Supplemental Information

Early increases in anti-SARS-CoV-2 antibody isotypes associated with organ dysfunction and mortality in patients hospitalized with COVID-19

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Supplemental Methods

Ethics & Patient Selection Criteria

This study was approved by the Providence Health Care and University of British Columbia Human Research Committee and by each of the contributing sites. Anonymized clinical data and use of discarded EDTA plasma from clinical blood tests were deemed low risk and informed consent was deemed not necessary for this research.

This study was a sub-study of adults hospitalized with acute COVID-19 who were included in a multi-centre Canadian pragmatic observational cohort study to examine safety and effectiveness of angiotensin receptor blockers (ARBs CORONA I; NCT04510623) (8). Individuals >18 years old admitted to hospital for acute COVID-19 (with PCR-confirmation) were included. We excluded acute COVID-19 readmissions, Emergency Department admissions and those admitted to hospital but not due to acute COVID-19. We selected patients from ARBs CORONA I who had plasma available on both days 4 and 7 because (i) we reasoned that most patients would have detectable antibodies by day 4, and (ii) we wanted to evaluate the acute change in antibody isotype concentrations early during hospitalization to determine whether they were associated with and cause changes in outcomes.

Quantification of plasma IgG, IgA and IgM

As previously described (9), we used the immunIQ COVID assay which is a quantitative assay that measures individual anti-SARS-CoV-2 isotypes (i.e., IgG, IgA, IgM, IgD and IgE) and subtypes in plasma. Plasma (4.2 μl) was added to RBD-coated magnetic beads and binding antibodies are eluted for reduction, alkylation and proteolytic digestion with trypsin. Analysis was performed by high performance liquid chromatography tandem mass spectrometry (Shimadzu LC 20AD LC system coupled to a SCIEX 5500 triple quadrupole mass spectrometer), where quantotypic and isotype-specific human immunoglobulin sequences are detected, and quantified via the inclusion of isotopically labeled internal standard peptides for each isotype. For the isotypes reported herein, the following quantotypic peptides were used: DTLMISR (for IgG), WLQGSQELPR (for IgA), and YVTSAPMPEPQAPGR (for IgM). For internal standards, the same sequences with stable isotope labels (^{13}C- and ^{15}N-labeled lysine and arginine) were used. The concentration of the internal standards was assigned against the ERM-DA470K certified reference material, following our previously published approach.(10) Data was analyzed using Analyst (SCIEX v.1.6) and Skyline (v.20.2).

LLMI is the term used instead of ‘lower limit of quantitation’ (LLOQ) when referring to mass spectrometric versus ligand binding protein assays (11). As anticipated, anti-RBD IgD and IgE antibodies were not detected in any sample. For IgG, IgM, and IgA isotypes, concentrations below the LLMI were imputed using the fill-in method (12), whereby the distribution (i.e., mean and variance) of the log-transformed concentration is first estimated from the observed data and then values are randomly drawn from that distribution that lie between log(zero) and log(LLMI). The sample values are exponentiated and then imputed into the dataset.

Baseline Characteristics, Therapies and Mortality

We recorded baseline characteristics (at study enrollment): age, sex, presence of heart failure, hypertension, chronic kidney disease, and diabetes (commonest COVID-19 co-morbidities associated with increased risk of ICU admission and death), heart rate, respiratory rate, temperature, blood pressure, arterial oxygen saturation (SaO_2), ratio of partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2), serum creatinine and bilirubin, use of oxygen, vasopressors, ventilation and renal replacement therapy (RRT) during the hospital stay, and 28-day and in-hospital mortality. We recorded use of COVID-19 vaccines and corticosteroids during the hospital course since they could modify antibody response to SARS-CoV-2 infection.

Outcomes

The primary outcome was 28-day mortality. We chose 28-day mortality as the primary outcome because it has been the primary outcome of many trials of new therapies in acute COVID-19. We recognize that in-hospital mortality is also a pertinent mortality outcome in COVID-19 because of the known association with prolonged ventilation and hospital admission. Secondary outcomes were in-hospital mortality and organ dysfunction, determined as invasion mechanical ventilation, vasopressors and RRT that was initiation after day 4 or 7. RRT was not formally analyzed because too few participants (n=7) received this therapy.

Statistical Analyses

Data were reviewed and validated for each patient by a team of coordinators. Across statistical models $\log_2$-concentrations of the antibodies were used. First, change in IgG, IgA and IgM concentrations from day 4 to day 7 were submitted to linear mixed models to estimate the amount of change over time. Second, logistic regression was used to predict death by day 28, death in hospital, vasopressor use, and the initiation of invasive mechanical ventilation using day 4 and day 7 antibody concentrations, while adjusting for participant sex and age, and prevalent cardiovascular disease, chronic kidney disease, hypertension, and diabetes. For each antibody isotype, two models were constructed: the first included the day 4 concentration value and the aforementioned covariates; the second included day 4 concentration, the change in concentration from day 4 to day 7, and the aforementioned covariates. The estimated associations between antibody concentrations and these binary outcomes are expressed as odds ratio (OR) and 95% confidence intervals (CI) around the estimated OR. As $\log_2$-concentrations were entered into these logistical regression models, the OR reflects the change in the odds of a given outcome for a one-unit difference in the $\log_2$ scale; this is equivalent to a doubling in the concentration in the raw scale. Statistical significance was defined at $p < 0.05$. We performed a complete case analysis, individuals with missing data were excluded.

Supplemental Results

From June 6, 2020 to February 23, 2021 inclusive, 137 patients from ARBs CORONA I met our sub-study inclusion/exclusion criteria. Baseline characteristics were typical of patients admitted to hospital with acute COVID-19 (**Table S1**). This subgroup was generally similar to the overall ARBs CORONA I population, except that a higher proportion of patients were admitted to ICU (**Table S2**). The 28-day mortality was 12.4% (**Table S2**), lower than the overall ARBs CORONA I cohort (17.4%) because in this sub-study we selected patients who still were alive and in hospital on day 7. Co-interventions are shown in **Table S3**.

Associations of anti-SARS-CoV-2 antibody isotype concentrations with 28-day mortality, mechanical ventilation, in-hospital death, and use of vasopressors are provided in in **Table S4**. We also did an adjusted analysis that included chronic obstructive pulmonary disease (COPD) and the findings are essentially unchanged. Median and interquartile range for plasma anti-RBD antibody isotype concentrations by outcome measures are found in **Table S5.**

Anti-SARS-CoV-2 antibody isotype concentrations, characteristics and outcomes of dexamethasone-treated and non-dexamethasone-treated patients are shown in **Tables S6** and **S7**. Patients who were treated with dexamethasone by day 1 had an in-hospital mortality of 20.4% (21/103), higher than the mortality of non-dexamethasone-treated patients 0% (0/19) ($p = 0.031$)".

Supplemental Discussion

There are four novel aspects of our study. First, we believe that there are no prior publications associating anti-SARS-CoV-2 binding antibody isotype concentrations with acute organ dysfunction and need for ventilation or vasopressors in patients hospitalized with acute COVID-19. We found larger increases in IgM from day 4 to day 7 in acute COVID-19 hospitalized patients who needed ventilation and vasopressors versus those that did not need that support. Second, our review of the literature shows that ours is the first study showing that use of dexamethasone is associated with increased concentration of binding antibodies to SAR-CoV-2.

Several aspects of the study have clinical relevance. First, there was a relatively high frequency of patients with low concentrations of anti-RBD antibodies at day 4 of hospitalization, as assessed by the percent of cases where antibody concentrations were below the assay's LLMI: IgG (45.3%), IgA (35.8%) and IgM (38%). Thus, patient antibody responses may be inadequate in some patients at admission for many reasons (e.g., immune response inadequacy, viral load differences, age, immuno-depressing drugs and conditions). Second, the greater increase in anti-RBD-antibodies observed in patients receiving dexamethasone early during hospitalization suggest further studies of dexamethasone in decreasing the need for ventilation and mortality in acute hospitalized COVID-19.

Early intervention in patients with severe-COVID-19 improves organ dysfunction and lowers mortality (14-16), and an early strong antibody response may be associated with survival in patients hospitalized with COVID-19 (6, 17). This is why we focused on the early hospitalization stage of COVID-19 and evaluated need for vital organ support (ventilation or vasopressors) and mortality outcomes.

We found that all anti-RBD isotypes (IgG, IgA and IgM) increased significantly from day 4 to day 7 of COVID-19 hospitalization. We focused our efforts on RBD-binding antibodies as potent neutralizing antibodies against multiple epitopes of SARS-CoV-2 spike protein (18) which are central to the adaptive immune response to SARS-CoV-2 (5). The RBD of the spike protein is the strongest initial (within the first week) and lasting (more than 6 months) natural antibody epitope and prevents SARS-CoV-2 entry into human cells (1-5). Following SARS-CoV-2 infection, though anti-RBD IgM is the first isotype to peak, IgG and IgA isotypes are also frequently detectable three to seven days after symptom onset (19, 20). This rapid production of all three isotypes is relatively unique to SARS-CoV-2 when compared to other coronaviruses.(21)

We found that patients who did not need ventilation or vasopressors had greater increase of IgM but not IgG or IgA from day 4 to 7. Shang and colleagues found that a lack of IgG response 21 days after symptom onset was associated with progression to critical illness (6). Lucas and colleagues identified that early anti-RBD neutralizing antibodies predicted outcome, whereby patients who were “early neutralizers” were less likely to require ICU admission, high flow oxygen, invasive mechanical ventilation, extracorporeal membrane oxygenation, glucocorticoid or vasopressor use (22). Anti-RBD IgG was associated with survival among ICU-admitted patients (7). In severe-COVID-19, early strong IgA and IgG increases correlated with disease severity (23). IgG concentrations were significantly higher in severely ill patients within 14 days of symptom onset compared to non-severely ill hospitalized patients (24) and anti-RBD IgG concentration (but not IgA and IgM) were predictive of survival in severe COVID-19 (7). There is also earlier seroconversion of IgG in patients with acute respiratory distress syndrome patients compared to patients who do not have acute respiratory distress syndrome (25, 26).

Our study is the first to show that early increases in plasma anti-RBD IgM are associated with and may cause less need for ventilation and vasopressors, markers of severity of acute COVID-19. Plasma anti-RBD IgM concentrations have not been correlated with disease severity (whereas IgA and IgG significantly increased in moderate and severe cases).(20, 27) Plasma concentrations of anti-RBD IgA were not associated with acute outcomes in our study. The role of IgA is less well defined in severe COVID-19.

Attenuated antibody response in COVID-19 is associated with disease severity.(28, 29) We add to the literature by showing that increasing IgG and IgM concentrations form day 4 to day 7 were associated with less use of ventilation and vasopressors (days alive and free of ventilation and vasopressors). This is very relevant for the patients by limiting their organ support days, for the

costs associated with such support and for clinicians to better understand a fundamental reason why some patients improve and others do not.

Dexamethasone use was associated with altered anti-RBD antibody concentrations in our study. The antibody concentrations at day 7 for those who had dexamethasone initiated by day 1 were significantly higher than those who were not on dexamethasone for the first 7 days, resulting in a significantly larger increase in antibody concentrations relative to day 4 in patients whose dexamethasone started on day 1. It is also possible that dexamethasone led to increase in viral replication and that in turn led to increased antibody response. The association of dexamethasone to increase response may not necessarily be a linear trajectory to recovery. Our findings regarding dexamethasone require validation in other studies and trials before making any changes of the use of dexamethasone in acute COVID-19. In previous trials, dexamethasone decreased mortality (30) and increased days alive and free of ventilation (31) in COVID-19 patients. A previous study found that dexamethasone did not delay or mitigate the antibody response in immunocompetent patients hospitalized with severe COVID-19 (32).

There are subsets of patients who present early after symptom onset and others who are late presenters post symptom onset, with differences in their respective outcomes. Unfortunately, we do not have information about when symptoms of acute COVID-19 bean prior to hospitalization.

There are limitations of our study. An association study cannot directly determine causation. We also note that the number of patients with the outcomes of interest was small and thus the results from the adjusted regression analysis should be interpreted with caution. Unfortunately, we did not collect information about secondary infections. Secondary infections could potentially confound the interpretation of the association between anti-RBD IgG concentrations and clinical outcomes. The initial plan for the ARBs CORONA I cohort was to limit sample size and create a closed cohort based on grant funding constraints and *a priori* plans. However, with the receipt of additional research funds and with the continuation of the COVID-19 pandemic, ARBs CORONA I evolved to an open acute COVID-19 cohort. We did a complete case analysis to minimize risk of bias due to missing data not at random—we recognize that as a limitation.

We did not have a severity of illness for COVID-19 and so could not adjust for acute COVID-19 severity of illness. This is a limitation because critically ill patients can develop an immunoparetic state; B-cell dysfunction and dysregulation is recognized in acute COVID-19. It is therefore possible that reverse causation is seen here, i.e., that the more severely ill patients were unable to mount an effective B-cell/immunoglobulin response and that this is associated with already being severely ill.

Although the finding of enhanced Ig response in patients receiving dexamethasone is interesting, as an observational study it is highly susceptible to confounding. It is likely that patients who did not receive dexamethasone were systematically different from those who did, perhaps having prior or

ongoing steroid administration for another indication or being immunocompromised—both of which could impact on seroconversion and production of anti-SARS-CoV-2 antibodies. Unmeasured confounding should be acknowledged as a potential weakness of our study. Total anti-RBD binding antibody concentrations were measured rather than neutralising antibody titres. While it is likely that an increased RBD-binding immunoglobulins leads to a similar increase in neutralising antibodies specifically, this was not directly assessed.

In conclusion, plasma concentrations of anti-RBD IgG, IgA and IgM increased significantly from day 4 to 7 of hospitalization for acute COVID-19. Increases in IgG and IgM from day 4 to day 7 were associated with lower mortality, and less use of ventilation and vasopressors, respectively. The dexamethasone results must be interpreted with caution because only 19 patients were treated with dexamethasone.

Supplemental Tables

Table S1. Baseline characteristics of patients admitted with acute COVID-19 that met our inclusion/exclusion criteria.

Variable	**All (n=137)**
Sex, n (%)	
Male	80 (58.4)
Female	57 (41.6)
Age	
Mean (SD)	66.3 (15.4)
Median (IQR)	67.0 (55.0, 78.0)
Co-morbidities, n (%) ^	
Any of the four below	95/136 (69.9)
Chronic cardiac disease	27/136 (19.9)
Chronic kidney disease	14/137 (10.2)
Hypertension	73/136 (53.7)
Diabetes	48/137 (35.0)
Chronic pulmonary disease (not asthma)	18/136 (13.2)
Moderate or severe liver disease	1/137 (0.7)
Mild liver disease	11/137 (8.0)
Chronic neurological disorder	7/136 (5.1)
Malignant neoplasm	5/133 (3.8)
Chronic hematologic disease	9/137 (6.6)
AIDS / HIV	1/135 (0.7)
Rheumatologic disorder	11/137 (8.0)
Dementia	8/136 (5.9)

Admitted to ICU on hospital admission day, n (%)	38 (27.7)
Organ support on admission day	
Invasive mechanical ventilation, n (%)	19/137 (13.9)
RRT or dialysis, n (%)	2/136 (1.5)
Vasopressors, n (%)	15/137 (10.9)
Temperature (°C), mean (SD)	37.5 (1.0)
Missing, n	3
Heart rate (beats per minute), mean (SD)	99.3 (20.2)
Respiratory rate (breaths per minute), mean (SD)	26.4 (9.3)
sBP, mean (SD)	131.4 (23.3)
dBP, mean (SD)	74.3 (13.0)
Weight, kg, mean (SD)	80.8 (21.5)
Oxygen saturation (SaO2; %), mean (SD)	89.7 (10.6)
Oxygen status, n (%)	
Room air	81 (60.4)
Oxygen therapy	53 (39.6)
WBC count (x10³/μL), median (IQR)	6.4 (4.8, 10.2)
Hemoglobin (g/L), median (IQR)	131.0 (117.0, 145.0)
Creatinine (μmol/L), median (IQR)	86.0 (68.0, 126.0)
Potassium (mEq/L), median (IQR)	3.8 (3.5, 4.1)
Platelets (x10⁹/L), median (IQR)	199.5 (160.0, 269.0)

Table S2. Baseline characteristics of patients in ARBs CORONA I population stratified by inclusion in current sub-study.

Variable	All (n=1680)	Not included (n=1543)	Included (n=137)	*P* value*
Sex, n (%)				0.546
Male	1021 (60.8)	941 (61.0)	80 (58.4)	
Female	658 (39.2)	601 (39.0)	57 (41.6)	
Age				0.632
Mean (SD)	65.6 (16.7)	65.6 (16.8)	66.3 (15.4)	
Co-morbidities, n (%) ^				
Any of the four below	1128/1674 (67.4)	1033/1538 (67.2)	95/136 (69.9)	0.522
Chronic cardiac disease	422/1667 (25.3)	395/1531 (25.8)	27/136 (19.9)	0.126
Chronic kidney disease	239/1675 (14.3)	225/1538 (14.6)	14/137 (10.2)	0.157
Hypertension	887/1673 (53.0)	814/1537 (53.0)	73/136 (53.7)	0.873
Diabetes	558/1673 (33.4)	510/1536 (33.2)	48/137 (35.0)	0.663
Chronic pulmonary disease (not asthma)	258/1670 (15.4)	240/1534 (15.6)	18/136 (13.2)	0.456
Moderate or severe liver disease	26/1669 (1.6)	25/1532 (1.6)	1/137 (0.7)	0.414
Mild liver disease	55/1671 (3.3)	44/1534 (2.9)	11/137 (8.0)	0.001
Chronic neurological disorder	170/1673 (10.2)	163/1537 (10.6)	7/136 (5.1)	0.043
Malignant neoplasm	118/1666 (7.1)	113/1533 (7.4)	5/133 (3.8)	0.119
Chronic hematologic disease	77/1673 (4.6)	68/1536 (4.4)	9/137 (6.6)	0.252
AIDS / HIV	10/1648 (0.6)	9/1513 (0.6)	1/135 (0.7)	0.576
Rheumatologic disorder	188/1673 (11.2)	177/1536 (11.5)	11/137 (8.0)	0.215
Dementia	154/1670 (9.2)	146/1534 (9.5)	8/136 (5.9)	0.160
Admitted to ICU on hospital admission day, n (%)	334 (19.9)	296 (19.2)	38 (27.7)	0.017
Organ support on admission day				
Invasive mechanical ventilation, n (%)	176/1680 (10.5)	157/1543 (10.2)	19/137 (13.9)	0.176
RRT or dialysis, n (%)	25/1663 (1.5)	23/1527 (1.5)	2/136 (1.5)	0.974
Vasopressors, n (%)	137/1680 (8.2)	122/1543 (7.9)	15/137 (10.9)	0.212
Temperature (°C), mean (SD)	37.4 (0.9)	37.3 (0.9)	37.5 (1.0)	0.033
Heart rate (beats per minute), mean (SD)	95.3 (20.4)	95.0 (20.4)	99.3 (20.2)	0.016
Respiratory rate (breaths per minute), mean (SD)	25.1 (8.6)	25.0 (8.5)	26.4 (9.3)	0.075
sBP, mean (SD)	129.2 (22.7)	129.0 (22.6)	131.4 (23.3)	0.234
dBP, mean (SD)	73.9 (12.7)	73.9 (12.7)	74.3 (13.0)	0.705
Weight, kg, mean (SD)	82.8 (22.8)	82.9 (22.9)	80.8 (21.5)	0.493
Oxygen saturation (SaO2; %), mean (SD)	90.4 (9.0)	90.4 (8.9)	89.7 (10.6)	0.399
Oxygen status, n (%)				0.373
Room air	1043 (64.0)	962 (64.3)	81 (60.4)	
Oxygen therapy	587 (36.0)	534 (35.7)	53 (39.6)	
WBC count (x10³/μL), median (IQR)	6.9 (5.1, 9.6)	6.9 (5.1, 9.6)	6.4 (4.8, 10.2)	0.544
Haemoglobin (g/L), median (IQR)	132.0 (119.0, 146.0)	133.0 (119.0, 146.0)	131.0 (117.0, 145.0)	0.450
Creatinine (μmol/L), median (IQR)	86.0 (69.0, 116.0)	86.0 (69.0, 116.0)	86.0 (68.0, 126.0)	0.498
Potassium (mEq/L), median (IQR)	3.9 (3.6, 4.3)	4.0 (3.6, 4.3)	3.8 (3.5, 4.1)	0.006
Platelets (x10⁹/L), median (IQR)	204.0 (159.0, 262.0)	204.0 (159.0, 261.0)	199.5 (160.0, 269.0)	0.926

* *P* value was based on Chi-square test, Fisher's exact test, t-test or Wilcoxon rank sum test as appropriate.

Table S3. Co-interventions and outcomes of patients admitted with acute COVID-19 that met our inclusion/exclusion criteria.

Variable	All (n=137)
28-day mortality, n (%)	17 (12.4)
In-hospital death, n (%)	23 (16.8)
Ever admitted to ICU, n (%)	87 (63.5)
Hospital length of stay, median (IQR)*	16.0 (10.0, 27.0)
Septic shock, n (%)	23/131 (17.6)
Acute respiratory distress syndrome, n (%)	49/132 (37.1)
Acute kidney injury, n (%)	49/129 (38.0)
Acute cardiac injury, n (%)	16/132 (12.1)
Co-intervention while hospitalized, n (%)	
Antiviral agent	27 (19.7)
Remdesivir	23 (16.8)
Antibiotic	129 (94.2)
Corticosteroid	122 (89.1)
Dexamethasone	121 (88.3)
Antifungal agent	12 (8.8)
Organ support while hospitalized, n (%)	
Invasive mechanical ventilation	58/137 (42.3)
RRT or dialysis	7/136 (5.1)
Vasopressors	55/137 (40.1)

* Among those who were discharged alive.

Table S4. Associations of plasma concentration, and changes in plasma concentration, of anti-RBD IgG, IgA and IgM in separate models. All models include age and sex, and prevalent CVD, CKD, hypertension, and diabetes; models with change scores as the primary predictor also include day 4 value as a covariate.

	28-day mortality		**Mechanical ventilation**	
Variable	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**
IgG, day 4	1.08 (0.80-1.47)	0.600	1.30 (0.93-1.84)	0.126
IgG, Δ*	0.56 (0.32-0.93)	0.033	0.50 (0.19-1.17)	0.122
IgA, day 4	0.90 (0.66-1.19)	0.468	0.88 (0.60-1.25)	0.496
IgA, Δ	1.11 (0.68-1.83)	0.664	1.15 (0.45-2.90)	0.768
IgM, day 4	0.86 (0.56-1.28)	0.471	1.17 (0.73-1.87)	0.499
IgM, Δ	0.90 (0.49-1.64)	0.739	0.15 (0.02-0.67)	0.026
	In-hospital death		**Vasopressor**	
IgG, day 4	1.06 (0.81-1.38)	0.680	1.08 (0.78-1.48)	0.646
IgG, Δ	0.68 (0.44-1.03)	0.078	0.69 (0.32-1.43)	0.323
IgA, day 4	0.91 (0.69-1.17)	0.485	0.95 (0.69-1.30)	0.770
IgA, Δ	1.19 (0.78-1.85)	0.421	1.27 (0.58-2.95)	0.552
IgM, day 4	0.90 (0.62-1.28)	0.563	0.93 (0.58-1.45)	0.753
IgM, Δ	0.88 (0.50-1.52)	0.648	0.17 (0.03-0.69)	0.026

* Change (delta) in concentration from day 4 to day 7.

Table S5. Median and interquartile range for plasma anti-RBD antibody isotype concentrations by outcome measures.

	28-day mortality		Mechanical ventilation	
Variable	**No (n = 120)**	**Yes (n = 17)**	**No (n = 79)**	**Yes (n = 58)**
IgG				
Day 4	2.6 [1.3, 7.8]	1.8 [0.8, 11.8]	1.8 [0.8, 5.4]	5.6 [1.7, 15.5]
Day 7	11.7 [3.2, 20.5]	6.8 [1.0, 20.2]	7.4 [2.1, 16.3]	16.0 [6.8, 26.8]
Δ*	5.7 [1.2, 12.5]	0.9 [-0.5, 6.7]	4.2 [1.1, 10.6]	7.5 [0.9, 13.6]
IgA				
Day 4	2.3 [0.9, 6.7]	1.1 [0.4, 4.5]	1.7 [0.7, 5.4]	3.3 [0.9, 6.5]
Day 7	5.6 [2.1, 13.1]	5.0 [2.5, 6.5]	4.5 [1.5, 9.8]	5.9 [3.1, 14.7]
Δ	1.8 [0.3, 4.5]	2.3 [0.4, 5.6]	1.9 [0.4, 4.5]	1.7 [0.0, 4.6]
IgM				
Day 4	3.1 [1.5, 5.1]	1.9 [0.9, 3.8]	2.0 [1.3, 3.9]	3.8 [1.9, 7.2]
Day 7	5.0 [2.3, 9.7]	4.1 [2.2, 5.6]	3.7 [1.9, 7.5]	6.4 [4.0, 11.6]
Δ	1.6 [0.4, 4.2]	1.5 [0.7, 2.9]	1.4 [0.4, 4.0]	2.2 [0.6, 4.1]
	In-hospital death		**Vasopressor use**	
	No (n = 114)	**Yes (n = 23)**	**No (n = 82)**	**Yes (n = 55)**
IgG				
Day 4	2.6 [1.2, 8.6]	1.8 [1.1, 11.4]	1.8 [0.9, 5.9]	4.5 [1.6, 15.8]
Day 7	11.8 [3.1, 21.6]	6.9 [1.1, 18.8]	8.3 [2.3, 17.9]	13.1 [6.3, 24.3]
Δ	5.9 [1.2, 12.5]	2.1 [-0.3, 7.1]	4.6 [1.2, 10.8]	6.7 [0.7, 12.9]
IgA				
Day 4	2.3 [0.9, 6.9]	1.1 [0.6, 5.4]	1.6 [0.7, 5.7]	3.3 [1.0, 6.5]
Day 7	5.6 [2.0, 13.8]	5.0 [2.8, 6.4]	4.7 [1.7, 10.9]	5.9 [2.9, 14.2]
Δ	1.8 [0.3, 4.5]	2.3 [0.4, 5.2]	1.9 [0.3, 5.3]	1.7 [0.0, 4.3]
IgM				
Day 4	3.0 [1.5, 5.1]	2.0 [1.2, 4.3]	2.0 [1.2, 4.2]	3.7 [1.9, 7.4]
Day 7	5.1 [2.3, 10.2]	4.1 [2.3, 6.1]	4.0 [1.9, 8.1]	6.3 [3.7, 11.2]
Δ	1.6 [0.4, 4.4]	1.6 [0.7, 2.6]	1.4 [0.4, 4.1]	1.9 [0.5, 4.1]

* Change in concentration from day 4 to day 7.

Table S6. Anti-RBD IgG, IgA and IgM in patients admitted to hospital with acute COVID-19 according to dexamethasone usage during hospitalization.

	Dexamethasone		
Variable	**No usage during first 7 days**	**Initiated by day 1**	***P* value***
Detectable at day 4, n (%)			
IgG	7/19 (36.8)	61/103 (59.2)	0.071
IgA	10/19 (52.6)	68/103 (66.0)	0.264
IgM	10/19 (52.6)	64/103 (62.1)	0.436
Median (IQR) concentration at day 4			
IgG	1.6 (0.8, 4.4)	2.9 (1.3, 8.4)	0.265
IgA	1.1 (0.6, 3.3)	2.3 (0.9, 6.8)	0.162
IgM	2.0 (1.3, 4.0)	3.0 (1.5, 5.1)	0.366
Detectable at day 7, n (%)			
IgG	10/19 (52.6)	85/103 (82.5)	0.004
IgA	12/19 (63.2)	88/103 (85.4)	0.020
IgM	11/19 (57.9)	86/103 (83.5)	0.011
Median (IQR) concentration at day 7			
IgG	1.9 (1.0, 15.9)	12.3 (3.3, 20.6)	0.057
IgA	2.4 (1, 6)	5.9 (2.7, 15.0)	0.015
IgM	2.5 (1, 6)	5.2 (2.4, 10.2)	0.029
Median (IQR) change in concentration from day 4 to 7			
IgG	2.7 (-0.4, 6.2)	6.1 (1.2, 13.1)	0.010
IgA	0.4 (-0.5, 2.3)	2.3 (0.5, 5.6)	0.013
IgM	0.4 (-0.2, 1.6)	1.8 (0.7, 4.5)	0.004

* From Wilcoxon rank sum test.

Table S7. Baseline characteristics and outcomes of dexamethasone-treated and non-dexamethasone-treated patients.

	Dexamethasone		
Variable	**No usage during first 7 days (n=19)**	**Started by day 1 (n=103)**	*P* value
Sex, n (%)			0.298
Male	9 (47.4)	62 (60.2)	
Female	10 (52.6)	41 (39.8)	
Age			
Mean (SD)	66.9 (16.9)	65.9 (15.1)	0.790
Co-morbidities, n (%) ^			
Any of the four below	15/19 (78.9)	67/102 (65.7)	0.256
Chronic cardiac disease	6/19 (31.6)	17/102 (16.7)	0.128
Chronic kidney disease	5/19 (26.3)	7/103 (6.8)	0.009
Hypertension	13/19 (68.4)	51/102 (50.0)	0.140
Diabetes	6/19 (31.6)	33/103 (32.0)	0.968
Chronic pulmonary disease (not asthma)	1/19 (5.3)	15/102 (14.7)	0.265
Liver disease	3/19 (15.8)	8/103 (7.8)	0.262
Moderate or severe	1/19 (5.3)	0/103 (0.0)	0.156
Mild	2/19 (10.5)	8/103 (7.8)	0.687
Chronic neurological disorder	1/19 (5.3)	6/102 (5.9)	0.915
Malignant neoplasm	0/18 (0.0)	3/100 (3.0)	1.000
Chronic hematologic disease	3/19 (15.8)	6/103 (5.8)	0.127
AIDS / HIV	0/19 (0.0)	1/101 (1.0)	1.000
Rheumatologic disorder	4/19 (21.1)	6/103 (5.8)	0.026
Dementia	1/19 (5.3)	6/102 (5.9)	0.915
Admitted to ICU on hospital admission day, n (%)	1 (5.3)	34 (33.0)	0.014
Organ support on admission day			
Invasive mechanical ventilation, n (%)	1/19 (5.3)	17/103 (16.5)	0.204
RRT or dialysis, n (%)	0/19 (0.0)	1/102 (1.0)	1.000
Vasopressors, n (%)	0/19 (0.0)	14/103 (13.6)	0.088
Temperature (°C), mean (SD)	37.1 (0.7)	37.6 (1.0)	0.039
Heart rate (beats per minute), mean (SD)	97.5 (20.8)	100.9 (19.6)	0.497
Respiratory rate (breaths per min), mean (SD)	21.4 (6.4)	27.4 (9.4)	0.008
sBP, mean (SD)	128.4 (27.5)	132.4 (22.0)	0.487
dBP, mean (SD)	73.6 (14.3)	75.2 (12.9)	0.628
Weight, kg, mean (SD)	81.7 (30.9)	81.9 (20.2)	0.980
Oxygen saturation (SaO2; %), mean (SD)	94.2 (4.7)	88.6 (11.6)	0.043
Oxygen status, n (%)			0.210
Room air	13 (72.2)	57 (56.4)	
Oxygen therapy	5 (27.8)	44 (43.6)	
WBC count (x10³/μL), median (IQR)	6.4 (4.6, 11.4)	6.4 (5.0, 10.3)	0.866
Haemoglobin (g/L), median (IQR)	101.0 (89.0, 145.0)	132.0 (120.0, 147.0)	0.003
Creatinine (μmol/L), median (IQR)	130.0 (73.0, 227.0)	84.0 (68.0, 110.0)	0.036
Potassium (mEq/L), median (IQR)	3.7 (3.5, 4.4)	3.8 (3.5, 4.1)	0.686
Platelets (x10⁹/L), median (IQR)	240.0 (121.0, 304.0)	199.0 (168.0, 268.0)	0.889

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