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

Richard D. Beger[1]*, Li-Rong Yu [1], Tao Han[1], Vikrant Vijay [1], Jinchun Sun[1], Mallikarjun Bidarimath[1], Elysia Masters[1], Thomas Schmitt[1], Lisa Pence[1], Jessica Hawes Oliphant[1], Heather S. Smallwood[2],

[1] National Center for Toxicological Research, Jefferson, AR; [2] Department of Pediatrics, University of Tennessee Health Science Center, TN

## Abstract

The severity outcome of COVID-19 resulting from SARS-CoV-2 infection has been shown to be related to underlying health conditions like diabetes and cancer. There remains an urgent need to gain mechanistic insights at the molecular level to understand factors that influence severity of the infection and discover early biomarkers that enable prediction of severity among COVID-19 patients. These insights could help ease the burden of care and aid in evaluation and development of treatments. In this study, COVID-19-positive patients were categorized into 3 symptom response categories using a 12-point patient response scoring system: mild (score 0-1), moderate (score 2-4), and severe (score 5-12). Blood samples from 93 COVID-19 patients collected at the time of initial diagnoses were processed to plasma and deidentified for proteomic and miRNA analyses. A total of 2,921 proteins and 2,083 miRNAs were analyzed in the plasma samples. Ingenuity Pathway Analysis (IPA) of significant proteins revealed that the top pathways were neutrophil degranulation, cytokine storm, role of osteoblasts in rheumatoid arthritis signaling, interleukin-10 signaling, and wound healing signaling in severe patients. Interleukin-6 (IL6) was involved in nine of the top ten IPA pathways. MicroRNA associations with protein targets were extracted from IPA using experimentally observed and highly predicted criteria. Proteomics and miRNA analyses of plasma from recently diagnosed COVID-19 patients revealed 801 protein and 365 miRNA biomarkers of severity response to COVID which are associated with preexisting health status and acute response to the COVID infection. Additional experiments are needed to validate the biomarkers.

## Experimental Design

Blood samples from 93 COVID-19 patients collected from August 2020 until July of 2022 were processed to plasma. The response to COVID was scored using a 12- point patient response scoring system described below and grouped into mild(0-1), moderate(2-4) and severe(5-12) responders depending on their score. Pooled QC samples were made prior to conducting proteomics and miRNA analysis and used to assess data quality. A total of 2,921 proteins were evaluated by OLINK and 2,083 miRNAs were analyzed by Firalis using HTG EdgeSeq technology. Univariate and multivariate analyses were conducted on the proteins and miRNAs. All the proteins were imported into Qiagen's web-based ingenuity pathway analysis (IPA) software. Core analysis of significantly (FC>1.5, p<0.05) altered proteins was performed to evaluate pathways enrichment using Fisher's exact test. Canonical pathways with -log10 p-value >1.3 were considered significantly enriched. IPA also calculates z-scores to predict the activation/inhibition of pathways based on fold changes of altered proteins. Z-score > 2 or z-score < -2 indicate predicted activation or inhibition of pathways, respectively. Information about miRNA and their target proteins was also obtained from IPA using miRNA target filter tool. This information also includes the source and confidence (experimental, highly-predicted or moderately-predicted) in miRNA-protein target relationship. The miRNA target protein relationships were compared with correlation analysis of significant miRNAs and proteins.

**Patient Response Scoring:**

- 2 points for hospital admission within 60 days of enrollment;
- 3 points for ICU admission within 60 days of enrollment;
- 1 point for mild symptoms: cough, fever, diarrhea, vomiting, headache, loss of taste or smell, sore throat, myalgias, fatigue, lymphadenopathy, and malaise;
- 2 points for symptoms: shortness of breath (dyspnea), wheezing, SpO2<92% on room air, respiratory rate (RR)>30, and new non-invasive oxygen requirement.
- 2 points for symptoms: invasive or positive-pressure oxygen requirement, acute kidney injury (Cr >1.5x upper limit normal for age or estimated glomerular filtration rate [eGFR] <60), elevated aspartate/alanine transaminase ((AST/ALT); ratio >2x normal), new elevation international normalized ratio (INR) >1.3, and altered mental status;
- 3 points for symptoms: acute respiratory distress syndrome (ARDS), shock requiring pressors, renal failure with dialysis, extracorporeal membrane oxygenation (ECMO) requirement, organ transplant, pulmonary embolism, deep venous thrombosis, and/or stroke.

## Results

**Table 1. Patient Demographics by Severity**

| | Severity | | | | | | |
|---|---|---|---|---|---|---|---|
| | Mild | Moderate | Severe | [1]P-value | [2]P-value | [3]P-value | Single P-value |
| N | 33 | 28 | 32 | | | | |
| Scoring | 0.79 ± 0.42 | 2.82 ± 0.98 | 8.50 ± 2.24 | 4.88E-12 | 1.89E-19 | 1.41E-16 | 1.00E-05 |
| Age | 39.3 ± 14.3 | 44.6 ± 14.4 | 52.2 ± 19.0 | 1.55E-01 | 3.20E-03 | 8.58E-02 | 7.10E-03 |
| Female | 15 (45.5%) | 16 (57.1%) | 16 (50.0%) | 3.63E-01 | 7.14E-01 | 5.80E-01 | 6.59E-01 |
| Black | 4 (12.1%) | 10 (35.7%) | 22 (68.8%) | 3.65E-02 | 3.27E-06 | 1.05E-02 | 9.28E-06 |
| White | 13 (39.4%) | 9 (32.1%) | 9 (28.1%) | 5.57E-01 | 3.37E-01 | 7.35E-01 | 6.21E-01 |
| Other Race | 0 | 2 (7.1%) | 1 (3.1%) | 2.07E-01 | 4.92E-01 | 5.94E-01 | 1.99E-01 |
| Race unknown | 16 (48.5%) | 7 (25.0%) | 0 | 5.93E-02 | 2.73E-06 | 3.10E-03 | 4.29E-06 |
| Diabetic | 4/13 (30.8%) | 5/15 (33.3%) | 11/27 (40.7%) | 1.00E+00 | 7.30E-01 | 6.36E-01 | 8.16E-01 |
| Hypertension | 6/13 (46.2%) | 10/16 (62.5%) | 20/27 (74.1%) | 3.79E-01 | 8.29E-02 | 4.24E-01 | 2.22E-01 |
| Cardiovascular diseases | 3/13 (23.1%) | 8/16 (50.0%) | 10/27 (37.0%) | 2.49E-01 | 4.84E-01 | 4.05E-01 | 3.49E-01 |
| Chronic renal disease | 1/13 (7.7%) | 0/14 | 7/27 (25.9%) | 4.82E-01 | 2.36E-01 | 7.45E-02 | 6.41E-02 |
| Severe obesity | 3/12 (25.0%) | 8/16 (50.0%) | 11/27 (40.7%) | 2.53E-01 | 4.77E-01 | 5.55E-01 | 4.50E-01 |

[1] Moderate vs mild; [2] Severe vs mild; [3] Severe vs moderate, Parenthesis is the percentage within group

**Volcano Plots**

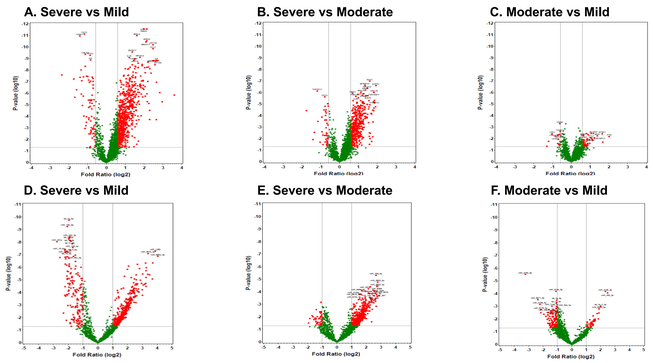


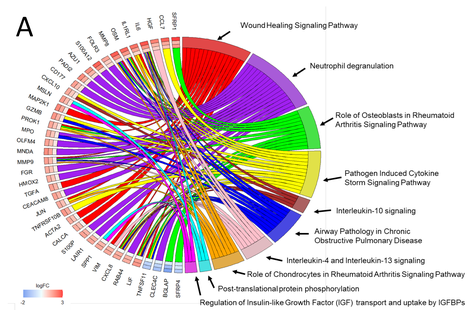

**Figure 1. Volcano plots of proteins in severe vs mild (A); severe vs moderate (B); moderate vs mild (C). Volcano Plots of miRNA in severe vs mild (D); severe vs moderate (E); moderate vs mild (F). Significant changes (FC> 1.5 (proteins) FC> 2.0 (miRNAs), p-value <0.05) in red, non-significant changes in green.**

**Table 2: Top 10 IPA Canonical Pathways**

| Ingenuity Canonical Pathways | Severe vs Mild -log (p-value) | Severe vs Mild z-score | Severe vs Moderate -log (p-value) | Severe vs Moderate z-score | Moderate vs Mild -log (p-value) | Moderate vs Mild z-score |
|---|---|---|---|---|---|---|
| Neutrophil degranulation | 24.5 | 7.14 | 22.1 | 6.66 | 0 | NA |
| Pathogen Induced Cytokine Storm Signaling Pathway | 15.2 | 4.72 | 14.6 | 3.45 | 2.41 | 2.26 |
| Regulation of Insulin-like Growth Factor (IGF) transport | 13.3 | 3.00 | 14 | 2.99 | 2.29 | NA |
| Post-translational protein phosphorylation | 12.9 | 2.71 | 13.2 | 2.68 | 2.47 | NA |
| Interleukin-10 signaling | 11.7 | 3.36 | 8.85 | 2.71 | 0.93 | NA |
| Role of Chondrocytes in Rheumatoid Arthritis Signaling | 11.1 | 3.67 | 9.09 | 2.83 | 1.23 | NA |
| Airway Pathology in Chronic Obstructive Pulmonary Disease | 11.1 | 2.65 | 9.45 | 2.24 | 0.55 | NA |
| Interleukin-4 and Interleukin-13 signaling | 10.8 | 3.58 | 11.9 | 2.83 | 1.42 | NA |
| Role of Osteoblasts in Rheumatoid Arthritis Signaling | 10.7 | 3.29 | 7.38 | 1.79 | 2.31 | 1 |
| Wound Healing Signaling Pathway | 10.4 | 3.77 | 13.1 | 3.16 | 0.80 | NA |

## Results

**Chord Diagrams**



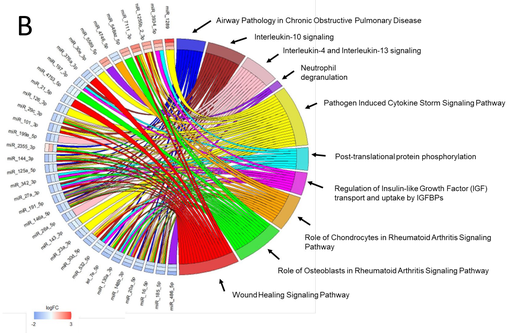




**Figure 2. A)Top 40 chord linkage to top 10 IPA Canonical pathways with z-score >2.0 B) Top 40 miRNAs with experimental observed or highly predicted confidence to mRNA of proteins in the top 10 Canonical pathways. Blue to red show the changes of the protein or miRNAs in severe vs mild, severe vs moderate and moderate vs mild.**

**Table 3: Correlation Between MiRNA and their Experimentally Observed Target Significant Proteins**

| Protein | MiRNA | Pearson Correlation Coefficient | Correlation P-Value | Top 10 Canonical Pathways Per Protein |
|---|---|---|---|---|
| CRIM1 | miR_20a_5p | -0.637 | 8.88E-12 | |
| TNFRSF10B | miR_30d_5p | -0.624 | 3.10E-11 | B |
| LMNB2 | miR_30d_5p | -0.598 | 3.15E-10 | |
| ADAMTS1 | miR_125a_5p | -0.583 | 1.04E-09 | |
| JUN | miR_144_3p | -0.558 | 7.45E-09 | B; G; J |
| CSF1 | miR_130a_3p | -0.550 | 1.32E-08 | E; D; C |
| VEGFA | miR_185_5p | -0.532 | 4.87E-08 | H; B; G; I; J |
| CD40 | miR_146a_5p | -0.532 | 4.90E-08 | |
| ARID4B | miR_486_5p | -0.530 | 5.37E-08 | |
| KRT19 | miR_125a_5p | -0.526 | 7.42E-08 | |
| ACTA2 | miR_21_5p | -0.521 | 1.04E-07 | J |
| JUN | miR_16_5p | -0.520 | 1.06E-07 | B; G; J |
| CD274 | miR_145_5p | -0.502 | 3.45E-07 | |
| ARID4B | miR_20a_5p | -0.478 | 1.41E-06 | |
| CCN2 | miR_19b_3p | -0.476 | 1.62E-06 | |
| S100A12 | miR_146a_5p | -0.474 | 1.86E-06 | A |
| VEGFA | miR_20a_5p | -0.473 | 1.91E-06 | H; B; G; I; J |
| SWAP70 | miR_145_5p | -0.471 | 2.15E-06 | |
| JUN | miR_30d_5p | -0.471 | 2.15E-06 | B; G; J |
| MAP2K1 | miR_16_5p | -0.471 | 2.16E-06 | J |
| GPR37 | miR_29c_3p | -0.462 | 3.60E-06 | |
| CXCL8 | miR_20a_5p | -0.452 | 6.00E-06 | F; E; H; B; G; I; J |
| MMP3 | miR_20a_5p | -0.452 | 6.07E-06 | H; G; I |
| TNFAIP2 | miR_30d_5p | -0.439 | 1.22E-05 | |
| CCN2 | miR_30d_5p | -0.434 | 1.56E-05 | |
| KRT19 | let_7e_5p | -0.432 | 1.70E-05 | |
| CXCL8 | miR_146a_5p | -0.430 | 1.85E-05 | F; E; H; B; G; I; J |
| IL6 | miR_191_5p | -0.426 | 2.35E-05 | F; E; H; B; D; C; G; I; J |
| CDCP1 | miR_30d_5p | -0.417 | 3.52E-05 | |
| VIM | miR_20a_5p | -0.417 | 3.60E-05 | H; J |
| LMNB1 | miR_23a_3p | -0.416 | 3.77E-05 | |
| PMS1 | miR_16_5p | -0.413 | 4.21E-05 | |
| AHNAK | miR_145_5p | -0.413 | 4.22E-05 | |
| PALM | miR_122_5p | -0.412 | 4.47E-05 | |
| AMOT | miR_139_5p | 0.405 | 6.12E-05 | |
| PGLYRP1 | miR_146a_5p | -0.402 | 7.14E-05 | A |
| BCL2L11 | miR_92b_3p | -0.400 | 7.94E-05 | |

**Caption/legend:** All the proteins are significantly (fold change > 1.5; p-value<0.05) altered between severe vs. mild response group. Information about miRNA targeting proteins and proteins' pathway enrichments were obtained from Qiagen's Ingenuity Pathway Analysis software.

**Canonical Pathways Key: A**, Neutrophil degranulation; **B**, Pathogen Induced Cytokine Storm Signaling Pathway; **C**, Regulation of Insulin-like Growth Factor (IGF) transport and uptake by IGFBPs; **D**, Post-translational protein phosphorylation; **E**, Interleukin-10 signaling; **F**, Airway Pathology in Chronic Obstructive Pulmonary Disease; **G**, Role of Chondrocytes in Rheumatoid Arthritis Signaling Pathway; **H**, Interleukin-4 and Interleukin-13 signaling; **I**, Role of Osteoblasts in Rheumatoid Arthritis Signaling Pathway; **J**, Wound Healing Signaling Pathway.

## Conclusion

- Black race was significant patient demographic determining severity for all three response groups and age is significant in severe vs mild.
- Many miRNAs were decreased in severe vs mild and conversely more proteins were increased in severe vs mild.
- The top two IPA canonical pathways are neutrophil degranulation and cytokine storm in severe vs mild and severe vs moderate.
- IL6 was involved in 9 of the top 10 IPA canonical pathways and miR_146a_5p was observed in 8 of the top 10 canonical pathways and has been experimentally observed to interact with two proteins in the neutrophil degranulation pathway.
- Significant correlation of miRNAs with proteins was found in the IPA miRNA confidence with experimental observed or highly predicted (not shown) and these miRNA-proteins could be biomarkers of COVID-19 response severity.
- Many of the protein and miRNA severity response biomarkers reported in the study are consistent with previous COVID-19 studies, and most of those studies did not have both proteomics and miR profiling. The study findings warrants further investigation in a larger study.