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# Trajectories of Host-Response Subphenotypes in Patients With COVID-19 Across the Spectrum of Respiratory Support

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**Additional information:** The e-Appendix, e-Figures, and e-Tables are available online under “Supplementary Data.”

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## Abstract

**BACKGROUND:** Hospitalized patients with severe COVID-19 follow heterogeneous clinical trajectories, requiring different levels of respiratory support and experiencing diverse clinical outcomes. Differences in host immune responses to SARS-CoV-2 infection may account for the heterogeneous clinical course, but we have limited data on the dynamic evolution of systemic biomarkers and related subphenotypes. Improved understanding of the dynamic transitions of host subphenotypes in COVID-19 may allow for improved patient selection for targeted therapies.

**RESEARCH QUESTION:** We examined the trajectories of host-response profiles in severe COVID-19 and evaluated their prognostic impact on clinical outcomes.

**STUDY DESIGN AND METHODS:** In this prospective observational study, we enrolled 323 inpatients with COVID-19 receiving different levels of baseline respiratory support: (1) low-flow oxygen (37%), (2) noninvasive ventilation (NIV) or high-flow oxygen (HFO; 29%), (3) invasive mechanical ventilation (27%), and (4) extracorporeal membrane oxygenation (7%). We collected plasma samples on enrollment and at days 5 and 10 to measure host-response biomarkers. We classified patients by inflammatory subphenotypes using two validated predictive models. We examined clinical, biomarker, and subphenotype trajectories and outcomes during hospitalization.

**RESULTS:** IL-6, procalcitonin, and angiopoietin 2 persistently were elevated in patients receiving higher levels of respiratory support, whereas soluble receptor of advanced glycation end products (sRAGE) levels displayed the inverse pattern. Patients receiving NIV or HFO at baseline showed the most dynamic clinical trajectory, with 24% eventually requiring intubation and exhibiting worse 60-day mortality than patients receiving invasive mechanical ventilation at baseline (67% vs 35%; $P < .0001$). sRAGE levels predicted NIV failure and worse 60-day mortality for patients receiving NIV or HFO, whereas IL-6 levels were predictive in all patients regardless of level of support ($P < .01$). Patients classified to a hyperinflammatory subphenotype at baseline (< 10%) showed worse 60-day survival ($P < .0001$) and 50% of them remained classified as hyperinflammatory at 5 days after enrollment.

**INTERPRETATION:** Longitudinal study of the systemic host response in COVID-19 revealed substantial and predictive interindividual variability influenced by baseline levels of respiratory support.

### Keywords

acute lung injury; acute respiratory failure; biomarkers; COVID-19; host response; longitudinal; subphenotypes

SARS-CoV-2 has infected > 676 million individuals and led to > 6.8 million deaths worldwide,[1] with > 1 million deaths in the United States[2] as of March 2023. Extensive research has shown that a dysregulated inflammatory response against the virus develops in patients with COVID-19 with severe illness requiring hospitalization, often leading to acute respiratory failure with parenchymal lung damage and impaired gas exchange.[3] Current care consists of two main elements: (1) provision of appropriate respiratory support (invasive or noninvasive options) to improve gas exchange and work of breathing, and (2) delivery of timely and effective antiviral and immunomodulatory therapies[4,5] to curtail the aberrant inflammatory response.

The provision of the first main element of care, appropriate respiratory support, is dynamic and responsive to clinical changes at the bedside. Provision of the second main element of care, antiviral and immunomodulatory agents, is based largely on cross-sectional assessments of respiratory failure severity and crude biomarkers that are available clinically (eg, C-reactive protein levels for anti-IL-6 treatment initiation). However, the systemic inflammatory response in severe COVID-19 is complex, with multiple pathways involved and differences compared with non-COVID ARDS.[6] Extensive research in non-COVID ARDS has shown replication validity of distinct host-response subphenotypes (eg, hyperinflammatory and hypoinflammatory), potentially offering new opportunities for targeted therapeutics.[7–10] Such biomarker-based subphenotypes also have been described in COVID-19 ARDS and may allow better targeting of immunomodulatory interventions. Enhanced understanding of the dynamic variability of the longitudinal systemic inflammatory response in patients with COVID-19 across the spectrum of respiratory failure severity may help to improve prognostication and patient selection for timely interventions.

In this prospective, observational study spanning the first 2 years of the SARS-CoV-2 pandemic, we collected longitudinal data in two independent cohorts of inpatients with COVID-19 requiring different levels of respiratory support. We characterized the clinical, biomarker, and subphenotype trajectories in COVID-19 and examined whether host-response biomarkers and subphenotypes had different prognostic value on patient outcomes depending on the baseline level of respiratory support.

## Study Design and Methods

Detailed methods are provided in e-Appendix 1.

### Clinical Cohorts

We prospectively enrolled hospitalized patients with COVID-19 in two independent, prospective cohort studies within the University of Pittsburgh Medical Center Health System (see e-Appendix 1 for details): the Acute Lung Injury Registry and Biospecimen

Repository enrolled critically ill patients with COVID-19 hospitalized in ICUs,[6] and the COVID INpatient Cohort enrolled moderately ill inpatients with COVID-19 hospitalized in dedicated inpatient wards.[11] We enrolled patients after admission to the hospital and obtained informed consent from the patients or their legally authorized representatives under study protocols STUDY19050099 and STUDY20040036 approved by the University of Pittsburgh Institutional Review Board.

## Biospecimen Collection

We collected baseline blood samples on enrollment (day 1) and at follow-up intervals (days 5 and 10) for those who remained hospitalized, and we measured host-response biomarkers.

## Clinical Data Collection

From the electronic medical record, we extracted data on demographics, comorbid conditions, vital signs, and laboratory test results at baseline, as well as immunomodulatory treatments received during hospitalization. We broadly classified baseline respiratory support in four ordinal categories of increased intensity, referred to as clinical groups: (1) low-flow oxygen (LFO), that is, patients with a conventional nasal cannula or oxygen mask; (2) noninvasive ventilation (NIV) or high-flow oxygen (HFO), that is, patients receiving either NIV (continuous or bilevel positive airway pressure) or humidified, heated HFO delivered via nasal cannula or mask; (3) invasive mechanical ventilation (IMV) via endotracheal intubation; and (4) extracorporeal membrane oxygenation (ECMO) support. We recorded the clinical group trajectories starting from date of symptom onset to date of positive polymerase chain reaction (PCR) testing, as well as hospital or ICU admission. We followed up patients prospectively for the type(s) of respiratory support required during follow-up and recorded escalation or de-escalation of support. Because NIV and HFO often were used in the pandemic as respiratory support measures to avoid intubation, we specifically labeled patients as NIV or HFO fail for those who were supported by NIV or HFO at baseline but ended up requiring IMV or ECMO or died during hospitalization, vs NIV or HFO success for those who never required intubation and survived hospitalization. The primary outcome was 60-day survival from hospital admission, and the secondary outcome was the result of NIV or HFO trials (success vs failure).

## Biomarker Measurements

With a Luminex panel, we measured plasma host-response biomarkers (e-Appendix 1) and focused analyses on four biomarkers with direct implications in COVID-19 biological features: (1) IL-6,[12] a target of approved immunomodulatory therapies for COVID-19; (2) procalcitonin,[13] as a plausible biomarker for secondary bacterial infections; (3) soluble receptor of advanced glycation end products (sRAGE),[14,15] a biomarker for alveolar epithelial injury; and (4) angiopoietin 2,[16,17] a biomarker for endothelial injury. From a subset of available plasma samples, we also quantified SARS-CoV-2 RNA with quantitative PCR (ie, RNA-emia).[18,19]

### Subphenotype Classifications and Statistical Analyses

We classified patients into host-response subphenotypes by applying two biomarker-based parsimonious logistic regression models that had been developed previously via latent class analyses: (1) the four-variable model by Drohan et al[7] (hereafter the Drohan model) using bicarbonate, procalcitonin, soluble tumor necrosis factor receptor 1, and angiopoietin 2 levels using the 0.5 probability threshold for subphenotype classification and (2) the three-variable model by Sinha et al[20] (hereafter the Sinha model) using bicarbonate, IL-6, and soluble tumor necrosis factor receptor 1 using the Youden index cutoff (0.274) per the previous application of this model in patients with COVID-19. Phenotype 2 by each model, characterized by elevated levels of biomarkers, was labeled as hyperinflammatory, whereas phenotype 1 was labeled as hypoinflammatory.

We compared continuous and categorical variables between respiratory support groups or subphenotypes with the Wilcoxon and Fisher exact tests, respectively. We performed $\log_{10}$-transformations of biomarker values for statistical analyses. We conducted sensitivity analysis by probable SARS-CoV-2 variant and background immunomodulatory therapies. We examined the dynamics of biomarker levels over time using mixed linear regression models against time from hospital admission with random patient intercepts and inclusion of interaction terms for time × type of respiratory support, as well as by comparing biomarker levels between sampling follow-up intervals (days 1, 5, and 10). For 60-day survival, we constructed Kaplan-Meier curves for time to event from hospital admission, as well as Cox proportional hazards models adjusted for age, time from hospital admission, and type of respiratory support at baseline. We tested for the proportionality assumptions graphically and with the Shoenfeld residual test. For 60-day mortality, we constructed logistic regression models adjusted for age, time from hospital admission, and type of respiratory support at baseline. To examine for differential prognostic effects for each biomarker or subphenotype by level of baseline respiratory support on 60-day mortality, we added interaction terms (eg, biomarker × type of respiratory support) in the logistic regression models and examined the statistical significance ($P < .05$) of the interaction terms. For the outcome of NIV or HFO trial, we constructed a logistic regression model adjusted for known clinical predictors of NIV or HFO success vs failure (age, sex, BMI, history of COPD, and immunosuppression).[21] We conducted all analyses in R version 4.2.0 software (R Foundation for Statistical Computing).

## Results

### Clinical Characteristics of Study Population

Between March 1, 2020, and March 29, 2022, we enrolled a total of 323 patients with COVID-19 (Table 1, e-Fig 1). Enrolled patients were predominantly male (57%) and White (78%), with a median age of 61.4 years. At baseline, we classified patients into the clinical groups of LFO (n = 120 [37%]), NIV or HFO (n = 92 [29%]), IMV (n = 88 [27%]), and ECMO (n = 23 [7%]). Patients managed with ECMO were younger, more often were White, and showed higher BMI than the other clinical groups (Table 1). Patients enrolled in this cohort with biospecimen acquisition showed similar distribution of demographics compared

with 11,429 patients with COVID-19 hospitalized across the University of Pittsburgh Medical Center system during a partially overlapping study period (e-Table 1).[22]

## SARS-CoV-2 Infection Timeline and Clinical Group Trajectories

Patients receiving ECMO at the time of enrollment showed a significantly longer time from index COVID-19 quantitative PCR positive results and onset of symptoms, followed by patients receiving IMV and NIV or HFO, overall indicating later stages of COVID-19 compared with patients receiving LFO (Table 1, e-Fig 2). This difference in COVID-19 phase of illness at time of enrollment was also supported by differences in plasma viral RNA load, with patients receiving NIV or HFO showing the highest viral RNA levels (Fig 1A), potentially indicating an earlier phase of SARS-CoV-2 infection with higher viral replication.

Across all groups, we found that patients requiring greater respiratory support at time of enrollment achieved worse 60-day survival (Fig 1B). We then examined the clinical group trajectories starting from baseline assignments to maximum level of respiratory support required during hospitalization (Fig 1C). The NIV or HFO group showed the most frequent clinical group changes, with 24% (n = 22) requiring escalation to IMV or ECMO. These patients who required escalation from NIV or HFO to IMV or ECMO showed the highest 60-day mortality (67.4%) compared with the rest of the cohort.

## Plasma Biomarker Trajectories by Clinical Group

In baseline comparisons, IL-6, procalcitonin, and angiopoietin 2 increased with each higher level of support from LFO to ECMO, whereas for sRAGE, the sickest patients receiving ECMO showed the lowest levels compared with the other groups (Fig 2A–D, e-Table 2). These differences between levels of support persisted in linear regression models adjusted for time from symptom onset (e-Table 3), as well as in both day 5 and day 10 comparisons (e-Fig 3). By constructing mixed linear regression models of biomarker levels from time of admission, we found different trajectories for the effects of time by type of respiratory support ($P < .001$ for interaction) for all four biomarkers. Specifically for sRAGE, we found declining trajectories (statistically significant negative β coefficients for effects of time of sampling from hospital admission) for patients receiving NIV or HFO, IMV, and ECMO (e-Fig 4, e-Table 4), suggesting that sRAGE is a biomarker that peaks earlier in the COVID-19 course.

## Subphenotype Trajectories by Clinical Group

The two parsimonious models (the Drohan and Sinha models) showed fair agreement in baseline subphenotypic classifications (area under the receiver operating characteristic curve, 0.64), with 8% and 7% of patients classified to the hyperinflammatory subphenotype, respectively (Fig 3A, e-Fig 5A). Patients classified to the hyperinflammatory subphenotype showed lower platelets levels, higher WBC counts, and worse renal function parameters ($P < .01$) (e-Table 5). For patients with available follow-up biospecimens, subphenotypic classifications from days 1 to 5 were overall stable for the hypoinflammatory subphenotype (with 2% and 8% transitions by the Drohan and Sinha models), but unstable for the day 1

hyperinflammatory subphenotype, with 50% of patients assigned as hypoinflammatory on day 5 by both models (Fig 3, e-Fig 6).

## Baseline Biomarker Levels and Subphenotypes Prognosticate Clinical Outcome

In serial comparisons of biomarker levels between 60-day survivors and nonsurvivors, we found that nonsurvivors showed higher levels of all four biomarkers at baseline compared with survivors, but only IL-6 and angiopoietin 2 levels persistently were elevated in nonsurvivors across all three time points (Fig 4A–D). We then examined whether baseline level of support modified the associations between biomarkers and 60-day mortality in logistic regression models that included interaction terms for each biomarker and clinical group assignment. We found that the prognostic effects for procalcitonin and sRAGE were modified (ie, significant interaction terms) by baseline respiratory support, whereas the prognostic effects for IL-6 and angiopoietin 2 were not affected by respiratory support level (e-Table 6). In an exploratory analysis, we compared biomarker levels of patients receiving NIV or HFO with successful vs failed noninvasive support trial and found that the latter group showed significantly higher sRAGE and procalcitonin levels ($P < .0001$) (Fig 4E–H). We then constructed logistic regression models for the outcome of noninvasive success vs failure adjusted for common clinical predictors and found that higher baseline sRAGE levels were independent predictors of noninvasive failure (e-Table 7).

Baseline subphenotypes by the two models also were strongly predictive of outcome, even after adjustments for the different baseline levels of respiratory support. We found that patients classified to the hyperinflammatory subphenotype at baseline showed significantly higher hazard ratios for death compared with patients classified as hypoinflammatory for both the Sinha model (adjusted hazard ratio [95% CI], 6.55 [3.45–12.43]) (Fig 3, e-Table 8) and the Drohan model (adjusted hazard ratio, 3.59 [95% CI, 1.81–7.12]) (e-Fig 5, e-Table 8). Notably, patients who were classified as hyperinflammatory by both models (concordant hyperinflammatory) achieved significantly worse outcomes than patients classified as hypoinflammatory by both models (concordant hypoinflammatory) as well as those with discordant classifications between models (e-Fig 7). In logistic regression models for the outcome of 60-day mortality, we found that the prognostic effects of each subphenotype were not modified by level of baseline respiratory support (ie, nonsignificant interaction terms), although we noted that no patient receiving LFO was classified as hyperinflammatory subphenotype by the Sinha model (Fig 3A). Subphenotypic classification transitions by day 5 were also prognostic of further outcomes among patients who survived to day 5 and whose biospecimens were available. Patients who persisted or emerged as hyperinflammatory subphenotype by day 5 showed significantly worse mortality (logistic regression OR, 2.62 [95% CI, 1.12–6.89]; $P = .04$) compared with patients who were classified as hypoinflammatory by day 5 (either stably hypoinflammatory from baseline or following resolution of the baseline hyperinflammatory classification by the Sinha model) (Fig 3D).

## Discussion

We demonstrated distinct clinical and biomarker trajectories that predicted patient outcomes in a prospective, observational study of hospitalized patients with COVID-19 across the spectrum of illness severity. Host-response biomarker associations with clinical outcomes depended on the level of respiratory support at time of sampling. sRAGE, a biomarker of alveolar epithelial injury, was predictive of outcome among patients receiving NIV or HFO, whereas IL-6 was predictive among patients receiving IMV or ECMO. sRAGE levels declined during hospitalization, whereas other biomarkers showed flat or rising trajectories. Synthesis of host-response profiles with subphenotypic classifications showed an overall low prevalence of the hyperinflammatory subphenotype in patients with COVID-19, but patients classified in the hyperinflammatory subphenotype showed markedly worse outcomes, independent of the level of respiratory support.

The clinical trajectory analyses revealed that patients at different levels of support early in the hospitalization may signify different phases of SARS-CoV-2 infection and the evolving host response. The highest plasma viral load detected in patients while receiving NIV or HFO suggests that such patients may be in a phase of more active viral replication than patients requiring more invasive support. Such distinctions could have implications for personalized treatment by targeting antivirals and immunomodulators based on the phase evolution of the infection. From a supportive care standpoint, we highlighted the clinical instability of patients enrolled in the NIV or HFO group. Whereas most patients receiving NIV or HFO were supported successfully without intubation, patients for whom this trial failed showed the worst 60-day mortality of all groups (67%). We found that patients with a failed NIV or HFO trial showed markedly higher sRAGE levels at the time of baseline sampling compared with those with a successful trial ($P < .0001$) (Fig 4). These findings raise the hypothesis that serial assessments of sRAGE levels in larger cohorts of patients with NIV or HFO support may inform decision-making in conjunction with bedside assessments as to which patients may benefit from continuation of a noninvasive trial vs those who should be intubated earlier.

The dynamic trajectories of sRAGE level offer new insights into its prognostic value. As a marker of alveolar epithelial injury, sRAGE level would be expected to track with COVID-19 severity, yet our analyses showed a seemingly paradoxical pattern, with the sickest patients receiving ECMO showing markedly lower levels. Low sRAGE levels in patients receiving ECMO may reflect that such patients receive ultraprotective, low tidal volume ventilation, perhaps mitigating further injury and release of sRAGE into the bloodstream. This hypothesis also is suggested by the extremely high levels of sRAGE in patients for whom NIV or HFO fails, in whom tidal volumes are difficult to regulate and may induce injurious tidal stretching. However, we did not have serial recordings of ventilatory mechanics, including tidal volumes and driving pressures, among patients receiving different levels of respiratory support, and therefore we could not evaluate directly for a possible relationship between sRAGE level and ongoing lung injury. Notably, we also found that sRAGE levels consistently decreased over time, a trajectory that was different from the other biomarkers and congruent with prior literature.[14] Given that patients earlier in the disease course showed higher plasma SARS-CoV-2 levels, it is possible that

sRAGE also may reflect more active viral replication and lung injury in earlier stages of COVID-19 pneumonia.[23] In patients with non-COVID ARDS, plasma sRAGE levels have been associated with worse radiographic severity, impairments in gas exchange and mechanics, and poor outcome.[24,25] Although plasma sRAGE levels may not be entirely specific to alveolar epithelial injury and can have other sources, our results raise the question as to whether serial sRAGE levels can offer a potential dynamic metric of patient self-induced or ventilator-induced lung injury, which can be examined in future studies.

Biomarker-based subphenotyping with validated models from cohorts with non-COVID ARDS and respiratory failure offered prognostic enrichment across the spectrum of COVID-19 severity. We used two different models that used partially different combinations of biomarkers to examine their relative prognostic value. Both models used soluble tumor necrosis factor receptor 1 and bicarbonate levels, whereas the Sinha model used IL-6 levels and the Drohan model used angiopoietin 2 and procalcitonin levels. Overall, we found low prevalence of the hyperinflammatory subphenotype (< 10%) by both models, but when patients were classified as hyperinflammatory by both models, these patients showed the worst outcome. Subphenotypic classifications were stable from baseline to middle interval for the hypoinflammatory subphenotype, but patients classified as hyperinflammatory demonstrated dynamic transitions, with 50% of them classified as hypoinflammatory on follow-up by both models used for assignments. Patients transitioning to the hyperinflammatory subphenotype on follow-up or those who persistently were classified as hyperinflammatory showed worse outcomes compared with patients classified as hypoinflammatory. Prior observations supported the stability of subphenotypes in non-COVID ARDS,[26] but our data in patients with COVID-19 with acute respiratory failure highlight the need for better understanding of the time-dependent prognostic value and drivers of subphenotypic transitions.

Our study has some noteworthy limitations. Our data set represents a single health care network, which may limit generalizability of our findings, although we enrolled patients from seven different units and inpatient wards from three different hospitals. Variability in timing of enrollment was present because of logistical constraints in obtaining consent from legally authorized patient representatives. Nonetheless, we obtained dedicated biospecimens for research and processed with the same standardized protocol immediately after blood draws,[27] thus mitigating biases related to use of clinical leftover plasma samples. Inevitably, some of the biospecimens were obtained later in the hospital course, and biomarker levels may have been influenced by administration of immunomodulatory treatments. Follow-up sample availability was limited by informative censoring, either because of rapid clinical improvement and discharge from the hospital or because of early mortality. Therefore, interpretation of the longitudinal data must be cautious and viewed within the context of persistent COVID-19 respiratory failure requiring prolonged hospitalization. We made concerted efforts to harmonize individual patient trajectories based on objective milestones of COVID-19 illness, such as timings of quantitative PCR testing, symptom onset, and hospitalization. For practical reasons, we merged patients receiving NIV or HFO into a single group. Therefore, we could not investigate differential effects of spontaneous positive pressure (NIV) vs negative pressure (HFO) ventilation on host innate immune and injury biomarkers. Despite the sample size of > 300 patients in the cohort, some

of the clinical subgroups were small, especially for subphenotype analyses in follow-up samples. Therefore, cautious interpretation is needed regarding outcome associations and inferences on generalizability of our findings to patients with ARDS resulting from non-COVID-19 causes. Finally, the observational nature of our study allowed for only prognostic assessments of biomarkers and subphenotypes on outcomes of interest, without the ability to examine for predictive enrichment and treatment effect heterogeneity by subphenotypes.[28]

## Interpretation

Longitudinal assessment of the systemic host response in hospitalized patients with COVID-19 revealed substantial and prognostic interindividual variability, which was influenced heavily by baseline levels of respiratory support. Future studies examining the prognostic value of biomarkers and subphenotypes in COVID-19 and acute respiratory failure need to control for clinical illness trajectory, timings of illness, and respiratory support methods. Robust predictive enrichment with biological subphenotyping of patients considered for enrollment in future clinical trials may allow for better targeting of host modulatory interventions and improved outcomes in critical illness.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## ABBREVIATIONS:

| | |
|---|---|
| **ECMO** | extracorporeal membrane oxygenation |
| **HFO** | high-flow oxygen |
| **IMV** | invasive mechanical ventilation |
| **LFO** | low-flow oxygen |
| **NIV** | noninvasive ventilation |
| **PCR** | polymerase chain reaction |
| **sRAGE** | soluble receptor of advanced glycation end products |

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## Take-home Points

**Study Question:**

We examined the trajectories of host-response profiles in severe COVID-19 and evaluated their prognostic impact on clinical outcomes.

**Results:**

Lung epithelial injury plasma biomarker sRAGE (soluble receptor of advanced glycation end products) predicted adverse outcome in patients supported by noninvasive respiratory methods earlier during the inpatient course of COVID-19, whereas systemic inflammation measured by plasma IL-6 levels was predictive across all time points and regardless of level of support. By synthesis of host-response biomarkers into subphenotypes, patients classified to a hyperinflammatory subphenotype either at baseline or during follow-up showed markedly worse survival than their counterparts classified to a hypoinflammatory subphenotype.

**Interpretation:**

Longitudinal study of host response in severe COVID-19 demonstrated prognostic interindividual variability influenced by baseline levels of respiratory support.

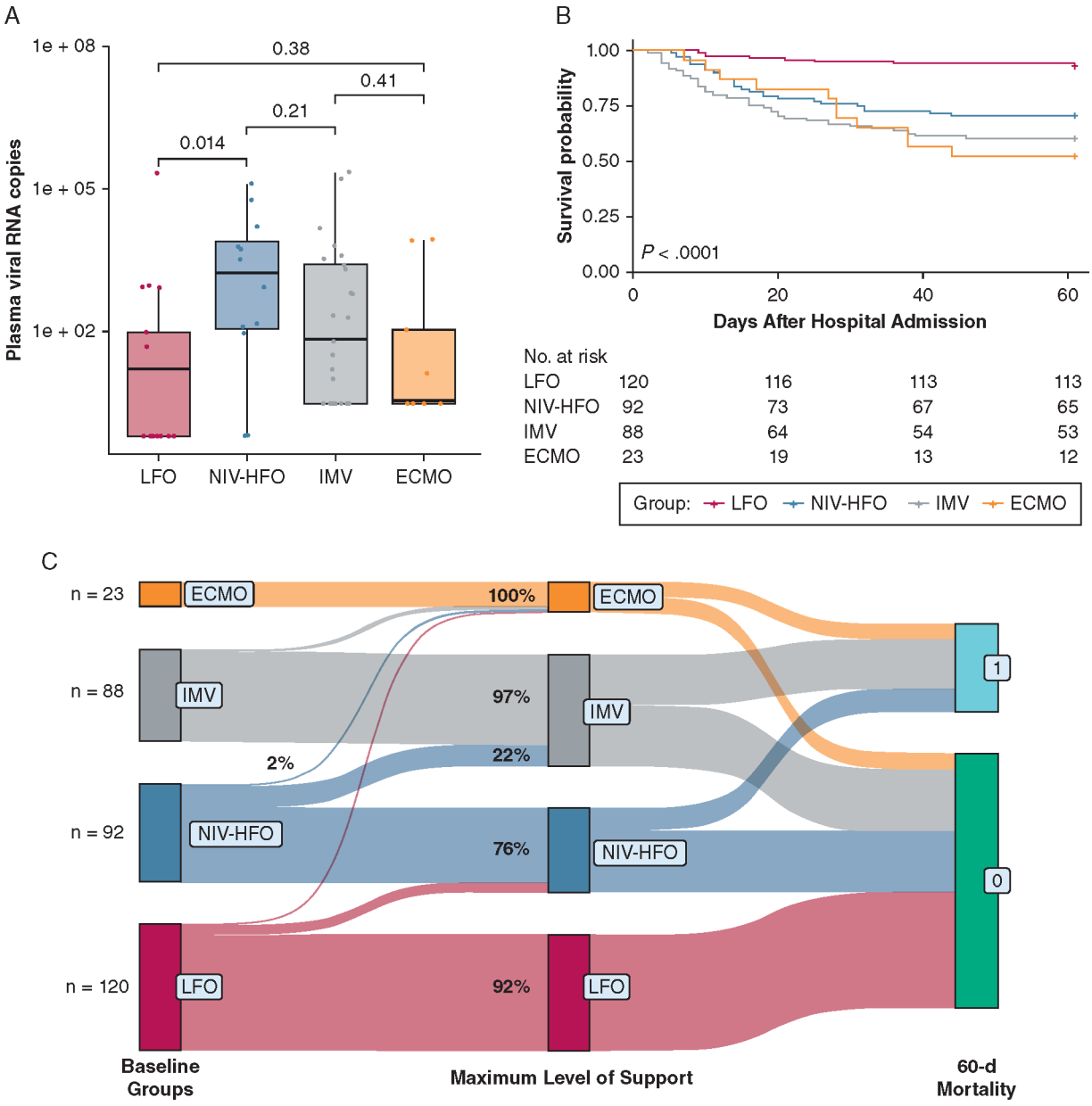


**Figure 1.**

SARS-CoV-2 infection timelines and clinical group trajectories. A, Box-and-whisker plot showing that patients receiving NIV or HFO had the highest levels of plasma viral RNA load (RNA-emia) than the other groups (patients with available viral RNA load measurements at baseline by clinical group: LFO, n = 17; NIV or HFO, n = 12; IMV, n = 27; ECMO, n = 9). B, Line graph showing 60-day survival curves by Kaplan-Meier analysis for the four clinical groups at baseline. Patients receiving LFO achieved markedly improved survival compared with the other three groups. C, Diagram showing transition of clinical groups from baseline assignments to the maximum level of respiratory support required during the inpatient stay and then to 60-day outcome (0 = survivors, 1 = nonsurvivors). The greatest proportion of transitions occurred in patients receiving NIV or HFO. ECMO = extracorporeal membrane

oxygenation; HFO = high-flow oxygen; IMV = invasive mechanical ventilation; LFO = low-flow oxygen; NIV = noninvasive ventilation.

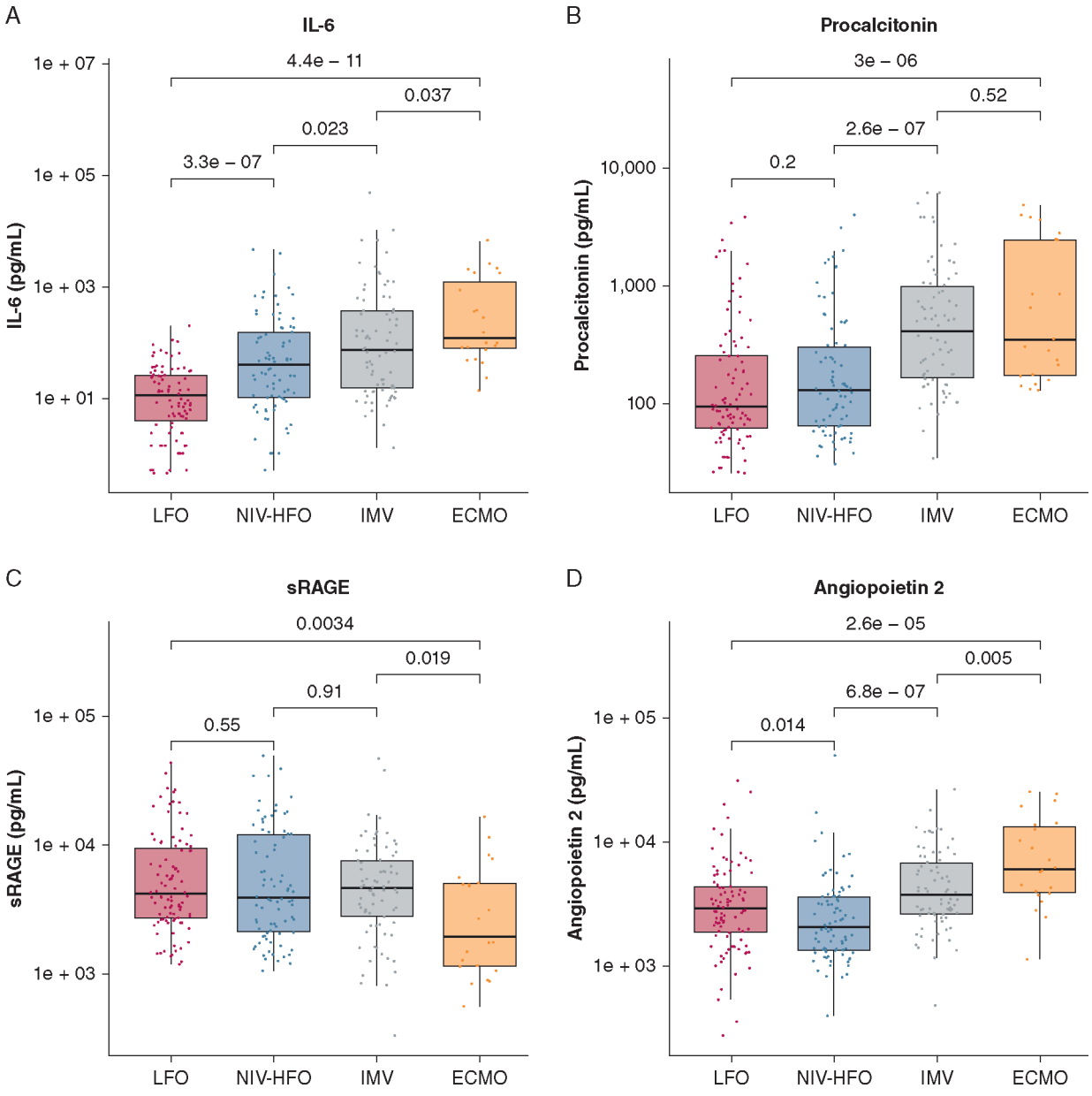


**Figure 2.**

A-D, Box-and-whisker plots showing baseline biomarker comparisons between clinical groups of different levels of respiratory support. Patients with higher levels of IL-6, procalcitonin, and angiopoietin 2 required increasing levels of respiratory support, whereas sRAGE levels were lower in patients receiving ECMO compared with the other groups. ECMO = extracorporeal membrane oxygenation; HFO = high-flow oxygen; IMV = invasive mechanical ventilation; LFO = low-flow oxygen; NIV = noninvasive ventilation; sRAGE = soluble receptor of advanced glycation end products.

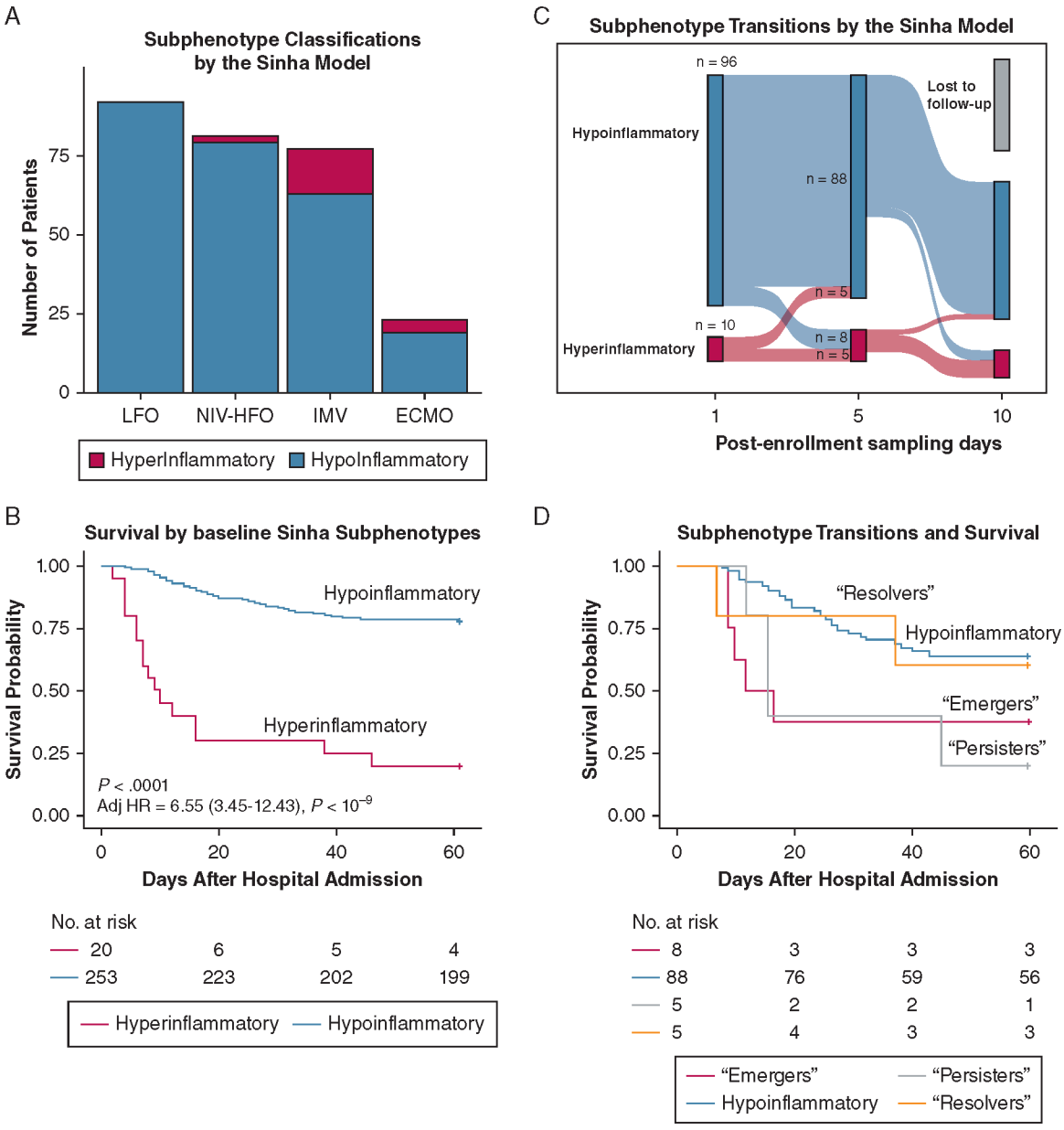


**Figure 3.**

Subphenotypic classifications at baseline, transitions over time, and prediction of outcome. A, Bar graph showing the proportion of hypoinflammatory (red) and hyperinflammatory (blue) subphenotypes by the Sinha model classified by level of respiratory support at baseline. B, Line graph showing that hyperinflammatory subphenotype patients by the Sinha model achieved worse survival in Kaplan-Meier curves and Cox proportional hazards models adjusted for age, time from hospital admission, and baseline level of respiratory support. C, Sankey plot showing transition of Sinha subphenotypes at each follow-up interval for patients with available follow-up samples on day 5. Overall, patients classified as hypoinflammatory remained stable (8% transitions), whereas 50% of patients classified as hyperinflammatory on day 1 were classified as hypoinflammatory by day 5. D, Line graph showing that among patients with both baseline (day 1) and follow-up (day 5)

biospecimens, comparison of subphenotypic classifications by the Sinha model recorded the following transition categories: (1) patients classified as hyperinflammatory in both time points, ie, persistently hyperinflammatory or “persisters”; (2) patients classified as hypoinflammatory on day 1 but who were classified as hyperinflammatory on day 5, ie, emerging hyperinflammatory or “emergers”; (3) patients who were classified as hyperinflammatory on day 1 and as hypoinflammatory on day 5, ie, resolving baseline hyperinflammatory subphenotype or “resolvers”; and (4) patients who were classified as hypoinflammatory stably at both time points, or “hypoinflammatory.” Emergers and persisters according to the Sinha model showed higher 60-day mortality compared with resolvers and hypoinflammatory: logistic regression OR, 2.62 (95% CI, 1.12–6.89; P = .04) for emergers or persisters vs resolvers or hypoinflammatory. ECMO = extracorporeal membrane oxygenation; HFO = high-flow oxygen; HR = hazard ratio; IMV = invasive mechanical ventilation; LFO = low-flow oxygen; NIV = noninvasive ventilation.

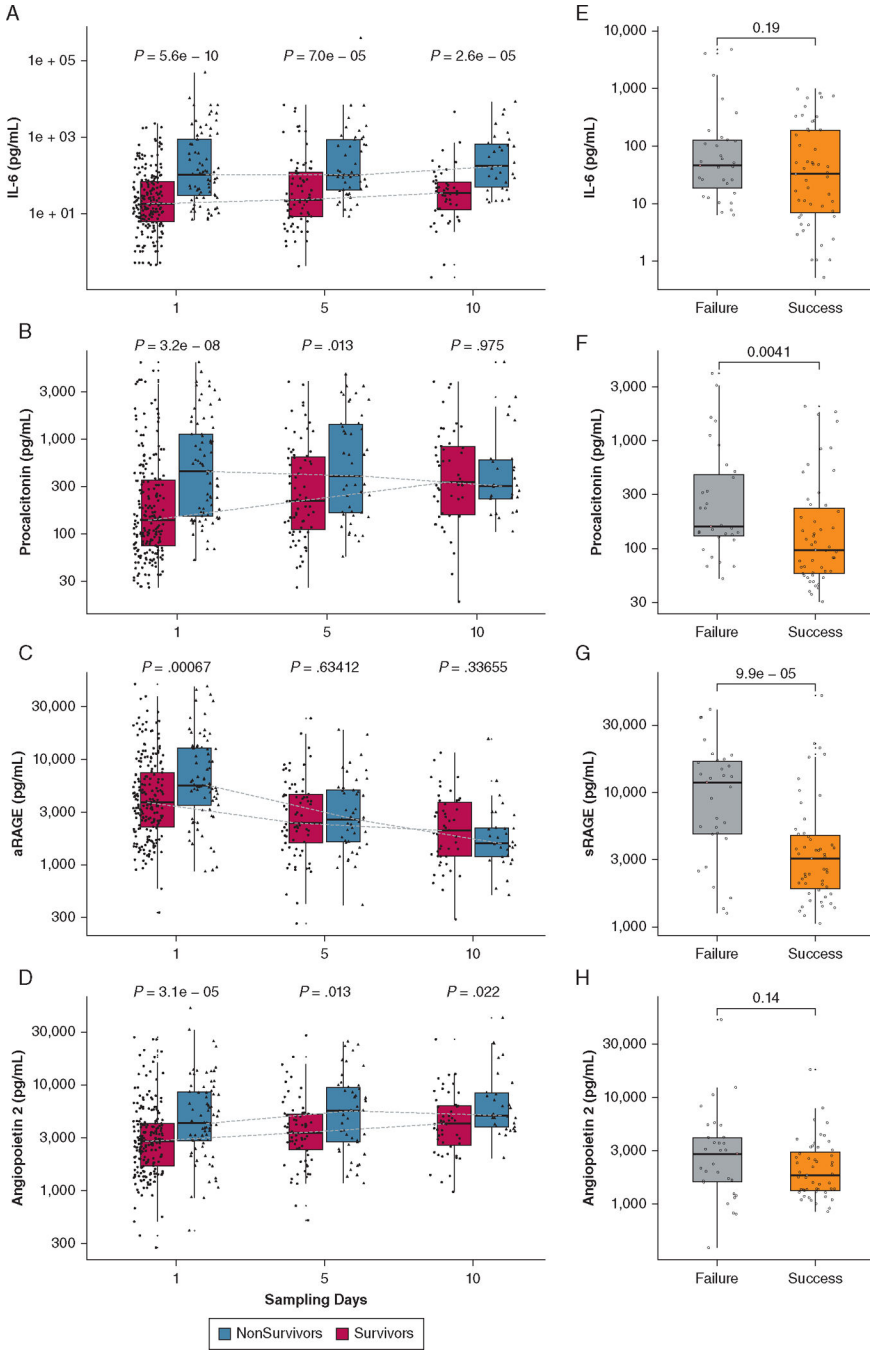


**Figure 4.**

Box-and-whisker plots showing biomarker levels by 60-day mortality and outcome of NIV or HFO trial. A-D, Nonsurvivors by 60 days showed higher baseline levels of IL-6, procalcitonin, sRAGE, and angiopoietin 2 compared with survivors, whereas during follow-up, nonsurvivors also showed higher levels of IL-6 and angiopoietin 2. E-H, Patients with successful trials of NIV or HFO showed lower levels of procalcitonin and sRAGE

compared with those for whom NIV or HFO trials failed. HFO = high-flow oxygen; NIV = noninvasive ventilation; sRAGE = soluble receptor of advanced glycation end products.

**TABLE 1**

Patient Characteristics and Outcomes

| Characteristic | LFO (n = 120) | HFO or NIV (n = 92) | IMV (n = 88) | ECMO (n = 23) | *P* Value |
|---|---|---|---|---|---|
| Demographics | | | | | |
| Male sex | 49 (40.8) | 61 (66.3) | 54 (61.4) | 19 (61.4) | < .01 |
| Age, y | 64.8 (53.8–71.2) | 61.1 (54.4–69.1) | 61.5 (50.3–70.2) | 53.1 (46–57.6) | < .01 |
| White race | 95 (79.2) | 69 (75.0) | 73 (83.0) | 20 (87.0) | .01 |
| BMI, $kg/m^2$ | 30.1 (26.5–36.4) | 31.7 (26.2–38.4) | 32.3 (28.3–37.1) | 36 (33.1–42.0) | < .01 |
| COPD | 22 (18.3) | 16 (17.4) | 10 (11.4) | 2 (8.7) | .4 |
| History of immunosuppression[a] | 31 (25.8) | 21 (22.8) | 24 (27.3) | 0 (0.0) | .04 |
| Current tobacco use | 5 (4.3) | 4 (5.3) | 3 (5.3) | 1 (5.3) | .99 |
| Biomarker sampling timeline, d | | | | | |
| Time from symptom onset | 10 (7.0–13.2) | 12 (7.0–16.0) | 12 (8.0–17.0) | 17 (11.0.-19.0) | < .01 |
| Time from positive PCR results for COVID-19 | 5 (3.0–8.0) | 8 (5.0–14.0) | 7.5 (4.0–13.0) | 12 (8.5–15.5) | < .01 |
| Time from admission | 3 (2.0–4.0) | 4 (2.0–7.0) | 4 (2.8–7.2) | 3 (2.0–5.0) | .01 |
| Hospitalization duration | 7.5 (4.0–13.0) | 10 (6.8–18.0) | 16 (10.0–30.5) | 31 (25.0–41.0) | < .01 |
| Laboratory variables | | | | | |
| WBC count, $\times 10^9$/L | 6.4 (4.3– 8.6) | 7.8 (5.6–11.8) | 10 (7.6–16.4) | 13.4 (10.4–14.5) | < .01 |
| Platelets, $\times 10^9$/L | 209 (163.0–282.0) | 234 (161.0–299.0) | 211.5 (161.0–270.5) | 139 (102.5–241.0) | .01 |
| Creatinine, mg/dL | 0.9 (0.7–1.5) | 0.9 (0.7–1.3) | 1 (0.8–1.9) | 0.8 (0.6–1.6) | .27 |
| Bicarbonate, mEq/L | 25 (22–28) | 25 (23–28) | 27 (24–30) | 29 (28–32.5) | < .01 |
| Plasma viral RNA, copies/μL | 16 (0.7–96.0) | 2112 (119.0–8554.0) | 70 (3.0–2607.5) | 3.4 (3.0–106.5) | .04 |
| Hyperinflammatory subphenotype | | | | | |
| Drohan model | 8 (8.7) | 4 (4.9) | 9 (11.7) | 2 (8.7) | < .01 |
| Sinha model | 0 (0.0) | 2 (2.5) | 14 (18.2) | 4 (17.4) | < .01 |
| Treatments | | | | | |
| Steroids | 73 (60.8) | 85 (92.4) | 77 (87.5) | 22 (95.7) | < .01 |
| Second immunomodulator[b] | 0 (0.0) | 25 (27.2) | 28 (31.8) | 9 (39.1) | < .01 |
| CRRT during hospitalization | 2 (1.7) | 12 (13.0) | 24 (27.3) | 12 (52.2) | < .01 |
| Outcome | | | | | |

| Characteristic | LFO (n = 120) | HFO or NIV (n = 92) | IMV (n = 88) | ECMO (n = 23) | *P* Value |
|---|---|---|---|---|---|
| 60-d mortality | 9 (7.5) | 27 (29.3) | 35 (39.8) | 11 (47.8) | < .01 |

Data are presented as No. (%) or median (interquartile range), unless otherwise indicated. CRRT = continuous renal replacement therapy; ECMO = extracorporeal membrane oxygenation; HFO = high-flow oxygen; IMV = invasive mechanical ventilation; LFO = low-flow oxygen; NIV = noninvasive ventilation; PCR = polymerase chain reaction.

[a]Defined as use of chronic steroids, alkylating agents, antimetabolites, calcineurin inhibitors, mycophenolate, biologics, active chemotherapy against solid tumor or hematologic malignancy, or diagnosis of primary immunodeficiency, such as common variable immunodeficiency or chronic granulomatous disease.

[b]Added to steroids per University of Pittsburgh Medical Center hospital guidelines and could include tocilizumab, sarilumab, or baricitinib, depending on drug availability and emerging evidence during the pandemic.