Original Research *Feature article*

Elevated SARS-CoV-2 in peripheral blood and increased COVID-19 severity in American Indians/Alaska Natives

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Impact Statement

Since the start of the pandemic, COVID-19 has disproportionately affected certain racial/ethnic groups, including American Indian or Alaska Native (AI/AN) populations who have experienced high rates of infection, hospitalization, and mortality. Despite national epidemiological trends identifying health disparities, the relationship between SARS-CoV-2 viral load (VL) dynamics and COVID-19 severity is largely unexplored in a diverse cohort of hospitalized patients, such as the one reported here. For patients enrolled in 2020 (May to October) with SARS-CoV-2 viral clades 20A, C, and G, the strongest predictor of severe COVID-19 was the mean VL in peripheral blood (PB). Self-reported race/ethnicity as AI/AN was associated with severe COVID-19 and significantly higher SARS-CoV-2 levels and frequency of detection in PB. These results offer evidence, at least in part, for the higher burden of severe disease. Treatment interventions targeting SARS-CoV-2 in PB may be an important strategy for improving patient outcomes, especially in vulnerable populations.

Abstract

Epidemiological data across the United States show health disparities in COVID-19 infection, hospitalization, and mortality by race/ethnicity. While the association between elevated SARS-CoV-2 viral loads (VLs) (i.e. upper respiratory tract (URT) and peripheral blood (PB)) and increased COVID-19 severity has been reported, data remain largely unavailable for some disproportionately impacted racial/ethnic groups, particularly for American Indian or Alaska Native (AI/AN) populations. As such, we determined the relationship between SARS-CoV-2 VL dynamics and disease severity in a diverse cohort of hospitalized patients. Results presented here are for study participants ($n=94$, ages 21–88 years) enrolled in a prospective observational study between May and October 2020 who had SARS-CoV-2 viral clades 20A, C, and G. Based on self-reported race/ethnicity and sample size distribution, the cohort was stratified into two groups: (AI/AN, $n=43$) and all other races/ethnicities combined (non-AI/AN, $n=51$). SARS-CoV-2 VLs were quantified in the URT and PB on days 0–3, 6, 9, and 14. The strongest predictor of severe COVID-19 in the study population was the mean VL in PB (OR = 3.34; $P=2.00\times10^{-4}$). The AI/AN group had the following: (1) comparable co-morbidities and admission laboratory values, yet more severe COVID-19 (OR = 4.81; $P=0.014$); (2) a 2.1 longer duration of hospital stay ($P=0.023$); and (3) higher initial and cumulative PB VLs during severe disease ($P=0.025$). Moreover, self-reported race/ethnicity as AI/AN was the strongest predictor of elevated PB VLs ($\beta=1.08$; $P=6.00\times10^{-4}$) and detection of SARS-CoV-2 in PB (hazard ratio = 3.58; $P=0.004$). The findings presented here suggest a strong relationship between PB VL (magnitude and frequency) and severe COVID-19, particularly for the AI/AN group.

Keywords: SARS-CoV-2, COVID-19, race/ethnicity, viral load, hospitalization, intensive care

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Introduction

Data collected by the Centers for Disease Control and Prevention (CDC) throughout the COVID-19 pandemic for five groups of race/ethnicity demonstrate that certain groups suffer a disproportionate disease burden.[1,2] Such data can help identify risk markers for underlying conditions that affect health such as socioeconomic factors, health-care access, and occupational exposure risk. Throughout the pandemic, American Indian or Alaska Native (AI/AN) populations have experienced the highest proportion (per 100,000) of COVID-19 hospitalizations and deaths, and along with Hispanics or Latinos, the highest proportions of incident cases.[1,2] As such, COVID-19 follows a similar pattern as that witnessed for other respiratory infections. This includes the high rate of mortality among AI/AN persons during the 1918 Spanish influenza pandemic, and more recently, during the 2009 influenza (H1N1) pandemic.[3–5] While many important drivers could explain elevated morbidity and mortality from communicable diseases in AI/AN populations, including social determinants of health, investigations presented here focus on the extent to which SARS-CoV-2 viral load (VL) dynamics relate to increased COVID-19 disease severity in this group.

Estimates early in the pandemic (2020) from 44,672 confirmed SARS-CoV-2 infections (non-variant) in China revealed that 81% of symptomatic individuals develop mild disease, 14% experience severe symptoms requiring hospitalization, and 5% are critical, necessitating intensive care unit (ICU) support.[6] In patients who progress to severe disease, the median interval between infection and development of dyspnea is 5–8 days, acute respiratory distress syndrome (ARDS) is 8–12 days, and ICU admission is 10–12 days.[7–10] In addition to ARDS, the inflammatory response to SARS-CoV-2 can also promote life-threatening sepsis and multiorgan injuries to the lung, heart, liver, kidneys, gastrointestinal tract, and immune system.[7,11,12]

The magnitude and duration of the SARS-CoV-2 viral burden in the upper respiratory tract (URT) appear to play a substantial role in COVID-19 disease severity. Non-variant SARS-CoV-2 VLs, measured by quantitative reverse transcription polymerase chain reaction (RT-qPCR), in the URT typically peak within the first week of disease onset.[13,14] A previous study showed that 90% of the individuals with mild disease had undetectable levels of SARS-CoV-2 in the URT beyond day 10, whereas 100% of the patients with severe disease had detectable virus after day 10 with levels up to 60-fold higher.[15] Elevated levels of SARS-CoV-2 in the URT are more prevalent with advanced age, severe disease, and mortality.[7,10,14,16–22] In addition, detection of SARS-CoV-2 in peripheral blood (PB), measured in either whole blood or fractionated portions (e.g. plasma or serum), is also associated with more severe COVID-19, including ICU admission, invasive mechanical ventilation, and mortality.[23–27] Collectively, these results illustrate high levels of prolonged SARS-CoV-2 in both the URT and PB are associated with an increase in COVID-19 disease severity and mortality.

Despite the disproportionate disease burden witnessed in certain populations, data are largely unavailable on the clinical course and pathogenesis of COVID-19 in hospitalized patients of AI/AN descent. To better understand the relationship between SARS-CoV-2 VLs and disease outcomes, we conducted a single-site, prospective observational study in a diverse cohort of hospitalized patients (≥18 years, $n = 94$) with COVID-19. Patients were recruited from the University of New Mexico Hospital (UNMH), a 618-bed tertiary care facility, serving as a referral center for the state and surrounding regions, including tribal lands. To determine if VL dynamics in hospitalized patients could provide insight into differing COVID-19 disease severities, we compared SARS-CoV-2 VLs ($\log_{10}$ copies/1000 cells) in the URT and PB between two groups: AI/AN and all other races/ethnicities combined (non-AI/AN).

Materials and methods

Study design and participants

Patients ($n = 94$, age 21–88 years) in the prospective observational study were recruited from the ICU and non-ICU wards at UNMH from 18 May to 20 October 2020. Only patients with a positive SARS-CoV-2 polymerase chain reaction (PCR) test were enrolled. Exclusion criteria included age <18 years, treatment with extracorporeal membrane oxygenation (ECMO) at study entry, and either individuals or their legally authorized representative (LAR) who did not have the decision capacity/competency to provide informed consent. Race/ethnicity was self-reported by the patient/LAR. The study was approved by the UNM Health Sciences Center Human Research Protection Office (protocol #20-194).

Co-morbidities were determined by a medical chart review and verbal interview with either the patient or LAR. Clinical and laboratory variables were measured daily from admission throughout hospitalization. SARS-CoV-2 VLs in the URT and whole blood (5–7 mL, EDTA-vacutainers) were measured in samples collected on days 0, 1, 2, 3, 6, 9, and 14. Sample collection starting on day 0 was within 24 h of enrollment. URT samples consisted of either nasal swabs (NSs) for non-intubated patients or nasopharyngeal swabs (NPs) for patients who were intubated. Swabs were placed into 2 mL of viral transport media (VTM) and centrifuged at 1,200*g*. Whole blood was obtained by either venipuncture or from an arterial line.

Disease severity was defined by admission to the ICU at any time throughout the two-week sampling period: non-severe (non-ICU) and severe (ICU). Patients who died during the two-week timeframe were classified as severe disease, regardless of admission to the ICU. Patients were also classified using a modified eight-category ordinal scale based on oxygen requirements.[28,29] Clinical outcome measures during hospitalization included invasive mechanical ventilation, renal replacement therapy, acute hepatic injury, heart failure, thrombotic events (i.e. stroke or venous thromboembolism, VTE), shock, and encephalopathy. Additional clinical outcome measures were the worst ordinal scale score throughout the two weeks, length of stay (days), and 14-day and in-hospital mortality.

Quantification of SARS-CoV-2 VLs in the URT and PB

Aliquots of VTM and whole blood were inactivated in DNA/RNA Shield™ (Zymo Research, Irvine, CA, USA) and stored at −80°C until batch processing. Viral RNA was isolated using the Quick-RNA Viral kit (Zymo Research). VL

was determined by RT-qPCR with N1 and RNase P (RP) primers and probes (CDC 2019-nCoV Real-Time RT-PCR diagnostic panel) under the recommended conditions using an Applied Biosystems StepOnePlus™ Real-Time PCR system. Samples with C_t (cycle threshold) values ⩾40 were considered negative.

Standard curves were generated from the C_t values of N1 using known concentrations of SARS-CoV-2 RNA (WA1-USA strain, BEI Resources) and RP using plasmids harboring the human RP gene fragment. VLs were quantified using the equation below. N1 was normalized to the respective RP and multiplied by a factor of 5×10^7 RnaseP/1000 cells.[30] Results were $\log_{10}$ transformed, yielding the final quantified VL ($\log_{10}$ copies/1000 cells).

The following equation presents the viral load quantification from N1 and RP C_t values

$$\text{VL in } \log_{10}\left(\frac{\text{Copies}}{1000\,\text{cells}}\right) = \log_{10}\left(\frac{\text{Copies of N1}}{\text{Copies of RNaseP}} \times \frac{5\times10^7\,\text{RNaseP}}{1000\,\text{cells}}\right)$$

Statistical analyses

For patient demographics, laboratory measures, co-morbidities, clinical events, and disease outcome measures, statistical comparisons between the AI/AN and non-AI/AN groups were analyzed using R version 4.0.3.[31] Statistical significance between group means (reported with SD) and counts (n, presented with percentage, %) was determined by Student's t-test and Fisher's exact test, respectively. Statistical comparisons of the group mean (SD) for VLs ($\log_{10}$ copies/1000 cells) on each day, and cumulatively, were performed using the Wilcoxon rank-sum test due to departure from normal distribution. For cumulative VLs across all the timepoints, mean VL (per patient) for all days was calculated to account for: (1) patients who were not hospitalized for the entire two-week sampling schedule, and (2) patients who died before 14 days. Statistical significance for all analyses was set at $P \leqslant 0.050$.

Logistic regression modeling was performed to determine factors that influenced admission to the ICU (severe disease) with the following co-variates: race/ethnicity (AI/AN and non-AI/AN), sex, age, days symptomatic before enrollment, body mass index (BMI), treatment with remdesivir and/or steroids, and all co-morbidities which occurred in >5 patients. Relevant factors were identified by forward-backward selection minimizing the Akaike information criterion (AIC). To quantify the influence of VL dynamics (both URT and PB) on disease severity, logistic regression was performed with the above co-variates, along with mean VLs and frequency of detection. Predictors of SARS-CoV-2 VL levels in PB were determined by fitting a linear regression using identical co-variates with the mean VL across the 14-day sampling period as the outcome variable. To further understand factors that influence VL dynamics in PB, a multiple-event-per-subject conditional proportional hazard model was fit,[32] as described above for the logistic regression models. An event was defined by detection of SARS-CoV-2 in blood (any level), while time-to-event was measured from the onset of symptoms prior to hospitalization (right-censored). The Bonferroni–Holm correction for multiple testing was applied for all models, disregarding the P value of the model intercept.

Results

Demographic and laboratory values upon admission

Demographic information and clinical laboratory values were obtained for all hospitalized patients on admission (Table 1). To establish if baseline characteristics differed according to race/ethnicity, hospitalized patients ($n = 94$; 40/94 female, ages 21–88 years) were stratified into two groups: AI/AN (45.7%, $n = 43$) and non-AI/AN (54.3%, $n = 51$). The distribution of self-reported race/ethnicity in the non-AI group was Hispanic/Latino (62.7%, $n = 32$), non-Hispanic White (NHW, 21.6%, $n = 11$), Black/African American (11.8%, $n = 6$), and Asian/Pacific Islander (3.9%, $n = 2$). The distribution of females and males, as well as pregnancy status in females, was similar between the two groups ($P = 0.145$ and $P = 1.00$, respectively). The AI/AN group was marginally younger than the non-AI/AN group ($P = 0.050$). Vital sign measures were comparable between the groups, as were baseline hematological values, except for an elevated neutrophil percentage in AI/AN group ($P = 0.030$). Several clinical laboratory values also differed with the AI/AN group having reduced prothrombin time ($P = 0.041$) and lower albumin levels ($P = 0.006$).

Oxygen requirements on admission were comparable between the two groups, as indicated by similar ordinal scale scores ($P = 0.458$). Duration of illness in the AI/AN and non-AI/AN patients upon admission was also similar based on days from the first positive SARS-CoV-2 RT-PCR test ($P = 0.546$) and days symptomatic prior to admission ($P = 0.146$). Thus, although the duration of illness and most laboratory values were comparable upon hospital admission, several factors known to increase the risk of severe disease were witnessed in AI/AN group, namely, elevated neutrophil percentages, and lower prothrombin time and albumin levels.[33–35]

Comorbid conditions

The co-morbidities are listed in Table 2. Several co-morbidities known to increase COVID-19 severity[36,37] were lower in the AI/AN group, such as cancer ($P = 0.036$), coronary artery disease ($P = 0.014$), and heart failure ($P = 0.005$), while all other co-morbidities were comparable. Since the presence of one or more co-morbid conditions is highly prevalent in patients with severe and fatal COVID-19,[38] documented co-morbidities were stratified into none, 1, and >1. Although the stratification for none, 1, or >1 co-morbidities did not reveal any significant differences between the groups, the mean number of co-morbidities was lower in the AI/AN group ($P = 0.031$).

Major clinical events and disease outcome measures during hospitalization

Major clinical events and disease outcome measures were recorded throughout the two-week study period (Table 3).

Table 1. Patient demographics and baseline laboratory characteristics upon admission to hospital.

Patient characteristics	All patients	AI/AN	Non-AI/AN	*P*
Total, *n* (%)	94 (100.0)	43 (45.7)	51 (54.3)	–
Race/ethnicity, *n* (%)				
American Indian/Alaska Native	43 (45.7)	43 (100)	–	–
Asian/Pacific Islander	2 (2.1)	–	2 (3.9)	–
Black/African American	6 (6.4)	–	6 (11.8)	–
Hispanic/Latino	32 (34.1)	–	32 (62.7)	–
Non-Hispanic White	11 (11.7)	–	11 (21.6)	–
Sex at birth, *n* (%)				
Male	54 (57.4)	21 (48.8)	33 (64.7)	0.145
Female	40 (42.6)	22 (51.2)	18 (35.3)	
Pregnant, *n* (%)	5 (5.3)	3 (7.0)	2 (3.9)	1
Age, years, mean (SD)	55.4 (15.6)	51.9 (12.7)	58.2 (17.3)	**0.050**
Temperature (°C)	36.8 (0.9)	36.7 (0.8)	37.0 (1.0)	0.107
Peripheral pulse (bpm)	90.7 (20.5)	89.6 (18.0)	91.6 (24.1)	0.666
Heart rate monitored (bpm)	90.8 (20.5)	90.5 (18.1)	91.0 (22.4)	0.920
Systolic pressure (mmHg)	130.0 (23.7)	125.9 (20.7)	133.5 (25.6)	0.125
Diastolic pressure (mmHg)	78.8 (19.4)	77.2 (18.4)	80.2 (20.3)	0.464
Mean arterial pressure (mmHg)	93.8 (17.9)	90.1 (14.0)	80.7 (14.2)	0.188
Respiratory rate (bpm)	21.5 (6.7)	21.5 (6.6)	21.5 (6.9)	0.993
pO_2 (mmHg)	93.2 (5.8)	93.6 (4.0)	92.8 (7.0)	0.503
WBC ($\times 10^3$/μL)	8.2 (4.7)	8.9 (5.0)	7.7 (4.5)	0.223
Monocytes ($\times 10^3$/μL)	0.6 (0.4)	0.5 (0.4)	0.6 (0.3)	0.283
Monocyte (%)	8.2 (9.4)	6.2 (4.9)	8.0 (4.6)	0.090
Neutrophils ($\times 10^3$/μL)	6.8 (5.3)	7.3 (5.0)	6.4 (5.5)	0.509
Neutrophil (%)	76.2 (10.8)	79.5 (8.7)	73.9 (11.5)	**0.030**
Lymphocyte ($\times 10^3$/μL)	1.1 (0.6)	1.0 (0.7)	1.1 (0.6)	0.617
Lymphocyte (%)	14.9 (8.1)	13.3 (7.1)	16.0 (8.7)	0.179
NLR (#)	9.0 (15.0)	9.7 (14.5)	8.5 (15.5)	0.739
Platelet count ($\times 10^3$/μL)	216.9 (88.6)	229.4 (92.8)	207.8 (85.1)	0.269
Hemoglobin (g/dL)	12.9 (2.5)	12.7 (2.5)	13.1 (2.5)	0.424
RDW (%)	14.2 (1.9)	14.5 (2.3)	14.0 (1.4)	0.197
Prothrombin time (s)	14.5 (2.9)	13.6 (2.1)	15.2 (3.4)	**0.041**
Procalcitonin (ng/mL)	3.1 (14.2)	4.0 (17.6)	2.3 (9.9)	0.685
BUN (mg/dL)	24.9 (21.3)	24.4 (20.8)	25.3 (21.8)	0.845
Creatinine (mg/dL)	1.7 (1.9)	1.9 (2.6)	1.4 (0.9)	0.189
Glucose (mg/dL)	149.5 (84.4)	155.2 (87.9)	145.0 (82.1)	0.577
Albumin (g/dL)	2.7 (0.6)	2.5 (0.5)	2.9 (0.6)	**0.006**
T-Bilirubin (mg/dL)	0.9 (1.1)	0.9 (0.8)	0.9 (1.3)	0.809
Lactate (mmol/L)	2.0 (2.1)	1.4 (0.6)	2.4 (2.6)	0.129
ALP (units/L)	110.1 (71.5)	121.7 (92.2)	100.1 (46.0)	0.214
ALT/SGPT (units/L)	189.0 (913.9)	40.5 (24.9)	317.5 (1241.2)	0.212
AST/SGOT (units/L)	219.7 (955.1)	59.6 (54.1)	358.1 (1295.4)	0.198
Ordinal scale score*	5.3 (1.0)	5.4 (1.0)	5.2 (1.0)	0.458
SARS-CoV-2 + test (days)†	6.5 (5.4)	6.1 (5.0)	6.8 (5.8)	0.546
Symptomatic (days)‡	8.1 (4.9)	7.3 (3.4)	8.8 (5.9)	0.146

WBC: white blood cell; NLR: neutrophil number ($\times 10^3$/μL) to lymphocyte number ($\times 10^3$/μL) ratio; RDW: red blood cell distribution width; BUN: blood urea nitrogen; ALP: alkaline phosphatase; ALT/SGPT: alanine aminotransferase; AST/SGOT: aspartate aminotransferase.
Data are presented as mean value (SD) unless otherwise noted. Baseline demographics, vital signs, and clinical laboratory measures were determined upon admission to hospital. Data are presented for all patients and patients stratified according to self-reported American Indian/Alaska Native (AI/AN) and non-AI/AN (all other races/ethnicities combined). Group means of the AI/AN and non-AI/AN were compared using Student's *t*-tests (two-sided). Homogeneity for categorical variables between AI/AN versus non-AI/AN was tested using Fisher's exact test (two-sided). Bold indicates statistical significance at $P \leqslant 0.05$.
*A modified eight-category ordinal scale was used to classify patient status according to oxygen requirements. Since only hospitalized patients were included in the study, the applicable categories ranged from 4 to 8: (4) room air, (5) low-flow supplemental oxygen delivery, (6) high-flow oxygen delivery or non-invasive positive pressure ventilation, (7) invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO), and (8) death.
†Number of days from first SARS-CoV-2 positive PCR-test until initial sample collection (day 0).
‡Number of days from initial presentation of COVID-19 symptoms until initial sample collection (day 0).

Although none of the clinical events differed significantly, the AI/AN group had a higher percentage of invasive mechanical ventilation (41.9% vs 25.5%), renal replacement therapy (9.3% vs 3.9%), shock (41.9% vs 33.3%), and encephalopathy (25.6% vs 19.6%). Univariate analysis of the proportion of patients who required ICU support revealed more severe disease in the AI/AN group ($P = 0.039$). This finding was confirmed by logistic regression modeling, accounting for

Table 2. Co-morbidities of the hospitalized patients.

Patient co-morbidities	All patients	AI/AN	Non-AI/AN	*P*
Total number	94	43	51	
Cancer	9 (9.6)	1 (2.3)	8 (15.7)	**0.036**
Cardiovascular disease				
Hypertension	47 (50.0)	19 (44.2)	28 (54.9)	0.408
Coronary artery disease	7 (7.4)	0 (0)	7 (13.7)	**0.014**
Heart failure*	12 (12.8)	1 (2.3)	11 (21.6)	**0.005**
Stroke	3 (3.2)	1 (2.3)	2 (3.9)	1
Chronic respiratory disease				
Asthma	11 (11.7)	4 (9.3)	7 (13.7)	0.542
COPD	6 (6.4)	1 (2.3)	5 (9.8)	0.214
Sleep apnea	12 (12.8)	4 (9.3)	8 (15.7)	0.537
Immunosuppression				
Solid organ transplant	3 (3.2)	0 (0)	3 (5.9)	0.247
Autoimmune disease†	3 (3.2)	2 (4.7)	1 (2.0)	0.591
Kidney disease				
Chronic (stages 1–4)	8 (8.5)	3 (7.0)	5 (9.8)	0.723
End-stage (stage 5)	7 (7.4)	3 (7.0)	4 (7.8)	1
Liver disease				
Cirrhosis	5 (5.3)	2 (4.7)	3 (5.9)	1
Other‡	2 (2.1)	2 (4.7)	0 (0)	0.206
Metabolic disease				
BMI, mean (SD)	33.6 (9.6)	34.5 (10.6)	32.8 (8.7)	0.410
Obesity (BMI $\geqslant$30)	52 (59.1)	23 (57.5)	29 (56.9)	0.836
Morbid obesity ($\geqslant$40)	18 (20.5)	7 (17.5)	11 (22.9)	0.604
Diabetes§	45 (47.9)	23 (53.5)	22 (43.1)	0.408
Controlled	23 (51.1)	10 (43.5)	13 (59.1)	0.379
Uncontrolled	22 (48.9)	13 (56.5)	9 (40.9)	
Hyperlipidemia	23 (24.5)	12 (27.9)	11 (21.6)	0.631
Hypothyroidism	7 (7.4)	1 (2.3)	6 (11.8)	0.121
Smoker				
Past	15 (16.0)	4 (9.3)	11 (21.6)	0.158
Current	13 (13.8)	5 (11.6)	8 (15.7)	0.766
Comorbidity summary				
None	4 (4.3)	1 (2.3)	3 (5.9)	0.261
1	18 (19.1)	11 (25.6)	7 (13.7)	
$>$1	72 (76.6)	31 (72.1)	41 (80.4)	
Co-morbidities, mean (SD)	3.2 (2.1)	2.7 (1.6)	3.6 (2.3)	**0.031**

COPD: chronic obstructive pulmonary disease; BMI: body mass index.
Data are presented as the number of patients (*n*) and percentage (%) in each category unless otherwise noted. Data are presented for all patients and patients stratified according to self-reported American Indian/Alaska Native (AI/AN) and non-AI/AN (all other races/ethnicities combined). Co-morbidities were determined by a medical chart review and a verbal interview with either the patient or a legally authorized representative. Homogeneity between the groups for categorical variables, *n* (%), was tested by Fisher's exact test (two-sided). Differences in group means between AI/AN and non-AI/AN were tested using Student's *t*-tests (two-sided). Bold indicates statistical significance at $P \leqslant 0.05$.
*Defined by a prior diagnosis of heart failure (preserved ejection fraction and reduced ejection fraction) and cardiomyopathy (ischemic and non-ischemic).
†Defined by a prior diagnosis of either lupus ($n=1$ in the overall group: $n=1$ in the AI/AN group and $n=0$ in the non-AI/AN group, $P=0.457$) or rheumatoid arthritis ($n=2$ in the overall group: $n=1$ in the AI/AN group and $n=1$ in the non-AI/AN group, $P=1$). Other autoimmune diseases were not reported in the population.
‡Defined by a prior diagnosis of either chronic hepatitis C ($n=1$ in the overall group: $n=1$ in the AI/AN group and $n=0$ in the non-AI/AN group, $P=0.457$) or non-alcoholic fatty liver disease ($n=1$ in the overall group: $n=1$ in the AI/AN group and $n=0$ in the non-AI/AN group, $P=0.457$).
§Controlled versus uncontrolled diabetes was defined by A1C levels: controlled ($<$7.0%) and uncontrolled ($\geqslant$7.0%).

race/ethnicity, sex, pregnancy status, age, days symptomatic prior to enrollment, BMI, treatment with remdesivir and/or steroids, and all co-morbidities that occurred in >5 patients. The highest risk factor for severe disease was self-reported race/ethnicity as AI/AN (OR = 4.81; 95% CI = 1.38–16.80; $P=0.014$), followed by being male (OR = 4.75; 95% CI = 1.57–14.29; $P=0.006$) with both factors remaining significant after multiple test correction (Figure 1). Consistent with having more severe disease, the AI/AN group had 2.1 times longer duration of hospitalization ($P=0.023$, Table 3). Moreover, the AI/AN group had a twofold higher in-hospital mortality ($P=0.074$), but 14-day mortality did not differ between the groups ($P=0.503$).

URT and PB VL dynamics

Since data on VL dynamics remain unreported for certain populations, SARS-CoV-2 VLs were initially compared between the AI/AN and non-AI/AN groups, without stratification according to disease severity. Although URT VLs were similar between the groups on each of the sampling days and across two weeks ($P=0.723$, Figure 2(A)), the

Table 3. Clinical events and disease severity.

Clinical events	All patients	AI/AN	Non-AI/AN	*P*
Total number	94	43	51	
Invasive mechanical ventilation	31 (33.0)	18 (41.9)	13 (25.5)	0.124
Renal replacement therapy*	6 (6.4)	4 (9.3)	2 (3.9)	0.395
Acute hepatic injury†	2 (2.1)	0 (0)	2 (3.9)	0.505
Heart failure	12 (12.8)	4 (9.3)	8 (15.7)	0.546
Thrombotic events				
Stroke	0 (0)	0 (0)	0 (0)	1
VTE‡	7 (7.4)	3 (7.0)	4 (7.8)	1
Shock§	35 (37.2)	18 (41.9)	17 (33.3)	0.196
Encephalopathy	21 (22.3)	11 (25.6)	10 (19.6)	0.326
Disease outcome measures				
Non-severe disease (non-ICU)	54 (57.4)	20 (46.5)	34 (66.7)	**0.039**
Severe disease (ICU)	40 (42.6)	23 (53.5)	17 (33.3)	
Worst ordinal scale score, mean (SD)‖	6.1 (1.2)	6.2 (1.2)	6.0 (1.2)	0.611
Length of stay (days), mean (SD)¶	16.9 (27.1)	23.8 (37.7)	11.2 (9.6)	**0.023**
Mortality (14-day)	10 (10.6)	5 (11.6)	5 (9.8)	0.503
Mortality (in-hospital)	19 (20.2)	12 (27.9)	7 (13.7)	0.074

AI/AN, American Indian/Alaska Native; ICU: intensive care unit; VTE: venous thromboembolism.
Data are presented as the number of patients (*n*) and percentage (%) in each category unless otherwise noted. Data are presented for all patients and patients stratified according to self-reported American Indian/Alaska Native (AI/AN) and non-AI/AN (all other races/ethnicities combined). Only those clinical events that occurred during the two-week sampling time were included. Disease outcome measures for non-severe (non-ICU) and severe (ICU) disease, and the worst ordinal scale score were defined across the two-week sampling time. Mortality was captured at 14 days and throughout hospitalization (in-hospital) from the start of the study. Homogeneity for categorical variables between AI/AN versus non-AI/AN was tested using Fisher's exact test (two-sided), except for non-severe and severe disease, and mortality in which a one-sided test was used since data for the United States and New Mexico illustrate that disease severity and mortality are worse in the AI/AN populations. Group means of the AI/AN and non-AI/AN were compared using Student's *t*-tests (two-sided). Bold indicates statistical significance at $P \leqslant 0.05$.
*Only patients with no history of renal replacement therapy (RRT) prior to hospitalization were included. RRT was defined by continuous renal replacement therapy (CRRT) and intermittent hemodialysis (IHD).
†Defined as aspartate transaminase >5× upper limit of normal (ULN) and alanine transaminase >5× ULN. ULN was defined as ⩽40 U/L for both laboratory measures.
‡Defined as deep vein thrombosis and/or pulmonary embolism events.
§Defined as mean arterial pressure (MAP) ⩽65 mmHg, obtained from cuff and intra-arterial measurements.
‖Defined as the highest (worst) ordinal scale score across the 14-day sampling period using the modified eight-category ordinal scale.
¶Defined as the amount of time spent (days) in hospital from admission to discharge/death.

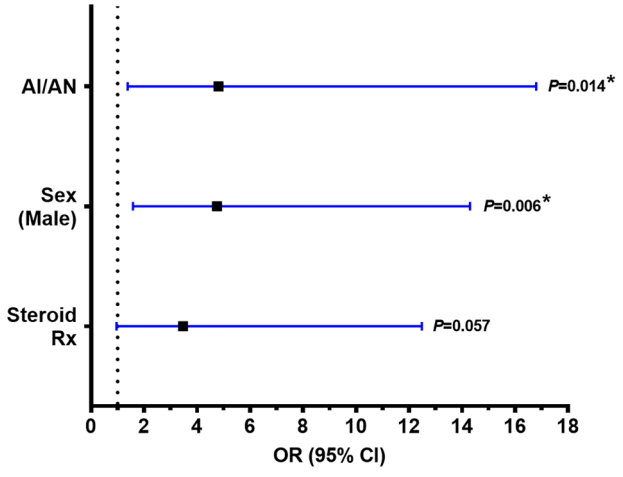

Figure 1. Factors associated with severe disease in hospitalized patients.
AI/AN: American Indian/Alaska Native; Rx: treatment.
Data presented as OR and 95% CI as determined by logistic regression modeling. The outcome variable was the development of severe disease across the 14-day sampling period. Co-variates in the model included race/ethnicity, sex, pregnancy status, age, days symptomatic prior to enrollment, BMI, treatment with remdesivir and/or steroids, and all co-morbidities that occurred in >5 patients. Statistical significance was set at $P \leqslant 0.05$.
*Indicates statistical significance after the multiple test correction.

AI/AN group had higher PB VLs on day 0 ($P = 0.012$), day 1 ($P = 0.004$), day 2 ($P = 0.005$), and cumulatively ($P = 0.009$, Figure 2(B)). To gain additional insight into VL dynamics, the two groups were further stratified by disease severity. URT VLs in non-severe patients were lower in the AI/AN group on day 3 ($P = 0.033$), but comparable cumulatively ($P = 0.368$, Figure 2(C)). URT VLs in patients with severe disease were similar at each timepoint and cumulatively ($P = 0.923$, Figure 2(D)). The same pattern emerged for VL measurements in the PB of patients with non-severe disease ($P = 0.368$, Figure 2(E)). However, among patients with severe disease, the AI/AN group had significantly higher PB VLs on day 0 ($P = 0.012$), day 1 ($P = 0.010$), and cumulatively ($P = 0.025$, Figure 2(F)).

Impact of SARS-CoV-2 in the URT and PB on disease severity

To further explore the relationship between VL dynamics and disease severity, logistic regression modeling was performed with identical co-variates listed above, with the addition of two metrics for both the URT and PB: mean VL and frequency of detection. The strongest predictor of severe disease was the mean VL in blood (OR = 3.34; 95% CI = 1.78–6.27; $P = 2.00 \times 10^{-4}$ Figure 3(A)), the only variable that remained significant after multiple test correction.

Since the mean PB VL was strongly associated with an increased risk of severe disease, whereas neither URT VL levels nor frequency of detection emerged as predictors, a linear regression model was performed with mean VL across the 14-day sampling period as the response variable, considering the full set of co-variates. After multiple test correction, race/ethnicity, hypertension, and hyperlipidemia

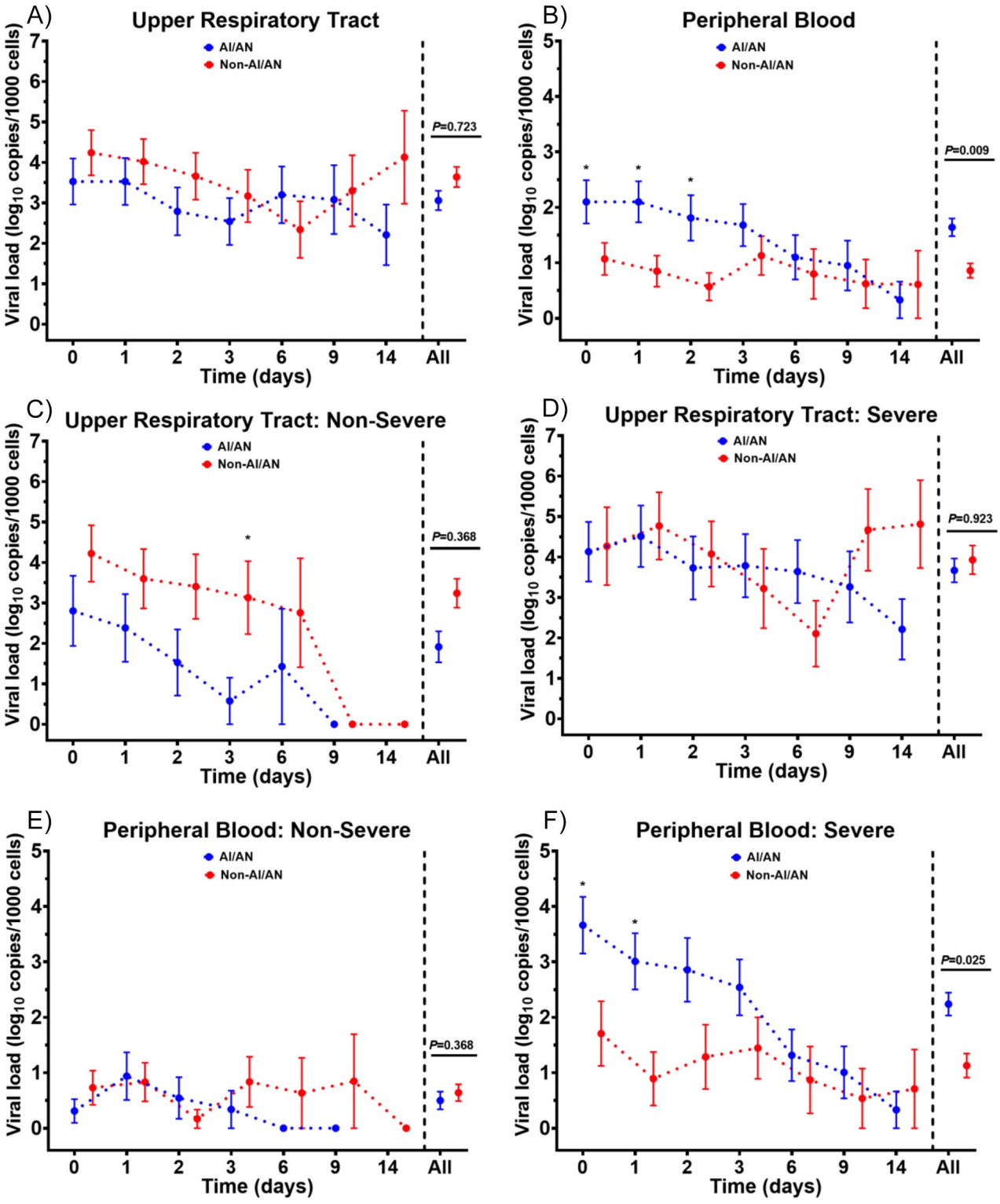

Figure 2. Viral load dynamics in hospitalized patients stratified according to race/ethnicity, and disease severity.
Viral loads (log10 copies/1000 cells) are presented as mean value and SD. Values are offset on each of the days to provide visual clarity. Significant differences between groups on each of the days were determined by the Wilcoxon rank-sum test. Cumulative viral loads (all) were determined by taking the mean value of each patient across all days, followed by comparison of the American Indian/Alaska Native (AI/AN) versus non-AI/AN groups with the Wilcoxon rank-sum test. (A) Upper respiratory tract and (B) peripheral blood viral levels for AI/AN ($n=43$) and non-AI/AN ($n=51$) patients. SARS-CoV-2 was detected in peripheral blood ($\geqslant$1 timepoint(s)) in 58.1% of the AI/AN and 33.3% of the non-AI/AN. (C) Upper respiratory tract viral loads in non-severe (AI/AN [$n=20$] and non-AI/AN [$n=34$]) and (D) severe (AI/AN [$n=23$] and non-AI/AN [$n=17$]) patients. (E) Peripheral blood viral loads in non-severe (AI/AN [$n=20$] and non-AI/AN [$n=34$]) and (F) severe (AI/AN [$n=23$] and non-AI/AN [$n=17$]) patients. SARS-CoV-2 was detected in peripheral blood ($\geqslant$1 timepoint(s)) in 25.0% of the AI/AN and 23.5% of the non-AI/AN with non-severe disease, whereas SARS-CoV-2 was detected in 77.0% of the AI/AN and 52.9% of the non-AI/AN with severe disease.
*Indicates $P \leqslant 0.05$.

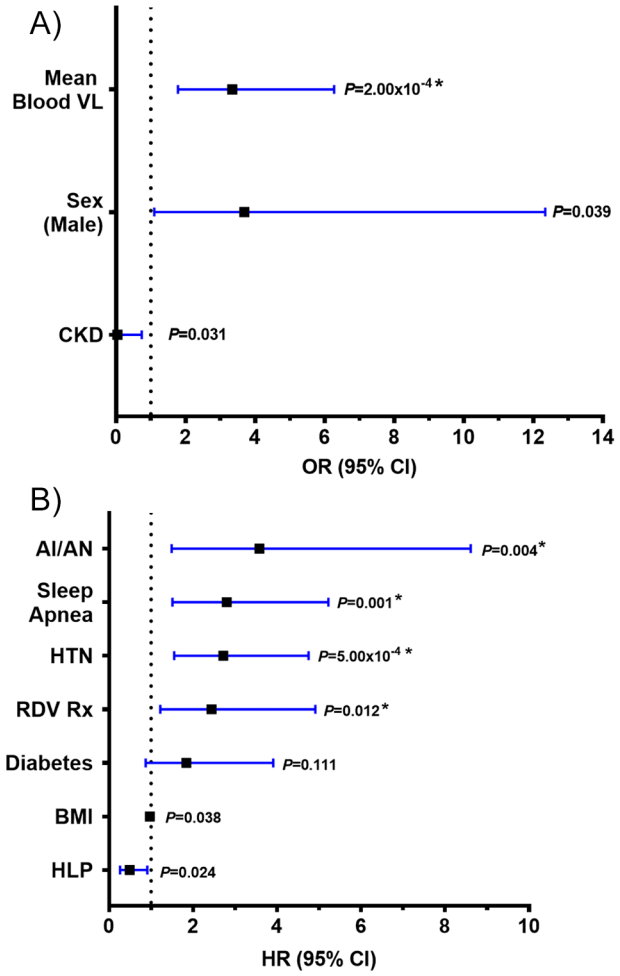

Figure 3. Demographic, clinical, and virological predictors of severe disease and detection of SARS-CoV-2 in peripheral blood in hospitalized patients. (A) Data presented as OR and 95% CI as determined by logistic regression modeling. The outcome variable was development of severe disease across the 14-day sampling period. Co-variates in the model included race/ethnicity, sex, pregnancy status, age, days symptomatic prior to enrollment, BMI, treatment with remdesivir and/or steroids, all co-morbidities that occurred in >5 patients, and two SARS-CoV-2 metrics for both the upper respiratory tract and peripheral blood: mean viral load and frequency of detection. (B) Data are presented as hazard ratios (HR) and 95% CI as determined by a multiple-event-per-subject conditional proportional hazard model. An event was defined as detection of SARS-CoV-2 in blood (any level) and time-to-event was defined by the onset of symptoms prior to hospitalization until the day of measurement (right-censored). Co-variates in the model included race/ethnicity, sex, pregnancy status, age, days symptomatic prior to enrollment, BMI, treatment with remdesivir and/or steroids, and all co-morbidities that occurred in >5 patients. Statistical significance was set at $P \leqslant 0.05$.
VL: viral load; CKD: chronic kidney disease; AI/AN: American Indian/Alaska Native; HTN: hypertension; RDV Rx: remdesivir treatment; BMI: body mass index; HLP: hyperlipidemia.
*Indicates statistical significance after multiple test correction.

remained significant (Table 4). The strongest predictor of elevated PB VLs was self-reported race/ethnicity as AI/AN ($\beta = 1.08$; standard error [SE] = 0.30; $P = 6.00 \times 10^{-4}$), followed by presence of hypertension ($\beta = 0.85$; SE = 0.30; $P = 0.007$), with hyperlipidemia showing protection against elevated PB VLs ($\beta = -1.02$; SE = 0.34; $P = 0.003$).

To further understand viral dynamics in PB, a multiple-event-per-subject conditional proportional hazard model was performed with the full set of co-variates. Among the retained co-variates, four emerged as significant after multiple test correction (Figure 3(B)). The highest risk factor for SARS-CoV-2 detection in PB was self-reported race/ethnicity as AI/AN (hazard ratio [HR] = 3.58; 95% CI = 1.49–8.61; $P = 0.004$), consistent with detection in PB being more frequent in the AI/AN (58.1%) than non-AI/AN (33.3%, $P = 0.022$). Additional factors that increased the risk of SARS-CoV-2 in PB were sleep apnea (HR = 2.80; 95% CI = 1.51–5.22; $P = 0.001$), hypertension (HR = 2.72; 95% CI = 1.55–4.75; $P = 5 \times 10^{-4}$), and treatment with remdesivir (HR = 2.44; 95% CI = 1.22–4.91; $P = 0.012$).

Discussion

Data collected in the United States throughout the COVID-19 pandemic clearly show that AI/AN populations have disproportionately higher cases, hospitalizations, and mortality. Since New Mexico is a uniquely diverse state with the highest percentage of Hispanic/Latino populations (49.3%) and the third-highest proportion of AI/AN populations (11.0%),[39] we can explore the pathogenesis of COVID-19 in populations who were under-represented in the previous studies. Since data presented are for study participants enrolled through October 2020, our findings are representative of earlier SARS-CoV-2 viral clades, as confirmed by our sequencing efforts that revealed the following viral clades: 20A, 20C, and 20G (see Figure S1 in the Supplementary Material).

For primary comparisons in this study, the overall cohort was stratified into AI/AN (45.7%) and non-AI/AN (54.3%). The non-AI/AN group was predominantly Hispanic/Latino (62.7%) and NHW (21.6%) with a smaller proportion of Black/African American (11.8%) and Asian/Pacific Islander (3.9%) individuals. Consistent with our previous study in New Mexicans,[40] analysis of ancestry informative markers in a subset of the cohort revealed high concordance between self-identified race/ethnicity and genetic ancestry estimates (see Table S1 in the Supplementary Material). Of potential importance, the AI/AN group was marginally younger ($P = 0.050$) and had a nearly equal proportion of females and males, whereas the non-AI/AN group had a 1.8-fold higher proportion of males, suggesting hospitalization at a younger age and equivalent susceptibility for both sexes in the AI/AN group. Our ongoing investigations accruing larger samples sizes in three primary groups (AI/AN, Hispanic/Latino, and NHW), along with additional studies by others, will aid in determining the generalizability of these findings.

Clinical laboratory measures upon admission were comparable for the two groups, except for elevated neutrophil (%), lower albumin levels, and reduced prothrombin time in the AI/AN group. This profile of characteristics early in the course of disease is consistent with eventual development of more severe COVID-19 as described in other populations.[33–35] To further assess the patient population, we compared co-morbidities known to influence COVID-19 disease outcomes.[36,37] These analyses revealed that the two groups were largely comparable, except for lower rates of cancer, coronary artery disease, and heart failure in individuals of AI/AN descent. Moreover, the AI/AN group had a significantly lower mean number of co-morbidities. While

Table 4. Predictors of SARS-CoV-2 viral load levels in peripheral blood.

Co-variates	β	SE	*z*	*P*
AI/AN	1.08	0.30	3.59	**6.00×10^{-4}***
Sleep apnea	1.07	0.40	2.65	**0.010**
Chronic kidney disease	1.02	0.54	1.89	0.063
Hypertension	0.85	0.30	2.79	**0.007***
Diabetes	0.78	0.35	2.24	**0.028**
Remdesivir Rx	0.71	0.29	2.44	**0.017**
BMI	–0.02	0.02	–1.55	0.125
Hyperlipidemia	–1.02	0.34	–3.04	**0.003***

AI/AN: American Indian/Alaska Native; Rx: treatment; BMI: body mass index.
Linear regression modeling for predictors of SARS-CoV-2 VL levels in PB. Data are ranked from the highest to lowest β-weight with accompanying standard error of the regression (SE) and *z*-value. The outcome variable was mean VL in blood across the 14-day sampling period with the following co-variates: race/ethnicity (AI/AN and non-AI/AN), sex, age, days symptomatic before enrollment, BMI, treatment with remdesivir and/or steroids, and all co-morbidities which occurred in >5 patients. Bold indicates statistical significance at $P \leqslant 0.05$.
*Indicates statistical significance after multiple test correction.

the underlying reason for this finding remains unclear, it may reflect undiagnosed conditions in the AI/AN group due to less access to care.[41] Since the prospective study began at hospital admission and prior outside records are unavailable, we cannot confirm if differences in comorbid conditions reflect differences in access to care.

Comparisons of major clinical events associated with COVID-19 disease severity across the two-week study period revealed that the AI/AN group had a 1.6-fold higher rate of invasive mechanical ventilation and a 2.4-fold higher rate of renal replacement therapy. Although neither of these results were significantly different, likely due to sample size, they suggest a trend toward more adverse clinical events during hospitalization in the AI/AN group and, therefore, require further exploration in a larger cohort of study participants.

While there are several valid categorization schemes for defining COVID-19 disease severity, we classified severe disease as those individuals who were admitted to ICU at any point throughout the two-week period, and individuals who died throughout the sampling period, irrespective of ICU admission. This definition of severe disease was used because it captures both the respiratory and systemic inflammatory nature of COVID-19 that require critical care. Univariate analysis revealed that the AI/AN group had a significantly higher proportion of severe disease. To account for the complex factors that influence disease severity, logistic regression was performed with a selection model that included all co-variates. The strongest predictor of severe disease was self-reported race/ethnicity as AI/AN, followed by being male. These findings are consistent with national statistics showing a higher proportion of hospitalizations in AI/AN populations, and studies reporting more severe disease in males in the general population.[2,36,37] Of importance, severe disease in females was 2.2 times higher in the AI/AN group (39.1%) than the non-AI/AN group (17.7%), suggesting the potential of increased susceptibility to severe disease in AI/AN women.

To further assess disease severity, we compared the duration (days) of hospitalization between the two groups. The AI/AN group was hospitalized 2.1 times longer, indicating enhanced disease severity that required supportive care for a longer duration, or alternately, a protracted timeframe of mortality. For example, while the 14-day mortality was comparable between the two groups, in-hospital mortality was twofold higher in AI/AN group, albeit non-significant ($P = 0.074$).

After establishing that the AI/AN group had more severe disease, we determined if VL dynamics in the URT and/or PB may explain, at least in part, the disproportionate disease burden. Since the duration of illness prior to hospitalization was comparable for the two groups (i.e., time since first positive SARS-CoV-2 PCR-test and initial presentation of symptoms), temporal VL measures began on day 0 for both groups and were not time-adjusted. Stratification based on race/ethnicity revealed comparable URT VLs across the sampling period. However, the AI/AN group had significantly higher VLs in PB during the initial phase of infection (days 0, 1, and 2) and cumulatively. Because disease severity could account for differences in VLs, the ancestral groups were further stratified into non-severe and severe disease. No differences were observed for URT VLs in either non-severe or severe disease, as was the case for PB VLs in patients with non-severe disease. However, in patients with severe disease, the AI/AN group had significantly higher PB VLs during the early phase of infection (days 0 and 1) and cumulatively, suggesting that the magnitude and frequency of SARS-CoV-2 in circulation may be an important factor for the higher burden of severe disease in the AI/AN group. This hypothesis is supported by the logistic and linear regression modeling showing: (1) mean blood VLs were the strongest predictor of severe disease, and (2) high blood VLs were most strongly associated with self-reported race/ethnicity as AI/AN. Further support comes from the multiple-event-per-subject conditional proportional hazard model in which self-reported race/ethnicity as AI/AN was the highest risk factor for recurrent time-to-event of SARS-CoV-2 in circulating blood.

Collectively, AI/AN participants in this study had more severe disease, despite comparable levels of comorbid conditions. Based on our findings, we propose that higher and more frequent detection of SARS-CoV-2 in the PB of AI/AN individuals may be an important factor for more severe COVID-19 observed in the study population. However, given the sample size of 94 individuals parsed into dichotomous groups, these findings will need to be confirmed with a larger sample size to make the interpretations more

generalizable. To address this need, we are currently examining URT and PB VLs in over 500 study participants with three primary groups (AI/AN, Hispanic/Latino, and NHW), and in the context of different viral variants. We acknowledge that VL dynamics likely represents only one of the many complex variables that influence the development of severe COVID-19, and other unmeasured factors in this study, such as socioeconomic status, could be a confounder. In addition, to explore potential biological factors for our findings, we are conducting temporal gene expression studies (i.e. entire expressed transcriptome) on the same sampling days for which VLs were measured. Based on findings presented here, we recommend that early treatment interventions that reduce and/or prevent SARS-CoV-2 in PB may improve patient outcomes, particularly in populations vulnerable to severe COVID-19.

AUTHORS' CONTRIBUTIONS

All authors contributed to conception or design of the work; or acquisition, analysis, or interpretation of data for the work; drafting the work or revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL APPROVAL

This project (protocol ID: 20-194) was approved by the University of New Mexico Health Sciences Human Research Protection Program. Written consent was obtained from all participants.

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SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

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