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Increased Prevalence of Myocardial Injury in Patients with SARS-CoV-2 Viremia

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ABSTRACT

BACKGROUND: Patients with coronavirus disease 2019 (COVID-19) have a high prevalence of detectable troponin and myocardial injury. In addition, a subset of patients with COVID-19 has detectable severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral loads. The objective of this study was to understand the relationship among SARS-CoV-2 viremia, detectable troponin, and myocardial injury in hospitalized patients with COVID-19.

METHODS: SARS-CoV-2 plasma viral load was measured in plasma samples drawn from patients hospitalized for COVID-19 at 2 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 (≥6 ng/mL) and myocardial injury (hs-cTnT ≥14 ng/mL; >99th percentile for assay).

RESULTS: A total of 70 hospitalized patients with COVID-19 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. All of the patients with viremia (100%) had detectable troponin during hospitalization compared with 59% of patients without viremia (P = 0.0003). Myocardial injury was seen in 76% of patients with viremia and 38% of those patients without viremia (P = 0.004).

CONCLUSIONS: Hospitalized patients with COVID-19 with SARS-CoV-2 viremia have a significantly higher prevalence of detectable troponin and myocardial injury during their hospitalization compared with patients who did not. This first report of the relationship among SARS-CoV-2 viremia, detectable troponin, and myocardial injury in patients with COVID-19 points to additional mechanistic pathways that require deeper study to understand the complex interplay among these unique findings, cardiovascular outcomes, and mortality in COVID-19.

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KEYWORDS: Cardiac injury; COVID-19; Myocardial injury; SARS-CoV-2; Viral load

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 patients hospitalized with COVID-19 and associated with increased morbidity and mortality.1−3 Beyond myocardial injury, detectable troponin is reported in patients with COVID-19 and correlated...
with abnormal cardiac magnetic resonance imaging findings in convalescent patients. Early data indicate that the presence of detectable troponin is associated with worse outcomes in patients with COVID-19, and the highest elevations correlate with the poorest outcomes. 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.

In the SARS pandemic caused by the related SARS-CoV-1, worse clinical outcomes including respiratory failure and death, were associated with serum viremia. 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 patients with COVID-19. 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 patients hospitalized with COVID-19 with SARS-CoV-2 viremia compared to those without viremia.

**METHODS**

**Patient Recruitment and Endpoint Ascertainment**

We received consent from and enrolled 70 patients hospitalized 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 medical records. SARS-CoV-2 viral load was measured from patient samples collected during the hospitalization using methods described herein. 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 nonviremic. 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 >99th percentile of assay (≥14 ng/mL) during hospitalization.

**SARS-CoV-2 Viral Load Quantification**

SARS-CoV-2 viral load was quantified using the US Centers for Disease Control and Prevention 2019-nCoV_N1 primers and probe set. Virions were pelleted from plasma by centrifugation at approximately 21,000 g for 2 hours at 4°C, and 750 μL of TRIzol-LS Reagent (ThermoFisher) was added to the pellets after supernatant removal and then incubated on ice. Following incubation, 200 μL chloroform (MilliporeSigma) was added and vortexed. Mixtures were separated by centrifugation at 21,000 g for 15 minutes at 4°C, and the aqueous layer removed and treated with an equal volume of isopropanol (Sigma). GlycoBlue Coprecipitant (ThermoFisher) and 100 μL 3M Sodium Acetate (Life Technologies) were added to each sample and incubated on dry ice until frozen. RNA was pelleted by centrifugation at 21,000 g for 45 min 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 probe. 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 2 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.

**Statistical Analysis**

Fisher exact and χ² tests were used as appropriate for statistical comparisons. A 2-sided P < 0.05 was considered statistically significant.

**RESULTS**

Among 70 patients hospitalized with COVID-19, 21 patients (30%) had detectable SARS-CoV-2 viremia. In those with viremia, median viral load was 2.4 log₁₀ RNA copies/mL (range 1.8-3.8 log₁₀ RNA copies/mL). Baseline characteristics of the cohort are presented in Table 1. Patients with viremia were significantly older (67 ± 13 years vs 54 ± 17 years, P = 0.001), with a trend toward fewer females with viremia compared to those without viremia (24% vs 45%, P = 0.1). There were no significant differences in race or body mass index between groups. Compared to patients without viremia, those with viremia had a trend toward more baseline cardiovascular comorbidities including diabetes (12 of 21 [57%] vs 17 of 49 [35%), P = 0.11), hypertension (15 of 21 [71%] vs 23 of 49 [47%], P = 0.072), and hyperlipidemia (13 of 21 [62%] vs 17 of 49 [35%], P = 0.064).

**CLINICAL SIGNIFICANCE**

- Association of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viremia and myocardial injury is currently unknown.
- Out of 70 hospitalized adults with coronavirus disease 2019 (COVID-19), 30% had SARS-CoV-2 viremia.
- SARS-CoV-2 viremia was associated with a significantly higher rate of detectable troponin.
- SARS-CoV-2 viremia was associated with a significantly higher rate of myocardial injury.
Next, we investigated the relationship among SARS-CoV-2 viremia, detectable troponin, and myocardial injury (Figure). During hospitalization, detectable troponin was measured in all patients with viremia (21 of 21, 100%) and in 59% (29 of 49) of those without viremia ($P = 0.0003$, Figure A). Myocardial injury was present in 16 of 21 (76%) patients with viremia and 18 of 49 (38%) patients without viremia ($P = 0.004$, Figure B).

### Table 1  Baseline Characteristics of the Enrolled Cohort of 70 Patients Hospitalized with COVID-19

| Characteristic                          | All participants (N = 70) | SARS-CoV-2 Viremia (N = 21) | No SARS-CoV-2 Viremia (N = 49) | $P$ Value* |
|----------------------------------------|---------------------------|----------------------------|-------------------------------|------------|
| Female (%)                             | 27 (39%)                  | 5 (24%)                    | 22 (45%)                      | 0.12       |
| 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%)                       | 0.16       |
| 50-60                                  | 16 (23%)                  | 1 (5%)                     | 15 (31%)                      | 0.072      |
| 60-70                                  | 17 (24%)                  | 10 (48%)                   | 7 (14%)                       | 0.064      |
| 70-80                                  | 11 (16%)                  | 5 (24%)                    | 6 (12%)                       | 0.36       |
| >80                                    | 4 (6%)                    | 2 (10%)                    | 2 (4%)                        | 1.0        |
| Race/ethnicity                         |                           |                            |                               |            |
| White                                  | 24 (34%)                  | 10 (48%)                   | 14 (29%)                      | 0.16       |
| Black                                  | 11 (16%)                  | 3 (14%)                    | 8 (16%)                       | 0.17       |
| Hispanic/Latino                        | 26 (37%)                  | 4 (19%)                    | 22 (45%)                      | 0.068      |
| Other or Unknown                       | 9 (13%)                   | 4 (19%)                    | 5 (10%)                       |            |
| Body mass index, mean (SD), kg/m$^2$   | 30 (7)                    | 28 (4)                     | 30 (8)                        | 0.096      |
| Diabetes (%)                           | 29 (41%)                  | 12 (57%)                   | 17 (35%)                      | 0.11       |
| 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%)                        | 0.74       |
| Chronic lung disease (%)               | 12 (17%)                  | 4 (19%)                    | 8 (16%)                       | 0.096      |
| Active cancer (%)                      | 2 (3%)                    | 2 (10%)                    | 0 (0%)                        |            |
| Beta-blocker (%)                       | 8 (11%)                   | 3 (14%)                    | 5 (10%)                       | 0.69       |
| Statin (%)                             | 31 (44%)                  | 13 (62%)                   | 18 (37%)                      | 0.068      |
| ACEi/ARB (%)                           | 22 (31%)                  | 7 (33%)                    | 15 (31%)                      | 1.0        |

*P value comparing patients with SARS-CoV-2 viremia to those without SARS-CoV-2 viremia. ACEi = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; COVID-19 = coronavirus disease 2019; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SD = standard deviation.
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 among 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 patients hospitalized with COVID-19 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 covariates and possible confounders. As a result, the exact relationship among patient age, sex, preexisting cardiovascular disease, SARS-CoV-2 viremia, and myocardial injury could not be completely assessed in the current study. Therefore, although these findings point toward a strong association between SARS-CoV-2 viremia and myocardial injury in patients hospitalized with COVID-19, 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 patients with COVID-19 is associated with significantly worse outcomes, including death, making it a key priority to understand the mechanism of this relationship. 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 among 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.

References

1. Attri D, Siddiqi HK, Lang JP, Naufual V, Morrow DA, Bohula EA. COVID-19 for the cardiologist. JACC Basic Transl Sci 2020;5 (5):518–36. https://doi.org/10.1016/j.jacbts.2020.04.002.

2. Bavishi C, Bonow RO, Trivedi V, Abbott JD, Messerli FH, Bhatt DL. Acute myocardial injury in patients hospitalized with COVID-19 infection: a review [e-pub ahead of print]. Prog Cardiovasc Dis. Accessed August 12, 2020

3. Shi S, Qin M, Shen B, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. JAMA Cardiol 2020;5(7):302. https://doi.org/10.1001/jamacardio.2020.0950.

4. Puntmann VO, Carere ML, Wieters I, et al. Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19) [e-pub ahead of print]. JAMA Cardiol. Accessed August 12, 2020.

5. Lala A, Johnson KW, Januzzi JL, et al. Prevalence and impact of myocardial injury in patients hospitalized with COVID-19 infection. J Am Coll Cardiol 2020;76(5):533–46. https://doi.org/10.1016/j.jacc.2020.06.007.

6. Lang JP, Wang X, Moura FA, Siddiqi HK, Morrow DA, Bohula EA. A current review of COVID-19 for the cardiovascular specialist. Am Heart J 2020;226:29–44. https://doi.org/10.1016/j.ahj.2020.04.025.

7. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of coronaviruses on the cardiovascular system: a review. JAMA Cardiol 2020;5(7):831. https://doi.org/10.1001/jamacardiol.2020.1286.

8. Hung IFN, Cheng VCC, Wu AKL, et al. Viral loads in clinical specimens and SARS manifestations. Emerg Infect Dis 2004;10(9):1550–7. https://doi.org/10.3201/eid1009.040058.

9. Xu D, Zhou F, Sun W, et al. Relationship between serum SARS-CoV-2 nucleic acid (RNAemia) and organ damage in COVID-19 patients: a cohort study [e-pub ahead of print]. Clin Infect Dis. Accessed August 12, 2020.

10. Chen X, Zhao B, Qu Y, et al. Detectable serum severe acute respiratory syndrome coronavirus 2 viral load (RNAemia) is closely correlated with drastically elevated interleukin 6 level in critically ill patients with coronavirus disease 2019. Clin Infect Dis 2020;71 (8):1937–42. https://doi.org/10.1093/cid/ciaa449.

11. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA 2020;323(18):1843–4. https://doi.org/10.1001/jama.2020.3786.

12. Centers for Disease Control and Prevention. Information for Laboratories about Coronavirus (COVID-19). Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html. Accessed August 9, 2020.

13. Palmer S, Wiegand AP, Maldarelli F, et al. New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. J Clin Microbiol 2003;41 (10):4531–6. https://doi.org/10.1128/JCM.41.10.4531-4536.2003.

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