**Diabetes mellitus and the risk of severe SARS-CoV-2 infection**

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Keywords

Word Count

**Abstract**

**Introduction**

Coronavirus Disease of 2019 (COVID-19), caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), was announced as a pandemic by the WHO at the beginning of 2020 due to its rapid communicability and disease severity. By October 2022, COVID-19 had caused over 6.5 million deaths. Primarily a condition that affects the respiratory system, it presents in patients with a wide range of symptoms, ranging from asymptomatic and mild to severe. In the most critical cases, patients may require ICU care and mechanical intubation, among other intensive interventions. A variety of risk factors are suggested to increase the risk for severe illness, including 65 years of age and older, hypertension, smoking, and diabetes.

Multiple meta-analyses of the clinical correlation between diabetes and SARS-CoV-2 have demonstrated that patients with diabetes are at higher risk for severe disease and mortality, reporting odds ratio as high as OR = 2.75 (95% CI: 2.09-3.62; p < 0.01) for severe disease. Diabetes has been previously implicated in other infectious conditions, including being associated with over a four-fold risk of ICU admission in patients with the Influenza A infection of 2009 (H1N1). Furthermore, diabetes has been observed to be associated with critical illness and identified as an independent risk factor for 90-day mortality in patients with Middle East respiratory syndrome coronavirus (MERS-CoV). Other studies further corroborate a bi-directional link between diabetes and COVID-19, including cases and systematic reviews that found a higher incidence rate of new-onset diabetes and hyperglycemia in patients previously infected by COVID-19. Despite the substantial data that supports diabetes as a risk factor for diabetes, the mechanism that mediates this risk is largely unknown.

Although poorly elucidated, the mechanism of disease severity in diabetes mellitus patients may be connected to angiotensin-converting enzyme 2 (ACE2) and cytokine/chemokine gene expression. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) uses the ACE2 receptor to enter host cells. Upon entry, there is a downregulation of surface ACE2 expression. Circulating angiotensin 2 (Ang-II) are elevated in COVID-19 patients compared to healthy controls providing evidence of renin-angiotensin system (RAS) imbalance in the disease. Increases in Ang-II leads to increases in a disintegrin and metalloproteinase 17 (ADAM17) activity and subsequent release of tumor necrosis factor α (TNF- α) and other inflammatory cytokines. Nuclear factor erythroid 2–related factor 2 (Nrf2) and Nrf2 related genes are regulators of cellular redox balance and are involved in the release of inflammatory cytokines and chemokines secondary to stress. Nrf2 activation downregulated a variety of cytokines reported to be elevated in COVID-19 suggesting reduced Nrf2 activity as a contributor to the “cytokine storm” seen in COVID-19.

Chemokines are an important secretory protein responsible for immune signaling and have been implicated in a variety of lung pathologies. For example, CCL2 [chemokine (C-C motif) ligand 2; monocyte chemoattractant protein-1, (MCP-1)] and its receptor CCR2 are involved in monocyte/macrophage migration, Th2 cell polarization, and the production of TGF-β and procollagen in fibroblast cells. This chemokine is associated with acute respiratory distress syndrome and pulmonary fibrosis7 – both observed in COVID-19. CCL2 elevation has also been found to be associated with severe SARS-CoV. A variety of chemokines have been reported to be elevated in COVID-19 infection, but there has not been an evaluation of differential patterns of expression in individuals with and without diabetes or other stratifications such as race.

Diabetes is also associated with renin-angiotensin system (RAS) dysregulation, ACE2 downregulation, low-grade inflammation, cytokine/chemokine upregulation, and altered Nrf2 activity. The combination of COVID-19 and diabetes may contribute to exaggerated ACE2 depletion and elevations in circulating Ang-II following by release of cytokines/chemokines mediated by Nrf2 as well as altered immune function. In terms of immune signaling, regulation of T cell function has been linked to Ang-II. ACE2 is expressed in leukocytes and the lungs, kidneys, heart, and gastrointestinal tract (all organs affected by COVID-19) subjecting these organs to damage from inflammation. Furthermore, individuals with diabetes are already at an increased risk for cardiovascular events; and myocardial inflammation associated with downregulation of ACE2 in COVID-19 has been previously reported representing additive risk. Collectively, there are many similarities between the COVID-19 and diabetes pathophysiology that suggest increased risk for worse outcomes. Moreover, the association between cytokine/chemokine gene expression patterns and disease severity may be used as a predictive biomarker.

The purpose of this study was to evaluate biomarker and gene expression patterns in individuals hospitalized with diabetes mellitus infected with SARS-CoV-2. In addition, the relationship between these patterns and disease severity was examined.

**Methods**

**Data source and patient collection**: We performed a single-center, IRB-approved, cohort study using data from electronic health records at a large community medical center. Inclusion/exclusion criteria.

**Data extraction and collection**: All data were extracted from the electronic health record (Epic Systems). Patient age, sex, race/ethnicity, comorbidities, vaccination status, concomitant anti-hyperglycemic medications, COVID treatment interventions, and other basic relevant laboratory data were extracted from the records. Patient comorbidities were identified using the International Classification of Diseases, tenth revision, clinical modification (ICD-10-CM) codes. Overall comorbidity status of patients was defined by the scoring of the Charlson-Deyo comorbidity Index (CCI). Patients’ baseline characteristics including demographics, vaccination information, comorbidities, medications, lab work, clinical scores and ELISA protein concentration were summarized in Table 1 by being diagnosed with COVID-19 and having any type of diabetes mellites (DM).

**RNA-Sequencing**

**Cytokine and chemokine multiplex assay**

**ACE2 and DPPIV ELISA**

**Outcomes**: Patients were stratified into those with COVID-19 and those without COVID-19 as well as those with diabetes and those without diabetes. The primary endpoint was differences in inflammatory mediator expression profile between COVID-19 patients with and without diabetes. Secondary endpoints include differences in inflammatory mediator expression profile between diabetes patients with and without COVID-19 and occurrence of severe illness (defined by mechanical ventilation, ICU admission, or mortality).

**Statistical Analysis**: Patient demographics were assessed using descriptive statistics. Continuous data are represented with means and standard deviation, while nominal data is represented with percentages and standard deviations. Differences in baseline characteristics were analyzed utilizing t-tests for continuous data and chi-squared or Fischer’s exact test for categorical data.

**Results**

Between January 1st 2022 and May 30th 2022, 183 adult patients were enrolled within the specified timeframe, of which 91 patients had blood samples extracted. Patients’ blood samples were obtained within 2 days of hospital admission.

Out of 110 patients admitted with COVID-19, 12 (10.9%) died in hospital compared to 3 out of 72 non-COVID-19 patients (4.2%). Three (3) out of the 12 COVID-19 patients that died in hospital were admitted for shortness of breath (ICD10 R06.02), unspecified fever (ICD-10 R50.9) or fatigue (ICD-10 R53.83). The 3 non-COVID in-hospital deaths occurred in patients admitted for pneumonia (ICD-10 J18.9) or acute respiratory distress (ICD-10 R06.03).

90-day mortality (after discharge) was recorded for 5 non-COVID patients who were initially admitted for acute ischemic heart disease (ICD-10 I24.9), pneumonia, acute respiratory distress, or intestinal obstruction (ICD-10 K56.609). Additionally, 26 non-COVID patients were readmitted to hospitals within 90 days of discharge. No follow-up information was available for the COVID-19 patients at the time of this report.

There was no significant association between DM and COVID-19 patients’ in-hospital death rate, with 6 DM and 6 non-DM COVID-19 patients dying in hospital (Chi-square test p-value = 0.236). Similarly, obesity and BMI were not found to be significant factors associated with in-hospital death (p-values of 0.760 and >0.999, respectively). However, the odds of in-hospital death were 21.5 times higher (95% CI = 5.2 to 88.3, p-value< 0.001) for the COVID patients admitted to the critical care unit (ICU) compared to those who were not admitted to ICU. Specifically, 7 out of 13 ICU-admitted patients died in hospital compared to 5 deaths occurring in 97 non-ICU patients.

The odds ratio of in-hospital death for patients admitted with COVID-19 versus non-COVID-19 patients was not statistically significantly different from 1 (OR=2.82, 95%CI = 0.86 to 12.70, p-value = 0.119). However, after adjusting for ICU, the association of COVID-19 diagnoses with death became significant (OR = 6.79, 95%CI = 1.73 to 36.07, p-value = 0.012).

COVID-19 severity was measured on World Health Organization Original Scale for Clinical Improvement (WHO OSCI) scale. COVID-19 patients were grouped by the WHO OSCI into Moderate (score < 5) and severe (>=5 and <8) cohorts. WHO OSCI score of 8 signified deaths. At the admission, 77 out of 110 COVID patients had WHO OSCI score of 5 or higher. Notable, 5 out of the 13 COVID patient admitted to the CC unit had the score of 5, and another 8 score of 6. At the same time, 64 out of 97 non-CCU patients (66.0%) were scored 5 or above at the admission. Additionally, all 12 COVID-19 patients that died in hospital had WHO OSCI score of 5 or 6 at the admission, and their scores did not decrease until their death except for a single patient whose score decreased from 5 to 4 on Day 3, just before death (Table 2).

On average, COVID patients were admitted for a slightly shorter period compared to non-COVID patients (mean+/-SEM = 7.3+/-0.9 and 8.8+/-1.1 days respectively). The patients who died in hospital were hospitalized for longer times compared to those discharged alive (12.5+/-2.4 vs 7.5+/-0.7, respectively). The difference between COVID and non-COVID patients’ length of stay was even larger for those who were not discharged on the day of admission (i.e., stayed for more than one day), with LOS of 10.1+/-1.2 days for non-Covid patients discharged alive vs. 7.4+/-0.9 days for the COVID patients discharged alive. For the patients who died in hospital, the LOS were 11.0+/-3.8 and 12.8+/-2.9 for non-COVID vs COVID patients, respectively.

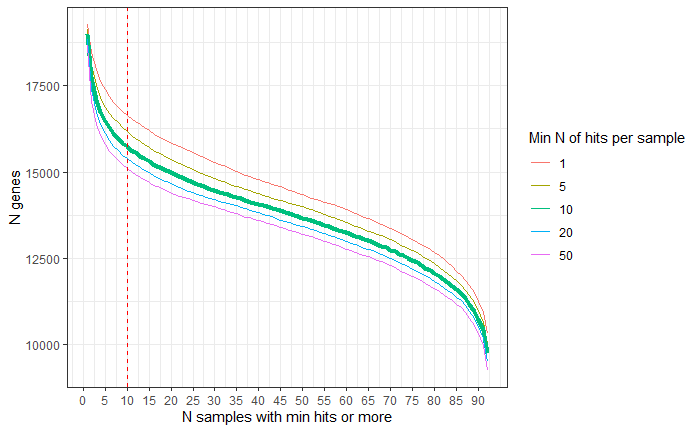
Patients’ comparison (medications, treatments administered, length of stay, vaccination status)

Outcomes (inflammatory mediator expression profile, statistical analysis)

Differential Gene Expression Analyses

In total, 58,708 protein coding and long noncoding genes and gene variants were found in the 92 RNA-seq samples. Out of these, 19,909 were protein coding genes. Note that 15 genes had 2 variants each. For these genes, the counts of the variants were added up within each sample.

Genes with a small number of hits were filtered out. Based on an examination of the number of genes remaining after filtering using varying the minimum number of hits per sample and the minimum number of samples with at least that many hits (Figure 1), it was decided to set both numbers to 10. Hence, 14,223 genes with at least 10 hits in at least 10 out of 92 samples were used in the analysis.



Next, we identify coding genes differentially expressed in COVID patients compared to controls (at least 2-fold change and adjusted p-value ≤ 0.05). 2 genes were downregulated and 16 upregulated in COVID patients compared to non-COVID. The list of the 18 genes and the estimates of the differences (on log2 scale and representing the number of 2-fold changes in COVID vs. non-COVID patients’ samples) is presented in Table 3. Figure 1 shows the total number of hits for each of the 18 genes in each sample.



Figure 1: differentially expressed genes in COVID vs non-COVID

One of the most striking differences found in this part of the analysis was the overexpression of Interferon Alpha Inducible Protein 27 (IFI27) coding gene, with a 2-fold difference of 2.37 (SEM = 0.49). The protein coded by IFI27 was previously shown to be associated with viral infections including Hepatitis C, respiratory syncytial virus (RSV) infection and Enterovirus 71 (EV71) hand foot and mouth disease. It has been proposed as a biomarker for an early prediction of COVID-19 outcomes ([Shojaei et al 2022](https://pubmed.ncbi.nlm.nih.gov/36685600/)). In this study, most of the patients that had gene sequencing data and died in hospital had their IFI27 expression levels elevated compared to those who were discharged alive (Figure 2 left). No obvious patterns were observed for critical care as an outcome (Figure 2 right).



Figure 2: IFI27 expression in COVID vs. non-COVID color-coded for in-hospital deaths (left) and critical care (right). The only patient who died in the hospital and had an RNA-seq results had IFI27 levels that exceeded all the non-COVID patients' readouts.

NOTE for Lu and Marshall: please check if my conclusions above make clinical sense, perhaps find more related studies showing IFI27 to be important for COVID, and what would be the mechanism of action. Also, are any of the rest of these genes are clinically interesting?

Next, DMs association with gene expressions was tested. In total, 431 genes were differentially expressed in DM patients vs. the controls (non-DM), with 45 genes upregulated by DM and 386 genes downregulated (Table 4.)

NOTE for Lu and Marshall: please check these genes for biological relevance for DM.

Out of all genes found to be differentially expressed in COVID/non-COVID and DM/non-DM patients, there were 5 genes in common: GRASP, KRT8, MYZAP, PRKG1 and SMIM24. The number of hits in the samples, grouped by COVID and DM diagnoses, are presented in Figure 3.



Figure 3: number of hits in samples, grouped by COVID and DM diagnoses.

For Davit and Vahe (1/18/2024):

1. Compare in-hospital death COVID/non-COVID patients’ gene expressions. Maybe add CCU (in-hospital death or CCU).
2. Javier’s suggestion: use Javier’s DMR package to aggregate omics data but weight each dataset. This way, gene expressions will not overwhelm the analysis.
3. Look only at the genes that coded for proteins in the ELISA dataset. High gene expression should correspond to high protein production unless there is a delay, mRNA gets blocked, or there are multiple transcriptomes that do not correspond to the observed proteins.

**Discussion**

Inflammatory mediators measured:

IFN- α, IFN- γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8 (CXCL8), IL10, IL-12p70, IL-17A (CTLA-8), IL-18, IP-10 (CXCL10), MCP-1 (CCL2), MIP-1α, MIP-1β, TNFα, and TNFβ

**References**