Early trajectories of virological and immunological biomarkers and clinical outcomes in patients admitted to hospital for COVID-19

an international, prospective cohort study

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Early trajectories of virological and immunological biomarkers and clinical outcomes in patients admitted to hospital for COVID-19: an international, prospective cohort study

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Summary

Background Serial measurement of virological and immunological biomarkers in patients admitted to hospital with COVID-19 can give valuable insight into the pathogenic roles of viral replication and immune dysregulation. We aimed to characterise biomarker trajectories and their associations with clinical outcomes.

Methods In this international, prospective cohort study, patients admitted to hospital with COVID-19 and enrolled in the Therapeutics for Inpatients with COVID-19 platform trial within the Accelerating COVID-19 Therapeutic Interventions and Vaccines programme between Aug 5, 2020 and Sept 30, 2021 were included. Participants were included from 108 sites in Denmark, Greece, Poland, Singapore, Spain, Switzerland, Uganda, the UK, and the USA, and randomised to placebo or one of four neutralising monoclonal antibodies: bamlanivimab (Aug 5 to Oct 13, 2020), sotrovimab (Dec 16, 2020, to March 1, 2021), amubarvimab-romlusevimab (Dec 16, 2020, to March 1, 2021), and tixagevimab-cilgavimab (Feb 10 to Sept 30, 2021). This trial included an analysis of 2149 participants with plasma nucleocapsid antigen, anti-nucleocapsid antibody, C-reactive protein (CRP), IL-6, and D-dimer measured at baseline and day 1, day 3, and day 5 of enrolment. Day-90 follow-up status was available for 1790 participants. Biomarker trajectories were evaluated for associations with baseline characteristics, a 7-day pulmonary ordinal outcome, 90-day mortality, and 90-day rate of sustained recovery.

Findings The study included 2149 participants. Participant median age was 57 years (IQR 46–68), 1246 (58·0%) of 2149 participants were male and 903 (42·0%) were female; 1792 (83·4%) had at least one comorbidity, and 1764 (82·1%) were unvaccinated. Mortality to day 90 was 172 (8·0%) of 2149 and 189 (8·8%) participants had sustained recovery. A pattern of less favourable trajectories of low anti-nucleocapsid antibody, high plasma nucleocapsid antigen, and high inflammatory markers over the first 5 days was observed for high-risk baseline clinical characteristics or factors related to SARS-CoV-2 infection. For example, participants with chronic kidney disease demonstrated plasma nucleocapsid antigen 424% higher (95% CI 319–559), CRP 174% higher (150–202), IL-6 173% higher (144–208), D-dimer 149% higher (134–165), and anti-nucleocapsid antibody 39% lower (60–18) to day 5 than those without chronic kidney disease. Participants in the highest quartile for plasma nucleocapsid antigen, CRP, and IL-6 at baseline and day 5 had worse clinical outcomes, including 90-day all-cause mortality (plasma nucleocapsid antigen hazard ratio (HR) 4·50 (95% CI 3·29–6·15), CRP HR 3·37 (2·30–4·94), and IL-6 HR 5·67 (4·12–7·80). This risk persisted for plasma nucleocapsid antigen and CRP after adjustment for baseline biomarker values and other baseline factors.

Interpretation Patients admitted to hospital with less favourable 5-day biomarker trajectories had worse prognosis, suggesting that persistent viral burden might drive inflammation in the pathogenesis of COVID-19, identifying patients that might benefit from escalation of antiviral or anti-inflammatory treatment.

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Introduction

Understanding the dynamics between SARS-CoV-2 infection, host immune response, and clinical manifestations in patients admitted to hospital has advanced substantially. High plasma nucleocapsid antigen and low neutralising anti-spike antibody at baseline have been associated with unfavourable clinical outcomes.[1,2] Abnormalities in other host biomarkers on admission, including C-reactive protein (CRP), IL-6, and D-dimer, have also been linked to disease severity.[3,4] Two randomised controlled trials have indicated that the presence of anti-spike antibody can identify patients admitted to

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Research in context

Evidence before this study

Understanding the dynamics of biomarkers of SARS-CoV-2 viral burden, antibody production, and inflammatory response over time in patients admitted to hospital with COVID-19 might help identify patients who could benefit from intensified treatment and supportive care. We used the search terms ("SARS-CoV-2" OR "COVID-19" OR "COVID") AND "biomarkers" AND ("serial measurement" OR "trajectory") to search PubMed for studies published since Jan 1, 2020 until May 1, 2023, without any further restrictions. We identified seven publications that examined the association between biomarker trajectories, baseline risk factors, and clinical outcomes in patients admitted to hospital with COVID-19. Two small studies focused on viral burden and serological response, mapping the kinetics of endogenous antibody production with viral clearance. These studies suggest that persistent SARS-CoV-2 viraemia is associated with more severe disease and increased mortality. A few studies evaluated the ability of host-response inflammatory biomarkers to predict risk of adverse outcomes. In an analysis of 810 patients, the ratio of measurements of C-reactive protein (CRP), D-dimer, total lymphocyte count, and lactate dehydrogenase at 48–72 h compared with baseline performed poorly to predict outcomes. A risk-prediction model that made use of changes in vital signs and biomarkers (CRP, D-dimer, glomerular filtration rate, and total lymphocyte count) from 1884 patients over time was predictive of discharge, death, and mechanical ventilation, but the dataset had inconsistent follow-up data. A smaller study of 37 patients found reduction of IL-6 and IL-10 at day 3 compared with baseline in survivors but not in non-survivors. Among 23 patients categorised by level of respiratory support and abnormal IL-6, procalcitonin, angiopoetin-2, and S-RAGE, those with persistently abnormal cytokines at day 5 had worse survival. In a study of 318 patients on mechanical ventilation, those with higher sequential organ failure assessment (SOFA) scores had worse 30-day outcomes. However, although high SOFA scores had higher laboratory markers of severity over 7 days after intubation, differences in biomarker trajectories were not significant. Lastly, serial measurement of cytokine panels in 219 patients showed unfavourable outcomes associated with hyperinflammatory pathways, such as chemotaxis and IL production. Overall, these studies suggest that persistently abnormal serial measurements of biomarkers are associated with worse clinical outcomes. However, the scarcity of consistent baseline and outcome data, missing biomarker values at follow-up days, and relatively small cohort sizes for many of these publications contribute to an increased risk of bias. Further, previous work did not evaluate markers of viral burden and inflammation in the same cohort. Additionally, adjustment of outcomes for baseline factors associated with risk was not done. We therefore used the large, international, and well characterised cohort from trials done under the TICO–ACTIV-3 master protocol to further examine these associations.

Added value of this study

This study represents the largest global, multisite cohort evaluating serial biomarkers for patients admitted to hospital with COVID-19. Additionally, the assessment of markers of serological response, viral burden, and inflammation in the same participants allowed these responses to be linked, demonstrating an association between abnormal viral clearance and inflammatory responses. This report connects abnormal trajectories with three key clinical outcomes and assesses the importance of these findings when controlled for baseline clinical characteristics and biomarker values, demonstrating that serial biomarker values during early infection of COVID-19 act as independent predictors of poor outcomes.

Implications of all the available evidence

We demonstrated patterns of abnormal 5-day trajectories of viral and host-response biomarkers that can help identify patients admitted to hospital with COVID-19 who have a worse prognosis and might benefit from further antiviral or immunomodulatory treatment. Our results suggest that both active viral replication and ongoing inflammation have a role in the pathogenesis of COVID-19 among patients who are admitted to hospital.

hospital who might benefit from neutralising monoclonal antibody (nMAb) treatments.[5,6]

Data are limited, however, on the temporal kinetics of virological and immunological markers in patients admitted to hospital.[7–14] Smaller studies, mainly retrospective and from single centres, suggest an association between persistent elevation of CRP, IL-6, and D-dimer, and worse outcomes,[7,9,11] although other studies did not demonstrate this finding.[12] A dysregulated immune response is often viewed as the primary cause of pathology in patients admitted to hospital, but some data suggest that continuous viral replication could fuel the inflammatory response and disease severity.[15,16] Analysis of the dynamics of biomarkers in this population can shed light on this important part of pathogenesis.

The Therapeutics for Inpatients with COVID-19 (TICO) platform trial within the Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV) programme did international, masked, randomised, placebo-controlled trials of four different nMAbs in patients admitted to hospital with COVID-19, none of which had an overall effect on the primary outcome of time to sustained recovery.[17–20] Plasma nucleocapsid antigen, anti-nucleocapsid antibody, anti-spike antibody, CRP, IL-6, and D-dimer were measured at baseline (day 0) before randomisation and at day 1, day 3, and day 5. We aimed to analyse the trajectories in these biomarkers, their associations with clinical outcomes, and to explore subpopulations for differences in trajectory patterns.

Methods

Study design and participants

We did a secondary analysis of data collected as part of the TICO–ACTIV-3 trials using a prospective cohort design. We

included participants enrolled in TICO–ACTIV-3 who required admission for COVID-19, had laboratory-confirmed SARS-CoV-2 infection, symptoms for 12 days or less, and no organ failure, at 108 sites in Denmark (ten sites), Greece (five sites), Poland (one site), Singapore (one site), Spain (eight sites), Switzerland (one site), Uganda (six sites), the UK (three sites), and the USA (73 sites). Patients were randomly assigned to placebo or one of four nMAbs: bamlanivimab (Aug 5 to Oct 13, 2020);[19] sotrovimab (Dec 16, 2020, to March 1, 2021),[18] amubarvimab combined with romlusevimab (Dec 16, 2020, to March 1, 2021),[18] and tixagevimab combined with cilgavimab (Feb 10 to Sept 30, 2021).[17] Patients receiving no oxygen or standard oxygen therapy via nasal cannula were eligible. Patients receiving high-flow nasal oxygen (HFNO), or non-invasive ventilation (NIV) were excluded in the sotrovimab and amubarvimab combined with romlusevimab studies,[18] and those requiring invasive mechanical ventilation were excluded from all trials. In addition, patients who previously received passive immunotherapy for COVID-19, pregnant women, and patients unwilling to abstain from sexual intercourse or practise appropriate contraception to day 90 were also excluded from all trials. Remdesivir was provided to all participants unless contraindicated.

We included all participants in our study who had measurements for plasma nucleocapsid antigen, anti-nucleocapsid antibody, anti-spike antibody, CRP, IL-6, or D-dimer at baseline (day 0 of trial enrolment) and at least one of days 1, 3, or 5.

Approvals for the TICO-ACTIV-3 trial protocols, including secondary analyses, were obtained from institutional review boards at all participating sites. The trials were registered with ClinicalTrials.gov (NCT04501978). Written informed consent for trial participation was obtained from each enrolled patient or a legally authorised representative, as applicable.

Outcomes

The primary outcome was time to sustained recovery, defined as return to home for 14 consecutive days, to day 90. Secondary outcomes included all-cause mortality to day 90 and a seven-category pulmonary ordinal scale assessed on day 7.[20]

Procedures

Demographic characteristics and information on comorbidities (appendix p 21), concomitant medications, vaccination status, and symptom duration were collected at the time of trial enrolment (baseline). Baseline disease severity was recorded on the seven-category pulmonary ordinal scale.

Samples were stored centrally at Advanced BioMedical Laboratories (Cinnaminson, NJ, USA), and analysed by the Frederick National Laboratory (Frederick, MD, USA).

Plasma nucleocapsid antigen was quantified using the Quanterix SARS-CoV-2 Nucleocapsid assay (Quanterix, Billerica, MA, USA), clinical cutoff for positive result 3 ng/L.

Anti-nucleocapsid antibody was measured using the BioRad SARS-CoV-2 assay (BioRad, Hercules, CA, USA). A signal-to-cutoff ratio (optical density divided by that of the control) higher than 1 was considered positive.

Neutralising anti-spike antibody was determined using the GenScript SARS-CoV-2 Surrogate Virus Neutralization assay (GenScript, Piscataway, NJ, USA). A positive result was defined as 30% or higher binding inhibition.[21]

CRP and IL-6 were measured using electrochemiluminescence (Meso Scale Discovery, Gaithersburg, MA, USA). D-dimer was measured by an enzyme-linked fluorescent assay (BioMerieux, Durham, NC, USA). 10 mg/L was used as the upper limit of normal for CRP, 2 ng/L for IL-6, and 0·5 mg/L for D-dimer.

Infection with the delta variant was established with baseline midturbinate nasal swabs collected from participants enrolled after May 1, 2021, using reverse transcriptase PCR. Participants enrolled before this date were considered to be infected with a non-delta variant.

Statistical analysis

The bivariate relationships between plasma nucleocapsid antigen, anti-nucleocapsid antibody, CRP, and D-dimer were visualised with heat maps, at baseline and each follow-up day. Each bivariate density was estimated using a two-dimensional-binned approach with a standard bivariate Gaussian kernel and bandwidths selected via the direct plugin method.[22]

To identify factors associated with differences in trajectories over time for each biomarker, we investigated subgroups by baseline factors, comprising age, sex, comorbidities, COVID-19 vaccination status, symptom duration, pulmonary ordinal scale, viral variant, plasma nucleocapsid antigen concentration, anti-spike antibody status, and trial allocation (nMAb or placebo).

Longitudinal differences in plasma nucleocapsid antigen, anti-nucleocapsid antibody, CRP, IL-6, and D-dimer between subgroups were estimated using linear mixed models, with fixed effects for subgroup, trial allocation (nMAb or placebo), visit (day 0, 1, 3, and 5) as a class factor, and a random term for intercept (patient effect). From this model the mean difference over visits between subgroups was estimated. Differences between subgroups were summarised as geometric means for the log-transformed measurements (plasma nucleocapsid antigen, CRP, IL-6, and D-dimer) and as the absolute difference (anti-nucleocapsid antibody and anti-spike antibody). Raw means or geometric means were plotted by subgroup and timepoint. A sensitivity analysis limited to participants randomly assigned to placebo was performed.

See Online for appendix

To explore differences in trajectories and associations with clinical outcomes, we defined five high-risk trajectory groups among participants who were alive on day 5 and had measurements available from both day 0 and day 5. Plasma nucleocapsid antigen, CRP, IL-6, and D-dimer high-risk groups were defined as being in the upper quartile of both the day 0 and the day 5 distributions. The anti-nucleocapsid

antibody high-risk group was defined by a negative result on day 0 and a concentration in the lower quartile on day 5. We identified risk factors for being in these high-risk trajectory groups using a multivariable logistic regression model. Models were selected using bidirectional stepwise variable selection minimising the Bayesian information criteria, and associations with baseline factors were characterised by the odds ratio of falling into the high-risk trajectory groups.[23] The maximal candidate model for the model selection procedure included all baseline characteristics: age, sex, geographical region, comorbidities, COVID-19 vaccination status, symptom duration, pulmonary ordinal scale, viral variant, plasma nucleocapsid antigen concentration of 1000ng/L or higher, anti-spike antibody status, and nMAb allocation in trial. Associations were only reported for variables that were selected into the final multivariable logistic regression model.

Associations between each high-risk trajectory group and clinical outcomes were then assessed. Given that biomarker values to day 5 were used to define the exposure variable, participants who died before day 5 were excluded. Time to sustained recovery was assessed with a Fine-Gray competing risk-hazard regression model to estimate the subhazard ratio for sustained recovery, accounting for the competing risk of death before recovery. Day-90 all-cause mortality was assessed with Cox proportional hazards models. Day-7 pulmonary ordinal scale was assessed with a proportional odds model, where the odds ratio reflects the odds of being in a better category. Additional adjusted models were constructed. The first models adjusted for age, geographical region, viral variant, chronic kidney disease, and being immunocompromised. To isolate any added predictive value of repeat measurement on day 5, the second models adjusted for baseline biomarker concentration and COVID-19 severity (as a pulmonary ordinal scale).

Nominal p values equal to or lower than 0·05 were considered significant. Statistical analyses were done using SAS (version 9.4) and R (version 4.1).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Our study included 2149 participants from 108 sites with measurements at baseline and at least one follow-up day (day 1, day 3, or day 5; appendix p 2). Median age was 57 years (IQR 46–68), 1246 (58·0%) of 2149 participants were male and 903 (42·0%) were female, 1792 (83·4%) had at least one comorbidity, and 1764 (82·1%) were unvaccinated (table 1). Mortality to day 90 was 172 (8·0%) of 2149 and 189 (8·8%) participants had sustained recovery.

Median symptom duration was 8 days (IQR 6–10), and 1596 (74·3%) participants required oxygen supplementation at enrolment, including a small proportion requiring HFNO or NIV. Use of remdesivir was reported in 1296 (60·3%) of 2149 before enrolment and 1990 (92·6%) after randomisation because this medication was provided as part of the trial. The baseline proportion receiving systemic corticosteroids was 1465 (68·2%) overall, decreasing to 1057 (49·2%) by day 5, and 1674 (77·9%) among participants

	Number of participants or results
All participants	2149
Age, years	57 (46–68)
Sex	
Female	903 (42·0%)
Male	1246 (58·0%)
Geographical region	
Africa	86 (4·0%)
Asia	24 (1·1%)
Europe	325 (15·1%)
USA	1714 (79·8%)
Ethnicity	
Asian	96 (4·5%)
Black	499 (23·2%)
Hispanic	406 (18·9%)
White	1071 (49·8%)
Others	77 (3·6%)
Comorbidities	
Cardiovascular disease	1033 (48·1%)
Chronic kidney disease	211 (9·8%)
Chronic lung disease	326 (15·2%)
Diabetes	618 (28·8%)
Hepatic impairment	36 (1·7%)
HIV	36 (1·7%)
Immunocompromise	328 (15·3%)
Obesity	1151 (53·7%)
Any comorbidity	1792 (83·4%)
Concomitant medications	
Corticosteroids*	1465 (68·2%)
Heparin, therapeutic dose	85 (4·0%)
Remdesivir†	1990 (92·6%)
Trial active agent	1178 (54·8%)
COVID-19 vaccination status	
Fully vaccinated‡	190 (8·8%)
Partially vaccinated	195 (9·1%)
Not vaccinated	1764 (82·1%)
Symptom duration, days	8 (6–10)
Pulmonary ordinal scale	
No supplementary oxygen	553 (25·7%)
<4 L oxygen per min	816 (38·0%)
≥4 L oxygen per min	582 (27·1%)
HFNO or NIV	198 (9·2%)
Viral variant	
Delta	658 (30·9%)
Other	1·474 (69·1%)
Nucleocapsid antigen result	
Positive	2033 (94·6%)
Positive, result ≥1000 ng/L	899 (41·9%)

(Table 1 continues on next page)

	Number of participants or results
(Continued from previous page)	
Antibody status	
Anti-nucleocapsid antibody positive	1330 (61·9%)
Anti-spike antibody positive	1065 (49·6%)
Biomarker results	
C-reactive protein, mg/L	31 (14–56)
IL-6, ng/L	6 (2–14)
D-dimer, mg/L	0·9 (0·6–1·4)

Dara are N, n (%), or median (IQR). All subcategory percentages do not add up to 100% because only the primary characteristics are included. HFNO=high-flow nasal oxygen. NIV=non-invasive ventilation. *10 mg or more of prednisolone or equivalent. †Started before enrolment or at time of enrolment. ‡Full primary vaccination course completed; symptoms started at least 14 days after the last dose.

Table 1: **Baseline characteristics**

who required oxygen supplementation. Only 85 (4·0%) received therapeutic heparin. The delta variant was detected in 658 (30·9%) participants.

We found a positive baseline anti-nucleocapsid antibody result for 1330 (61·9%) participants (mean signal-to-cutoff ratio 2·4, SD 1·9) and positive anti-spike antibody-neutralising activity for 1065 (49·6%; mean binding inhibition 38·1%, SD 34·1). Most participants had detectable plasma nucleocapsid antigen (2033 [94·6%] of 2149, median 1·510 ng/L, IQR 252–4709).

Biomarker concentrations from baseline to day 5 were evaluated individually on longitudinal box-whisker plots (appendix p 3), and relationships between markers at each timepoint were explored with heat maps of bivariate distributions (figure 1). This approach outlined general trends over time and allowed visual identification of subpopulations with atypical trajectories for further quantification and hypothesis testing in subsequent analyses.

Most participants followed a general trend of steady decline from baseline to day 5 for plasma nucleocapsid antigen (median 1510 ng/L, IQR 252–4709, to 7 ng/L, 4–30) and CRP (median 30·8 mg/L, IQR 14·2–55·9, to 6·6 mg/L, 2·6–17·9), although IL-6 (median of 5·93 ng/L, IQR 2·35–14·29, to 3·76 ng/L, 1·42–12·47) and D-dimer (median 0·93 mg/L, IQR 0·63–1·44, to 0·73 mg/L, 0·44–1·43) declined more slowly (appendix p 3). The overall anti-nucleocapsid antibody trajectory increased over the same timepoints from a median signal-to-cutoff ratio of 2·97 (IQR 0·21–4·07) to 4·01 (3·52–4·37). An expected marked increase from baseline (median 29·1% binding inhibition, IQR 8·0–69·0) to day 1 (median 96·0% binding inhibition, 85·1–97·7) was observed for anti-spike antibody, reflecting interaction between nMAb and the assay, and we therefore did not further analyse anti-spike antibody trajectories.

The participant-level relationship of plasma nucleocapsid antigen to anti-nucleocapsid antibody showed an overall decrease in plasma nucleocapsid antigen towards the threshold of detection by day 5 with simultaneous increase in anti-nucleocapsid antibody (figure 1). A notable subpopulation demonstrated persistently higher plasma nucleocapsid antigen despite normal increasing concentrations of anti-nucleocapsid antibody (figure 1). Interestingly, another subpopulation demonstrated persistently lower anti-nucleocapsid antibody. Although some of these participants followed the standard declining plasma nucleocapsid antigen trajectory, others showed persistently higher plasma nucleocapsid antigen, suggesting inability to effectively clear the virus. Some participants, but not all, with higher plasma nucleocapsid antigen by day 5 also had a higher degree of inflammation measured by CRP (figure 1). IL-6 induces CRP production,[24] and we found both CRP and IL-6 generally declined together towards normal values by day 5, but there were subpopulations where they remained elevated (figure 1). More than half of the cohort had D-dimer concentrations higher than the upper limit of normal (0·5 mg/L) at day 5 (figure 1). Importantly, although some of these also had persistent inflammation (CRP higher than the upper limit of normal, 10 mg/L) at day 5, the majority had normalised CRP at that timepoint, pointing to a persistent coagulopathic response independent of ongoing inflammation.

We hypothesised that participants with atypical virological and immunological biomarker trajectories observed in bivariate analysis (figure 1) would have baseline characteristics previously associated with poor outcomes. We summarised key trends in longitudinal models comparing biomarkers of six selected subgroups, comprising age, male sex, chronic kidney disease (illustrating similar trends across other comorbidities such as cardiovascular disease and immunocompromise; figure 2; appendix pp 4–20, 22), and baseline factors related to the SARS-CoV-2 infection (symptom duration, pulmonary ordinal scale, and viral variant).

In 11 of 17 subgroups, we observed elevated mean plasma nucleocapsid antigen over 5 days relative to their complemental subgroups. For example, plasma nucleocapsid antigen concentrations for participants with chronic kidney disease were 424% (95% CI 319–559) higher than in those without chronic kidney disease. Sensitivity analysis restricted to the placebo group showed similar results, demonstrating minimal effects of nMAb treatment (appendix p 23).

Anti-nucleocapsid antibody concentrations to day 5 were lower for many of the same subgroups that had higher plasma nucleocapsid antigen concentrations. Notable exceptions were participants requiring HFNO and NIV, for whom 39% (95% CI 17–60) higher anti-nucleocapsid antibody concentrations were observed compared with those with lower supplemental oxygen requirements, and participants who were not fully vaccinated who had 142% (121–163) higher concentrations than participants who were vaccinated. Importantly, participants who are vaccinated also had markedly higher neutralising anti-spike antibody concentrations (12·9% higher binding inhibition, 95% CI 7·0–18·9) when restricting the analysis to participants randomly assigned to placebo (appendix p 23).

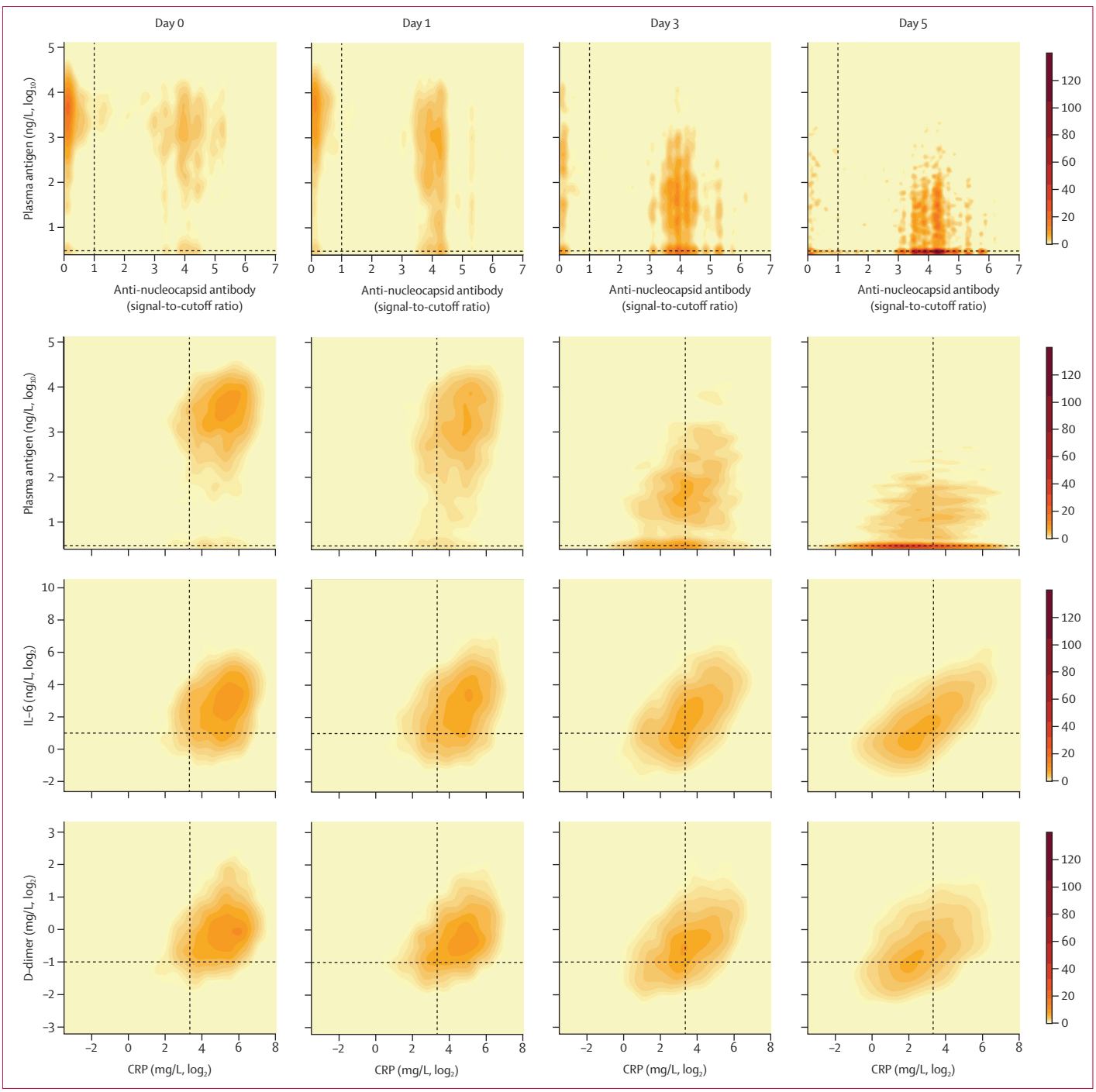

Figure 1: **Bivariate distributions of biomarkers of viral infection and immune response from baseline (day 0) to day 5**
Dashed lines represent the lower limit of detection (plasma nucleocapsid antigen), threshold of positivity (anti-nucleocapsid antibody), or upper limit of normal (CRP, IL-6, and D-dimer). Colour scale reflects the observed density relative to a fully uniform distribution across the displayed area. CRP=C-reactive protein.

Associations of subgroups with CRP, IL-6, and D-dimer largely mirrored the associations with plasma nucleocapsid antigen, although fewer reached statistical significance. Interestingly, when comparing markers of inflammation in participants requiring HFNO and NIV to those with lower oxygen requirements, we observed higher measurements over time of both CRP and IL-6, but the pooled difference was much higher for IL-6 (360%, 95% CI 300–433) than

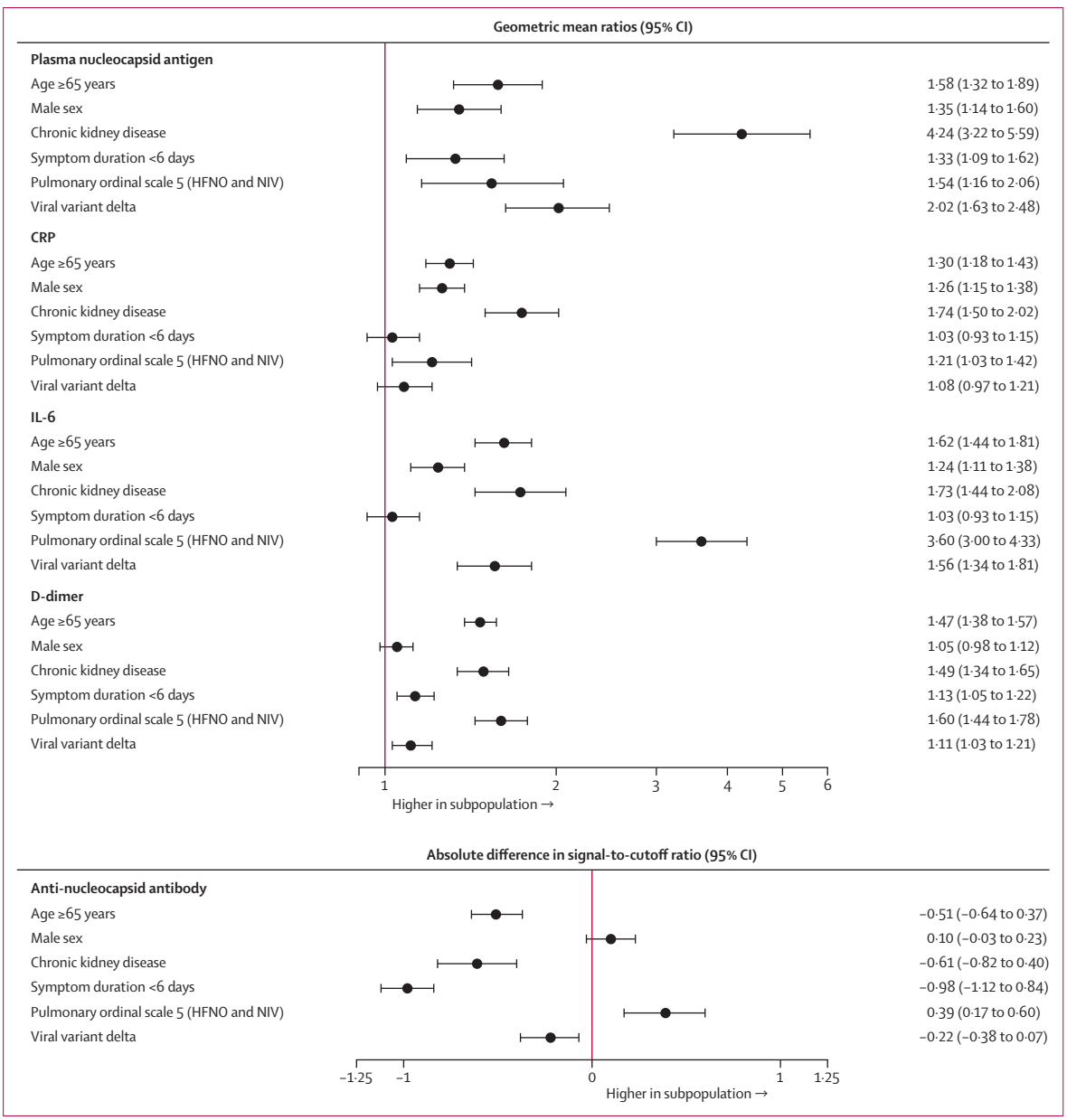

Figure 2: **Difference in trajectory of plasma nucleocapsid antigen, CRP, IL-6, D-dimer, and anti-nucleocapsid antibody, in selected subpopulations compared with the complemental subpopulation**

CRP=C-reactive protein. HFNO=high-flow nasal oxygen. NIV=non-invasive ventilation.

for CRP (121%, 103–142). This discrepancy between the magnitude of the IL-6 and CRP responses was not seen for other subgroups.

To look more directly at biomarker dynamics over 5 days, we defined five high-risk trajectory groups as persistently high nucleocapsid antigen, CRP, IL-6, and D-dimer (highest quartile at baseline and day 5) or persistently low anti-nucleocapsid antibody (negative at baseline and lowest quartile at day 5; figure 3A) To add potential clinical applicability, we used these high-risk trajectory groups to explore associations with clinical outcomes. These definitions were generally consistent with observations in bivariate analysis (figure 1).

First, we identified clinical characteristics most predictive of the high-risk trajectories in univariable and multivariable analysis (appendix pp 24–29) and found those with at-risk clinical subgroups identified in the longitudinal analysis (figure 2; appendix pp 22–23) were consistently part of the high-risk trajectory groups. Chronic kidney disease (odds ratio [OR] 4·19, 95% CI 2·86–6·12), higher baseline pulmonary ordinal scale (OR 4·66, 2·72–7·98; for HFNO and NIV *vs* no supplementary oxygen), and negative anti-spike

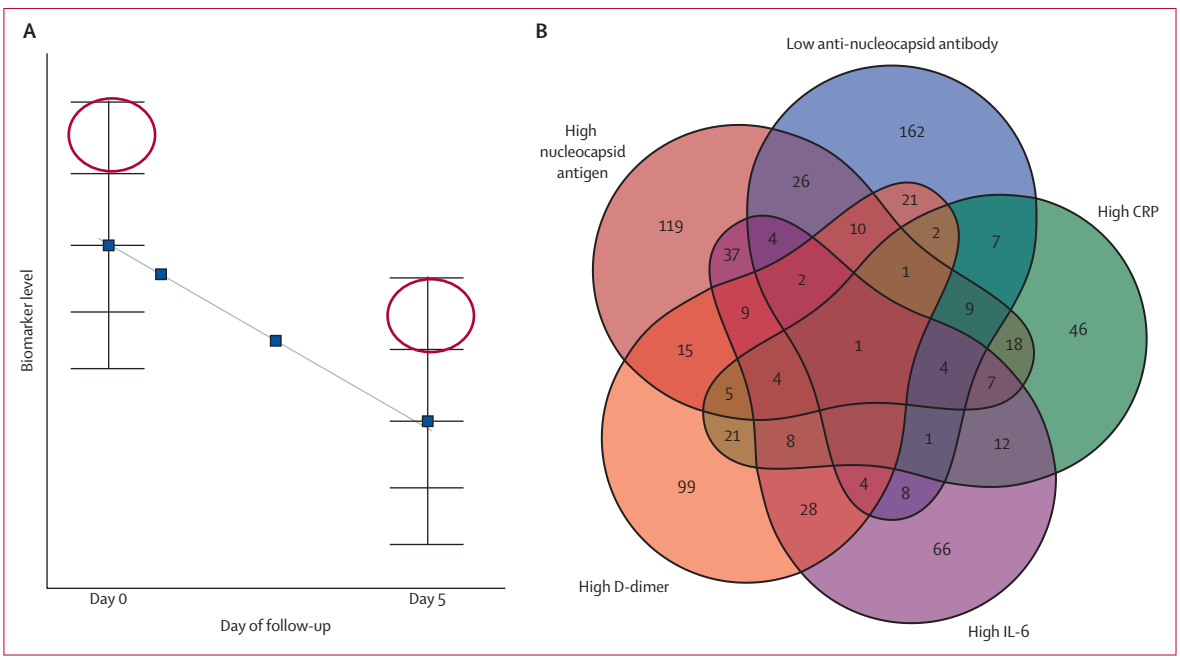

Figure 3: **Five high-risk trajectory groups and overlap between groups in the study cohort**
High-risk trajectory groups are defined as participants in the upper quartile on both day 0 and day 5 (red circles), except for the group with persistently low anti-nucleocapsid antigen, which was defined by having a negative result on day 0 and a concentration in the lower quartile on day 5 (A). Overlap between the four high-risk trajectory groups. Numbers in the represent number of participants (B). CRP=C-reactive protein.

antibody serostatus (OR 6·72, 4·88–9·40) were most predictive of persistently high plasma nucleocapsid antigen. By contrast, immunocompromise (OR 2·21, 1·54–3·17), lower baseline pulmonary ordinal scale indicating lower oxygen requirement (OR 0·29, 0·15–0·53; for HFNO and NIV *vs* no supplementary oxygen) and negative anti-spike antibody serostatus (OR 6·44, 4·45–9·55) were the strongest predictors of persistently low anti-nucleocapsid antibody. HFNO and NIV requirement was also associated with persistently high IL-6 (OR 4·97, 3·08–8·12; for HFNO and NIV *vs* no supplementary oxygen), but not persistently high CRP. The high-risk D-dimer trajectory was the only group with a difference among geographical regions (OR 9·22, 5·58–15·21; for Africa *vs* the USA). 101 (34·7%) of 291 participants in the persistently elevated plasma nucleocapsid antigen trajectory overlapped with high-risk inflammatory trajectories (CRP, IL-6, or both), and persistently low anti-nucleocapsid antibody most commonly overlapped with elevated plasma nucleocapsid antigen (21·8%; figure 3B, appendix p 29).

Participants in the high-risk trajectory groups with persistently high plasma nucleocapsid antigen, CRP, IL-6, and D-dimer all had significantly higher risk of worse early outcome (higher day-7 pulmonary ordinal scale), worse day-90 all-cause mortality, and worse rate of sustained recovery to day 90 in the unadjusted analysis (table 2). Most of these associations persisted after adjustment for baseline factors associated with poor clinical outcomes, and after adjustment for baseline value of the specific biomarker and disease severity (table 2). The association with day-90 all-cause mortality was statistically significant in both adjusted models for the plasma nucleocapsid antigen high-risk group (hazard ratio [HR] 3·00, 95% CI 2·15–4·17, after adjustment for baseline factors, and HR 1·72, 1·15–2·58, after adjustment for baseline biomarker concentration and disease severity), the CRP high-risk group (HR 2·06, 1·38–3·06, after adjustment for baseline factors, and HR 1·73, 1·11–2·69, after adjustment for baseline biomarker concentration and disease severity), and the IL-6 high-risk group (HR 3·30, 2·32–4·70, after adjustment for baseline factors, and HR 1·56, 1·00–2·43, after adjustment for baseline biomarker concentration and disease severity). Similar associations for plasma nucleocapsid antigen, CRP, and IL-6 were observed with the rate of sustained recovery to day 90. Participants in the anti-nucleocapsid antibody high-risk group did not have worse pulmonary ordinal scale on day 7, but this group was associated with higher day-90 all-cause mortality (HR 1·59, 1·01–2·51) and lower rate of sustained recovery (RRR 0·75, 95% CI 0·63–0·89) in the analysis adjusted for baseline biomarker concentration and disease severity. The high-risk group with persistently high D-dimer was associated with all three outcomes in the unadjusted analysis, but these associations did not reach statistical significance when adjusted for baseline biomarker concentration and disease severity.

Discussion

In this population of people admitted to hospital with COVID-19, we evaluated biomarkers associated with viral burden (plasma nucleocapsid antigen), endogenous

antibody production (anti-nucleocapsid antibody), inflammatory response (CRP and IL-6), and D-dimer, and observed important patterns in their dynamics. In addition to the linked general trends over time of decreasing plasma nucleocapsid antigen, CRP, IL-6, and D-dimer, and increasing anti-nucleocapsid antibody, we found small subpopulations with atypical trajectories. Previous evaluation of the kinetics of plasma nucleocapsid antigen, serological status, and inflammatory markers during COVID-19 have focused on severity of illness and mortality outcomes.[7,9–13] This study characterises atypical biomarker trajectories early in hospital admission for subgroups identified by age, sex, comorbidities, and factors associated with SARS-CoV-2 infection (eg, baseline antibody status), lending important insight into how these biomarkers behave over time in specific populations. Interestingly, characteristic patterns of less favourable biomarker response (higher viral burden, lower antibody production, and higher inflammation) were found for many subgroups with well established higher risk, such as patients with chronic kidney disease. We further defined high-risk trajectories and found that persistently abnormal markers of viral clearance (plasma nucleocapsid antigen and anti-nucleocapsid antibody) or inflammation (CRP and IL-6) at day 5 demonstrated worse recovery and mortality by day 90 even when adjusting for baseline clinical characteristics and biomarker concentrations. These data suggest day-5 biomarker measurements add predictive value beyond baseline clinical characteristics and biomarker concentrations.

We found a subgroup of participants with persistent plasma nucleocapsid antigen together with elevated CRP and IL-6 through 5 days of measurement, implicating ongoing viral burden as a driver for persistent inflammation. Participants with the highest values of any of these biomarkers at day 5 were at particularly high risk for poor outcomes. This finding is consistent with previous studies showing that persistence of SARS-CoV-2 RNAemia during early infection is associated with severity of disease,[14] and several reports indicating the patients with the most severe COVID-19 have persistent inflammation and improved outcomes with immunomodulatory agents.[12,13] The idea of a viral pathogen acting as a catalyst for persistent inflammation is not a new concept. For example, reduction of viral burden in chronic HIV with early initiation of antivirals led to reduction of chronic inflammatory signalling and reduction of AIDS, serious non-AIDS events, and mortality.[25] On the basis of our findings, it could be postulated that early escalation of antivirals in patients with COVID-19 with evidence of persistent viraemia could reduce inflammatory signalling. In addition, our data demonstrate that participants with more advanced disease and need for HFNO and NIV had higher concentrations of day-5 plasma nucleocapsid antigen and IL-6, but not CRP and D-dimer. Adding immunomodulators to dexamethasone, particularly inhibitors of IL-6, reduces mortality in patients requiring HFNO and NIV.[26]

	Pulmonary ordinal scale on day 7, OR (95% CI)	Day-90 all-cause mortality, HR (95% CI)	Day-90 sustained recovery, RRR (95% CI)
Persistently high plasma nucleocapsid antigen			
Unadjusted	6·02 (4·76–7·64)	4·50 (3·29–6·15)	0·41 (0·36–0·46)
Adjustment for baseline factors*	4·81 (3·76–6·17)	3·00 (2·15–4·17)	0·46 (0·40–0·52)
Adjustment for baseline biomarker and disease severity†	1·43 (1·05–1·94)	1·72 (1·15–2·58)	0·65 (0·56–0·76)
Persistently low anti-nucleocapsid antibody			
Unadjusted	0·81 (0·64–1·02)	1·41 (0·96–2·08)	0·87 (0·76–0·99)
Adjustment for baseline factors*	0·96 (0·75–1·22)	1·32 (0·88–1·97)	0·80 (0·69–0·92)
Adjustment for baseline biomarker and disease severity†	0·91 (0·66–1·25)	1·59 (1·01–2·51)	0·75 (0·63–0·89)
Persistently high CRP			
Unadjusted	3·17 (2·34–4·31)	3·37 (2·30–4·94)	0·52 (0·43–0·63)
Adjustment for baseline factors*	2·53 (1·84–3·48)	2·06 (1·38–3·06)	0·57 (0·46–0·70)
Adjustment for baseline biomarker and disease severity†	1·65 (1·13–2·41)	1·73 (1·11–2·69)	0·73 (0·59–0·91)
Persistently high IL-6			
Unadjusted	6·65 (5·05–8·78)	5·67 (4·12–7·80)	0·37 (0·31–0·43)
Adjustment for baseline factors*	3·92 (2·94–5·23)	3·30 (2·32–4·70)	0·48 (0·41–0·57)
Adjustment for baseline biomarker and disease severity†	1·40 (0·96–2·03)	1·56 (1·00–2·43)	0·80 (0·65–0·98)
Persistently high D-dimer			
Unadjusted	2·04 (1·60–2·62)	4·04 (2·93–5·58)	0·54 (0·46–0·62)
Adjustment for baseline factors*	1·56 (1·20–2·03)	2·19 (1·55–3·09)	0·70 (0·60–0·82)
Adjustment for baseline biomarker and disease severity†	1·38 (0·95–2·01)	1·25 (0·79–1·98)	0·82 (0·66–1·01)

Persistently high trajectory groups indicate a concentration in the upper quartile of the distribution both at baseline and at day 5. Persistently low trajectory groups indicate negative concentration at baseline and in the lower quartile of the distribution at day 5. Estimates are presented unadjusted and for two adjusted models. CRP=C-reactive protein. HR=hazard ratio. OR=odds ratio. RRR=recovery-rate ratio. *Adjusted for age, geographical region, viral variant, chronic kidney disease, and immunocompromise. †Adjusted for baseline pulmonary ordinal scale and baseline level of the corresponding biomarker.

Table 2: **Association between high-risk trajectory groups and clinical outcomes**

We observed persistently low anti-nucleocapsid antibody over 5 days predominantly among patients with at least one comorbidity, most commonly chronic kidney disease, diabetes, and immunosuppression (appendix p 25). Adjustment for baseline clinical factors attenuated differences in pulmonary ordinal scale at 7 days and 90-day mortality for the group with persistently low anti-nucleocapsid antibody, indicating that poorer outcomes were largely driven by high-risk baseline clinical factors. Participants with persistently low anti-nucleocapsid antibody trajectories included a small group of fully vaccinated individuals (59 [24·2%] of 285) who also had associated higher concentrations of neutralising anti-spike antibody (appendix p 14), similar to a previous study on a non-hospitalised population.[27] We found no difference in plasma nucleocapsid antigen trajectory in the vaccinated study population with breakthrough infection compared with patients who were unvaccinated, suggesting adequate viral clearance.

Baseline measurements of biomarkers and clinical characteristics have helped predict risk of progression and are proposed as a guide to initial therapeutics in patients admitted to hospital with COVID-19.[1–6] Our findings add to this knowledge by demonstrating that serial biomarker

measurements identify patients with worse prognosis who might benefit from treatment intensification before clinical decline. However, further evaluation will be necessary to facilitate clinical translatability and guidance. For example, although an upper quartile plasma nucleocapsid antigen in this cohort portends higher risk of poor outcomes, a cutoff to be used across the general population is less clear, especially in the setting of evolving variants.

Our study has important strengths and limitations. International, multicentre recruitment with a large sample size ensured generalisability. Because data were collected in a randomised trial, clinical outcomes were ascertained reliably and consistently across individual studies, with minimal loss to follow-up. However, plasma nucleocapsid antigen concentrations might lag actual viral replication and might therefore not be an ideal real-time proxy for viral kinetics, and it is also unclear whether plasma nucleocapsid antigen can differentiate ongoing viral replication from non-viable shedding from cell death. The universal use of the antiviral remdesivir could also have affected the results. Not all original trial participants had follow-up biomarker measurements. This characteristic was mostly caused by early discharge before planned blood sampling, which could have introduced selection bias towards a population with more severe symptoms of COVID-19. However, absence of a follow-up biomarker measurement was not associated with observed clinical outcomes when adjusting for baseline biomarker and disease severity, which supports generalisability of those adjusted analyses to the broader trial population. Finally, analyses are exploratory given that we considered many outcomes without adjustment for inflation of type 1 error.

In summary, we have identified specific patterns in viral, immunological, and inflammatory-biomarker trajectories that are associated with certain patient characteristics at baseline and poor clinical outcomes. These data suggest a link between persistent viral burden and inflammation. Serial measurements of plasma nucleocapsid antigen and CRP, in particular, have the potential to identify patients who might benefit from more intensive monitoring or additional antiviral treatment, although specific cutoffs remain to be identified.

Contributors

TOJ, GAG, MKJ, TAM, BG, MAM, KS-S, and ERK, conceived of the study. All authors participated in its design and interpretation of the results. TAM and GAG led the statistical analysis and have directly accessed and verified the underlying data. TOJ drafted the manuscript, and all other authors contributed to revisions. All authors read and approved the final manuscript and have final responsibility for the decision to submit the manuscript for publication. All authors accept responsibility to submit for publication.

Declaration of interests

ALG reports receiving institutional funding from Novavax, institutional partnership with AstraZeneca, and being personally named as inventor on a patent used by AstraZeneca. DDM reports receiving grants from the Danish National Research Foundation. DL reports participation in a study funded by Gilead with no salary support. EM reports payments to his institution received from SciClone Pharmaceuticals, Regeneron Pharmaceuticals, Pfizer, Chemic Labs and KODA Therapeutics, and Cidara, and payment for participating in an advisory board for Basilea. KW reports honorarium for Acute Respiratory Distress Syndrome (ARDS) International Conference in 2023, and travel support from University of California San Francisco. MAM reports institutional funding by the US Department of Defence, Roche-Genetech, and Quantum Health, and personal consulting fees from Gilead, Novartis, and Johnson & Johnson. MKJ reports grants received from Regeneron, Laurent, and Gilead, honoraria from Gilead, and a leadership role with the HIV Medicine Association Board of Directors. PC reports consulting fees from Eli Lilly and Regeneron. All other authors declare no competing interests. All authors were not precluded from accessing data in the study.

Data sharing

De-identified data from TICO trials will be made available 1 year after publication of final results from the platform. Supporting documents will be made available, including the protocol, statistical analysis plan, informed consent document, and data dictionary. Data will be made available to researchers after approval of a proposal for use of the data. Proposals for data use should be submitted using the Research Proposal Form on the INSIGHT website, www.insight-trials.org.

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