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Immune biomarkers associated with COVID-19 disease severity in an urban, hospitalized population

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ABSTRACT

Objectives: We sought to identify immune biomarkers associated with severe Coronavirus disease 2019 (COVID-19) in patients admitted to a large urban hospital during the early phase of the SARS-CoV-2 pandemic.

Design: The study population consisted of SARS-CoV-2 positive subjects admitted for COVID-19 (n = 58) or controls (n = 14) at the Los Angeles County University of Southern California Medical Center between April 2020 through December 2020. Immunologic markers including chemokine/cytokines (IL-6, IL-8, IL-10, IP-10, MCP-1, TNF-α) and serologic markers against SARS-CoV-2 antigens (including spike subunits S1 and S2, receptor binding domain, and nucleocapsid) were assessed in serum collected on the day of admission using bead-based multiplex immunoassay panels.

Results: We observed that body mass index (BMI) and SARS-CoV-2 antibodies were significantly elevated in patients with the highest COVID-19 disease severity. IP-10 was significantly elevated in COVID-19 patients and was associated with increased SARS-CoV-2 antibodies. Interactions among all available variables on COVID-19 disease severity were explored using a linear support vector machine model which supported the importance of BMI and SARS-CoV-2 antibodies.

Conclusions: Our results confirm the known adverse association of BMI on COVID-19 severity and suggest that IP-10 and SARS-CoV-2 antibodies could be useful to identify patients most likely to experience the most severe forms of the disease.

1. Introduction

The pathophysiology of SARS-CoV-2 infection and the associated clinical illness known as COVID-19 is a subject of major interest. SARS-CoV-2 starts primarily as an infection of epithelial cells in the nasal cavity which then descends the respiratory tract resulting in a viral pneumonia [1,2]. Patients may have mild constitutional symptoms (e.g., low grade fever, mild cough) which then progress to

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Abbreviations

IFN	interferon
DSS	disease severity score
STROBE	Strengthening of Observational Studies in Epidemiology
S1	SARS-CoV-2 spike subunit S1
S2	SARS-CoV-2 spike subunit S2
RBD	SARS-CoV-2 receptor binding domain
N	SARS-CoV-2 nucleocapsid
MFI	mean fluorescent intensity
AUROC	Area Under the Receiver-Operator Characteristic curve
BMI	body mass index
OR	odds ratio
SVM	support vector machine model
WBC	white blood cells count
DBP	diastolic blood pressure
Plt	platelet count
CRP	C-reactive protein
TRAIL	TNF-related apoptosis-inducing ligand

more significant symptoms of fever, chills, cough, dyspnea, and severe malaise/fatigue which typically abates within 14 days. Some patients resolve the infection without exhibiting any symptoms. Early observations in an unvaccinated population suggested that about 80% of patients exhibit a mild/moderate form of COVID-19 which resolves without any specific treatment [3]. However, in approximately 20% of unvaccinated patients, the illness progresses to a "cytokine storm" involving pulmonary (diffuse viral pneumonia, hypoxemia, respiratory failure) and extra-pulmonary (coagulopathy, hepatic, renal, neurologic, cardiac) manifestations requiring hospitalization and intensive care level support [4]. Severe COVID-19 is associated with acute respiratory distress syndrome, multiple organ system involvement, and a significant risk for mortality.

Blood-based protein biomarkers have been used to monitor the hyper-inflammatory host immune response to SARS-CoV-2. IL-6 is the prototypic pro-inflammatory cytokine secreted by many cell types in response to acute infection and tissue damage and has been proposed as a biomarker and therapeutic target in severe COVID-19 [5]. Increases in circulating levels of IL-6 and other pro-inflammatory cytokines (including IL-1, IL-2, and IL-8) have been associated with severity of COVID-19 [6–10]. Acute SARS-CoV-2 infection is initially associated with a blunted type I interferon (IFN) antiviral response, which is eventually overcome to allow for brisk upregulation of IFN-response genes [11]. Circulating levels of type I interferons (IFN-α2, IFN-γ) are found to be below the level of detection in most studies, but changes in IFN-I responsive genes (e.g., IP-10/CXCL10, MCP-1/CCL2) can often be detected in plasma [12]. Finally, the presence and titer of SARS-CoV-2 specific antibodies have also been used to diagnose and monitor immunologic response to viral infection [8,10,13].

We hypothesized that differences in immune response to SARS-CoV-2 infection underlie the clinical heterogeneity of COVID-19. Further, we hypothesized that we could identify clinically relevant immune markers at the time of initial hospital admission that could be used to predict the course of COVID-19 illness. This study incorporates an assessment of the blood-based immune markers discussed above (e.g., cytokines, chemokines, and antibodies) along with basic clinical factors obtained on the day of hospital admission in an unvaccinated population during the first year of the pandemic.

2. Materials and methods

2.1. Subject selection and study design

The study population consisted of 58 patients admitted for SARS-CoV-2 to the Los Angeles County-USC Medical Center between April 2020 through December 2020 who prospectively consented to participate in the USC Keck COVID-19 Biorepository study (USC IRB: HS-20-00322). The control population consisted of 14 subjects admitted to the same facility for non-COVID-19 related lower respiratory tract infection who also gave prospective consent for clinical data and specimen acquisition. SARS-CoV-2 status for all subjects and controls was determined by standard clinical grade PCR testing. Clinical data were abstracted for all 72 subjects from medical records. Disease severity score (DSS) was assessed throughout hospitalization according to the WHO Illness Severity Scale [14] with the maximal value assigned to each subject. Positive cases were further dichotomized as mild (DSS$\leq$4) and moderate/severe (DSS$\geq$5). Blood was collected and available for analysis of immune markers in 53 of the 58 COVID-19-positive subjects and all 14 controls. The patients were enrolled and their samples collected before widespread availability of COVID-19 vaccines, and therefore the study population is considered as unvaccinated for COVID-19. This report follows the Strengthening of Observational Studies in Epidemiology (STROBE) reporting guideline for observational studies [15].

2.2. Quantification of cytokines and SARS-CoV-2 antibodies

Serum was obtained from all subjects on hospital day 1, processed, and stored at −80 °C until testing. Individual aliquots were used on the day of testing and analyzed according to the manufacturer's instructions. In brief, samples were allowed to thaw to room temperature, mixed by vortexing, and centrifuged immediately prior to use. Cytokines and chemokines were measured in each sample using a custom Milliplex panel (HCYTA-60K-10; IFN-α2, IFN-γ, IL-1β, IL-2 IL-6, IL-8, IL-10, IP-10/CXCL10, MCP-1/CCL2, TNF-α; MilliporeSigma, Burlington, MA USA). Serum IgG and IgM were detected using the Milliplex MAP SARS-CoV-2 Antigen Panel 1 IgG (HC12SERG-8k5K) and IgM (HC19SERM-85K) against spike subunits (S1 and S2), receptor binding domain (RBD), and nucleocapsid (N) proteins according to the manufacturer's instructions. The Milliplex assays used in this study are intended for research use and were not validated for clinical use. Information from the assay manufacturer indicates intra-assay precision within 15% CV and inter-assay precision within 20% CV. A study that evaluated the Milliplex SARS-CoV-2 antibody tests confirmed these precision results and also found moderate and strong correlations with the Roche Elecsys anti-SARS-CoV-2 S test (detecting Pan-Ig anti-RBD) and the Siemens SARS-CoV-2 IgG test (sCOVG; detecting IgG anti-S1/RBD), respectively [16]. For our study, all samples were analyzed in duplicate, with the sample mean used for subsequent data analysis.

2.3. Data analysis

For cytokines, values obtained for most analytes were computed using mean fluorescent intensity (MFI) in individual samples fit to a standard curve using a five-parameter logistic formula with power law variance. For IP-10, the logistic formula had poor curve fitting, and therefore logarithmic fitting was used instead. Missing values were defined by MFI below the quantification limit set by the standard curve. For some downstream data analyses, missing values were imputed using a single-imputation method with one-half the lower limit of detection. Of the ten cytokines tested, four (IL-2, IFN-α2, IFN-γ, and IL-1β) were below the limit of detection in more than 20% of samples and were thus excluded from further analysis, as imputation for variables with ≥20% missingness can introduce bias [17]. Anti-SARS-CoV-2 serology was analyzed using MFI values for IgG and IgM against each antigen normalized to the negative controls [18]. To categorize subjects as IgG positive or negative, the IgG MFI values for the four distinct antigens were collapsed into a single value by performing a Principal Component Analysis using the FactoMineR version 2.4 package in R [19] and assigning the first principal component projection value to each subject. Subjects whose value was at or above the median were classified as "High IgG," and those below as "Low IgG."

Comparison of patient demographic, clinical, cytokines and immunoglobulins characteristics between mild versus moderate/severe COVID-19 groups were conducted using Wilcoxon tests for continuous variables and Chi-square tests for categorical variables. Similarly, descriptive comparisons between the control, mild, and moderate/severe groups were conducted using Kruskal-Wallis tests for continuous variables and Chi-square tests for categorical variables. Comparisons between High and Low IgG groups were conducted with Welch two-sample t-tests. Multiple linear logistic regression models were used to assess the effect of covariates on COVID-

Table 1
Distribution of demographics, clinical, and laboratory characteristics by COVID-19 status and severity.

	COVID-19 Severity		Control n = 14	p-value	
	Mild n = 32	Moderate/Severe n = 26		Mild vs Moderate/Severe[a]	Overall[b]
Baseline Characteristics					
Age, years (mean (SD))	51.9 (11.3)	54.8 (12.9)	54.6 (14.5)	0.365	0.636
Sex, No. (%)				0.890	
Female	7 (21.9)	7 (26.9)	6.0 (42.9)		0.341
Male	25 (78.1)	19 (73.1)	8.0 (57.1)		
Body Mass Index[c] (mean (SD))	27.9 (5.3)	31.3 (6.9)	31.2 (5.1)	0.042*	0.074
Systolic Blood Pressure[d] (mean (SD))	128.1 (19.1)	119.4 (18.7)	125.1 (23.3)	0.085	0.251
Diastolic Blood Pressure[d] (mean (SD))	72.3 (10.9)	73.2 (11.0)	70.2 (18.8)	0.752	0.777
Hemoglobin[e] (mean (SD))	12.1 (2.4)	13.0 (2.3)	11.2 (2.3)	0.174	0.074
White Blood Cells[f] (mean (SD))	7.7 (3.9)	9.8 (5.4)	9.8 (5.5)	0.084	0.178
Platelet Count[f] (mean (SD))	255.6 (148.3)	258.0 (139.8)	226.5 (114.0)	0.950	0.767
Blood Urea Nitrogen[g] (median [IQR])	15.0 [8.8, 17.5]	15.5 [12.3, 23.5]	20.0 [9.8, 33.5]	0.180	0.273
Creatinine[g] (median [IQR])	0.8 [0.6, 0.9]	0.8 [0.6, 1.0]	0.9 [0.7, 1.3]	0.702	0.627
Outcomes					
Hospital Stay, days (median [IQR])	3.5 [1.0, 22.0]	11.0 [1.0, 63.0]	4.0 [2.0, 53.0]	<0.001*	0.001*
Intensive Care Unit (ICU) (%)				<0.001*	
Yes	7.0 (21.9)	21.0 (80.8)	5.0 (35.7)		<0.001*
No	25.0 (78.1)	5.0 (19.2)	9.0 (64.3)		
ICU Stay, days (median [IQR])	0.0 [0.0, 11.0]	7.0 [0.0, 62.0]	0.0 [0.0, 21.0]	0.001*	<0.001*

SD: standard deviation, IQR: interquartile range.
[a]Wilcoxon p-values.
[b]Kruskal-Wallis p-values.
Units of measurements: [c] kg/m^2, [d] mmHg, [e] g/dL, [f] 10e3 cells/mcL, [g] mg/dL.

19 severity. Due to the small study sample size, separate models were used to iteratively assess the impact of patient demographics, clinical cytokines, and immunoglobulins measurements on COVID-19 severity. All statistical tests were two-sided, conducted at a confidence level of alpha = 0.05, and performed using R [20].

2.4. Supervised learning for predicting COVID-19 severity

Linear support vector machine models were fitted to perform the binary classification task of predicting mild vs. moderate/severe COVID-19 using the python library scikit-learn [21]. Data were first split into training (75%) and testing (25%) sets. Data were pre-processed with centering, scaling, and imputing with the feature mean. The pre-processing procedure was first applied to the training data before the same method was applied without re-calculation to the validation and test sets. To estimate model accuracy and tune the model's regularization hyperparameter, we performed leave-one-out cross validation with grid search. The range of values searched through was [0.01, 10]. After cross validation, we fitted a final trained model using the entirety of the training set. We evaluated the performance of the final model with AUROC (Area Under the Receiver-Operator Characteristic curve) of the held-out test set. For interpretability, we extracted and plotted the final model's coefficients.

3. Results

3.1. Study population

The study population included 72 subjects admitted to a single large, urban hospital between April 2020 and December 2020 (58 patients diagnosed with COVID-19 and 14 controls). Baseline demographic variables are summarized in Table 1. Maximal COVID-19 severity was assessed for all patients to characterize them as mild (DSS$\leq$4, n = 32) or moderate/severe (DSS$\geq$5, n = 26) [14]. Groups were well matched for most baseline clinical and laboratory variables. Mean (SD) age in years was 51.9 (11.3) for mild, 54.8 (12.9) for moderate/severe cases, and 54.6 (14.5) for controls. The proportion of female patients was 21.9% for mild, 26.9% for moderate/severe, and 42.9% for controls. Body mass index (BMI) was found to be significantly lower in the mild versus moderate/severe COVID-19 groups (mean (SD) BMI 27.9 (5.3) kg/m^2 in mild versus 31.3 (6.9) kg/m^2 in moderate/severe, p = 0.042).

3.2. COVID-19 immune signature

Serum from hospital day 1 was available from 67 subjects (53 SARS-CoV-2-positive patients and 14 controls) and analyzed for IL-6, IL-8, IL-10, TNF-α, MCP-1, IP-10, and anti-SARS-CoV-2 (S1, S2, RBD, N) IgM and IgG (summarized in Table 2 and Fig. 1). Median IP-10 was significantly higher in COVID-19 patients versus controls: 602.9 (interquartile range: 35.5–38,228.8) for all COVID-19 patients combined versus 259.9 (79.2–437.6) pg/mL for controls, p = 0.002. However, IP-10 was not significantly different between mild and moderate/severe cases, and no other significant differences in cytokines were observed between groups. As expected, mean MFI for all SARS-CoV-2 antibodies were significantly elevated in COVID-19-positive subjects versus controls. Of greatest interest, IgG against all antigens (S1, S2, RBD, N) and IgM anti-S1 were significantly elevated in the moderate/severe group relative to the mild disease group.

Next, we examined the interaction between cytokines and SARS-CoV-2 antibodies at the individual patient level. A PCA projection

Table 2
Distribution of cytokines and immunoglobins by COVID-19 status and severity.

Serum Biomarkers	COVID-19 Severity		Control n = 14	p-value	
	Mild n = 29	Moderate/Severe n = 24		Mild vs Moderate/Severe[a]	Overall[b]
Cytokines[c] (median [IQR])					
IL-6	25.8 [0.8, 212.4]	19.5 [3.0, 1423.0]	22.8 [0.8, 395.2]	0.872	0.983
IL-8	17.8 [1.2, 261.6]	19.2 [6.8, 897.9]	14.7 [2.8, 125.5]	0.543	0.660
IL-10	20.5 [0.4, 966.6]	9.1 [0.4, 184.7]	7.3 [0.0, 126.6]	0.204	0.359
IP-10	640.8 [35.5, 33,978.8]	493.3 [115.8, 38,228.8]	259.9 [79.2, 437.6]	0.381	0.005*
MCP-1	35.4 [2.2, 963.8]	489.1 [231.0, 1968.9]	465.5 [212.6, 973.7]	0.943	0.350
TNF-α	35.4 [2.1, 963.8]	43.7 [2.2, 1939.7]	37.0 [2.1, 855.6]	0.353	0.470
Immunoglobulins[d] (median [IQR])					
IgM N	4733.0 [284.5, 15,147.0]	7680.5 [662.0, 17,060.8]	1913.2 [149.3, 7531.0]	0.231	0.001*
IgM RBD	7461.0 [824.0, 15,086.5]	8599.5 [919.7, 17,655.8]	1508.3 [226.0, 7138.0]	0.260	<0.001*
IgM S1	3273.5 [36.5, 12,469.0]	5879.8 [193.2, 14,267.5]	90.3 [3.5, 7783.3]	0.037*	<0.001*
IgM S2	4827.2 [126.5, 14,040.0]	6285.1 [699.7, 15,828.0]	743.9 [65.3, 3437.4]	0.509	<0.001*
IgG N	11,325.4 [566.0, 20,196.0]	15,410.2 [8896.7, 20,982.2]	1532.2 [530.0, 18,523.8]	0.032*	<0.001*
IgG RBD	9936.0 [158.8, 19,081.2]	15,547.4 [7840.2, 18,413.4]	952.3 [302.3, 17,814.0]	0.007*	<0.001*
IgG S1	7451.7 [19.8, 18,378.7]	14,709.6 [3839.7, 19,549.9]	107.0 [2.8, 15,807.3]	0.003*	<0.001*
IgG S2	16,497.0 [2642.8, 22,820.7]	19,564.6 [5626.5, 24,951.3]	3376.4 [660.0, 24,058.0]	0.015*	<0.001*

SD: standard deviation, IQR: interquartile range.
[a]Wilcoxon p-values.
[b]Kruskal-Wallis p-values.
Units of measurements: [c] pg/mL, [d] mg/dL.

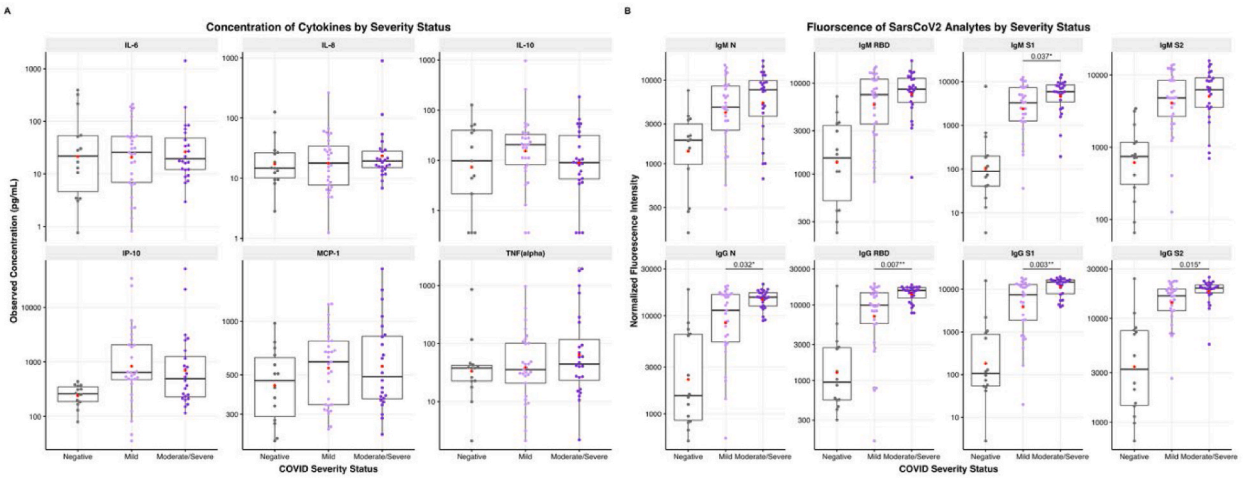


Fig. 1. Comparison of Cytokine and SarsCoV-2 Antibodies by COVID-19 Severity Status Boxplots denote median (black horizontal line) and first and third quartiles (boxes) and the highest and lowest values within 1.5 * inter-quartile range (whiskers). Mean is denoted by red dot. Subjects of different COVID-19 severity status show different (A) concentrations (pg/mL) of cytokines and (B) normalized mean fluorescence intensity (MFI) of SARS-CoV-2 antibodies. P-values were computed using Wilcoxon tests. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

method was used to combine specific anti-SARS-CoV-2 serologic markers into a single variable to classify subjects as either anti-SARS-CoV-2 "High IgG" or "Low IgG." The results showed a significant difference in IP-10 between the high and low IgG groups (mean(SD) 904(1032) pg/ml in high and 5644(10,885) pg/ml in low, $p = 0.036$) (Fig. 2). No significant differences were observed in the other cytokines/chemokines examined.

3.3. *Effect of clinical, laboratory and immunological variables on COVID-19 disease severity*

Linear logistic regression models were used to estimate the odds ratio (OR) for the effect of individual markers on COVID-19 acuity controlling for age and sex. In considering the effect of basic demographic and clinical variables on COVID-19 disease severity, we observed that only BMI exhibited a significant association with worse disease severity (OR of BMI for moderate/severe vs mild COVID-19 of 1.15 (95%CI: 1.00–1.33, $p = 0.044$). Next, we evaluated the effect of the immunologic biomarkers on COVID-19 severity. The

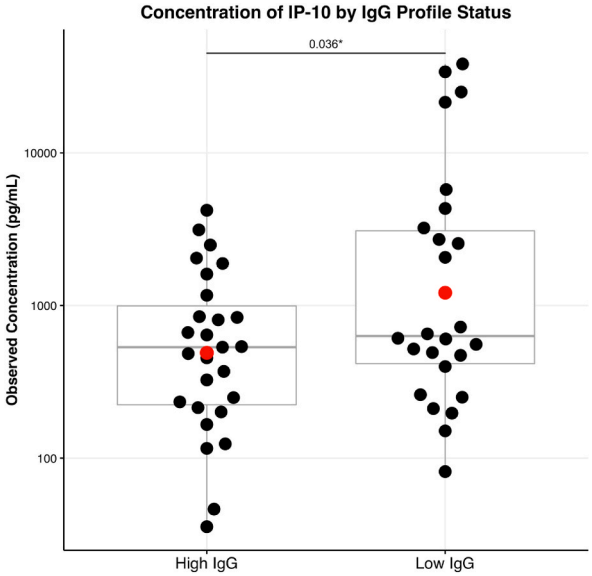
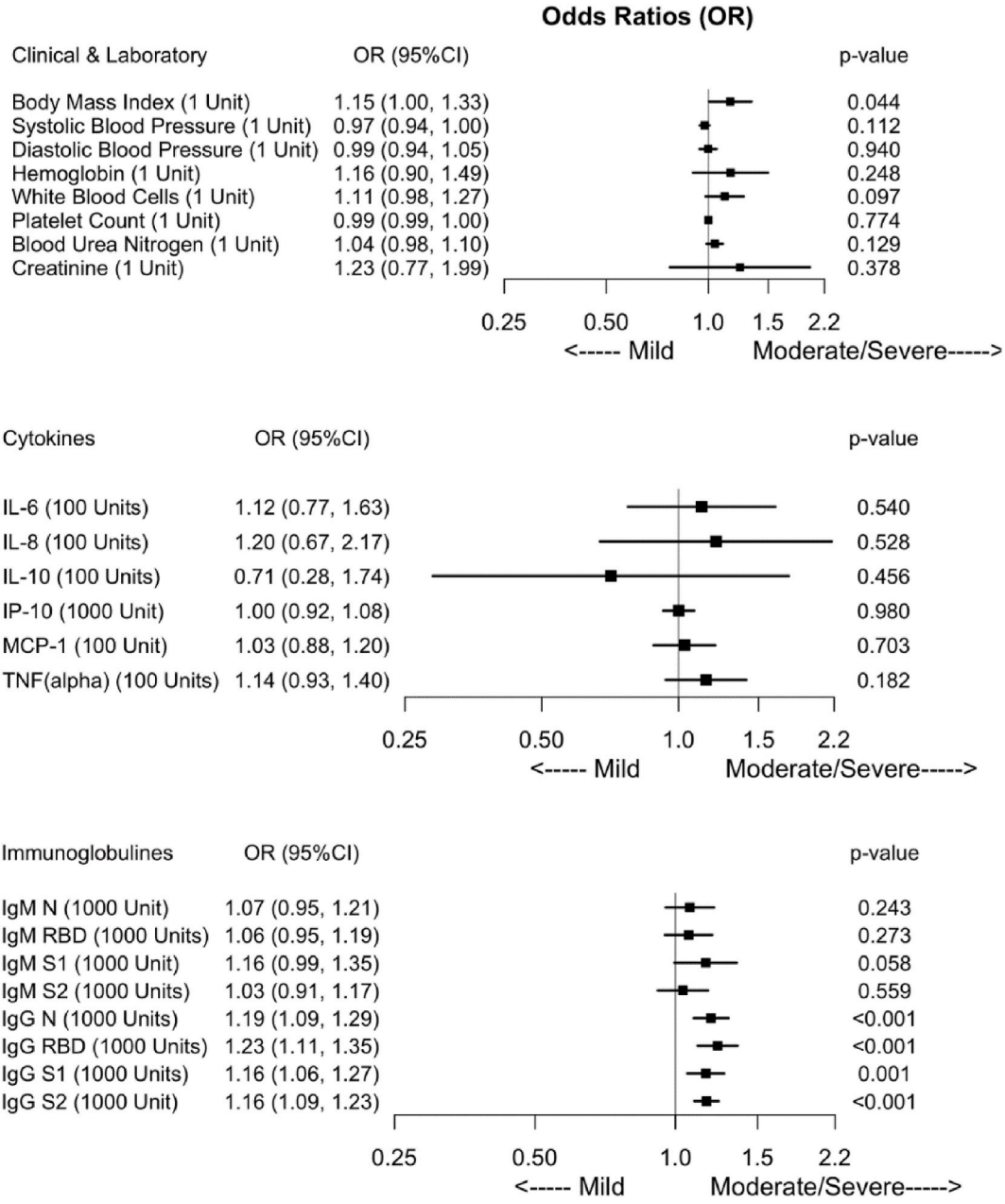


Fig. 2. Comparison of IP-10 and anti-SARS-CoV-2 antibody levels. Boxplots denote median and first and third quartiles (boxes) and the highest and lowest values within 1.5 * inter-quartile range (whiskers). Mean is denoted in red. P-value was computed using the Welch two sample *t*-test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

ORs for mild versus moderate/severe COVID-19 for the evaluated cytokines were not statistically significant (Fig. 3). However, we did observe a strong trend for increased ORs for IgM and a statistically significant effect for increased IgG for each of the SARS-CoV-2 antibodies examined. We conclude that SARS-CoV-2 antibody concentrations, particularly of the IgG isotype, are significantly associated with COVID-19 disease severity.

3.4. *Supervised learning for predicting COVID-19 severity*

Finally, we used a supervised learning approach to analyze the combined data set and provide additional insights into the combination of clinical and biological markers associated with COVID-19 infection and disease severity. We chose linear support vector machine models for their robustness and interpretability. A linear support vector machine model (SVM) was constructed (Fig. 4). Overall, the linear support vector machine achieved an accuracy of 64% and an AUROC of 0.81 in predicting COVID-19 severity status.



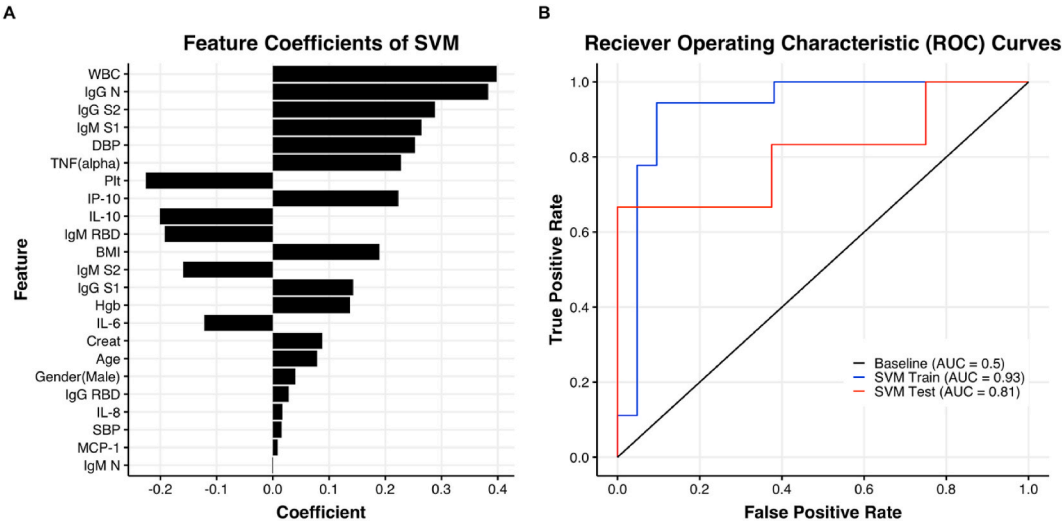


Fig. 4. A. Features coefficients extracted from the trained SVM COVID-19 severity prediction model. Features with higher absolute coefficient magnitudes are more likely to be significant predictors of severity. B. Receiver operator characteristic curves (ROC) of the SVM model evaluated on training data (blue), the SVM model evaluated on testing data (red), and a theoretical baseline model that predicts purely randomly (black). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Feature weights of this model represent the hyperplane that best separates the classes. As such, the weights provide additional insights into serological markers of biological significance and allow for assessment of the role of each variable independently. The results suggest that the most important clinical variables for predicting disease severity are white blood cells count (WBC), diastolic blood pressure (DBP), and platelet count (Plt), while the most important serologic markers are IgG anti-SARS-CoV-2 N, S1, S2, TNF-α, IP-10, and IL-10. Notably, this supervised learning approach also demonstrated that anti-SARS-CoV-2 IgGs are significant markers of COVID-19 severity.

4. Discussion

The primary goal of this study was to identify biomarkers associated with COVID-19 severity based on subjects admitted to a single, large urban hospital. Our approach focused on a combined analysis of chemokines, cytokines, and immunologic markers along with basic clinical and demographic variables, all of which could be assessed at the time of admission. As the patients were enrolled before the widespread availability of a SARS-CoV-2 vaccine, the data is particularly relevant to an unvaccinated population sampled during a time before SARS-CoV-2 Delta or later variants were in circulation [22].

Consistent with prior studies, we found that BMI correlated with an increased risk for higher COVID-19 disease severity [23,24]. However, other routine clinical or laboratory markers did not influence this risk. Further, in contrast to other studies, we did not detect an increased risk for more severe COVID-19 with elevation in IL-6 or other chemokines/cytokines at admission. Our data contrasts with other reports which identified IP-10, IL-10, and IL-6 elevation at admission as a “severity triad” associated with clinical deterioration [8].

Of the cytokine/chemokine markers assessed in this study, only IL-6 is commercially available as a single marker test for diagnostic use based on a FDA Emergency Use Authorization in the setting of the current COVID-19 pandemic. Its intended use is to assist in identifying severe inflammatory response in patients with confirmed COVID-19 illness to aid in determining the risk of intubation with mechanical ventilation, in conjunction with clinical findings and the results of other laboratory testing. Its emergency use authorization was supported by studies such as by Herold et al., who demonstrated that the maximal IL-6 level was strongly associated with the need for mechanical ventilation in a cohort of 89 patients hospitalized with COVID-19 [25]. In that study, the AUC for IL-6 (0.97) outperformed that of C-reactive protein (CRP) (0.86), an inflammatory marker routinely available in hospital laboratories. IL-6 testing has been further adopted by some hospitals that treat COVID-19 with IL-6 inhibitors such as tocilizumab, which is FDA-approved for various rheumatologic conditions and has been proposed to mitigate hyperinflammation associated with COVID-19, with varying outcomes [26]. IL-6 levels may aid in identifying patients who will respond well to tocilizumab therapy [27,28].

Interestingly, our data did not demonstrate significant differences in IL-6 levels between patient groups, and IP-10 was the sole cytokine to demonstrate significance between COVID-19 patients and controls. In this regard, our results are similar to those of Yang et al., who found that IP-10, but not IL-6, could predict the progression of COVID-19 [29]. IP-10, also known as CXCL10, has been shown to increase in patients with multiple types of respiratory viruses and has been proposed as a potential biomarker for the diagnosis of a viral infection and/or severity of infection [30]. While IP-10 alone is not currently available as an FDA-cleared test in clinical settings, it is part of the recently-cleared MeMed BV test (MeMed, Ltd.), which is an automated semi-quantitative immunoassay that simultaneously measures TNF-related apoptosis-inducing ligand (TRAIL), IP-10, and CRP in serum samples and results with a

single score that is intended to aid in differentiating bacterial from viral infections [31]. A separate study from our institution examined the temporal dynamics of host immune response in COVID-19 severity using MeMed BV and found the most significance between severe and non-severe patients with higher IP-10 levels on day 3 of hospitalization [32].

Our data showed the strongest correlation between elevations in SARS-CoV-2 antibodies and higher disease severity. Several types of SARS-CoV-2 antibody tests are widely available for clinical use by FDA Emergency Use Authorization, though their clinical utility is currently limited [33]. Unlike SARS-CoV-2 RNA tests, which have sufficient sensitivity in the early stages of infection, antibody-based tests typically take at least two or more weeks after the onset of symptoms to become positive and thus are not useful to rule in or out an infection [34]. Current serologic tests may be useful in limited situations, such as for symptomatic patients presenting >9–14 days post symptom onset who test negative by molecular testing, or in cases of suspected multisystem inflammatory syndrome in children (MIS-C) [35]. Current assays typically target either the nucleocapsid or the spike proteins of the virus and may be available as "total" antibody tests or may target IgG only. Results may be qualitative (positive/negative) or semi-quantitative, but the interpretation of quantitative measurements is not well understood, with most studies to date focusing on vaccine response or epidemiology of past infections rather than following natural infection courses [36]. Our results suggest that further elucidation and quantitation of SARS-CoV-2 antibodies specific to the S1, S2, RBD, and N proteins may provide insights into disease progression and severity.

Strengths of this study include a combined analysis of both cytokine/chemokine and immune response (antibody) biomarkers. However, we also acknowledge several important limitations to our approach. First, we acknowledge the relatively small size of our study and emphasize that our subjects were enrolled at a time before widespread vaccination against SARS-CoV-2. While we did observe a strong association between elevations in anti-SARS-CoV-2 antibodies against all antigens tested (including those that would only indicate "natural" infection) with worse outcome, this result may be blunted or obscured in a vaccinated population. Second, as our study relied on retrospective passive data collection, some important clinical variables, such as day of symptom onset, and additional severity details about the COVID-negative control patients, were not available and not included in our analysis. Third, as the patient population was enrolled during the early phases of the pandemic, patients did not receive antiviral medications and antibody therapy currently available for patients admitted for more severe forms of COVID-19. These treatments may blunt the effects on biomarkers we observed in this population. Fourth, while we were successful in constructing a SVM model that achieved an accuracy of 64% and an AUROC of 0.81 for predicting COVID-19 disease severity, this model was only tested on our single, institution data set. Further studies would be needed to more fully explore any associations, demonstrate the clinical utility, and validate any such model on a much larger, independent sample set.

5. Conclusions

In summary, our data support prior observations associating obesity (higher BMI) with worse COVID-19 disease outcome and suggest that methods to accurately quantify SARS-CoV-2 antibody and IP-10 concentrations in the blood could help to identify patients with an elevated risk of developing more severe forms of COVID-19. An important negative observation in our data is that it does not support the routine use of IL-6 as a prognostic marker in patients admitted for COVID-19. We suggest that future studies continue to jointly explore both cytokines/chemokines and antibody responses to SARS-CoV-2 infection to better understand and treat COVID-19.

Author contributions

Allison B. Chambliss: Conceptualization, Methodology, Funding acquisition, Writing. **Mayada Aljehani**: Formal analysis, Writing. **Brian Tran**: Investigation, Data curation. **Xingyao Chen**: Formal analysis. **Elizabeth Elton**: Formal analysis. **Carolina Garri**: Investigation, Data curation. **Nolan Ung**: Formal analysis. **Naim Matasci**: Formal analysis. **Mitchell E. Gross**: Conceptualization, Methodology, Funding acquisition, Writing.

Ethics

This study was approved by the Institutional Review Board of the University of Southern California in Los Angeles, CA USA (USC IRB: HS-20-00322). All procedures involving human samples conformed to the principles outlined in the Declaration of Helsinki. Prospective informed consent was obtained in all cases prior to sample acquisition.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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