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Hasan K. Siddiqi MD , Brittany Weber MD PhD ,
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Marcelo DiCarli MD , Jonathan Z. Li MD , Deepak L. Bhatt MD

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Increased prevalence of myocardial injury in patients with SARS-CoV-2 viremia.

Hasan K. Siddiqi MD^{1*}, Brittany Weber MD PhD^{1,2*}, Guohai Zhou PhD³, James Regan BS⁴,
 Jesse Fajnzylber BA⁴, Kendyll Coxen BS⁴, Heather Corry MS⁴, Xu G. Yu MD^{4,5,6}, Marcelo
 DiCarli MD^{1,2}, Jonathan Z. Li MD^{4#}, Deepak L. Bhatt MD^{1#%}

*Contributed equally; #Co-senior authors

¹Heart and Vascular Center, Division of Cardiovascular Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

²Cardiovascular Imaging Program, Departments of Medicine and Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

³Center for Clinical Investigation, Brigham and Women's Hospital, Boston, MA.

⁴Division of Infectious Diseases, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

⁵Massachusetts General Hospital, Harvard Medical School, Boston, MA.

⁶Ragon Institute of MGH, MIT and Harvard University, Boston, MA.

%Corresponding author: Deepak L. Bhatt, MD, MPH, FACC, FAHA, FSCAI, FESC
 Executive Director of Interventional Cardiovascular Programs,
 Brigham and Women's Hospital Heart & Vascular Center
 Professor of Medicine, Harvard Medical School
 75 Francis Street, Boston, MA 02115
 Email: dbhatt@bwh.harvard.edu
 Phone: 1-857-307-1992
 Fax: 1-857-307-1955

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Abstract:

Background: Patients with COVID-19 have a high prevalence of detectable troponin and myocardial injury. In addition, a subset of COVID-19 patients have detectable SARS-CoV-2 viral loads. The objective of this study is to understand the relationship between SARS-CoV-2 viremia, detectable troponin, and myocardial injury in hospitalized COVID-19 patients.

Methods: SARS-CoV-2 plasma viral load was measured in plasma samples drawn from patients hospitalized for COVID-19 at two academic medical centers. Baseline characteristics and clinically obtained high-sensitivity cardiac troponin T (hs-cTnT) values were abstracted from the

medical record. The main outcome was detectable hs-cTnT ($\geq 6\text{ng/mL}$) and myocardial injury (hs-cTnT $\geq 14\text{ng/mL}$; $>99\text{th}$ percentile for assay).

Results: 70 hospitalized COVID-19 patients were included in this study, with 39% females and median age 58 +/- 17 years. 21 patients (30%) were found to have detectable SARS-CoV-2 viral load and were classified in the viremia group. Patients with viremia were significantly older than those without viremia. 100% of viremic patients had detectable troponin during hospitalization, compared to 59% of non-viremic patients ($p=0.0003$). Myocardial injury was seen in 76% of viremic patients and 38% of non-viremic patients ($p=0.004$).

Conclusions: Hospitalized COVID-19 patients with SARS-CoV-2 viremia have a significantly higher prevalence of detectable troponin and myocardial injury during their hospitalization, compared to non-viremic patients. This first report of the relationship between SARS-CoV-2 viremia, detectable troponin and myocardial injury in COVID-19 patients points to additional mechanistic pathways that require deeper study to understand the complex interplay between these unique findings, cardiovascular outcomes and mortality in COVID-19.

Clinical Significance/Highlights:

- Association of SARS-CoV-2 viremia and myocardial injury is currently unknown.
- Out of 70 COVID-19 positive hospitalized adults, 30% had SARS-CoV-2 viremia.
- SARS-CoV-2 viremia was associated with a greater rate of detectable troponin.
- SARS-CoV-2 viremia was associated with a greater rate of myocardial injury.

Main Text:

Background

Myocardial injury is a common feature in patients with Coronavirus Disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome-CoronaVirus-2 (SARS-CoV-2), a finding reported in at least 20% of hospitalized COVID-19 patients and associated with increased morbidity and mortality.¹⁻³ Beyond myocardial injury, detectable troponin is reported in COVID-19 patients and correlated with abnormal cardiac magnetic resonance imaging findings in convalescent patients.⁴ Early data indicate that the presence of detectable troponin is associated with worse outcomes in COVID-19 patients, and the highest elevations correlate with the poorest outcomes.⁵ Mechanisms of myocardial injury and detectable troponin in patients with COVID-19 are currently unknown, with proposed hypotheses invoking several possibilities including direct viral myocardial injury and immune-mediated cardiac injury.^{1,6,7}

In the SARS pandemic caused by the related SARS-CoV-1, worse clinical outcomes including respiratory failure and death were associated with serum viremia.⁸ The detection of plasma viral SARS-CoV-2 RNA has been described in limited reports and may be associated with worse in-hospital mortality in COVID-19 patients.⁹⁻¹¹ However, the relationship between SARS-CoV-2 viremia and cardiovascular injury is currently unknown. We hypothesized that there would be a higher prevalence of detectable troponin and myocardial injury in hospitalized COVID-19 patients with SARS-CoV-2 viremia compared to those without viremia.

Methods:

Patient recruitment and endpoint ascertainment:

We consented and enrolled 70 hospitalized patients with COVID-19 in a prospective cohort study with appropriate institutional review board approval. Baseline characteristics and clinically obtained high-sensitivity cardiac troponin-T (hs-cTnT) values during hospitalization were extracted from the medical record. SARS-CoV-2 viral load was measured from patient

samples collected during the hospitalization using methods described below. SARS-CoV-2 viral loads below 40 RNA copies/mL were categorized as undetectable. Patients with detectable plasma SARS-CoV-2 RNA were classified in the viremic group and all others classified as non-viremic. Detectable troponin was defined as hs-cTnT concentration at or above the lowest level of detection (≥ 6 ng/ml) at any time point during admission. Myocardial injury was defined as a peak hs-cTnT concentration $>99^{\text{th}}$ percentile of assay (≥ 14 ng/ml) during hospitalization.

SARS-CoV-2 Viral Load Quantification

SARS-CoV-2 viral load was quantified using the US CDC 2019-nCoV_N1 primers and probe set.¹² Virions were pelleted from plasma by centrifugation at approximately 21,000g for 2 hours at 4°C. 750 μ L of TRIzol-LS™ Reagent (ThermoFisher) was added to the pellets after supernatant removal, and then incubated on ice. Following incubation, 200 μ L chloroform (MilliporeSigma) was added and vortexed. Mixtures were separated by centrifugation at 21,000g for 15 minutes at 4°C, aqueous layer removed and treated with an equal volume of isopropanol (Sigma). GlycoBlue™ Coprecipitant (ThermoFisher) and 100 μ L 3M Sodium Acetate (Life Technologies) were added to each sample and incubated on dry ice until frozen. RNA was pelleted by centrifugation at 21,000xg for 45 minutes at 4°C. Supernatant was discarded and RNA washed with cold 70% ethanol. RNA was resuspended in DEPC-treated water (ThermoFisher).

Each reaction contained extracted RNA, 1X TaqPath™ 1-Step RT-qPCR Master Mix, CG (ThermoFisher), CDC N1 forward and reverse primers, and probe¹². Viral copy numbers were quantified using N1 qPCR standards in 16-fold dilutions to generate a standard curve. The assay was run in triplicate for each sample and two non-template control wells (negative controls). Importin-8 (IPO8) housekeeping gene RNA level was quantified to determine quality

of respiratory sample collection. An internal virion control (RCAS) was spiked into each sample and quantified to determine RNA extraction and qPCR amplification efficiency.¹³

Statistical analysis:

Fisher's exact and X^2 tests were used as appropriate for statistical comparisons. A two-sided $p < 0.05$ was considered statistically significant.

Results:

Amongst 70 hospitalized COVID-19 patients, 21 patients (30%) had detectable SARS-CoV-2 viremia. In those with viremia, median viral load was $2.4 \log_{10}$ RNA copies/mL (range $1.8 - 3.8 \log_{10}$ RNA copies/mL). Baseline characteristics of the cohort are presented in Table 1. Viremic patients were significantly older (67 ± 13 years vs. 54 ± 17 years, $p = 0.001$), with a trend towards fewer females with viremia compared to those without viremia (24% vs. 45%, $p = 0.1$). There were no significant differences in race or BMI between groups. Compared to non-viremic patients, those with viremia had a trend towards more baseline cardiovascular comorbidities including diabetes [12/21 (57%) vs. 17/49 (35%), $p = 0.1$], hypertension [15/21 (71%) vs. 23/49 (47%), $p = 0.07$], and hyperlipidemia [13/21 (62%) vs. 17/49 (35%), $p = 0.06$].

Next, we investigated the relationship between SARS-CoV-2 viremia, detectable troponin, and myocardial injury (Figure 1). During hospitalization, detectable troponin was measured in all patients with viremia (21/21, 100%) and in 59% (29/49) of those without viremia ($p = 0.0003$, Figure 1A). Myocardial injury was present in 16/21 (76%) patients with viremia and 18/49 (38%) patients without viremia ($p = 0.004$, Figure 1B).

Conclusion:

Our study is the first to examine the relationship between SARS-CoV-2 viremia and cardiovascular injury. We report a uniquely high rate of troponin positivity and myocardial injury amongst hospitalized individuals with detectable SARS-CoV-2 viremia, in contrast to patients without detectable viremia. Patients with viremia were older, more likely to be male, and tended to have more baseline cardiovascular comorbidities than those without viremia. These findings suggest that the most vulnerable hospitalized COVID-19 patients are more likely to have SARS-CoV-2 viremia and have a greater degree of ensuing myocardial injury.

This study must be assessed in the context of its limitations. The major limitation is the small cohort size and therefore a limited ability to control for co-variables and possible confounders. As a result, the exact relationship between patient age, sex, pre-existing cardiovascular disease, SARS-CoV-2 viremia, and myocardial injury could not be completely assessed in the current study. Therefore, while these findings point towards a strong association between SARS-CoV-2 viremia and myocardial injury in hospitalized COVID-19 patients, they do not provide insight into the possible mediators and mechanism of this relationship.

Despite these limitations, to our knowledge, this study is the first to demonstrate a high prevalence of cardiovascular injury in the setting of SARS-CoV-2 viremia. Cardiovascular injury in COVID-19 patients is associated with significantly worse outcomes including death, making it a key priority to understand the mechanism of this relationship.^{3,5} Plasma viremia, with its associated systemic and immunologic disturbances, could be a mediator of this relationship. Further large-scale studies are necessary to investigate the complex relationships between SARS-CoV-2 viremia, immune response, risk of cardiac injury, and clinical outcomes. Insights into these relationships may open new avenues of diagnosis, prognostication, and therapy in patients with COVID-19.

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Figure 1 Title: Prevalence of detectable troponin and myocardial injury among patients with SARS-CoV-2 viremia.

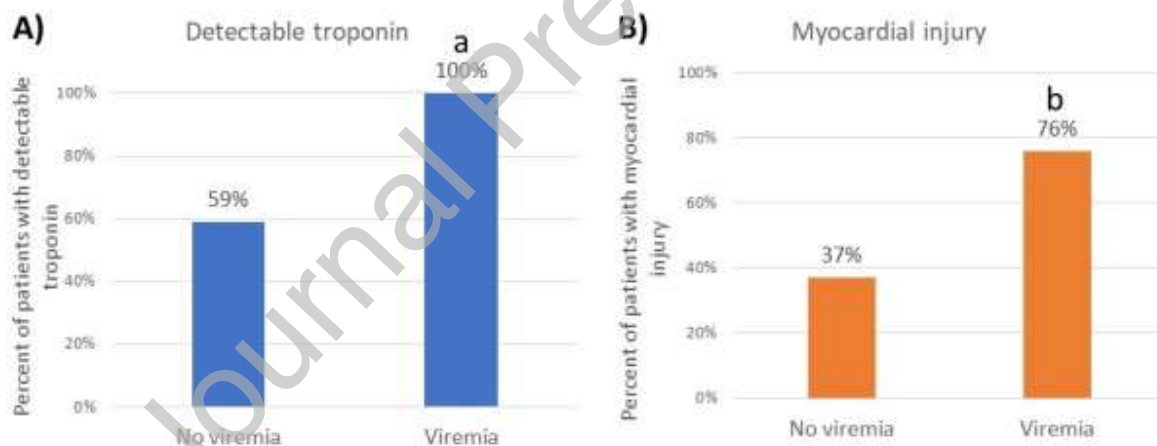


Figure 1 Legend: Prevalence of a) detectable troponin and b) myocardial injury among patients without and with SARS-CoV-2 viremia. A significantly higher proportion of hospitalized patients with SARS-CoV-2 viremia had a) detectable troponin and b) myocardial injury compared with hospitalized patients without SARS-CoV-2 viremia. ^ap=0.0003 for comparison with no viremia group; ^bp=0.004 for comparison with no viremia group.

Table 1: Baseline characteristics of the enrolled cohort of 70 hospitalized patients with COVID-19.

Characteristic	All participants (N=70)	SARS-CoV-2 Viremia (N=21)	No SARS-CoV-2 Viremia (N=49)	p-value ^a
Female (%)	27 (39%)	5 (24%)	22 (45%)	0.115
Age, mean (SD), years	58 (17)	67 (13)	54 (17)	0.001
Age distribution:				
<40	12 (17%)	1 (5%)	11 (22%)	0.006
40-50	10 (14%)	2 (10%)	8 (16%)	
50-60	16 (23%)	1 (5%)	15 (31%)	
60-70	17 (24%)	10 (48%)	7 (14%)	
70-80	11 (16%)	5 (24%)	6 (12%)	
>80	4 (6%)	2 (10%)	2 (4%)	
Race/ethnicity				
White	24 (34%)	10 (48%)	14 (29%)	0.1623
Black	11 (16%)	3 (14%)	8 (16%)	
Hispanic/Latino	26 (37%)	4 (19%)	22 (45%)	
Other or Unknown	9 (13%)	4 (19%)	5 (10%)	
Body mass index, mean (SD), kg/m ²	30 (7)	28 (4)	30 (8)	0.174
Diabetes (%)	29 (41%)	12 (57%)	17 (35%)	0.113
Hypertension (%)	38 (54%)	15 (71%)	23 (47%)	0.072
Hyperlipidemia (%)	30 (43%)	13 (62%)	17 (35%)	0.064
Coronary artery disease (%)	6 (9%)	3 (14%)	3 (6%)	0.355
Chronic lung disease (%)	12 (17%)	4 (19%)	8 (16%)	0.743
Active cancer (%)	2 (3%)	2 (10%)	0 (0%)	0.087
Beta blocker (%)	8 (11%)	3 (14%)	5 (10%)	0.689
Statin (%)	31 (44%)	13 (62%)	18 (37%)	0.068
ACEi/ARB (%)	22 (31%)	7 (33%)	15 (31%)	1.0

^ap-value comparing SARS-CoV-2 viremic and non-viremic patients

SD = standard deviation; ACEi = Angiotensin converting enzyme inhibitor; ARB = Angiotensin receptor blocker