Phenotypes of disease severity in a cohort of hospitalized COVID-19 patients: Results from the IMPACC study

Al Ozonoff,[a,1] Joanna Schaenman,[b,1] Naresh Doni Jayavelu,[c,1] Carly E. Milliren,[a,1] Carolyn S. Calfee,[d] Charles B. Cairns,[e] Monica Kraft,[f] Lindsey R. Baden,[g] Albert C. Shaw,[h] Florian Krammer,[i] Harm van Bakel,[i] Denise A. Esserman,[h] Shanshan Liu,[a] Ana Fernandez Sesma,[i] Viviana Simon,[i] David A. Hafler,[h] Ruth R. Montgomery,[h] Steven H. Kleinstein,[h] Ofer Levy,[g] Christian Bime,[f] Elias K. Haddad,[e] David J. Erle,[d] Bali Pulendran,[j] Kari C. Nadeau,[j] Mark M. Davis,[j] Catherine L. Hough,[k] William B. Messer,[k] Nelson I. Agudelo Higuita,[l] Jordan P. Metcalf,[l] Mark A. Atkinson,[m] Scott C. Brakenridge,[m] David Corry,[n] Farrah Kheradmand,[n] Lauren I.R. Ehrlich,[o] Esther Melamed,[o] Grace A. McComsey,[p] Rafick Sekaly,[p] Joann Diray-Arce,[a] Bjoern Peters,[q] Alison D. Augustine,[r] Elaine F. Reed,[b] Matthew C. Altman,[c,2] Patrice M. Becker,[r,2] and Nadine Rouphael,[s,2]* the IMPACC study group members

[a]Clinical & Data Coordinating Center (CDCC); Precision Vaccines Program, Boston Children's Hospital, Boston, MA, United States
[b]David Geffen School of Medicine at the University of California Los Angeles, Los Angeles, CA, United States
[c]Benaroya Research Institute, Seattle, WA, United States
[d]University of California San Francisco School of Medicine, San Francisco, CA, United States
[e]Drexel University/Tower Health Hospital, Philadelphia, PA, United States
[f]University of Arizona, Tucson, AZ, United States
[g]Boston Clinical Site: Precision Vaccines Program, Boston Children's Hospital, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA, United States
[h]Yale School of Medicine, and Yale School of Public Health, New Haven, CT, United States
[i]Icahn School of Medicine at Mount Sinai, New York, NY, United States
[j]Stanford University, Palo Alto, CA, United States
[k]Oregon Health Sciences University, Portland, OR, United States
[l]Oklahoma University Health Sciences Center, Oklahoma, OK, United States
[m]University of Florida, Gainesville and University of South Florida, Tampa, FL, United States
[n]Baylor College of Medicine, and the Center for Translational Research on Inflammatory Diseases, Michael E. DeBakey, Houston, TX, United States
[o]The University of Texas at Austin, Austin, TX, United States
[p]Case Western Reserve University, Cleveland, OH, United States
[q]La Jolla Institute for Immunology, La Jolla, CA, United States
[r]National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States
[s]Emory University, Atlanta, GA, United States

Summary

Background Better understanding of the association between characteristics of patients hospitalized with coronavirus disease 2019 (COVID-19) and outcome is needed to further improve upon patient management.

Methods Immunophenotyping Assessment in a COVID-19 Cohort (IMPACC) is a prospective, observational study of 1164 patients from 20 hospitals across the United States. Disease severity was assessed using a 7-point ordinal scale based on degree of respiratory illness. Patients were prospectively surveyed for 1 year after discharge for post-acute sequalae of COVID-19 (PASC) through quarterly surveys. Demographics, comorbidities, radiographic findings, clinical laboratory values, SARS-CoV-2 PCR and serology were captured over a 28-day period. Multivariable logistic regression was performed.

Findings The median age was 59 years (interquartile range [IQR] 20); 711 (61%) were men; overall mortality was 14%, and 228 (20%) required invasive mechanical ventilation. Unsupervised clustering of ordinal score over time revealed distinct disease course trajectories. Risk factors associated with prolonged hospitalization or death by day

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*Corresponding author.
E-mail address: nroupha@emory.edu (N. Rouphael).
[1] Co-first authors.
[2] Co-senior authors.

28 included age ≥ 65 years (odds ratio [OR], 2.01; 95% CI 1.28−3.17), Hispanic ethnicity (OR, 1.71; 95% CI 1.13−2.57), elevated baseline creatinine (OR 2.80; 95% CI 1.63− 4.80) or troponin (OR 1.89; 95% 1.03−3.47), baseline lymphopenia (OR 2.19; 95% CI 1.61−2.97), presence of infiltrate by chest imaging (OR 3.16; 95% CI 1.96−5.10), and high SARS-CoV2 viral load (OR 1.53; 95% CI 1.17−2.00). Fatal cases had the lowest ratio of SARS-CoV-2 antibody to viral load levels compared to other trajectories over time (p=0.001). 589 survivors (51%) completed at least one survey at follow-up with 305 (52%) having at least one symptom consistent with PASC, most commonly dyspnea (56% among symptomatic patients). Female sex was the only associated risk factor for PASC.

Interpretation Integration of PCR cycle threshold, and antibody values with demographics, comorbidities, and laboratory/radiographic findings identified risk factors for 28-day outcome severity, though only female sex was associated with PASC. Longitudinal clinical phenotyping offers important insights, and provides a framework for immunophenotyping for acute and long COVID-19.

Funding NIH.

Keywords: COVID-19; SARS-CoV-2; Viral load; Antibody

Research in context

Evidence before this study

We did a systematic search of the PubMed database from January 1st, 2020 until April 24th, 2022 using the search terms: "hospitalized" AND "SARS-CoV-2" OR "COVID-19" AND "Prospective" AND "Antibody" OR "PCR" OR "long term follow up" and applying the following filters: "Multicenter Study" AND "Observational Study". No language restrictions were applied. While clinical, laboratory, and radiographic features associated with severe COVID-19 in hospitalized adults have been described, description of the kinetics of SARS-CoV-2 specific assays available to clinicians (e.g. PCR and binding antibody) and their integration with other variables is scarce for both short and long term follow up. The current literature is comprised of several studies with small sample size, cross-sectional design with laboratory data typically only recorded at a single point in time (e.g., on admission), limited clinical characteristics, variable duration of follow up, single-center setting, retrospective analyses, kinetics of either PCR or antibody testing but not both, and outcomes such as death or, mechanical ventilation that do not allow delineation of variations in clinical course.

Added value of this study

In our large longitudinal multicenter cohort, the description of outcome severity, was not limited to survival versus death, but encompassed a clinical trajectory approach leveraging longitudinal data based on time in hospital, disease severity by ordinal scale based on degree of respiratory illness, and presence or absence of limitations at discharge. Fatal COVID-19 cases had the lowest ratio of antibody to viral load levels over time as compared to non-fatal cases. Integration of PCR cycle threshold and antibody values with demographics, baseline comorbidities, and laboratory/radiographic findings identified additional risk factors for outcome severity over the first 28 days. However, female sex was the only variable associated with persistence of symptoms over time. Persistence of symptoms was not associated with clinical trajectory over the first 28 days, nor with antibody/viral loads from the acute phase.

Implications of all the available evidence

The described calculated ratio (binding IgG/PCR Ct value) is unique compared to other studies, reflecting host pathogen interactions and representing an accessible approach for patient risk stratification. Integration of SARS-CoV-2 viral load and binding antibody kinetics with other laboratory as well as clinical characteristics in hospitalized COVID-19 patients can identify patients likely to have the most severe short-term outcomes, but is not predictive of symptom persistence at one year post-discharge.

Introduction

A comprehensive understanding of the interplay between pathogen, and host in modulating the disease course will enable identification of potential biomarkers, and host-directed therapeutic interventions against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and post-acute sequalae of coronavirus disease 2019 (COVID-19) (PASC). Mobilization of the global scientific community has produced significant findings

with unprecedented speed.[1] However, these studies had limitations including small sample size, cross-sectional design with laboratory data typically only recorded at a single point in time (e.g., on admission), limited clinical characteristics, variable duration of follow up, single-center setting, retrospective analyses, and basic outcomes (e.g. death, mechanical ventilation). Integration of longitudinal data is an attractive approach to characterize disease severity, and better understand patient outcomes by encompassing the totality of the disease course in terms of patient complications, persistence of symptoms and resource utilization. In contrast to categorical analysis of outcomes at a prespecified timepoint, using a longitudinal approach provides insights into mechanisms underlying the diverse clinical course observed in a large group of geographically, and demographically diverse hospitalized patients with COVID-19.

The National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) prospective longitudinal cohort study, ImmunoPhenotyping Assessment in a COVID-19 Cohort (or IMPACC [NCT04378777]), enrolled symptomatic, molecularly confirmed hospitalized COVID-19 patients from 20 different hospitals across the US. IMPACC prospectively collected clinical, laboratory, and radiographic data along with longitudinal biologic sampling of blood, and respiratory secretions for in-depth immunologic, and virologic testing with a one year follow up post discharge.[2]

Methods

Ethics

NIAID staff conferred with the Department of Health and Human Services Office for Human Research Protections (OHRP) regarding potential applicability of the public health surveillance exception [45CFR46.102(l)(2)] to the IMPACC study protocol. OHRP concurred that the study satisfied criteria for the public health surveillance exception, and the IMPACC study team sent the study protocol, and participant information sheet for review, and assessment to institutional review boards (IRBs) at participating institutions. Twelve institutions elected to conduct the study as public health surveillance, while 3 sites with prior IRB-approved biobanking protocols elected to integrate and conduct IMPACC under their institutional protocols (University of Texas at Austin, IRB 2020-04-0117; University of California San Francisco, IRB 20-30497; Case Western reserve university, IRB STUDY20200573) with informed consent requirements. Participants enrolled under the public health surveillance exclusion were provided information sheets describing the study, samples to be collected, and plans for data de-identification, and use. Those that requested not to participate after reviewing the information sheet were not enrolled. In addition, participants did not receive compensation for study participation while inpatient, and subsequently were offered compensation during outpatient follow-ups.

Study design and setting

The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting observational studies.[3] The design of the IMPACC study has been previously published[2] (see online supplement).

Study participants

Patients 18 years and older admitted to 20 US hospitals (affiliated with 15 academic institutions) were enrolled within 48 h of hospital admission. Only symptomatic cases with confirmed positive SARS-CoV-2 PCR were followed longitudinally.

Data collection, study variables, and biologic samples

Specific data elements were acquired via review of electronic medical records: participant demographic characteristics, and comorbidities, presenting signs, or symptoms, and onset, medications, diagnostic investigations (predefined laboratory values, and radiographic findings), and relevant clinical outcomes (oxygen- and ventilatory-support requirement, medications used, complications). Biologic samples consisted of blood, and mid-turbinate nasal swabs (staff or self collected; with step-by-step instructions provided to both staff and study participants). The timepoints were as follows: enrollment (day 1), and days 4, 7, 14, 21, and 28 post hospital admission (and if feasible, in discharged patients, days 14, and 28). Data lock on the data collected through day 28 from admission was performed on November 11th, 2021. Selected information was available after discharge for self-reported vaccination status, recurrent SARS-CoV2 infection, and persistence of symptoms, as well as mortality in patients with at least one follow-up at 3, 6, 9 and/or 12 months after discharge. Data lock on the survey data was performed on April 7, 2022. The full study data collection forms are provided in the online supplement and deidentified data is available upon request.

Outcomes

Clinical severity of illness was assessed using a 7-point ordinal scale (OS) adapted from the World Health Organization COVID-19, and NIAID disease ordinal severity scales,[4] (OS1= Not hospitalized, no limitations; OS2= Not hospitalized, activity limitations, or requires home O_2; OS3= Hospitalized, not requiring supplemental O_2; OS4= Hospitalized, requiring O_2; OS5= Hospitalized on

non-invasive ventilation, or high-flow O_2; OS6= Hospitalized on invasive mechanical ventilation, and/or extracorporeal membrane oxygenation (ECMO); OS7=Death). The 7-point OS for respiratory status was calculated at each inpatient timepoint. Length of hospital stay, complications, and other protocol-defined outcomes were assessed over 28 days and persistence of symptoms, reinfections as well as 28 day and delayed mortality were assessed for the duration of the study.

SARS-CoV-2 PCR

SARS-CoV-2 viral load was assessed by a central laboratory from nasal swab samples at each time point by RT-PCR of the viral N1, and N2 genes (see online supplement).[5]

Viral sequencing

Viral sequencing was performed by a central laboratory from one or more nasal swab samples per patient, ranked by lowest Ct value, to obtain at least one complete genome per patient. This yielded a total of 660 available genomes from 453 patients (see online supplement).[6]

Serology

At the time of publication, anti-SARS-CoV-2 spike (S), and receptor binding domain (RBD) antibodies, quantitated by enzyme-linked immunosorbent assay (ELISA) in serum,[7] were available for the first 891 participants (see online supplement).

Statistics

Longitudinal measures of ordinal scale over time were clustered using group-based trajectory modeling, a likelihood-based approach commonly used to group time series of clinical data. These models were implemented in an unsupervised fashion using SAS PROC TRAJ, using either 3, 4, 5, or 6 groups. For each specification of group number, polynomial curves up to cubic degree were allowed for each group. All models included timing of symptom onset relative to hospital admission as fixed effect. The best-fitting model, with respect to number of clusters and polynomial order, was selected using Akaike Information Criteria (AIC). A detailed description of the model implementation is provided in the online supplement.

Demographic, and clinical characteristics are reported as mean (standard deviation [SD]), median (interquartile range [IQR]), or frequency (percentage) compared across clinical trajectory groups using one-way ANOVA, Kruskal-Wallis tests, or chi-square tests as appropriate. Where distributions were skewed, continuous laboratory values were log-transformed prior to parametric analysis. In addition, clinical laboratory values were dichotomized to normal and abnormal as defined based on site-specific normal range. For several of the laboratory data considered, the proportion of missing values made imputation infeasible. Further, because labs were collected for clinical care rather than per protocol, it is likely that the missingness mechanism is associated with measurement value. For these reasons, labs that were not drawn were assumed to be normal (as not believed to be informative for clinical care) rather than implement a probabilistic imputation approach. The extent of missing data is noted where applicable. A series of multivariable logistic regression models was fit to identify demographic, and clinical risk factors associated with disease severity. All models included random intercept by enrollment site, and covariates were selected through a multi-step selection process (see Supplement). The covariates selected for the final model and included as fixed effects (see Figure 4) are: age, sex, race, Hispanic ethnicity, diabetes, chronic respiratory disease, presence of infiltrate on chest x-ray, lymphocyte count, creatinine level, troponin level, and N1 CT value. Ordinal and logistic regressions identified laboratory variables associated with the overall ordinal clinical trajectory trend, and discriminative of two different trajectory groups at baseline. A mixed-effect generalized additive model identified variables whose 28 day longitudinal values differ across clinical trajectory groups. This model included fixed effects for age and sex, and random effects for enrollment site and participant. Further details of the model specifications, and approaches for model selection are provided in the online supplement.

All analyses were performed in SAS (v9.4, Cary, NC), and R (v4.1, XXX) using an alpha-level of 0.05.

Role of the funders

The study was funded by project grants and cooperative agreements awarded by the National Institute of Allergy and Infectious Disease (NIAID). NIAID project scientists participated collaboratively in study design, data analyses, interpretation, and writing of the report but not in data collection. The manuscript content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases, the National Institutes of Health or any other Agency of the United States Government.

The authors are solely responsible for the study design, data collection, interpretation, manuscript preparation, and decision to submit the manuscript.

Results

Between May 5th, 2020 and March 19th, 2021, 1,286 patients were enrolled across 20 US hospitals; 1243 met eligibility criteria, and 1164 were included in the final

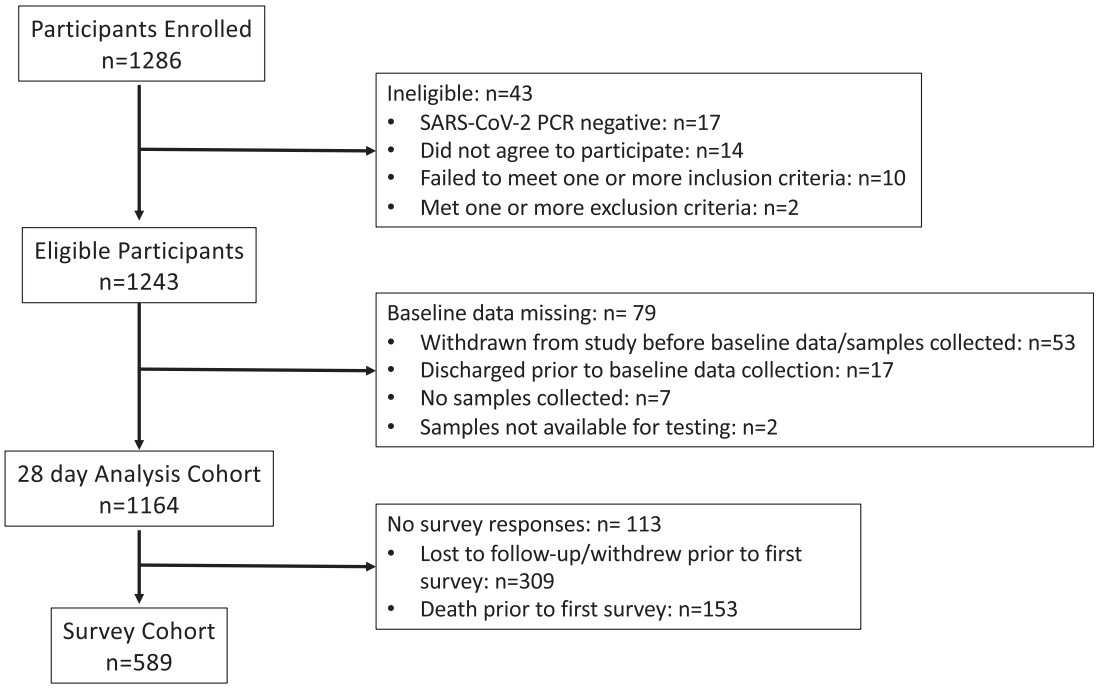

Figure 1. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Cohort Diagram.

analysis cohort with 589 being included in the post-hospitalization survey cohort (Figure 1). Clinical characteristics, baseline radiographic findings, and laboratory testing of the study cohort are provided in Table 1. The median age was 59 years (IQR 20), and 711 (61%) were men. 259 (22%) were Black/African American, and 363 (31%) were Hispanic/Latinx. 1096 (94%) had at least one comorbidity, including hypertension (677; 58%), diabetes (427; 37%), chronic respiratory disease (234; 20%), asthma (174; 15%), chronic cardiac disease (315; 27%), and chronic kidney disease (178; 15%). Current, or prior history of smoking, or vaping was reported in 377 (32%) patients. 58 (5%) were organ transplant recipients, and 21 (2%) were patients living with HIV. The median body mass index (BMI) was 31.3 kg/m^2 (IQR 9.6). Most patients (872; 75%) presented within 2 weeks of onset of symptoms. 276 (24%) were admitted directly to the intensive care unit (ICU) with 137 (12%) initially requiring mechanical ventilation, or ECMO. Almost half of the cohort presented with elevated D- dimer (>0.5 mg/L) (596; 51%), and C-reactive protein (CRP) (≥10 mg/L) (499; 43%). 139 (12%) patients had severe lymphopenia (<500 cells/microliter), and 59 (5%) had thrombocytopenia (<100,000 cells/microliter) at enrollment. A subset presented with evidence of renal insufficiency (creatinine ≥1.5 mg/dL; 186; 16%), liver dysfunction (alanine transaminase (ALT) ≥1.5 site specific upper limit of normal; 203; 17%), and/or myocardial injury (95 out of 445 available values with troponin >0.4 ng/mL; 8%). Most patients had opacities consistent with pneumonia on chest imaging on enrollment (800; 72%). The median SOFA (Sequential Organ Failure Assessment) score at baseline was 1 (IQR: 2).

The median length of hospital stay was 6 days (IQR 7). Twenty-eight day mortality was 9%, and did not differ across the duration of the study (divided into quarters; p=0.84) (Figure 1 Supplement); the overall mortality 12 months post hospital discharge was 14%. At day 28, 70 (6%) remained hospitalized. During their hospitalization, 228 (20%) required invasive mechanical ventilation, 176 (15%) experienced ICU level care escalation, and 958 (82%) had at least one pre-specified complication (Table 1 Supplement) including acute renal injury/failure (249; 21%), shock requiring use of pressors (173; 15%), bacteremia (113; 10%). 791 (68%) received systemic steroids (786; 68%), and 725 (62%) received remdesivir (Table 2 Supplement).

To capture the dynamics of clinical course of disease, we analyzed patient characteristics through an unsupervised clustering of respiratory OS over time. Five disease course trajectories were identified (Figure 2): brief length of stay (trajectory 1: n=258; 22%); intermediate length of stay (trajectory 2: n=310; 27%); intermediate length of stay with discharge limitations (trajectory 3: n=276; 24%); prolonged hospitalization (trajectory 4: n=212;18%); and fatal (trajectory 5: n=108; 9%).

Selected demographic characteristics, key comorbidities, radiographic, and laboratory findings were significantly associated with trajectory groups by univariate analysis (Table 1). In terms of symptoms at presentation, shortness of breath (p<0.001), and altered mental status (p<0.001) were associated with more severe

		Overall (*n*=1164)	Trajectory 1 (*n*=258)	Trajectory 2 (*n*=310)	Trajectory 3 (*n*=276)	Trajectory 4 (*n*=212)	Trajectory 5 (*n*=108)	Overall *p*-value
Age, median (IQR) in years		59 (20)	55 (22)	56 (22)	59.5 (21)	61.5 (17)	70 (18)	<.001
Sex, No. (%)	Male	711 (61)	148 (57)	195 (63)	151 (55)	143 (67)	74 (69)	0.012
Race, No. (%)[a]	White	559 (48)	113 (44)	138 (45)	144 (52)	98 (46)	69 (64)	<.001
	Black	257 (22)	78 (30)	78 (25)	62 (22)	25 (12)	16 (15)	
	Asian	51 (5)	10 (4)	14 (5)	14 (5)	9 (4)	4 (4)	
Ethnicity, No. (%)[c]	Non-Hispanic	753 (65)	186 (72)	212 (68)	183 (66)	102 (48)	70 (65)	<.001
	Hispanic	363 (31)	67 (26)	85 (27)	83 (30)	99 (47)	29 (27)	
Comorbidities, No. (%)	None	68 (6)	14 (5)	18 (6)	22 (8)	11 (5)	3 (3)	
	Hypertension	677 (58)	138 (53)	184 (59)	149 (54)	129 (61)	77 (71)	0.013
	Diabetes	427 (37)	97 (38)	96 (31)	92 (33)	91 (43)	51 (47)	0.006
	Chronic lung disease[i]	234 (20)	29 (11)	57 (18)	70 (25)	38 (18)	40 (37)	<.001
	Asthma	174 (15)	43 (17)	48 (15)	38 (14)	34(16)	11(10)	0.542
	Chronic cardiac disease	315 (27)	63 (24)	81 (26)	77 (28)	46 (22)	48 (44)	<.001
	Chronic kidney disease	178 (15)	35 (14)	38 (12)	48 (17)	28 (13)	29 (27)	0.004
	Chronic liver disease	58 (5)	13 (5)	18 (6)	10 (4)	13 (6)	4 (4)	0.647
	Chronic neurologic disorder	143 (12)	28 (11)	37 (12)	40 (14)	21 (10)	17 (16)	0.395
	Organ Transplantation	58 (5)	10 (4)	17 (5)	16 (6)	11 (5)	4 (4)	0.807
	HIV/AIDS	21 (2)	8 (3)	5 (2)	5 (2)	2 (1)	1 (1)	0.424
	Malignancy	119 (10)	26 (10)	28 (9)	30 (11)	18 (8)	17 (16)	0.299
	Drug, or alcohol abuse	88 (8)	30 (12)	22 (7)	16 (6)	13 (6)	7 (6)	0.084
	Smoking/Vaping[j]	377 (32)	84 (33)	96 (31)	88 (32)	61 (29)	48 (44)	0.068
BMI category in kg/m^2, No. (%)[c]	Overweight (25.1-29.9)	300 (26)	76 (29)	79 (25)	61 (22)	58 (27)	26 (24)	0.023
	Class 1-2 Obesity (30−39.9)	473 (41)	103 (40)	140 (45)	104 (38)	86 (41)	40 (37)	
	Class 3 Obesity (40+)	165 (14)	36 (14)	38 (12)	45 (16)	31 (15)	17 (16)	
Symptom onset to hospitalization (days), median (IQR)		7 (7)	7 (7)	7 (6)	7 (7)	6 (5.5)	6 (8)	0.862
Symptom onset to hospitalization in days, No (%)[b]	≤3	242 (21)	55 (21)	64 (21)	60 (22)	36 (17)	27 (25)	0.026
	4−7	325 (28)	66 (26)	96 (31)	62 (22)	74 (35)	27 (25)	
	8−14	305 (26)	77 (30)	92 (30)	73 (26)	42 (20)	21 (19)	
	>14	81 (7)	15 (6)	16 (5)	20 (7)	20 (9)	10 (9)	
Level of respiratory support, No. (%)	Mechanically ventilated, or ECMO (OS=6)	137 (12)	1 (0)	9 (3)	3 (1)	84 (40)	40 (37)	<.001
	Non-invasive ventilation, or high flow nasal O_2 (OS=5)	267 (23)	13 (5)	53 (17)	60 (22)	104 (49)	37 (34)	

Table 1 *(Continued)*

		Overall (*n*=1164)	Trajectory 1 (*n*=258)	Trajectory 2 (*n*=310)	Trajectory 3 (*n*=276)	Trajectory 4 (*n*=212)	Trajectory 5 (*n*=108)	Overall *p*-value
	Supplemental oxygen (not high flow) (OS=4)	491 (42)	93 (36)	196 (63)	159 (58)	22 (10)	21 (19)	
	None (OS=3)	267 (23)	150 (58)	52 (17)	53 (19)	2 (1)	10 (9)	
SpO_2/FiO_2 ratio category, No. (%)[c]	235, or lower	271 (24)	2 (1)	35 (11)	31 (11)	138 (65)	65 (60)	<.001
	236−315	179 (15)	14 (5)	66 (21)	61 (22)	26 (12)	12 (11)	
	315, or higher	653 (56)	230 (89)	196 (63)	170 (62)	34 (16)	23 (21)	
SOFA Score, median (IQR)		1 (2)	0 (1)	0 (2)	0 (2)	3 (6)	3 (7.5)	<.001
Radiographic findings	No infiltrate	296 (27)	99 (42)	83 (28)	67 (26)	28 (14)	19 (18)	<.001
on chest imaging, No. (%)	Unilateral infiltrates	103 (9)	21 (9)	37 (13)	24 (9)	13 (6)	8 (8)	
	Bilateral infiltrates	697 (63)	113 (48)	174 (59)	167 (64)	165 (80)	78 (74)	
Decreased lymphocyte count No. (%)[d]	<500/microliter	139 (12)	13 (5)	28 (9)	30 (11)	37 (17)	31 (29)	<.001
Decreased platelet count, No. (%)[c]	<100,000/microliter	59 (5)	9 (3)	9 (3)	16 (6)	11 (5)	14 (13)	<.001
D-dimer[e], No. (%)	>0.5 mg/L	596 (51)	130 (50)	156 (50)	135 (49)	109 (51)	66 (61)	0.288
ALT[d], No. (%)	>1.5x site-specific upper limit of normal	203 (17)	30 (12)	71 (23)	41 (15)	48 (23)	13 (12)	<.001
Creatinine[c], No. (%)	>=1.5 mg/dL	186 (16)	28 (11)	35 (11)	40 (14)	45 (21)	38 (35)	<.001
CRP[e], No. (%)	>=10 mg/L	499 (43)	90 (35)	142 (46)	119 (43)	94 (44)	54 (50)	0.036
Troponin[f], No. (%)	>0.4 ng/mL	95 (8)	20 (8)	25 (8)	15 (5)	15 (7)	20 (19)	<.001
Detectable SARS-CoV-2 RBD IgG, %[g]		59	59	65	55	63	46	0.153
Detectable SARS-CoV-2 S IgG, %[g]		82	78	84	78	86	82	0.318
SARS-CoV-2 N1 CT value, median (IQR)[h]		27.4 (8.7)	29.1 (8.9)	28 (8.1)	27.4 (7.8)	26 (9.2)	25.5 (6.8)	0.005

***Table 1*: Demographics, clinical characteristics, comorbidities, radiographic findings, and laboratory testing of cohort participants at baseline (*N*=1164).**

[a] 29% other/declined/unknown/missing.
[b] less than 10% missing data.
[c] less than 5% missing data.
[d] less than 20% missing data.
[e] less than 40% missing data.
[f] 62% did not have troponin level on admission.
[g] available on 891 participants.
[h] available on 1010 participants.
[i] not including asthma.
[j] current, or former.

p-value from chi-square test for categorical variables, and Kruskal-Wallis test for continuous variables (age, SOFA score).
Trajectory 1= brief length of stay; trajectory 2= intermediate length of stay; trajectory 3= intermediate length of stay with discharge limitations; trajectory 4= prolonged hospitalization; trajectory 5= fatal.

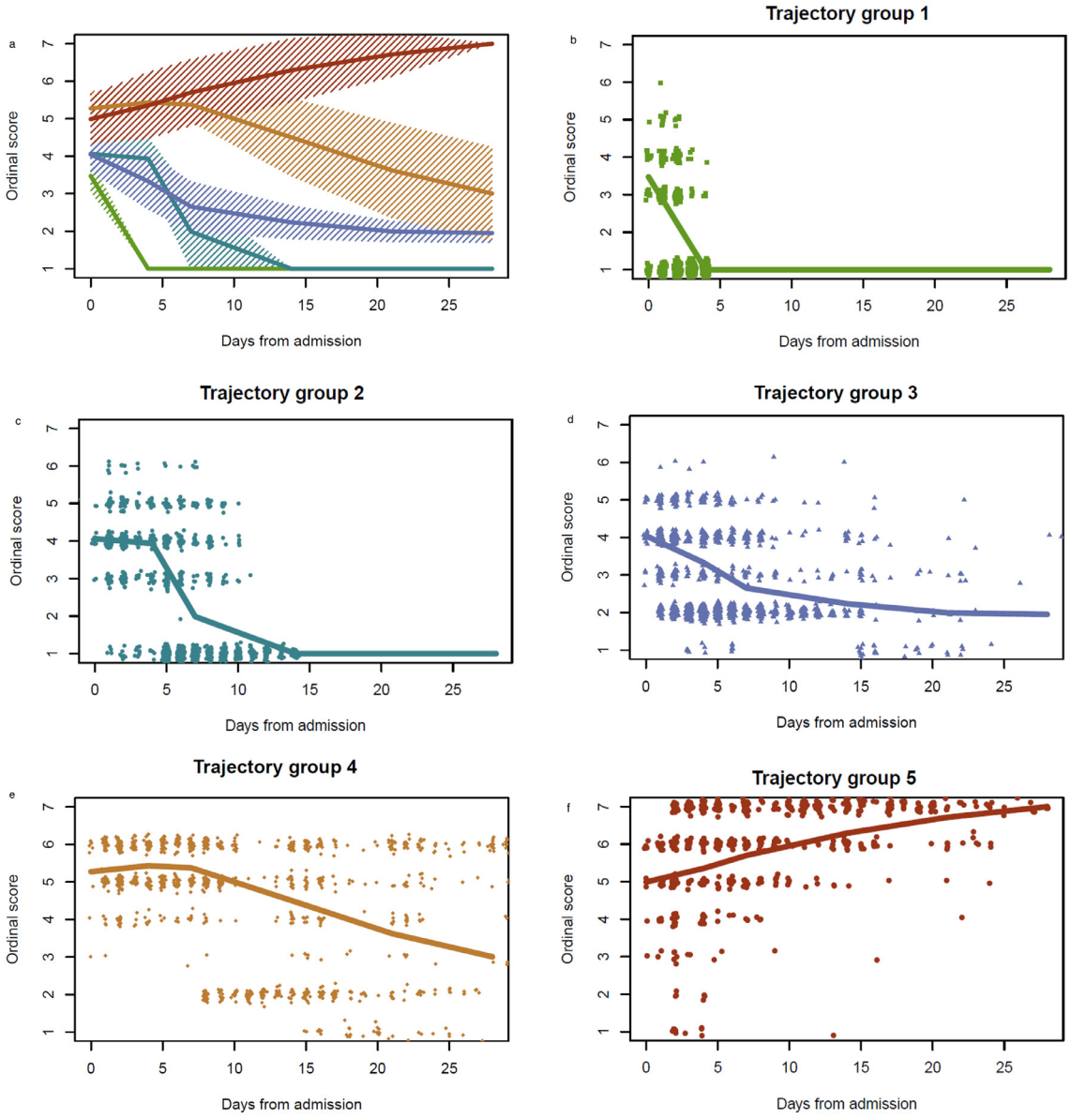

Figure 2. Classification of clinical trajectories.

Longitudinal trajectories of clinical respiratory status were classified into one of five groups. Mean ordinal score for each group are shown with IQR determined by normal approximation (i.e. group-specific mean +/− 0.674 SD) (panel a), estimated at each of the six visit timepoints as determined by study protocol. Separate group-specific mean plots are shown (panels b-f) with points representing observed participant-level (jittered) ordinal scores by days from admission.

Trajectory 1= brief length of stay (green); trajectory 2= intermediate length of stay (teal); trajectory 3= intermediate length of stay with discharge limitations (blue); trajectory 4= prolonged hospitalization (orange); trajectory 5= fatal (red).

Ordinal scores: OS1= Not hospitalized, no limitations; OS2= Not hospitalized, activity limitations, or requires home O_2; OS3= Hospitalized, not requiring supplemental O_2; OS4= Hospitalized, requiring O_2; OS5= Hospitalized on non-invasive ventilation, or high-flow O_2; OS6= Hospitalized on invasive mechanical ventilation, and/or ECMO; OS7=Death. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

disease, while gastrointestinal symptoms were associated with milder trajectories ($p<0.001$). The interval between onset of symptoms and hospitalization was not consistently associated with worsening outcome (Table 1). Higher markers of inflammation (i.e., CRP, lactate dehydrogenase, ferritin) differed by trajectories, but did not differ significantly between trajectories 4, and 5. An increased number of complications was noted with trajectories associated with higher OS (Table 1 Supplement).

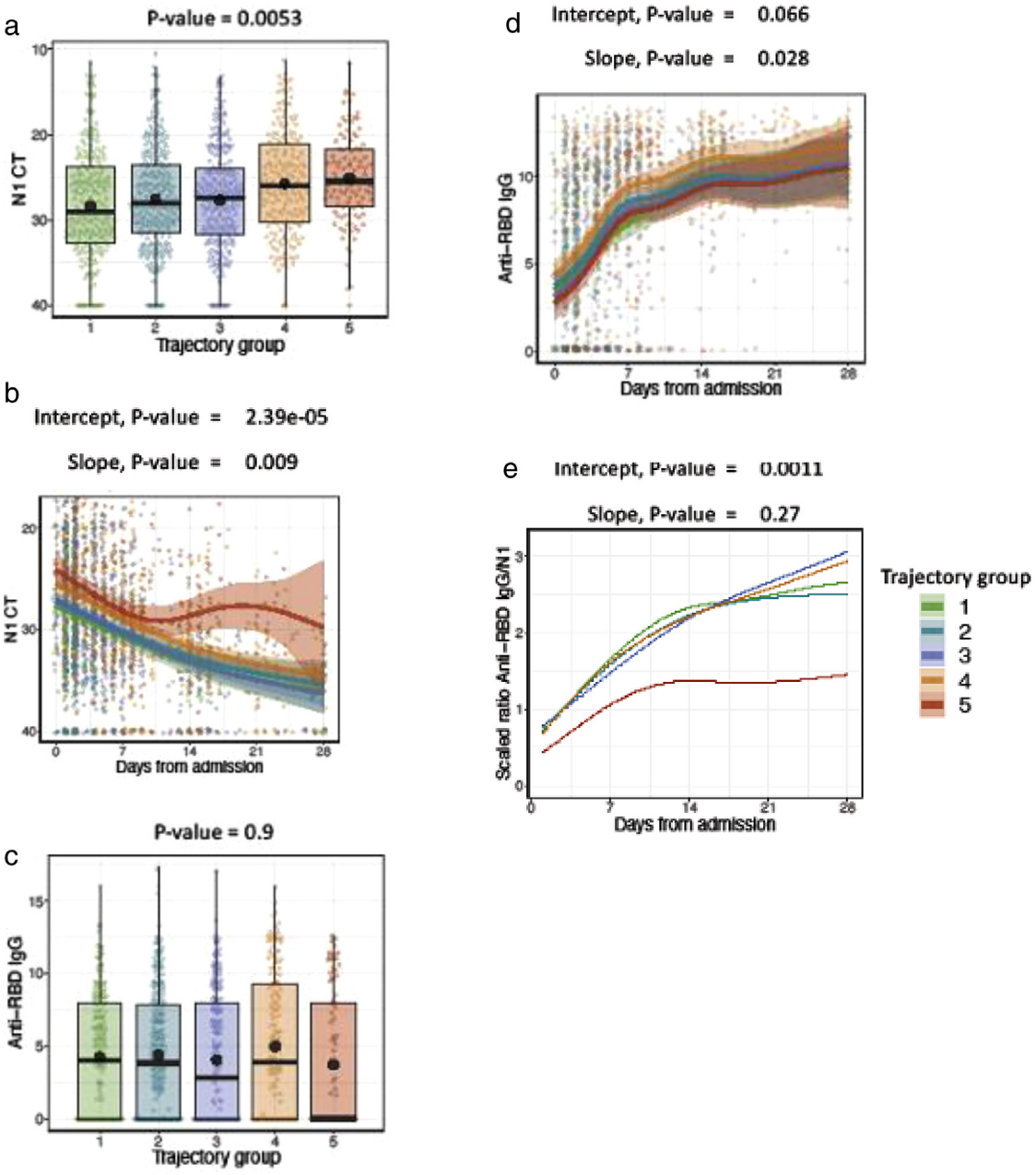

Figure 3. (a) N1 Ct baseline: Shown are the SARS-CoV-2 N1 gene Ct values during the first study collection timepoint which occurred at any time from admission until up to approximately 48 h after admission. Shown are median values (horizontal lines), mean values (black points), interquartile ranges (boxes), and 1.5 IQR (whiskers), as well as all individual points.

(b) N1 Ct over time: Shown are the SARS-CoV-2 N1 gene Ct values collected during the first 28 days of hospital admission. Trend lines for each trajectory represent the fit of a generalized additive model (GAM) to capture non-linear trends in the data.

(c) RBD IgG baseline: Shown are the anti-RBD IgG values during the first study collection timepoint which occurred at any time from admission until up to approximately 48 h after admission. Shown are median values (horizontal lines), mean values (black points), interquartile ranges (boxes), and 1.5 IQR (whiskers), as well as all individual points.

(d) RBD IgG over time: Shown are the RBD IgG values collected during the first 28 days of hospital admission. Trend lines for each trajectory represent the fit of a generalized additive model (GAM) to capture non-linear trends in the data.

(e) Ratio anti-RBD IgG/N1 Ct over time: Shown are the trends of the scaled ratio of anti-RBD IgG values divided by scaled SARS-CoV-2 N1 gene Ct values for time points collected during the first 28 days of hospital admission, emphasizing the divergence of trajectory 5 from the other 5 trajectories in this measure.

Trajectory 1= brief length of stay (green); trajectory 2= intermediate length of stay (teal); trajectory 3= intermediate length of stay with discharge limitations (blue); trajectory 4= prolonged hospitalization (orange); trajectory 5= fatal (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

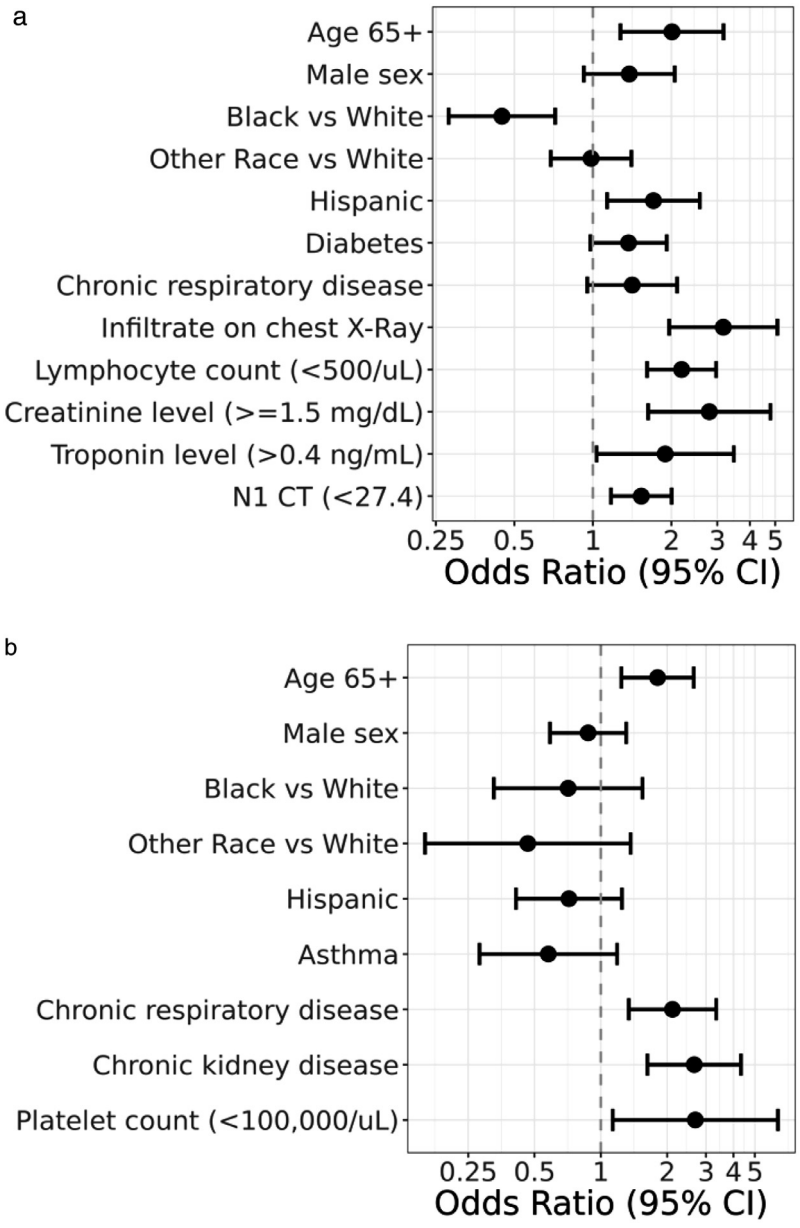

Figure 4. Forest Plot Models.

(a) Adjusted odds ratios for factors associated with more severe (groups 4, 5) versus less severe (groups 1,2,3) disease. (*N*=1164).

(b) Adjusted odds ratios for factors associated with 28-day mortality (group 5) versus prolonged hospitalized course (group 4) (*N*=320).

Trajectory 1= brief length of stay; trajectory 2= intermediate length of stay; trajectory 3= intermediate length of stay with discharge limitations; trajectory 4= prolonged hospitalization; trajectory 5= fatal.

N1 SARS-CoV-2 PCR median cycle threshold (Ct) values at enrollment were significantly lower (indicating higher viral RNA levels) in patients with a more severe disease course (Figure 3a) with progressively lower Ct values from trajectory 1 through 5 (p=0.0053). This pattern persisted throughout the 28 day-period; namely, more severe trajectories maintained higher viral RNA levels (Figure 3b) (p<0.001). In the majority of trajectories, we observed a steady decline in viral RNA levels in the first week after hospitalization. However, uniquely in trajectory 5, the rate of decline was attenuated between the first, and second week of hospitalization, and plateaued at a Ct value below 30 during the 2nd week of hospitalization (p = 0.009), indicating

persistence of viral RNA. In the other 4 trajectories, viral RNA levels continued to decline between 14, and 28 days after hospitalization. Viral RNA levels followed a similar pattern when analyzed by days from symptom onset S (Figure 4 Supplement). Similar observations were also seen with N2 SARS-CoV-2 PCR (Figure 2 Supplement, Figure 4 Supplement). Adjustment for administration of remdesivir, or systemic glucocorticoids during the hospital course did not alter these findings (Figure 3 Supplement). Viral sequencing, available in less than half of the cohort, revealed few variants of concern or interest that circulated during the study conduct [7 patients with alpha (B.1.1.7), 12 with epsilon (B.1.427 and B.1.429) and 4 with iota (B1.429)] (Figure 5 Supplement).

Anti-S, and RBD IgG antibodies (Figure 3c, d, and Figure 2 Supplement) were detectable on admission in 81.6%, and 58.8% of patients, respectively (Table 1). As expected, antibody levels were higher in patients who presented more than 7 days after symptom onset compared to those who presented within 7 days of symptom onset (Figure 4 Supplement). In the subset of individuals presenting more than 7 days after symptom onset, significantly lower levels of anti-RBD antibody were observed in trajectory 5 compared to other trajectories (p=0.03−0.04). We observed a rapid increase in anti-RBD, and anti-S IgG levels in the first 7 days after admission followed by a moderate rise between 7, and 21 days, reaching a plateau towards the end of the 28-day period.

Analysis of the relationship between antibody levels and antibody to viral load ratio revealed associations with clinical trajectories. We calculated the scaled ratio of anti-RBD antibody levels to Ct values, and observed that trajectory 5 had significantly lower values of this ratio compared to the other four trajectories over the 28-day window period (p = 0.001) (Figure 3e).

Multivariable analyses comparing patients with short or intermediate length of stay (trajectories 1 to 3) to patients who had prolonged hospitalization or died (trajectories 4, and 5), suggested that those with more severe illness were more likely to be 65 years, or older (odds ratio [OR], 2.01; 95% CI 1.28−3.17), of Latinx ethnicity (OR, 1.71; 95% CI 1.13−2.57), had elevated baseline creatinine (OR 2.80; 95% CI 1.63− 4.80), or troponin (OR 1.89; 95% 1.03−3.47), baseline lymphopenia (OR 2.19; 95% CI 1.61−2.97), presence of infiltrate by chest imaging (OR 3.16; 95% CI 1.96−5.10), and high SARS-CoV-2 viral burden (Ct < 27.4 (median value) (OR 1.53; 95% CI 1.17−2.00) (Figure 4). In multivariate analyses comparing patients who died (trajectory 5) with those with a prolonged hospitalization (trajectory 4), death was independently associated with older age (OR 1.81; 95% CI 1.24−2.64), chronic respiratory disease (OR 2.11; 95% 1.34−3.34), chronic kidney disease (OR 2.65; 95% CI 1.63−4.33), and low baseline platelet count (OR 2.68; 95% CI 1.13−6.36) (Figure 4).

589 participants completed at least one quarterly survey post discharge and did not differ from non-respondents based on baseline demographics or period of enrollment but did per trajectory group assignment (Table 3 Supplement). 108 deaths were reported prior to day 28 and 59 deaths were reported after day 28 mostly in patients in trajectory group 4 (n=36, 61%; p<0.001). Ten (2%) patients reported 11 SARS-CoV-2 reinfections post discharge. Vaccines were not available in the US for the general population until after study enrollment had largely been completed. 62% of patients reported receiving a COVID-19 primary vaccination series after discharge with 36% reporting booster doses, mostly with mRNA based vaccines (52% Pfizer BioNtech, 40% Moderna and 8% Johnson and Johnson for the primary series and 60% Pfizer BioNtech, 39% Moderna and 1% Johnson and Johnson products for the boost). 305 respondents (52%) reported symptoms after discharge. In those reporting any symptom after discharge, the median number of symptoms or organ systems affected was 1.0 (IQR=1.5). Among those reporting any symptoms, the symptoms were as follows: dyspnea (56%), muscle aches/myalgia (40%), cough (38%), headache (37%), fatigue/malaise (35%), loss of smell or taste (27%), red eye (26%), sore throat (14%), nausea or vomiting (13%) and fever, chills in less than 10%. The frequency of symptoms did not change during the follow up period (Figure 6 Supplement). The presence of symptoms at follow up was only associated with female sex in a univariate analysis (p=0.02) and not with trajectory group, Ct values, or antibody levels (Figure 7 Supplement).

Discussion

We identified five longitudinal clinical phenotypes based on respiratory OS in our cohort of hospitalized COVID-19 in hospitalized patients, and explored the relationship between demographic data, clinical features, readily available laboratory testing (immunologic, hematological, biochemical, and virologic), and radiographic findings with disease trajectories. Similar to other cohorts, we found that age ≥65 years,[8] Latinx ethnicity, certain comorbidities, presence of chest radiographic infiltrate, and selected biomarkers at baseline were associated with more severe disease course, and worse outcomes. While many of these risk factors have previously been linked with mortality in other cohorts,[9−12] our data is based on a multi-center prospective longitudinal study to comprehensively identify risk factors associated with trajectory of disease course over time. The clinical trajectory approach has advantages over the conventional cross-sectional approach by fully leveraging longitudinal data to analyze patient outcomes based on time in hospital, and disease severity.

In addition, our data demonstrate that higher SARS-CoV-2 viral burden at presentation is associated with

worse disease severity, consistent with prior reports in small case series.[12,13] Our report is unique in validating this observation in a larger cohort, and in demonstrating the association between longitudinal assessment of viral load, and clinical disease course. Cohort patients with fatal outcome had delayed clearance of detectable virus by PCR as well as the lowest levels of anti-RBD, and anti-S IgG, suggesting that impaired functional antiviral immune response necessary to clear the virus, may play a key role in fatal cases.The described calculated ratio (IgG/Ct value) is unique compared to other studies,[14–16] and may represent an accessible approach for patient risk stratification based on clinical laboratory testing.

The IMPACC cohort was diverse, with 31% participants being Hispanic/Latinx, and 22% black/African American, reflecting communities disproportionately affected by COVID-19,[12] and with wide geographic distribution within the USA. Consistent with prior reports, Hispanic/Latinx ethnicity was associated with increased risk for more severe disease, although ultimately neither race nor ethnicity were associated with death when assessing multivariable risk between the more severely ill groups (trajectories 4, and 5). Our data are consistent with prior retrospective cohorts which failed to demonstrate increased mortality in hospitalized disadvantaged minority populations,[17] unlike population based data on increased overall mortality in these communities.[12]

The overall 28-day mortality was 9%,and remained stable through the duration of the study. This finding is consistent with other reports[18] where mortality remained relatively unchanged after the first 2 months of widespread circulation of SARS-CoV-2 in the US (March, and April 2020), but suggests no significant impact of care interventions for hospitalized patients with COVID-19 on mortality during the period of this study. In contrast to the hospitalized COVID-19 prospective patient cohort studied early during the pandemic in the UK (ISARIC WHO CCP-UK), our IMPACC study population showed lower overall mortality (9% vs 26%)[10] though the characteristics of the study populations were different (with more major comorbidities noted in the IMPACC cohort and an older age in the ISARIC WHO CCP-UK cohort).

Our prospective study did not demonstrate an association of obesity with poor outcome as was reported by others.[10,19] This could in part be due to the fact that the majority of the patients (940; 81%) in IMPACC had a BMI above 25 kg/m^2. It could be that impaired metabolic health[20] (characterized by dyslipidemia, and insulin resistance) may be more predictive than BMI *per se*. A recent meta-analysis suggested that the effects of BMI on COVID-19 severity were less evident.[21] Additionally, although chronic respiratory disease tended to be more prevalent in patients with more severe illness, asthma was not a major confounding disease for COVID-19 severity in our cohort, possibly due to a previously reported protective role of type 2 immune inflammation.[22] Our ongoing longitudinal immunologic analyses may identify pathways distinguishing the impact of comorbidities such as diabetes, obesity, and asthma on disease outcome from COVID-19.

While other small cohorts provide conflicting data on effects of antivirals[23] and glucocorticoids[24] on viral clearance, we did not find any relationship between viral clearance, and the use of remdesivir, or glucocorticoids.[25] Within the two most severe groups, viral RNA levels were highest in patients who received glucocorticoids, potentially reflecting nonrandom assignment of this therapy to more severe cases. In trajectory group 5, modest differences were observed in the slope of viral RNA levels potentially reflecting greater persistence of virus in individuals not treated with glucocorticoids, but this result is difficult to evaluate given that few patients were still alive at day 28.

Surveys conducted post-discharge in our study demonstrated that 51% of patients suffered from at least one symptom of PASC, which is in line with results from multiple other studies.[26,27] Female sex was a risk factor for PASC though the overall cohort was male predominant, reflecting a higher risk associated with male sex for hospitalization due to COVID-19. Though all symptoms improved compared to the acute presentation, conjunctivitis was more frequent at follow up than at presentation, as described by others.[28,29] Some studies indicate that the risk of developing PASC may be diminished by vaccination.[30] However, our cohort completed enrollment prior to the nationwide rollout of vaccination, and therefore cannot address the characteristics of PASC in vaccine breakthrough cases.[31]

Caveats and limitations

Though IMPACC was racially, ethnically, and geographically diverse, the cohort excluded pregnant women known to be at risk for severe COVID-19,[32] and children with multisystem inflammatory syndrome.[33] The cohort only recruited symptomatic, hospitalized patients and therefore our findings are not generalizable to asymptomatic or outpatient cases. We also did not collect information on socioeconomic status, thought to be a contributor of mortality in COVID-19.[34] Additionally, PCR cannot distinguish between viable, and non-viable virus.[35] Prior studies have shown that the interval between symptom onset to viral clearance in culture did not exceed 12 days, and was much shorter than clearance of viral nucleic acid materials by PCR.[36] In addition, particularly early in the pandemic, missed collection of nasal samples at days 14, and 28 in patients discharged from the hospital before these time points, and absence of further sampling once patients died could affect estimates of viral load, or serologies over time. While most participants were vaccinated by the time of follow-up; self-reporting of re-infection and

small numbers precluded assessment of vaccine effectiveness. Our cohort was also fully enrolled prior to the widespread circulation of the SARS-CoV-2 B.1.617.2 (Delta), and B.1.1.529 (Omicron) variants which are associated with higher transmission, and possibly different severity of COVID-19. We assessed binding antibody levels rather than neutralizing antibody levels, though other studies have shown binding, and neutralizing antibody titers correlate well.[37] Finally, the convalescent survey, planned at the beginning of the pandemic, did not include an extensive list of symptoms currently associated with PASC. While the severity of symptoms was not captured, ongoing efforts aim to analyze the impact of these persistent symptoms on quality of life, collected prospectively using patient-reported outcome measures.

Conclusion

In this large, diverse, prospective cohort study of hospitalized patients in 20 sites across the U.S., we found that high baseline viral load, and its persistence were associated with more severe disease. Fatal cases were associated with the lowest levels of anti-RBD and S IgG. These findings suggest an impaired functional antiviral immune response necessary to clear the virus may play a key role in short-term mortality. The described calculated ratio (binding IgG/PCR Ct value) is unique compared to other studies, reflecting host pathogen interactions and appears to be an accessible approach for patient risk stratification. Immunophenotyping of blood, upper, and lower respiratory samples collected from this cohort is ongoing, and will enable identification of immune endotypes associated with severity of illness and/or persistence of symptoms. Furthermore, this effort will identify predictive and prognostic characteristics and generate hypotheses regarding the cellular and molecular basis of disease and recovery.

Contributors

All authors read and approved the final version of the manuscript.

M.C.A, P.M.B and N.R. have verified the underlying data

– Conceptualization: A.O, P.M.B, M.C.A, N.R, A.A, L.B, C.S.C, G.M, C.B.C, V.S, C.L.H, K.C.N, J.S, A.C.S, D.C, N.I.A.H, C.B, S.B, E.M.
– Data curation: C.M, N.D.J, H.VB, S.L, F.KR
– Formal analysis: A.O, C.M, N.D.J, H.VB, M.C.A, D.E, S.L, F. KR
– Funding acquisition: O.L, R.S, E.H, A.F.S, W.M, M. D, B.PU, E.F, D.J.E, R.M, D.H, F.KH, J.M, M.K, M.A.A, L.I.E
– Investigation: L.B, C.S.C, G.M, C.B.C, N.R, V.S, C.H, K.C.N, J.M, A.C.S, D.C, N.I.A.H, C.B, S.B, E.M
– Methodology: A.O, C.M, N.D.J, H.VB, M.C.A, D.E, S. L, F.KR, P.M.B, N.R
– Project administration: P.M.B, A.A, A.O, S.K, B.PE, N.R, E.R, J.M
– Resources: A.O, J.S, N.D.J, C.M, C.S.C, C.B.C, M.K, L.B, A.C.S, F.KR, H.VB, D.E, S.L, A.F.S, V.S, D.H, R.M, S.K, O.L, C.B, E.H, D.E, B.PE, K.C.N, M.D, C.L.H, W. M, N.I.A.H, J.M, M.A.A, S.B, D.C, F.KH, L.I.E, E.M, G. M, R.S, J.D-A, B.PU, A.A, E.R, M.C.A, P.M.B, N.R
– Software: C.M, N.D.J, H.VB, S.L, A.O, J.M
– Supervision: P.M.B, A.O, M.C.A, N.R
– Validation: A.O, C.M, N.D.J, H.VB, M.C.A, D.E, S.L, F.KR
– Visualisation: A.O, C.M, N.D.J, H.VB
– Writing-original draft: A.O, C.M, M.C.A, J.S, N.R and P.M.B
– Writing review & editing: A.O, J.S, N.D.J, C.M, C.S.C, C.B.C, M.K, L.B, A.C.S, F.KR, H.VB, D.E, S.L, A.F.S, V. S, D.H, R.M, S.K, O.L, C.B, E.H, D.J.E, B.PU, R.K.N, M. D, C.H, W.M, N.I.A.H, J.M, M.A.A, S.B, D.C, F.KH, L.I. E, E.M, G.M, R.S, J.D-A, B.PE, A.A, E.R, M.C.A, P.M.B, N.R

Replication of analyses: A.O,C.M, N.D.J, M.C.A, H.VB

IMPACC Study Group

Steering Committee: as above
Clinical & Data Coordinating Center (CDCC): Data curation
IMPACC Data Analysis Group: Formal analysis
IMPACC Site Investigators: Investigation
IMPACC Core Labs: Methodology
IMPACC Study Team: Investigation

Data sharing statement

The IMPACC Data Sharing Plan is designed to enable the widest dissemination of data, while also protecting the privacy of the participants and the utility of the data by de-identifying and masking potentially sensitive data elements. This approach is fully compliant with the NIH public data sharing policy. The study protocol and clinical dataset are deposited at the Immunology Database and Analysis Portal (ImmPort), a NIAID Division of Allergy, Immunology, and Transplantation-funded data repository, under study accession SDY1760. After publication, it will be available to appropriate academic parties upon request and submission of a suitable study protocol, analysis plan, and signed data use agreement subject to NIAID approval via AccessClinicalData@NIAID (https://accessclinicaldata.niaid.nih.gov/study-viewer/clinical_trials). Please contact ImmPort_Helpdesk@immport.org to view data for review purposes. All codes for the analyses and tables generated by this study are available in the Bitbucket repository.

Declaration of interests

J.S reports funds paid to institution by the National Institute of Health - National Institute of Allergy and Infectious Diseases for the completion of this manuscript.

C.S.C reports funds from NHLBI, has grants/contracts from Bayer, Roche-Genentech, Quantum Leap Healthcare Collaborative and received consulting fees from Vasomune, Genıe Life Sciences, Cellenkos, Janssen.

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D. E reports funds paid to institution for grants or contracts from the National Institute of Health. D.E is a statistician on the Data Safety Monitoring Board for the COLSTAT Trial. D.E is on the board of Directors for the Society for Clinical Trials.

V.S is listed as a co-inventor on a patent filed relating to SARS-CoV-2 serological assays (the "Serology Assays").

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The remaining authors declare that they have no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104208.

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