disease (1, 26). Furthermore, elevated ferritin levels decreased significantly after tocilizumab administration in patients with severe COVID-19 (12), suggesting a possible mechanism underlying the predictive value of ferritin for tocilizumab treatment effects in this patient population. Further investigation of downstream mechanisms of IL-6 and ferritin signaling interaction, such as single-cell analysis of pulmonary cells, might elucidate how elevated ferritin levels predict benefit from inhibition of IL-6 activity in patients with severe COVID-19.

Although IL-6 and CRP demonstrated strong prognostic value as disease biomarkers in COVID-19, elevated levels of IL-6 or CRP at baseline were not predictive for tocilizumab treatment effects in the overall COVACTA population. IL-6 only demonstrated a trend for predictive value among the subgroup of patients who required high-flow oxygen and noninvasive or invasive mechanical ventilation (ordinal scale categories 4 and 5) at baseline. Similarly, baseline CRP using a cutoff of 75 mg/L, the same cutoff used in RECOVERY, was predictive for tocilizumab effect on time to hospital discharge but not for other endpoints. CRP was not predictive in continuous or tertile analyses. This is consistent with the findings of the REMAP-CAP study, in which CRP levels at baseline did not predict tocilizumab efficacy (19). These results might be counterintuitive given that tocilizumab inhibits IL-6 signaling; however, it is unclear how IL-6 activity in blood is associated with tocilizumab efficacy in complex immunologic diseases (27, 28). For patients with COVID-19, investigation into how peripheral IL-6 and CRP levels are associated with tocilizumab effects on hyperinflammation and dysregulated immune responses in affected tissue, in particular the lung, is warranted.

Because the COVACTA trial did not meet its primary endpoint of improved clinical status in patients with COVID-19-associated pneumonia or its key secondary endpoint of reduced patient mortality, identifying a subgroup of patients who might have derived clinical benefit (patients with elevated ferritin levels at baseline) also revealed a subgroup potentially harmed (patients without highly elevated ferritin levels at baseline); however, data interpretation was limited because of the relatively small sample size. In RECOVERY, a large platform study, mortality benefit was observed consistently in the tocilizumab group in all patients

with hypoxia, regardless of their level of respiratory support (17). Additional studies are required to confirm the predictive value of ferritin for tocilizumab effects in patients with COVID-19 and to identify a clinically useful threshold at which ferritin levels could predict potential enhanced benefit. These studies, however, must consider factors such as variability between patient cohorts and assay methods. Another limitation of this study is that laboratory results were measured locally, entailing more variability than uniform test runs in a central laboratory. Local laboratory results for ferritin, however, were confirmed by retesting all samples at a central laboratory using an in vitro diagnostic method (Roche Cobas; Roche Diagnostics).

In conclusion, markers of hyperinflammation, macrophage activation, and dysregulated immune cells appear to be important prognostic biomarkers in COVID-19. Ferritin, as an acute-phase protein and a macrophage activation marker, might have potential as a predictive biomarker to identify patients with severe COVID-19 who respond to tocilizumab. Results from ongoing studies of tocilizumab in COVID-19 are needed to validate these findings.

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REFERENCES

1. Vabret N, Britton GJ, Gruber C, et al; Sinai Immunology Review Project: Immunology of COVID-19: Current state of the science. *Immunity* 2020; 52:910–941
2. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, et al: Complex immune dysregulation in COVID-19 patients with

- severe respiratory failure. *Cell Host Microbe* 2020; 27:992–1000.e3
3. Mazzone A, Salvati L, Maggi L, et al: Impaired immune cell cytotoxicity in severe COVID-19 is IL-6 dependent. *J Clin Invest* 2020; 130:4694–4703
 4. Zhu J, Pang J, Ji P, et al: Elevated interleukin-6 is associated with severity of COVID-19: A meta-analysis. *J Med Virol* 2020; 93:35–37
 5. Kaye AG, Siegel R: The efficacy of IL-6 inhibitor tocilizumab in reducing severe COVID-19 mortality: A systematic review. *PeerJ* 2020; 8:e10322
 6. Guaraldi G, Meschiari M, Cozzi-Lepri A, et al: Tocilizumab in patients with severe COVID-19: A retrospective cohort study. *Lancet Rheumatol* 2020; 2:e474–e484
 7. Rosas IO, Bräu N, Waters M, et al: Tocilizumab in hospitalized patients with severe COVID-19 pneumonia. *N Engl J Med* 2021; 384:1503–1516
 8. National Institutes of Health: Coronavirus Disease 2019 (COVID-2019) Treatment guidelines. Available at: <https://www.covid19treatmentguidelines.nih.gov/>. Accessed April 29, 2021
 9. Herold T, Jurinovic V, Arnreich C, et al: Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. *J Allergy Clin Immunol* 2020; 146:128–136.e4
 10. 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; 395:1054–1062
 11. Lohse A, Klopfenstein T, Balblanc JC, et al: Predictive factors of mortality in patients treated with tocilizumab for acute respiratory distress syndrome related to coronavirus disease 2019 (COVID-19). *Microbes Infect* 2020; 22:500–503
 12. Conrozier T, Lohse A, Balblanc JC, et al: Biomarker variation in patients successfully treated with tocilizumab for severe coronavirus disease 2019 (COVID-19): Results of a multidisciplinary collaboration. *Clin Exp Rheumatol* 2020; 38:742–747
 13. Luo P, Liu Y, Qiu L, et al: Tocilizumab treatment in COVID-19: A single center experience. *J Med Virol* 2020; 92:814–818
 14. Lok LSC, Farahi N, Juss JK, et al: Effects of tocilizumab on neutrophil function and kinetics. *Eur J Clin Invest* 2017; 47:736–745
 15. Gibiansky L, Frey N: Linking interleukin-6 receptor blockade with tocilizumab and its hematological effects using a modeling approach. *J Pharmacokinetic Pharmacodyn* 2012; 39:5–16
 16. Fonseca JE, Santos MJ, Canhão H, et al: Interleukin-6 as a key player in systemic inflammation and joint destruction. *Autoimmun Rev* 2009; 8:538–542
 17. RECOVERY Collaborative Group: Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): A randomised, controlled, open-label, platform trial. *Lancet* 2021; 397:1637–1645
 18. Stone JH, Frigault MJ, Serling-Boyd NJ, et al; BACC Bay Tocilizumab Trial Investigators: Efficacy of tocilizumab in patients hospitalized with COVID-19. *N Engl J Med* 2020; 383:2333–2344
 19. Salama C, Han J, Yau L, et al: Tocilizumab in patients hospitalized with COVID-19 pneumonia. *N Engl J Med* 2021; 384:20–30
 20. Gordon AC, Mouncey PR, Al-Beidh F, et al: Interleukin-6 receptor antagonists in critically ill patients with COVID-19. *N Engl J Med* 2021; 384:1491–1502
 21. Henry BM, de Oliveira MHS, Benoit S, et al: Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): A meta-analysis. *Clin Chem Lab Med* 2020; 58:1021–1028
 22. Ye W, Chen G, Li X, et al: Dynamic changes of D-dimer and neutrophil-lymphocyte count ratio as prognostic biomarkers in COVID-19. *Respir Res* 2020; 21:169
 23. 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 Respir Med* 2020; 8:1233–1244
 24. Kappert K, Jahić A, Tauber R: Assessment of serum ferritin as a biomarker in COVID-19: Bystander or participant? Insights by comparison with other infectious and non-infectious diseases. *Biomarkers* 2020; 25:616–625
 25. Ruscitti P, Di Benedetto P, Berardicurti O, et al: Pro-inflammatory properties of H-ferritin on human macrophages, ex vivo and *in vitro* observations. *Sci Rep* 2020; 10:12232
 26. Schulte-Schrepping J, Reusch N, Paclik D, et al; Deutsche COVID-19 OMICS Initiative (DeCOI): Severe COVID-19 is marked by a dysregulated myeloid cell compartment. *Cell* 2020; 182:1419–1440.e23
 27. Teachey DT, Lacey SF, Shaw PA, et al: Identification of predictive biomarkers for cytokine release syndrome after chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Cancer Discov* 2016; 6:664–679
 28. Choy EH, De Benedetti F, Takeuchi T, et al: Translating IL-6 biology into effective treatments. *Nat Rev Rheumatol* 2020; 16:335–345