

# Defining Potential Therapeutic Targets in Coronavirus Disease 2019: A Cross-Sectional Analysis of a Single-Center Cohort

**OBJECTIVES:** Multiple mechanisms have been proposed to explain disease severity in coronavirus disease 2019. Therapeutic approaches need to be underpinned by sound biological rationale. We evaluated whether serum levels of a range of proposed coronavirus disease 2019 therapeutic targets discriminated between patients with mild or severe disease.

**Design:** A search of ClinicalTrials.gov identified coronavirus disease 2019 immunological drug targets. We subsequently conducted a retrospective observational cohort study investigating the association of serum biomarkers within the first 5 days of hospital admission relating to putative therapeutic biomarkers with illness severity and outcome.

**Setting:** University College London, a tertiary academic medical center in the United Kingdom.

**Patients:** Patients admitted to hospital with a diagnosis of coronavirus disease 2019.

**Interventions:** None.

**Measurements and Main Results:** Eighty-six patients were recruited, 44 (51%) with mild disease and 42 (49%) with severe disease. We measured levels of 10 cytokines/signaling proteins related to the most common therapeutic targets (granulocyte-macrophage colony-stimulating factor, interferon- $\alpha$ 2a, interferon- $\beta$ , interferon- $\gamma$ , interleukin-1 $\beta$ , interleukin-1 receptor antagonist, interleukin-6, interleukin-7, interleukin-8, tumor necrosis factor- $\alpha$ ), immunoglobulin G antibodies directed against either coronavirus disease 2019 spike protein or nucleocapsid protein, and neutralization titers of antibodies. Four-hundred seventy-seven randomized trials, including 168 different therapies against 83 different pathways, were identified. Six of the 10 markers (interleukin-6, interleukin-7, interleukin-8, interferon- $\alpha$ 2a, interferon- $\beta$ , interleukin-1 receptor antagonist) discriminated between patients with mild and severe disease, although most were similar or only modestly raised above that seen in healthy volunteers. A similar proportion of patients with mild or severe disease had detectable spike protein or nucleocapsid protein immunoglobulin G antibodies with equivalent levels between groups. Neutralization titers were higher among patients with severe disease.

**Conclusions:** Some therapeutic and prognostic biomarkers may be useful in identifying coronavirus disease 2019 patients who may benefit from specific immunomodulatory therapies, particularly interleukin-6. However, biomarker absolute values often did not discriminate between patients with mild and severe disease or death, implying that these immunomodulatory treatments may be of limited benefit.

**KEY WORDS:** acute respiratory distress syndrome; coronavirus disease 2019; critical care; cytokine; immunomodulation; interleukin-6

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Patients with coronavirus disease 2019 (COVID-19) demonstrate a heterogeneous clinical course ranging from mildly symptomatic disease through to acute respiratory distress syndrome (ARDS) and death (1). Hospital mortality in patients admitted to U.K. critical care units during the first surge of the COVID-19 pandemic was 42% (2). The short- and long-term morbidity burden is also significant (3). There is clearly a need for further effective therapies targeting both virus and host response to improve outcomes.

The approximate 10-day delay between COVID-19 symptom onset and development of critical illness (4, 5) provides an important window of opportunity to intervene. While understanding of disease pathobiology has improved, it remains far from complete. This has not deterred academics and industry from trialing multiple approaches against a myriad of targets or nonselective approaches (6). Strategies range from immunomodulatory drugs to convalescent plasma, mesenchymal stem cells, monoclonal antibodies, and extracorporeal mediator removal. Unfortunately, a number of randomized controlled trials to date have failed to show outcome benefit. This may relate in part to the absence of a direct-acting antiviral, a sound biological rationale, and/or suboptimal selection of patients. An appropriate host response provides important protection against pathogens, whereas an exaggerated, dysregulated response leads to organ dysfunction and possibly death (7). Suppressing or removing mediators where blood levels are only mild-to-moderately elevated, or boosting endogenously raised levels of mediators to supranormal values, may prove futile or even detrimental. The same mistakes made over decades for sepsis may be repeated (8).

Our objectives were to ascertain the range of immunomodulatory therapies being trialed in COVID-19, to evaluate if therapeutic biomarkers discriminated between patients with mild and severe disease or those who subsequently died, and to allow identification of plausible therapeutic strategies and patients who might benefit.

## METHODS

### Literature Search

A search of ClinicalTrials.gov was conducted on November 1, 2020. All studies related to COVID-19 or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were screened, with the search restriction of “intervention (clinical trials)” only (T.A.C.S).

Immunomodulatory treatments were identified either by intervention type (Biological or Device), or if listed as “Drug” or “Other,” manual identification. All interventional clinical trials using specific immunomodulatory therapies were recorded.

### Approval

Ethical approval was granted by the London-Westminster Research Ethics Committee, the Health Research Authority and Health and Care Research Wales on July 2, 2020 (Research Ethics Committee reference 20/HRA/2505, Integrated Research Application System Identity 284088). The SARS-CoV-2 Acquisition in Frontline Healthcare Workers—Evaluation to Inform Response (SAFER) study protocol was approved by the National Health Service Health Research Authority (reference 20/SC/0147) on March 26, 2020. The South-Central Berkshire Research Ethics Committee provided ethical oversight.

### Inclusion and Exclusion Criteria

Blood samples taken from patients greater than or equal to 18 years old within 5 days of admission through the Emergency Department of University College London Hospitals with a pneumonic illness between March 1, 2020, and June 30, 2020, were used for analysis. A positive real-time reverse transcription-polymerase chain reaction (PCR) test for SARS-CoV-2 RNA from a combined nose-throat swab tested with an in-house developed method was required for all patients (9). Any samples taken from patients receiving immunomodulatory agents were excluded.

### Clinical Data and Definitions

Patient demographics, clinical data (including date of symptom onset, admission tissue oxygen saturation, respiratory rate, temperature, etc.), treatments, and outcome were recorded retrospectively from electronic healthcare records on a standardized data collection form. Outcome was determined utilizing the World Health Organization (WHO) COVID-19 ordinal severity scale, with a score of 1 defined by no limitation of activities, rising to 6 for those requiring noninvasive ventilation and additional organ support, and 10 for death (10, 11). Patients were divided into two cohorts: WHO severity scale less than 6 (mild) and 6–10 (severe).

## Cytokine Measurements

Data were recorded retrospectively from routine blood tests measured by automated laboratory analyzers including creatinine, C-reactive protein (CRP), lymphocyte count, and platelets. Blood was centrifuged within 4 hours of collection, separated, and sera frozen at  $-80^{\circ}\text{C}$  before batch analysis. Electrochemiluminescent immunoassays were performed according to the manufacturer's instructions (Meso Scale Discovery, Rockville, MD). For analysis, two 10-plasma exchange inflammatory marker panels including those related to common therapeutic targets (granulocyte-macrophage colony-stimulating factor [GM-CSF], interferon [IFN]- $\alpha$ 2a, IFN- $\beta$ , IFN- $\gamma$ , interleukin [IL]-1 $\beta$ , IL-1 receptor antagonist, IL-6, IL-7, IL-8, tumor necrosis factor [TNF]- $\alpha$ ) were used. The normal ranges for measured serum cytokines were determined using seven healthy healthcare worker volunteers enrolled in the SAFER study (12).

## Semi-Quantitative SARS-CoV-2 Anti-Spike Protein and Nucleocapsid Protein Immunoglobulin G Antibody Titer and Neutralization Assays

Levels of SARS-CoV-2 anti-spike protein (S1) and nucleocapsid protein (N) immunoglobulin G (IgG) concentrations were ascertained using enzyme-linked immunosorbent assay as previously described (13). Additionally, ex vivo pseudotype neutralization assays were conducted to assess the ability of SARS-CoV-2 antibodies to neutralize the activity of a pseudotyped virus against infecting angiotensin converting enzyme-2 receptor expressing HeLa cells, as described previously (13). The neutralization titer ( $\text{ID}_{50}$ ) was defined as the reciprocal of the serologic reagent dilution that produced a 50% reduction in luminescence (as a proxy of infection) compared with untreated virus control wells.

## Statistical Analysis

Analysis was performed using anonymized data. Clinical data were collated with viral loads, levels of SARS-CoV-2-specific antibodies, and plasma cytokines and chemokines. Continuous and categorical variables are reported as median (interquartile range) and  $n$  (%), respectively. Mann-Whitney  $U$  tests without post hoc correction for comparison between subgroups were performed for comparison of continuous

variables between groups. Categorical data were compared using the chi-square test. The association between biomarkers and clinical severity was assessed using area under the receiver operating characteristic curve (AUROC). Pearson's correlation coefficient was used to assess correlation between various clinical and therapeutic biomarkers). Graphs were constructed, and statistical analysis performed using SPSS Version 26.0 (IBM Corp, Armonk, NY) and GraphPad Prism (GraphPad Software, La Jolla, CA). Further details on methods are included in **Supplementary data** (<http://links.lww.com/CCX/A716>).

## RESULTS

### Literature Search

The ClinicalTrials.gov search identified 477 randomized trials assessing immunomodulatory therapies being given for the treatment of COVID-19. These included targets against 83 different immune pathways and used 168 different therapies. The greatest number of registered clinical trials related to the use of convalescent plasma ( $n = 87$ ), anti-IL-6 monoclonal antibodies ( $n = 43$ ), mesenchymal stem cells ( $n = 46$ ), IFN- $\alpha$ , IFN- $\beta$ , agonists ( $n = 14$ ), and IL-1 $\beta$  antagonism ( $n = 16$ ) (**Supplementary Table 1**, <http://links.lww.com/CCX/A715>). Other biological targets included TNF- $\alpha$ , IL-7, IL-8, IFN- $\gamma$ , and therapies either augmenting or inhibiting GM-CSF.

### Demographic, Clinical, and Routine Laboratory Data

Eighty-six patients were included in the final analysis. Demographic details are shown in **Table 1**. There were similar numbers of patients with mild disease (WHO scale  $< 6$ ) (44; 51%) and severe disease (WHO scale 6–10) (42; 49%) during their hospital stay. The time from hospital admission to blood sample collection was shorter in patients with mild disease and those with severe disease (0.5 [0–1] vs 1 [0–2.5];  $p = 0.012$ ). Healthy volunteers consisted of four Caucasian patients and three patients from Black and Asian backgrounds. None of the patients had premorbid illness and age of the patients was 34 years (28–49 yr).

There were no differences in the proportions of sex or underlying comorbidities between mild and severe groups. Compared with patients with mild disease, patients with severe illness were older, presented

**TABLE 1.**  
**Clinical Data of Patients With Coronavirus Disease 2019**

Clinical Data	Total, n = 86	Mild, n = 44	Severe, n = 42	p (Mild vs Severe)
Age (yr)	61 (48–73)	59 (46–69)	67 (52–75)	0.04
Body mass index (kg/m <sup>2</sup> )	25 (23–29)	25 (23–29)	25 (23–30)	0.87
Time from symptoms to hospital (d)	7 (4–11)	10 (5–14)	5 (3–8)	0.02
Time from symptoms to blood sample (d)	1 (0–2)	0.5 (0–1)	1 (0–2.5)	0.012
Oxygen saturation: FiO <sub>2</sub> ratio	438 (378–462)	448 (424–462)	395 (157–452)	< 0.001
Respiratory rate (breaths/min)	26 (20–32)	24 (19–31)	28 (21–36)	0.06
Temperature (°C)	37.5 (36.9–38.4)	37.2 (36.8–38.1)	37.7 (37.0–38.8)	0.22
Threshold cycle value	37 (32–40)	38 (35–40)	34 (28–39)	0.01
Male (%)	55 (64)	24 (56)	31 (74)	0.08
Diabetes mellitus (%)	18 (21)	9 (21)	9 (21)	0.91
Hypertension (%)	30 (35)	15 (34)	15 (38)	0.70
Smoker (%)	7 (8)	5 (11)	2 (5)	0.26
Creatinine (micromol/L)	88 (68–114)	81 (62–100)	94 (73–140)	0.02
C-reactive protein (mg/L)	114 (52–197)	78 (32–121)	180 (106–266)	< 0.001
Albumin (g/L)	39 (33–41)	40 (35–42)	37 (33–40)	0.04
Bilirubin (micromol/L)	10 (7–13)	10 (7–12)	10 (8–13)	0.48
Hemoglobin (g/L)	129 (113–140)	126 (113–141)	132 (114–139)	0.89
Lymphocyte count (10 <sup>9</sup> /mL)	0.93 (0.62–1.36)	1.14 (0.68–1.52)	0.75 (0.53–1.16)	0.04
Neutrophil count (10 <sup>9</sup> /mL)	6.42 (4.40–9.08)	5.28 (4.11–7.88)	7.18 (5.56–9.58)	0.03
Platelet count (10 <sup>9</sup> /mL)	238 (164–290)	245 (174–298)	232 (142–288)	0.33
Steroid use (%)	7 (8)	1 (2)	6 (14)	0.05
Antiviral drug use (%)	2 (2)	0 (0)	2 (5)	0.14
Continuous positive airway pressure (%)	34 (40)	–	34 (81)	–
Mechanical ventilation (%)	13 (15)	–	13 (31)	–
Vasopressors (%)	13 (15)	–	13 (31)	–
Renal replacement therapy (%)	3 (3)	–	3 (7)	–
Hospital mortality (%)	–	–	21 (24)	–

Continuous data are presented as median (interquartile range). Mann-Whitney *U* test and  $\chi^2$  used to assess differences between patients with mild disease and patients with severe disease or who subsequently died. Dashes indicate not applicable.

earlier to hospital, had worse oxygenation, and a higher viral load (defined by a lower threshold cycle value). Patients with severe disease had higher admission values of serum creatinine, CRP and neutrophil counts and lower values of albumin and lymphocyte count (Table 1).

Adjunctive therapies (for this March 2020–June 2020 cohort) throughout the entire length of hospitalization included steroid use (7/86, 8%), antibiotics (63/86, 73%), and antiviral medications (2/86; 2%). Fourteen patients (16%) needed invasive mechanical

ventilation and a further 22 patients (26%) required continuous positive airways pressure. Patients with severe disease were more likely to receive antibiotics and steroids, albeit not in the first week of presentation (Table 1). Twenty-one patients (24%) did not survive to hospital discharge.

### Biomarker and Antibody Data

Healthy volunteers were antibody and swab PCR negative for COVID-19. We measured levels of 10

cytokines including those related to the most common therapeutic targets (GM-CSF, IFN- $\alpha$ 2a, IFN- $\beta$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-1 receptor antagonist, IL-6, IL-7, IL-8, TNF- $\alpha$ ), IgG antibodies against the COVID-19 S1 or N, and neutralization antibodies titers within the first 5 days of hospital admission.

The ability of routinely measured biochemical variables (creatinine, C-reactive protein, albumin, neutrophil counts, and lymphocyte count) to predict corresponding biomarker levels was limited; the strongest correlation was between CRP and IL-6 (Spearman correlation coefficient 0.66;  $p < 0.001$ ).

Levels of IFNs were elevated in patients with COVID-19 compared with healthy controls. Proinflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$  were lower than seen in controls, albeit within the normal range. Similarly, levels of IL-8 and GM-CSF were lower than seen in healthy controls. Soluble IL-1 receptor antagonist, however, was significantly elevated in COVID-19 patients compared with controls, as was IL-7, a promoter of lymphocyte development and proliferation.

Between patients with mild or severe disease, levels of GM-CSF, IFN-gamma, TNF- $\alpha$ , and IL-1 $\beta$  were similar. Six biomarkers (IL-6, IL-7, IL-8, IFN- $\alpha$ , IFN- $\beta$ , IL-1 receptor antagonist) and neutralizing antibody titers were higher in patients with severe compared with mild disease (all  $p < 0.05$ ) (**Supplementary Fig. 1**, <http://links.lww.com/CCX/A713>; **Table 2**). IL-6 provided the greatest discrimination between patients with mild and severe disease (AUROC, 0.78; 95% CI, 0.68–0.88;  $p < 0.001$ ) (**Supplementary Fig. 2**, <http://links.lww.com/CCX/A714>; **Table 2**).

A similar proportion of patients with mild or severe disease had detectable S1 or N IgG antibodies (70% vs 59%;  $p = 0.18$ ). Among patients with detectable antibodies, there was no difference in S1 ( $p = 0.72$ ) or N ( $p = 0.69$ ) IgG values between patients with mild or severe disease. Among patients who seroconverted, those with severe disease had a higher serum ID<sub>50</sub> compared with patients with mild disease ( $p = 0.046$ ).

## DISCUSSION

Multiple mechanisms have been proposed to explain disease severity in COVID-19 including an impaired host response to the virus and a dysregulated host inflammatory response including immunosuppression,

endothelial injury and a pro-thrombotic state. A search of ClinicalTrials.gov on November 1, 2020, identified 477 randomized clinical trials assessing immunomodulatory therapies. These include targets against 83 different immune pathways and 168 different drugs or therapies.

We assessed plasma levels of twelve of the most frequently investigated targets. Five (TNF- $\alpha$ , IL-1 $\beta$ , GM-CSF, IFN- $\gamma$ , and anti-SARS-CoV-2 antibodies) did not differentiate between patients with mild or severe disease, challenging the validity of modulating these immune mediators in the treatment of COVID-19, and potentially increasing patient risk. Seven (IFN- $\alpha$ , IFN- $\beta$ , IL-6, IL-7, IL-8, IL-1 receptor antagonist, and neutralizing antibody titers) were increased in patients with severe disease. However, despite some cytokines being significantly higher among patients with severe disease, the absolute change in cytokines and chemokines above that seen in healthy individuals was modest in many cases. Inflammatory cytokine elevations in patients with severe or critical COVID-19 disease were markedly lower than those reported in patients with sepsis, ARDS unrelated to SARS-CoV-2 infection, and chimeric antigen receptor T cell-induced cytokine release syndrome (14).

In our patient cohort, IL-1 receptor antagonist levels were significantly higher in the severe patient subset, while levels of IL-1 $\beta$  did not differentiate between mild or severe COVID-19. Of interest, anakinra, a recombinant and modified version of the human IL-1 receptor antagonist protein, is being investigated in 16 trials. IFN- $\beta$ 1 levels were also similar in our mild and severe disease groups. The Solidarity trial recently reported no survival benefit from IFN- $\beta$ 1 in 4,100 patients (15). As a further example of scientific ambiguity, we also detected no differences in GM-CSF levels between mild and severe groups, yet ongoing studies are directly conflicting, either giving exogenous GM-CSF or blocking its effects (16).

While more biological rationale might be attached to a target that does show severity-related differences, this is not a *sine qua non*. A raised biomarker level may simply be an epiphenomenon, reflecting the underlying disease process but with no impact on survival. It is also uncertain if raised serum levels of an inflammatory mediator represent an adaptive/protective host response, especially when levels are only modestly elevated. In this case, targeted blockade may be ineffective

**TABLE 2.**  
**Association With Disease Severity With Different Biological Targets**

Therapeutic Target	Mechanism of Therapeutic Agent	Levels in Mild vs Severe Disease	Levels in Mild Disease	Levels in Severe Disease	<i>p</i> (Mild vs Severe)	Area Under the Receiver Operating Characteristic Curve (95% CI) (Mild vs Severe)
IL-6	Inhibitor	Higher in severe disease	13 (4–29)	22 (14–42)	< 0.001	0.78 (0.68–0.88)
IL-1 receptor antagonist	Inhibitor	Higher in severe disease	5,974 (3,418–12,033)	7,155 (3,642–19,990)	0.002	0.70 (0.59–0.81)
Neutralizing antibody	Agonist	Higher in severe disease	823 (190–1,983)	1,612 (810–5,551)	0.046	0.66 (0.52–0.81)
IL-8	Inhibitor	Higher in severe disease	9 (4–25)	13 (5–26)	0.045	0.66 (0.54–0.78)
IL-7	Agonist	Higher in severe disease	196 (120–268)	183 (128–263)	0.027	0.64 (0.52–0.76)
IFN- $\beta$	Agonist	Higher in severe disease	95 (0–201)	142 (42–224)	0.035	0.63 (0.51–0.75)
IFN- $\alpha$ 2a	Agonist	Higher in severe disease	27 (14–49)	42 (22–81)	0.043	0.63 (0.51–0.74)
Tumor necrosis factor- $\alpha$	Inhibitor	No difference	1.6 (0.9–2.8)	1.0 (0.8–2.1)	0.246	0.62 (0.50–0.74)
Granulocyte-macrophage colony-stimulating factor	Inhibitor agonist	No difference	0.12 (0.05–0.25)	0.07 (0.04–0.16)	0.398	0.60 (0.48–0.72)
IFN- $\gamma$	Inhibitor	No difference	44 (20–163)	34 (18–86)	0.700	0.58 (0.46–0.70)
IL-1 $\beta$	Inhibitor	No difference	0.6 (0.0–6.2)	0.0 (0.0–3.0)	0.999	0.54 (0.42–0.66)
Convalescent serum	Agonist	No difference				
Anti-nucleocapsid protein IgG			3.1 (1.3–18.2)	3.8 (1.2–24.6)	0.924	0.53 (0.37–0.69)
Anti-spike protein IgG			1.8 (0.6–5.7)	2.5 (0.5–7.3)	0.793	0.53 (0.38–0.68)

IFN = interferon, IgG = immunoglobulin G, IL = interleukin.

Continuous data presented as median (interquartile range). Units of cytokines (pg/mL), anti-nucleocapsid protein IgG, and anti-spike protein IgG (in microgram/mL). The neutralization titer was defined as the reciprocal of the serologic reagent dilution that produced a 50% reduction in luminescence (as a proxy of infection) compared with untreated virus control wells.

or even counter-productive. A similar approach of targeting mediators associated with mortality in sepsis has not yielded any successful therapies (17).

As confirmed by others, levels of IL-6 are elevated among patients with severe COVID-19 (18), yet these are often 1–2 log-orders lower than other causes of ARDS, sepsis or critical illness, and often barely elevated above values measured in normal subjects (14). Despite this, IL-6 was able to discriminate between

patient with mild and severe disease, and levels of IL-6 were not significantly higher in patients with mild disease compared with severe disease. Furthermore, observational reports describing the physiologic response to tocilizumab in COVID-19 patients support the biological plausibility of tocilizumab use in COVID-19 (19).

IL-6 is a key regulator of CRP production and fever. The well-established association between elevated

CRP and illness severity in COVID-19 (20) raises the possibility of a mortality benefit with IL-6 blockade in the sickest patients. Indeed, a mortality benefit of IL-6 blockade was seen in the Randomised Evaluation of COVID-19 Therapy (RECOVERY) study (21), and a Randomised, Embedded, Multi-factorial, Adaptive Platform Trial for Community-Acquired Pneumonia study in which ICU admission and advanced respiratory support was a pre-requisite for trial enrollment (22).

The association between higher viral load and disease severity has been reported elsewhere (23). The higher viral load among our patients with severe disease or who subsequently die supports early diagnosis and the early use of a direct-acting antiviral especially in individuals with risk factors as shown in our data. With the emergence of the B.1.1.7 variant, which appears linked to infections with higher virus load, our observation is of critical importance, although further data will be required to confirm it (24).

We found type I IFN levels were as expected, elevated in critically ill patients with higher viral loads. Critically ill patients have a higher viral load and higher IFN levels, the latter which may be an adaptive response. However, neutralizing IgG autoantibodies against type I IFNs have been described in a proportion of critically ill COVID-19 patients, which may render elevated IFN levels ineffective (25). Further augmenting this host response in all COVID-19 patients is thus of questionable benefit.

Several studies have also highlighted an association between higher SARS-CoV-2 reactive antibody responses and disease severity; however, these have predominantly compared mild or asymptomatic infection to severe disease (13, 26). Furthermore, the trend toward higher titers in severe disease could be a result of an increased duration of infection leading to greater antibody maturation (27). Importantly, our study covers an earlier window (~10 d of infection) than most other studies, and we saw no evidence of an association between anti-N or anti-S1 responses and disease severity in this cohort (28). Thus, while early antibody levels do not predict outcome in this cohort, it remains an unanswered question as to whether disease severity and associated higher antigen load drives higher antibody titers or vice versa at later stages of the disease. Clinical trials investigating convalescent plasma in

COVID-19 have not demonstrated any clinical benefit, even among studies with a minimum threshold of specific SARS-CoV-2 IgG antibody titers in infused plasma (29, 30).

Among patients who seroconverted, the 50% inhibitory dilution factors ( $ID_{50}$ ) against SARS-CoV-2 pseudotyped virus was higher among patients with severe illness than in patients with mild illness, which may reflect greater antigen burden and thus more extensive antibody maturation. With monoclonal antibody trials ongoing and while REGN-COV2, an antibody cocktail containing two SARS-CoV-2-neutralizing antibodies, had no clinical benefit in nonhospitalized patients with COVID-19 (31), its potential benefit in preventing seronegative hospitalized patients from progressing to critical illness is unknown.

Limitations of our study include the relatively small number of patients and the lack of serial data to evaluate the association between biomarker trajectory and outcome. Cytokine differences between patients with mild illness and those with critical illness or who died may reflect the expected trajectory of inflammatory markers rather than the nature of disease. Published studies on proinflammatory cytokine trajectory demonstrate that the highest levels are seen in the first few days following presentation (32). The samples measured represent a subset of our entire patient cohort and were selected based on availability of residual serum. The time from hospital admission to blood sample collection was shorter in patients with mild disease and those with severe disease by 0.5 days. Although statistically significant, a difference in 0.5 days is unlikely to have any clinical significance. The numbers of healthy volunteers are small and not matched to the patient demographics. However, our main comparison is between patients with mild disease and those who progresses to critical illness or death. Data from healthy volunteers were included to provide context to patient data.

Furthermore, our S1 specific antibody titers were generated using the S1 subunit not whole Spike, assessment of which may provide additional information regarding different outcomes (33). However, the  $ID_{50}$  were generated against virus pseudotyped with whole Spike. Our findings are consistent with those of others that include modest elevations in cytokine levels among COVID-19 patients compared with other conditions (14). The effect of viral load on immune

responses and cytokine levels requires further evaluation. Our study included patients prior to publication of the RECOVERY dexamethasone study results. A total of 477 randomized trials, including 168 different therapies against 83 different pathways, were identified (34). Therefore, the majority of patients did not receive steroids and the effect of corticosteroid therapy on biomarkers measured in COVID-19 is unknown.

The functional impact of pleiotropic cytokines, including IL-6, may not be reflected in the absolute level of the cytokine measured in serum (35). Further understanding of the impact of soluble mediators in the context of their diverse immune and nonimmune functions remains a challenge. Understanding of the pathogenic mechanisms underlying impaired viral clearance and the development of organ failure should precede well-meaning efforts to intervene. Use of therapeutic and prognostic biomarkers may identify appropriate therapeutic targets, patients most likely to benefit (e.g., those individuals with markedly elevated and potentially pathologic cytokine levels), and to subsequently monitor treatment effects.

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Dr. Arulkumaran, Dr. Snow, Prof. Nastouli, and Dr. Spyer designed the study. Dr. Snow, Dr. Kulkarni, Dr. Brealey, Dr. Rickman, Ms. Rees-Spear, Dr. Spyer, Dr. Heaney, Dr. Garr, Dr. Cherepanov, Dr. Kassiotis, Dr. Lunn, Dr. Houlihan, Dr. McCoy, and Prof. Nastouli acquired study data. Dr. Arulkumaran, Dr. Snow, and Dr. McCoy analyzed the data. Dr. Arulkumaran, Dr. Snow, and Dr. McCoy wrote the article. Prof. Singer, Dr. Lunn, Dr. Williams, and Prof. Nastouli critically reviewed the article. All authors approved the final version.

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The study protocol and data are available upon reasonable request submitted to the corresponding author.

## REFERENCES

1. Wu Z, McGoogan JM: Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: Summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA* 2020; 323:1239–1242
2. Richards-Belle A, Orzechowska I, Gould DW, et al; ICNARC COVID-19 Team: COVID-19 in critical care: Epidemiology of the first epidemic wave across England, Wales and Northern Ireland. *Intensive Care Med* 2020; 46:2035–2047
3. McCue C, Cowan R, Quasim T, et al: Long term outcomes of critically ill COVID-19 pneumonia patients: Early learning. *Intensive Care Med* 2021; 47:240–241

4. Wang D, Hu B, Hu C, et al: Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 2020; 323:1061–1069
5. Yang X, Yu Y, Xu J, et al: Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: A single-centered, retrospective, observational study. *Lancet Respir Med* 2020; 8:475–481
6. Snow TAC, Singer M, Arulkumaran N: Immunomodulators in COVID-19: Two sides to every coin. *Am J Respir Crit Care Med* 2020; 202:1460–1462
7. Singer M, Deutschman CS, Seymour CW, et al: The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016; 315:801–810
8. Fink MP, Warren HS: Strategies to improve drug development for sepsis. *Nat Rev Drug Discov* 2014; 13:741–758
9. Grant PR, Turner MA, Shin GY, et al: Extraction-free COVID-19 (SARS-CoV-2) diagnosis by RT-PCR to increase capacity for national testing programmes during a pandemic. *bioRxiv* Preprint posted online April 9, 2020. doi: 10.1101/2020.04.06.028316
10. World Health Organisation: Blueprint Novel Coronavirus COVID-19 Therapeutic Trial Synopsis. 2020. Available at: <https://www.who.int/teams/blueprint/covid-19>. Accessed May 21, 2021
11. WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection: A minimal common outcome measure set for COVID-19 clinical research. *Lancet Infect Dis* 2020; 20:e192–e197
12. Houlihan CF, Vora N, Byrne T, et al; Crick COVID-19 Consortium; SAFER Investigators: Pandemic peak SARS-CoV-2 infection and seroconversion rates in London frontline health-care workers. *Lancet* 2020; 396:e6–e7
13. O’Nions J, Muir L, Zheng J, et al: SARS-CoV-2 antibody responses in patients with acute leukaemia. *Leukemia* 2021; 35:289–292
14. 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
15. Pan H, Peto R, Henao-Restrepo AM, et al; WHO Solidarity Trial Consortium: Repurposed antiviral drugs for Covid-19 - interim WHO solidarity trial results. *N Engl J Med* 2021; 384:497–511
16. Lang FM, Lee KM, Tejjaro JR, et al: GM-CSF-based treatments in COVID-19: Reconciling opposing therapeutic approaches. *Nat Rev Immunol* 2020; 20:507–514
17. Remy KE, Brakenridge SC, Francois B, et al: Immunotherapies for COVID-19: Lessons learned from sepsis. *Lancet Respir Med* 2020; 8:946–949
18. Wang C, Fei D, Li X, et al: IL-6 may be a good biomarker for earlier detection of COVID-19 progression. *Intensive Care Med* 2020; 46:1475–1476
19. Strohhahn GW, Heiss BL, Rouhani SJ, et al: COVIDOSE: A phase II clinical trial of low-dose tocilizumab in the treatment of noncritical COVID-19 pneumonia. *Clin Pharmacol Ther* 2021; 109:688–696
20. Manson JJ, Crooks C, Naja M, et al: COVID-19-associated hyperinflammation and escalation of patient care: A retrospective longitudinal cohort study. *Lancet Rheumatol* 2020; 2:e594–e602
21. RECOVERY Collaborative Group: Tocilizumab in patients admitted to hospital with COVID-19: A randomised, controlled, open-label, platform trial. *Lancet* 2021; 397:1637–164
22. 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; 384:1491–1502
23. Fajnzylber J, Regan J, Coxen K, et al; Massachusetts Consortium for Pathogen Readiness: SARS-CoV-2 viral load is associated with increased disease severity and mortality. *Nat Commun* 2020; 11:5493
24. Rambaut A, Loman N, Pybus O, et al: Preliminary Genomic Characterisation of an Emergent SARS-CoV-2 Lineage in the UK Defined by a Novel Set of Spike Mutations. 2020. Available at: <https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563>. Accessed May 21, 2021
25. Bastard P, Rosen LB, Zhang Q, et al: Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science* 2020; 370:eabd4585
26. Long QX, Liu BZ, Deng HJ, et al: Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* 2020; 26:845–848
27. 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; 26:1623–1635
28. Atyeo C, Fischinger S, Zohar T, et al: Distinct early serological signatures track with SARS-CoV-2 survival. *Immunity* 2020; 53:524–532.e4
29. Farias DLC, Prats J, Cavalcanti AB, et al: Rationale and design of the “Tocilizumab in patients with moderate to severe COVID-19: An open-label multicentre randomized controlled” trial (TOCIBRAS). *Rev Bras Ter Intensiva* 2020; 32:337–347
30. Simonovich VA, Burgos Prats LD, Scibona P, et al; PlasmAr Study Group: A randomized trial of convalescent plasma in Covid-19 severe pneumonia. *N Engl J Med* 2021; 384:619–629
31. Weinreich DM, Sivapalasingam S, Norton T, et al; Trial Investigators: REGN-COV2, a neutralizing antibody cocktail, in outpatients with Covid-19. *N Engl J Med* 2021; 384:238–251
32. Remy KE, Mazer M, Striker DA, et al: Severe immunosuppression and not a cytokine storm characterizes COVID-19 infections. *JCI Insight* 2020; 5:140329
33. Ng KW, Faulkner N, Cornish GH, et al: Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. *Science* 2020; 370:1339–1343
34. RECOVERY Collaborative Group: Dexamethasone in hospitalized patients with Covid-19. *N Engl J Med* 2021; 384:693–704
35. McElvaney OJ, Curley GF, Rose-John S, et al: Interleukin-6: Obstacles to targeting a complex cytokine in critical illness. *Lancet Respir Med* 2021; 9:643–654