Association Between SARS-CoV-2 RNAemia and Post-Acute Sequelae of COVID-19.

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Abstract

Determinants of Post-Acute Sequelae of COVID-19 are not known. Here we show that 75% of patients with viral RNA in blood (RNAemia) at presentation were symptomatic in the post-acute phase. RNAemia at presentation successfully predicted PASC, independent of patient demographics, initial disease severity, and length of symptoms.

Keywords

SARS-CoV-2, RNAemia, PASC, long COVID
Background

The determinants of COVID-19 severity and extrapulmonary complications have now been well studied, and RNAemia (viral RNA in blood) has emerged as an important factor (1,2). Much less is known about the determinants of Post-Acute Sequelae of COVID-19 (PASC), the persistence or development of new symptoms after the acute phase of infection, recently reported to affect as many as 87.4% of COVID-19 patients (3,4) primarily with moderate or worse severity (5,6). Recent evidence suggested persistent clotting protein pathology with elevated levels of antiplasmin (7) and non-classical monocytes (8) in patients with PASC. Discovery of SARS-CoV-2 S1 protein in these non-classical monocytes and fragmented SARS-CoV-2 RNA in peripheral blood mononuclear cells in a PASC patient 15 months post infection further exhibited the persistence of viral particles (8). Given the importance of RNAemia in disease severity and its persistence in the blood, we describe the relationship between RNAemia at presentation and post-acute symptoms at least three weeks after symptom onset.

Methods

We studied the clinical trajectories of 155 patients enrolled in the IRB-approved (eP-55650) Stanford Hospital Emergency Department (ED) COVID-19 Biobank between April and November 2020 with completed follow-ups. We assessed symptoms and severity (based on a modified WHO scale) (1) on the date of enrollment (median = 4, range = 0 – 44 days after symptom onset), and at least three weeks after symptom onset (median = 35, range = 21 – 79 days).
We measured SARS-CoV-2 RNAemia at the time of enrollment, using the definitions of our earlier study (1). We compared the proportions of initially RNAemic and non-RNAemic patients with persistent or new symptoms in the post-acute phase using a 2-sample chi-squared test with continuity correction. We estimated the association between RNAemia at enrollment and PASC at follow-up in a logistic model controlling for disease severity at enrollment, patient demographics (age and gender), presence of any symptom at enrollment (anxiety, dizziness, fatigue, hair loss, palpitations, rash, insomnia, chest pain, chills, cough, decrease in sense of taste, fever, nausea/vomiting/diarrhea, headache, loss of smell, myalgia, new confusion, shortness of breath), and durations of symptoms. We also compared the median number of PASC symptoms for RNAemic and non-RNAemic patients using the Wilcoxon rank-sum test with continuity correction. We performed all analyses in R (version 4.0.3).

Results

49.0% (76/155) of patients were women, and the median age was 45 years (IQR 34 – 60). At enrollment, 27.1% (42/155) of patients had mild disease severity, 67.7% (105/155) moderate, and 5.2% (8/155) severe. Patients had a median of six symptoms (IQR = 4–8): 72.3% (112/155) had a cough, 65.3% (101/155) had shortness of breath, and 64.5% (99/155) had fever. In the post-acute phase, 52.3% (81/155) had one or more new (24.5% [38/155]) or persistent (37.4% [58/155]) symptoms, of which the most common were cough, dizziness, and loss of smell (Table 1). 1.3% (2/155) of patients developed anxiety that was not present at enrollment.
75.0% (27/36) of initially RNAemic patients were symptomatic in the post-acute phase, compared to 43.7% (52/119) of non-RNAemic patients (difference = 31.3% [95% CI, 12.8% - 49.8%], p=0.002). RNAemic patients had a median of one symptom in the post-acute phase compared to zero in non-RNAemic patients (p=0.014, Wilcoxon rank-sum test). RNAemia at presentation predicted PASC, conditional on patient demographics and initial disease severity (OR 1.31 [95% CI, 1.08 – 1.59], p=0.007 (Supplement)). The association was strongest for patients with moderate disease severity at presentation (Figure), with 78.6% (22/28) of initially RNAemic patients symptomatic in the post-acute phase, compared to 45.5% (35/77) of non-RNAemic patients (difference = 33.1% [95% CI, 11.8% - 54.4%], p=0.005). This difference was due almost entirely to persistent or new respiratory symptoms (difference in proportions = 28.2% [95% CI, 8.4% - 47.9%], p=0.002).

Discussion

To our knowledge, this study describes the first reported association between SARS-CoV-2 RNAemia and PASC. RNAemia at presentation was associated with new or persistent symptoms at least 21 days after symptom onset independent of initial patient severity and the association was strongest among patients with moderately severe clinical presentations requiring hospital admission. This finding adds to the growing literature on SARS-CoV-2 RNAemia’s role in disease severity and extrapulmonary complications in the acute phase of illness, as well as the association between hospitalization and PASC (1,2,5,6). The incidence of PASC was lower in this single-center study than in reports from Italy and the UK (3,4), but similar to that reported in a recent study from the US (9). The potential contributions of patient characteristics, study
methodologies, and viral variants to these discrepancies merit further study. Though the mechanisms underlying RNAemia’s contributions to multi-system pathology in both the acute and post-acute phases, when persistent, remain to be elucidated, mounting evidence for its predictive value suggests that testing for SARS-CoV-2 RNAemia at presentation may help guide the triage, management, and prognosis of COVID-19.

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References

1. Ram-Mohan N, Kim D, Zudock EJ, Hashemi MM, Tjandra KC, Rogers AJ, et al. SARS-CoV-2 RNAemia predicts clinical deterioration and extrapulmonary complications from COVID-19. Clinical Infectious Diseases. 2021 May 5;ciab394.

2. The Massachusetts Consortium for Pathogen Readiness, Fajnzylber J, Regan J, Coxen K, Corry H, Wong C, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. Nat Commun. 2020 Dec;11(1):5493.

3. Carfì A, Bernabei R, Landi F, for the Gemelli Against COVID-19 Post-Acute Care Study Group. Persistent Symptoms in Patients After Acute COVID-19. JAMA. 2020 Aug 11;324(6):603.

4. Arnold DT, Hamilton FW, Milne A, Morley AJ, Viner J, Attwood M, et al. Patient outcomes after hospitalisation with COVID-19 and implications for follow-up: results from a prospective UK cohort. Thorax. 2021 Apr;76(4):399–401.
5. Lund LC, Hallas J, Nielsen H, Koch A, Mogensen SH, Brun NC, et al. Post-acute effects of SARS-CoV-2 infection in individuals not requiring hospital admission: a Danish population-based cohort study. The Lancet Infectious Diseases. 2021 May;S1473309921002115.

6. Hirschtick JL, Titus AR, Slocum E, Power LE, Hirschtick RE, Elliott MR, et al. Population-based estimates of post-acute sequelae of SARS-CoV-2 infection (PASC) prevalence and characteristics. Clinical Infectious Diseases. 2021 May 19;ciab408.

7. Pretorius E, Vlok M, Venter C, Bezuidenhout JA, Laubscher GJ, Steenkamp J, et al. Persistent clotting protein pathology in Long COVID/ Post-Acute Sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin [Internet]. Infectious Diseases (except HIV/AIDS); 2021 May [cited 2021 Aug 30]. Available from: http://medrxiv.org/lookup/doi/10.1101/2021.05.21.21257578

8. Patterson BK, Francisco EB, Yogendra R, Long E, Pise A, Rodrigues H, et al. Persistence of SARS CoV-2 S1 Protein in CD16+ Monocytes in Post-Acute Sequelae of COVID-19 (PASC) Up to 15 Months Post-Infection [Internet]. Immunology; 2021 Jun [cited 2021 Aug 28]. Available from: http://biorxiv.org/lookup/doi/10.1101/2021.06.25.449905

9. Chopra V, Flanders SA, O’Malley M, Malani AN, Prescott HC. Sixty-Day Outcomes Among Patients Hospitalized With COVID-19. Ann Intern Med. 2020 Nov 11;M20-5661.
### Table: Progression of COVID-19 symptoms

| Symptom                          | On enrollment | Persistent at follow-up | New at follow-up |
|----------------------------------|---------------|-------------------------|------------------|
| **Cardiovascular**               |               |                         |                  |
| Chest Pain                       | 34.2% (53/155)| 11.3% (6/53)            | 0.7% (1/149)     |
| Palpitations                     | 0.6% (1/155)  | 0 (0)                   | 1.3% (2/155)     |
| **Dermatologic**                 |               |                         |                  |
| Rash                             | 1.3% (2/155)  | 0 (0)                   | 0.6% (1/155)     |
| Hair Loss                        | 0 (0)         | 0 (0)                   | 0.6% (1/155)     |
| **Gastrointestinal**             |               |                         |                  |
| Nausea/vomiting/diarrhea         | 43.8% (68/155)| 7.3% (5/68)             | 0.7% (1/150)     |
| **Constitutional**               |               |                         |                  |
| Fever                            | 64.5% (100/155)| 1.0% (1/100)         | 0 (0)            |
| Chills                           | 32.9% (51/155)| 3.9% (2/51)             | 0.6% (1/153)     |
| Myalgia                          | 41.2% (64/155)| 15.6% (10/64)          | 4.1% (6/145)     |
| Fatigue                          | 36.7% (57/155)| 14.0% (8/57)           | 8.2% (12/147)    |
| **Neuropsychiatric**             |               |                         |                  |
| Loss of Taste                    | 41.2% (64/155)| 14.0% (9/64)           | 0 (0)            |
| Loss of Smell                    | 29.6% (46/155)| 19.5% (9/46)           | 0 (0)            |
| Confusion                        | 2.5% (4/155)  | 0 (0)                   | 2.6% (4/155)     |
| Headache                         | 22.5% (35/155)| 2.8% (1/35)            | 5.2% (8/154)     |
| Dizziness                        | 5.8% (9/155)  | 22.2% (2/9)             | 2.6% (4/153)     |
| Insomnia                         | 0.6% (1/155)  | 0 (0)                   | 0.6% (1/155)     |
| Anxiety                          | 0 (0)         | 0 (0)                   | 1.3% (2/155)     |
| **Respiratory**                  |               |                         |                  |
| Cough                            | 72.2% (112/155)| 25.8% (29/112)        | 3.9% (5/126)     |
| Shortness of breath              | 65.1% (101/155)| 18.8% (19/101)        | 3.7% (5/136)     |
Figure. Rate of post-acute sequelae of SARS-CoV-2 infection, by RNAemia and clinical severity on enrollment. Overall, 75.0% (27/36) of initially RNAemic patients had one or more post-acute symptoms at follow-up, compared to 43.7% (52/119) of non-RNAemic patients (difference = 31.3% [95% CI: 12.8% - 49.8%], p=0.002). Conditional on severity at enrollment (mild = discharged from ED [n=42], moderate = hospitalized, requiring no more than oxygen by nasal cannula [n=105], severe = hospitalized, requiring high-flow nasal cannula or mechanical ventilation [n=8]), RNAemia on presentation was associated with significantly higher rates of
PASC for presentations of moderate severity (difference = 33.1% [95% CI, 11.8% - 54.4%], p=0.005). * indicates p-value<0.05.