Soluble exhaustion markers are associated with persistent SARS-CoV-2 viremia and disease severity

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Background

1. SARS-CoV-2 viremia is associated with adverse outcomes in COVID-19[1,2]. The immunologic mediators of this relationship remain under-explored.

2. There has been studies showing the correlation between humoral immunity, T cell immunity and COVID-19 outcome, but the correlation between innate immunity, especially immune exhaustion, remains under-characterized.

3. In this study, we aimed to evaluate the correlation between immune exhaustion markers, SARS-CoV-2 viremia clearance and clinical outcomes.

Methods

1. 126 participants with confirmed SARS-CoV-2 infection presenting to MGH Emergency Department (ED) in Boston, MA in early 2020.

2. Plasma samples from days 0, 3, and 7 were available for analyses. The plasma SARS-CoV-2 viral load was determined by RT-qPCR.

3. Proteomics data were generated using the Olink platform and neutralization level was assessed using a pseudovirus neutralization assay[3].

4. Viremia persistence:

Baseline viremia <1000 copies/ml	Baseline viremia ≥1000 copies/ml
>40 copies/ml (detection limit) at Day 7	>100 copies/ml (quantification limit) at Day 7

5. Partial least-squares discriminant analysis (PLS-DA) was used to select exhaustion markers that could distinguish viremia persistence and clearance. Exhaustion score was generated based on features selected by PLS-DA and was divided into four quartiles.

Results

1. In 126 pariticpants 63 (50%) were >65 years'old, 52 (41%) were female, 72 were non-Caucasion (57%), and 15 (12%) were morbidly obese.

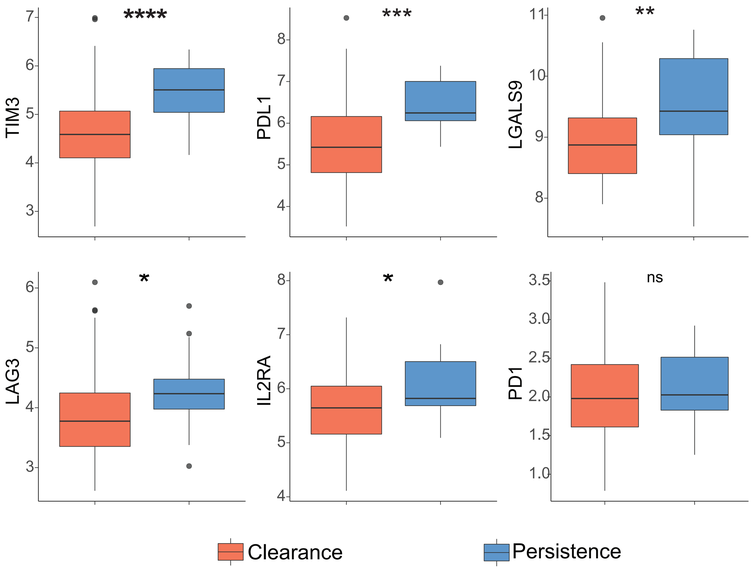

Figure 1. Viremia persistence was associated with higher levels of exhaustion markers. Wilcoxon Rank Sum test was used to determine the significance of differences. Y axis indicates Normalized Protein eXpression (NPX). *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001

Main findings

1. Certain soluble immune exhaustion markers, including TIM3, PDL1, LGALS9, LAG3, and IL2RA, are associated with SARS-CoV-2 viremia persistence.

2. In addition to being highly correlated with viremia, TIM3, PDL1 and LGALS9 were also associated with:
(a) Tissue damage, monocyte, neutrophil and proinflammatory markers.
(b) Clinical outcomes (severe disease, death)
(c) Upregulation in IL-18 signaling, innate immunity evasion, apoptosis, and lung fibrosis signaling.

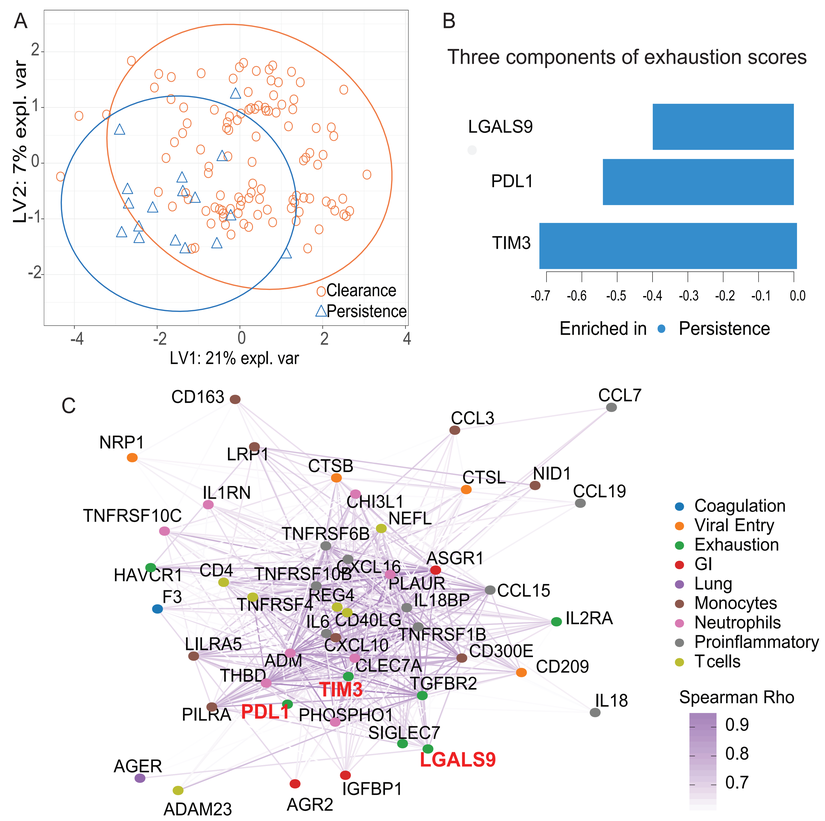

Figure 2. We developed an Exhaustion score based on TIM3, PDL1, and LGALS9 that was associated with viremia persistence, tissue damage and hyperinflammatory state. (A) PLS-DA plot demonstrating difference between Clearance and Persistence groups with feature down-selection (ROC AUC=0.83, 95%CI 0.73-0.93). (B) Loading coefficients for features selected in Component 1. (C) Correlation between selected immune exhaustiion markers and other soluble markers. Only correlations with a rho>0.6 and p value<0.05 were shown.

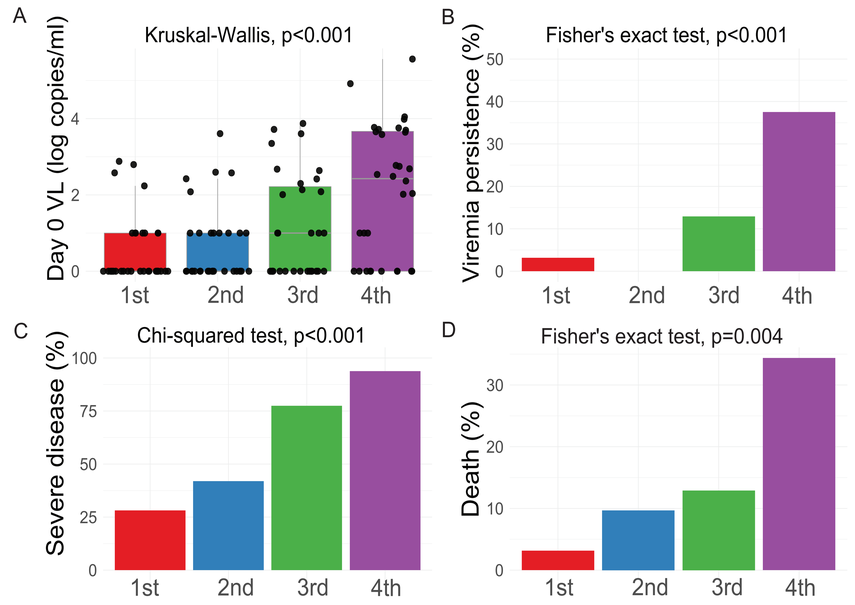

Figure 3. Exhaustion scores generated by TIM3/PDL1/LGALS9 were correlated to initial viremia levels (A), viremia clearance (B), COVID-19 severity (C) and mortality (D) within 28 days from presentation. We categorized exhaustion score derived from TIM3, PDL1, and LGALS9 into 4 quartiles, with 4th quartile the highest level of immune exhaustion.

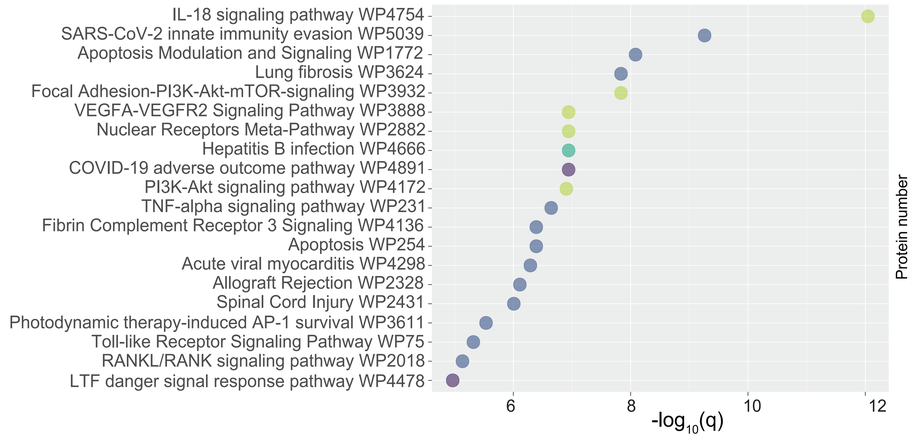

Figure 4. A high exhaustion level measured by soluble markers are associated with key pathways including IL-18 signaling, innate immunity evasion, apoptosis, lung fibrosis, and angiogenesis. We identified differentially expressed proteins (DEPs) between groups with 4th and 1st quartiles of the exhaustion score. Then we queried the Wiki Pathway database using upregulated DEPs in 4th quartile group.

Acknowledgement. We thank all the participants in this study. We thank all the clinical staff who made sample collection possible. Funding sources included Mark and Lisa Schwartz; NIH (U19AI082630); the American Lung Association; the Executive Committee on Research at MGH; the Chan Zuckerberg Initiative; Arthur, Sandra, and Sarah Irving for the David P. Ryan, MD, Endowed Chair in Cancer Research; an EMBO Long-Term Fellowship (ALTF 486-2018); a Cancer Research Institute/Bristol Myers Squibb Fellowship (CRI2993); the Harvard Catalyst/Harvard Clinical and Translational Science Center (National Center for Advancing Translational Sciences, NIH awards UL1TR001102 and UL1TR002541-01); MassCPR, and by the Harvard University CFAR (National Institute of Allergy and Infectious Diseases, 5P30AI060354).

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