Biomarker elevation during COVID-19: Differences between ambulatory and hospitalized individuals

Paul W. Blair, MD MHS MSPH[1,2,3], Charlotte Lanteri, PhD[4], Deborah Striegel, PhD[2,3], Brian K. Agan, MD[3,4], Ryan C. Maves, MD[5], Josh Chenoweth, PhD[2,3], Derek Larson, MD[6], Katrin Mende, PhD[3,4,7], Rhonda E. Colombo, MD, MHS[3,4,8], David A. Lindholm, MD[7], Anuradha Ganesan, MBBS, MPH[3,4,9], Stephanie A. Richard, PhD, MHS[3,4], Christopher J. Colombo, MD, MA[8], Cristian Madar, MD[10], Nikhil Huprikar, MD[9], David R. Tribble, MD, DrPH[4], John S. Dumler, MD, PhD[1], Timothy Burgess, MD, MPH[4], Danielle Clark, PhD[2,3]

[1]Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD; [2]Austere Environments Consortium for Enhanced Sepsis Outcomes (ACESO), Bethesda, MD; [3]The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, MD; [4]Infectious Disease Clinical Research Program, Department of Preventive Medicine and Biostatistics, Uniformed Services University of the Health Sciences, Bethesda, MD; [5]Naval Medical Center, San Diego, CA; [6]Fort Belvoir Community Hospital, Fort Belvoir, VA; [7]Brooke Army Medical Center, JBSA San Antonio, TX; [8]Madigan Army Medical Center, Tacoma WA; [9]Walter Reed National Military Medical Center, Bethesda, MD; [10]Tripler Army Medical Center, Honolulu, HI

Background

While the majority of illness due to COVID-19 does not require hospitalization, little has been described about the host inflammatory response in the ambulatory setting. Differences in the levels of inflammatory signaling proteins between outpatient and hospitalized populations could identify key maladaptive immune responses during COVID-19. We hypothesized: Differences in the levels of inflammatory signaling proteins between outpatient and hospitalized populations could identify maladaptive immune responses during COVID-19.

Methods

- Samples collected from 344 participants enrolled in ongoing, prospective COVID-19 cohort at 7 military treatment facilities (Table 1).
- Analysis restricted to those with positive SARS-CoV-2 RT-PCR testing
- Severity markers were measured (pg/mL) from longitudinal plasma samples using the Ella immunoassay and natural log transformed.
  - IL6, D-dimer, procalcitonin, ferritin, ICAM-1, IL5, lipocalin, RAGE, TNFR, VEGFA, IFNγ, IL1β, C-reactive protein (CRP)
- Levels were compared by highest level of care (i.e. outpatient, inpatient no ICU requirement, inpatient with ICU requirement) using a Student's T-test after restricting to first samples collected within the first two weeks since symptoms onset.
- Using the full marker panel, we performed a Principal Component Analysis (PCA) to determine directions of maximal variance in the data. Pearson's correlation coefficient was determined between analytes and each axis.

Results

Table 1. Baseline demographics

Characteristic	Total (n=344)	Outpatient (n=251)	Inpatient without ICU requirement (n=63)	Inpatient with ICU requirement (n=30)
Gender—no.(%)				
Female	121(35.2%)	93(37.1%)	22(34.9%)	6(20%)
Age—median, interquartile ratio (IQR)	43 (30, 56)	38 (28, 51)	56 (45, 65)	56 (47, 69)
Duration of symptoms—median, IQR	35 (27, 43)	36 (32, 43)	21 (10, 39)	20 (9, 40)

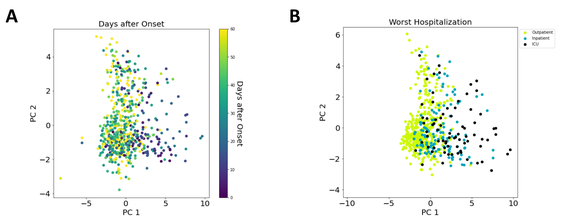

Figure 1. PCA of biomarkers stratified by **(A)** date of onset and **(B)** highest level of care.

Results

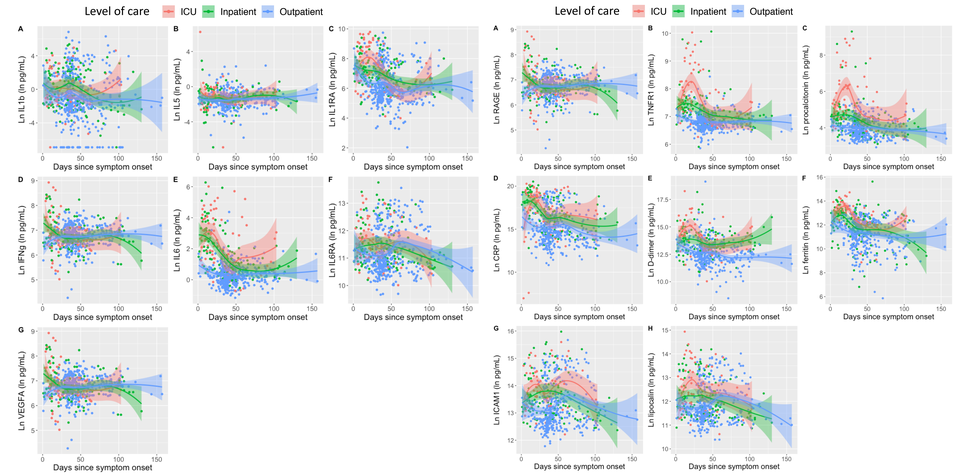

Figure 2. Cytokine biomarker kinetics over time divided by highest level of care.

Figure 3. Chemokine and acute phase reactant biomarker kinetics over time divided by highest level of care.

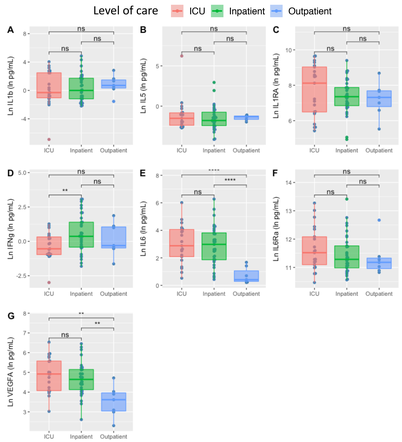

Figure 4. Cytokine biomarker levels during first two weeks of illness by highest level of care. (Student's T-test *p <0.05; ** p <0.005)

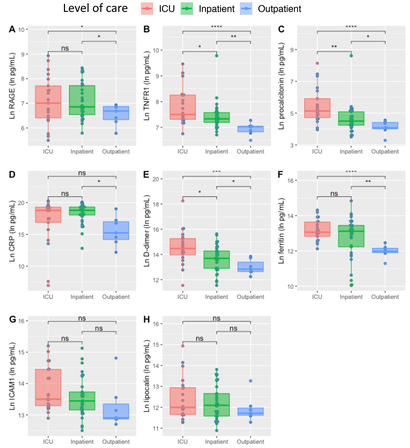

Figure 5. Chemokine and acute phase reactant biomarker levels during first two weeks of illness by highest level of care. (Student's T-test *p <0.05; ** p <0.005)

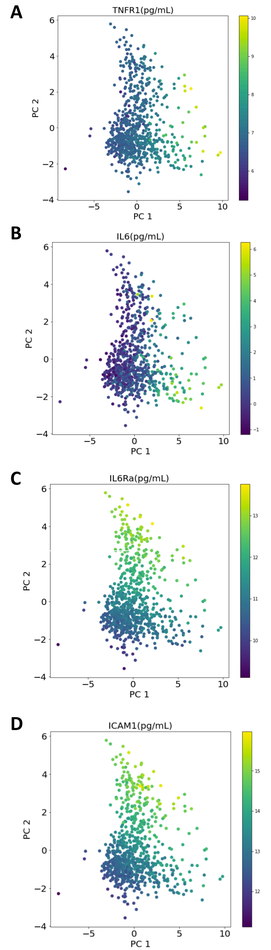

Figure 6. (A) TNFR1 and **(B)** IL6 highly correlated analytes with PC1. **(C)** IL6Ra and **(D)** ICAM1 highly correlated with PC2. Values are log-transformed.

Results

Table 2. Pearson Correlations between analytes and PC1 and PC2 .

Analyte	Pearson Correlation with PC1	Analyte	Pearson Correlation with PC2
TNFR1	0.824	IL6Ra	0.926
IL6	0.785	ICAM1	0.865
Procalcitonin	0.749	Lipocalin	0.808
CRP	0.663	CRP	0.057
Illra	0.633	RAGE	-0.023
VEGFA	0 .632	IL1b	-0.036
DDimer	0.617	TNFR1	-0.047
Lipocalin	0.509	VEGFA	-0.075
Ferritin	0.497	Procalcitonin	-0.082
IL1b	0.423	IL6	-0.111
ICAM1	0.414	IL5	-0.118
IFNγ	0.410	IFNg	-0.137
IL6Ra	0.273	IL1Ra	-0.406
IL5	0.206	D-dimer	-0.415
RAGE	0 .143	Ferritin	-0.428

- Both duration of symptoms and severity were noted to align with PCA axis 1 when stratifying by date of onset (Figure 1A) and hospitalization status at time of collection (Figure 1B).
- Longitudinal biomarker trajectories were plotted over time with a separation of confidence intervals noted during the first few weeks followed by a confluence of levels over time in most biomarkers (Figure 2 and Figure 3). However, D-dimer remained elevated in those with hospital and/or ICU-level of care. Additionally, procalcitonin and IL6 remained elevated in patients requiring ICU-level of care for up to 100 days post-symptom onset.
- Biomarkers levels during first 14 days of illness among participants with COVID-19 differed by level of care (Figure 4 and Figure 5), most markedly with ferritin, TNFR1, IL6, and VEGFA.
- Both time and severity were noted to align with PCA axis 1 when stratifying by days after onset (Figure 1A) and hospitalization status at time of collection (Figure 1B). Proinflammatory cytokines and immune response biomarkers including TNFR1, IL6, and procalcitonin aligned with this PCA 1 axis (Table 2; Figure 6A-B). PCA axis 2 most correlated with IL6Ra. ICAM1, and lipocalin (Table 2; Figure 6C-D).

Conclusions

TNFR1 and IL6Ra levels correlated with differences in the proinflammatory states between hospitalized and non-hospitalized individuals including time points late in the course of illness. Further analysis of these preliminary findings is needed to determine the immunologic underpinnings contributing to stages and severity of illness.

Acknowledgements

This project has been funded by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, under Inter-Agency Agreement Y1-AI-5072, the Defense Health Program, U.S. DoD, under award HU0001190002, and the Defense Health Agency, U.S. DoD, under awards HU00012020070 and W911QY-20-9-0006 .

Disclaimer: The view(s) expressed herein are those of the author(s) and do not reflect the official policy or position of Uniformed Services University of the Health Sciences, Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., National Institutes of Health or the Department of Health and Human Services, the Departments of the Air Force, Navy, or Army, U.S. DoD, or the U.S. Government. The investigators have adhered to the policies for protection of human subjects as prescribed in 45 CFR 46. This research has been approved the USU IRB.