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Evaluation of Plasma Proteome and miRNA Changes Related to COVID-19 Patient Severity Response

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Abstract

COVID-19 remains a worldwide pandemic where underlying health conditions like diabetes, cancer, obesity, high blood pressure, asthma, and smoking are all risk factors related to the severity of outcome from a SARS-CoV-2 infection. Therefore, gaining mechanistic insights at the molecular level to understand the differences in severity of the infection and discovering early biomarkers that enable prediction of outcomes among COVID-19 patients remains urgently needed. These insights could help ease burden of care and aid in evaluation of treatments or development of cures. Blood samples from 93 COVID-19 patients collected at the time of initial diagnoses were processed to plasma and deidentified for proteomic and miRNA analysis. COVID-19 positive patients were categorized into 3 symptom response categories: mild, out-patient; moderate, hospitalization without intensive care; and severe, hospitalization with intensive care. A total of 2939 proteins and 2097 miRNAs were analyzed in the plasma samples. Student's t-test was used for statistical significance with a p-value < 0.05 and fold change (FC) > 2.0 as the criteria. The number of significantly changed proteins was 369 (13%) for severe vs. mild%), 135 (5%) for severe vs. moderate, and 23 (1%) for moderate vs. mild. The number of significantly changed miRNAs was 578 (28%) for severe vs. mild, 386 (18%) for severe vs. moderate, and 122 (6%) for moderate vs. mild. Many of the most significant protein differences in patients with severe COVID-19 (p-value < 10-5, FC > 5) were involved in inflammation and cardiac injury, which confirms earlier reports of "cytokine storm" and cardiovascular events. Further investigations will be conducted, including analyzing more samples, evaluating metabolites and lipids to discover other outcome biomarkers, and, most importantly, aligning omics data with demographics, and clinical endpoints. The combination of omics and clinical data will be further evaluated for pathways analysis.

Introduction

COVID-19 is a worldwide pandemic and patients are overburdening hospitals in terms of beds, personal protective equipment (PPE), and ventilators. Age, gender, underlying health conditions like diabetes, cancer, obesity, high blood pressure, asthma and smoking are all risk factors related to the severity of outcome from SARS-CoV-2 infection (1). Therefore, gaining mechanistic insights at the molecular level to understand 1) the differences in severity of the infection, 2) differences in response to treatment and 3) to predict factors related to better or worse outcomes among the COVID-19 patients is urgently needed. These insights can help ease burden of care and aid in development of cures. Patients exposed to SARS-COV-2 infection are expected to develop an immune response. Their immune response can be observed by changes in plasma profiles of proteins and miRNAs. SAR-CoV-2 damages the blood vessels, causes inflammation and can infect all parts of the body. It is expected that the SARS-COV-2 virus initiates an immune response that may differ from patient to patient resulting in a wide range of outcomes to its infection and treatment. We propose profiling the proteins and miRNA in blood plasma from 90 COVID-19 positive patients that exhibit these various degrees of response to infection by this virus in order to investigate the associated biological mechanisms involved and discover potential biomarkers.

Materials and Methods

UTHSC has collected plasma samples for COVID-positive patients under the IRB approval of the clinical study of adults and children with COVID-19 at UTHSC titled "Immunologic and Virologic Features of Covid-19 (CIVIC-19) in Memphis, Tennessee" (20-07345-FB). The plasma samples that were deactivated were shipped to NCTR. Deidentified samples were labeled with a generic ID and a classification based on the following three COVID-19 patient response groups:

1. **Mild**: Out-patients: Symptoms that do not require hospitalization
2. **Moderate**: Hospitalization without intensive care:
3. **Severe**: Hospitalization with intensive care

miRNA Method:

The thermally deactivated plasma samples were thawed and used for miRNA extraction in an enhanced BSC 2 hood using Qiagen (Germantown, MD) miRNeasy Serum/Plasma Kits following methods previously published. The plasma samples and pooled QC plasma samples were sent to a service provider for miRNA profiling using Illumina NextGen sequencer. Aligned bam files from miRNA profiling dataset will be used for downstream statistical analysis to compute changes of miRNA expression level in COVID-19 patients.

Proteomics Method for shipment to OLINK:

COVID-19 samples and pooled samples (80 µL each) were transferred to 96-well plates. The sample plates with covers will then be shipped to Olink (https://www.olink.com/ , Boston, MA) on dry ice. Over 3000 proteins were analyzed from biomarker panels that include cardiometabolic, cardiovascular II, cardiovascular III, development, immune response, inflammation, metabolism, and organ damage. The OLINK protein data was in the arbitrary unit normalized protein expression (NPX) on a log2 scale, which was transformed to protein concentration by using the formula 2NPX.

Statistical Analysis and Ingenuity Pathway Analysis (IPA):

The miRNA and protein data was compared between the 3 response categories by using generalized linear model (glm) procedure of SAS 9.4. Fold changes (FC), p-values and false discovery rates (FDR, a method for adjusting p-values of multiple comparison) were calculated. MiRNAs or proteins were considered differentially expressed if the p-values of comparison was less than 0.05 and absolute FC was greater than 2. These differentially expressed miRNAs and proteins were uploaded in IPA to identify enriched canonical pathways for different comparisons. IPA predicts the activity of various pathways based on positive (increased activity) and negative (decreased activity) z-scores.

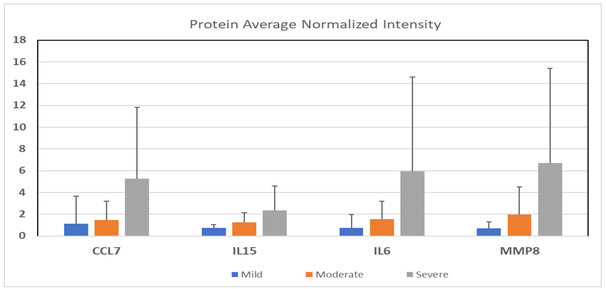
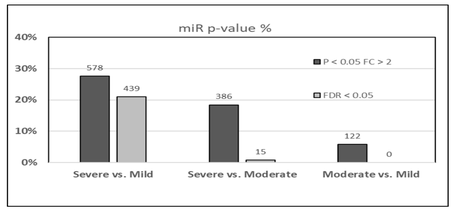


Figure 1. The bar plot figure above shows the normalized intensity of C-C motif chemokine 17 (CC17), Interleukin 15 (IL15), Interleukin 6 (IL6), and matrix metalloproteinase-8 (MMP8) that were found to increase in an apparent severity-dependent manner.

Results and Discussion

Figure 2 shows the number of significantly changed proteins with a FC > 2 was 369 (13%) for severe vs. mild, 135 (5%) for severe vs. moderate, and 23 (1%) for moderate vs. mild cases, while the number of significant FDR-based protein changes was 1003 (34%) for severe vs. mild and 354 (12%) for severe vs. moderate. The number of significantly changed miRNAs with FC of greater than 2 was 578 (28%) for severe vs. mild, 386 (18%) for severe vs. moderate, and 122 (6%) for moderate vs. mild cases, and the number of significant FDR-based miRNA changes was 439 (21%) for severe vs. mild and 15 (1%) for severe vs. moderate. The results show that even though the time when the first sample was taken may vary for the time from initial infection for patients in each response category, there were numerous significant changes in protein and miRNA expression levels of patients with severe and moderate COVID-19.

Figure 3 shows canonical pathway analysis of the protein and miRNA data using IPA was conducted and a z-Score is calculated for each pathway. Z-score is a statistical assessment that tells you how far away from the mean or average of your data lies in a normally distributed sample. Therefore, a high positive Z-Score (bright orange) indicates that the pathway is increased more than expected based on the overall dataset and a negative z-score (blue) indicates that the pathway is less activated or repressed. The IPA canonical pathway analysis showed that pathogen induced cytokine storm, wound healing, airway pathology in chronic obstructive pulmonary disease, role of chondrocytes in rheumatoid arthritis signaling, IL17 signaling, and multiple other pathways were significantly altered in the severe vs. mild cases.



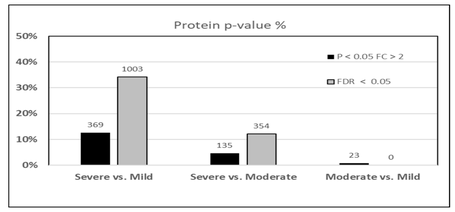


Figure 2. The bar plots above show the percentage of miRNAs and proteins that are significantly changed, using p-value <0.05 and fold change (FC) > 2.0 in black and false discovery rate (FDR) < 0.05 in grey

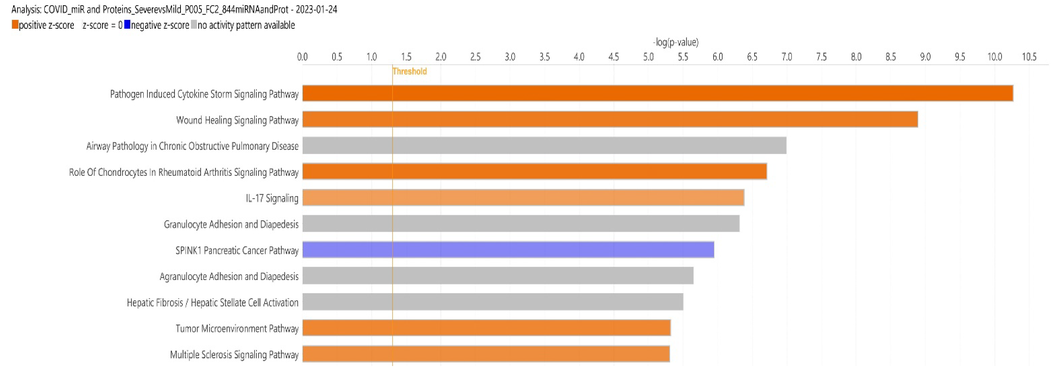


Figure 3. Canonical pathway analysis of the protein and miRNA data using IPA was conducted and a z-Score is calculated for each pathway.

Conclusions and Disclaimer

Changes in plasma proteins and miRNAs were observed in relation to COVID-19 severity as shown is Figures 1 and 2. Increases in IL6 are consistent with its role as both a pro-inflammatory cytokine and an anti-inflammatory myokine, as well as previous reports that it may be a biomarker of poor prognosis for COVID-19. Increases in the small chemokine CCL7 are consistent with its role in innate immunity, including recruitment of neutrophils to sites of bacterial and viral infection.

Disclaimer: This poster reflects the view of the authors and should not be construed to represent FDA's views or policies.