Genetic and inflammatory signatures associated with worse prognosis in hospitalized patients with severe SARS-CoV-2 infection with and without diabetes.

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# 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.1 Primarily a condition that affects the respiratory system, the disease presents in patients with a wide range of symptoms, ranging from asymptomatic and mild to severe. In the most critical cases, patients may require intensive critical care (ICU) and mechanical intubation, among other intensive interventions.1 A variety of risk factors are suggested to increase the risk for severe illness, including age greater than 65 years, hypertension, smoking, and diabetes.3

Multiple meta-analyses of the clinical correlation between diabetes and SARS-CoV-2 have demonstrated that individuals with diabetes are at higher risk for severe disease and mortality, reporting odds ratios as high as OR = 2.75 (95% CI: 2.09-3.62; p < 0.01) for severe disease.1,4,5 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).6 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).7 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-19.8,9 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.10 Upon entry, there is a downregulation of surface ACE2 expression. Circulating angiotensin 2 (Ang-II) is elevated in COVID-19 patients compared to healthy controls, providing evidence of renin-angiotensin system (RAS) imbalance in the disease.11 Increases in Ang-II lead to increases in disintegrin and metalloproteinase 17 (ADAM17) activity and subsequent release of tumor necrosis factor α (TNF- α) and other inflammatory cytokines.10 Nuclear factor erythroid 2–related factor 2 (NRF2) and NRF2-related genes regulate cellular redox balance and release inflammatory cytokines and chemokines secondary to stress. NRF2 activation downregulated a variety of cytokines that were reported to be elevated in COVID-19, suggesting reduced NRF2 activity as a contributor to the “cytokine storm” seen in COVID-19.12

Chemokines are an important secretory protein responsible for immune signaling and have been implicated in various 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.13,14 This chemokine is associated with acute respiratory distress syndrome and pulmonary fibrosis15 – both observed in COVID-19. CCL2 elevation has also been found to be associated with severe SARS-CoV.16 Various chemokines have been reported to be elevated in COVID-19 infection, but differential expression patterns have not been evaluated in individuals with and without diabetes17. This study evaluated gene and protein expression patterns in individuals hospitalized with diabetes mellitus infected with SARS-CoV-2 and examined the relationship between these patterns and disease severity.

# Methods

Data source and sample collection

We performed a single-center, IRB-approved cohort study using data from electronic health records and leftover clinical specimens at a large community medical center. All subjects 18 years of age or older with remnant clinical blood specimens within 48 hours of hospital admission were eligible for inclusion. Pregnant patients and those discharged directly from the Emergency Department were excluded. An aliquot of leftover whole blood specimens collected in EDTA tubes was immediately frozen for each patient. The remaining whole blood was centrifuged at 3000 x *G* for 10 minutes, and plasma was drawn off. All samples were stored at -80°C until analysis.

## Data extraction and collection

All data were extracted from the electronic health record (EPIC Systems; Wisconsin, USA). Patient age, sex, race/ethnicity, comorbidities, vaccination status, concomitant medications, COVID-related treatment interventions, and other relevant clinical 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. The overall comorbidity status of patients was defined by the scoring of the Charlson-Deyo comorbidity Index (CCI).

## RNA-Sequencing

RNA sequencing was performed “fee-for-service” by Singulomics (Bronx, NY). RNA was purified using poly-T oligo-attached magnetic beads. After fragmentation, the first strand cDNA was synthesized using random hexamer primers, followed by the second strand cDNA synthesis using either dUTP for directional library or dTTP for non-directional library. For the non-directional library, it was ready after end repair, A-tailing, adapter ligation, size selection, amplification, and purification.

## Cytokine and chemokine multiplex assay

Plasma cytokine and chemokine concentrations were measured using ProcartaPlexTM Human Cytokine Storm 21-Plex (Invitrogen; EPX210-15850-901) on the Luminex platform. The multiplex panel measured the plasma concentration of 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. Briefly, 25 µL of plasma and internal controls were plated on a 96-well plate, incubated with magnetic beads, and washed before adding 25 μL of detection antibody. The plate was then incubated for 30 minutes, followed by adding 50 μL of Streptavidin-PE to each well. The concentration of analytes was reported as pg/mL.

## ACE2 and DPP-IV ELISA

Circulating ACE2 and DPP-IV were measured by sandwich ELISA (Invitrogen; EH489RB; InvitrogenTM; EHDPP4). Briefly, 100 μL of standards and 100 μL of diluted plasma samples using the assay-specific dilient were plated on a 96-well plate. After a series of washes, 100 μL of biotin was added to each well, followed by a 1-hour incubation period at room temperature with gentle shaking. The solution was discarded, the plate was washed, and 100 μL of streptavidin-HRP was added. The plate was incubated for 45 minutes with gentle shaking. After the solution was discarded and the plate washed, 100 μL of TMB substrate was added and incubated for 30 minutes. Once the stop solution was added, the plate was read at an absorbance of 450 nm, and an assay-specific standard curve was used to obtain the protein concentrations. The plasma ACE2 and DPP-IV concentrations were reported as ng/mL and pg/mL, respectively.

## Primary and Secondary 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 the identification of differentially expressed genes between individuals with and without COVID-19 stratified by diabetes status. Secondary endpoints include differences in inflammatory mediator expression, circulating ACE2 circulating DPPIV, and clinical outcomes such as death, length of hospital stay, and WHO-OSCI score.

## Statistical Analysis

Statistical analysis and data visualization were performed using *R* 4.3.1 software.18 All data are presented with summary statistics. Categorical variables are represented as proportions, and continuous data are represented by means and standard deviations or standard errors. Differences in baseline characteristics were analyzed utilizing a t-test or analysis of variance (ANOVA) for continuous data and chi-squared test for categorical data. RNA-seq data were analyzed with the DESeq2 R package based on the negative binomial distribution for differential gene expression analysis.19

# Results

The study included 182 hospitalized adult patients with an admitting diagnosis of COVID-19 (n=110) and control subjects admitted for any other reason (n=72). All available remnant blood samples were obtained within 48 hours of hospital presentation. **Table 1** summarizes patients’ baseline characteristics, including demographics and comorbidities, stratified by COVID-19 and diabetes mellitus (DM) diagnosis. Overall, individuals with DM, regardless of COVID-19 status, had higher comorbidity, as described by the Charlson Deyo Index. Available clinical laboratory values are summarized in **Table 2**. A significant proportion of laboratory values were missing for the no COVID-19 group, likely due to the patient diagnosis and the necessity for a specific laboratory order. Individuals with COVID-19 were more likely to be on remdesivir, tocilizumab, and corticosteroids (**Table 3**). In terms of background diabetes therapeutics, all individuals with DM, regardless of COVID-19 status, received similar medications. Individuals with DM were more likely to receive anticoagulants, and those with COVID-19 were more likely to be on full-dose anticoagulation (81.8% versus 72.2%).

## 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. Of these, 19,909 were protein-coding genes. Note that 15 genes had two variants each. For these genes, the variant counts were added up within each sample.

Genes with a small number of hits were filtered out. After examining the number of genes remaining after filtering using varying minimum numbers 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 ten hits in at least 10 out of 92 samples were used in the analysis.

Next, weidentified 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 4**. **Figure 2** shows the total number of hits for each of the 18 genes in each sample.

One of the most striking differences found in this part of the analysis was the overexpression of the Interferon-Alpha Inducible Protein 27 (IFI27) coding gene, with more than a a 2-fold difference (Ratio = 2.37, SEM = 0.49). In this study, most patients who had gene sequencing data and died in hospital had their IFI27 expression levels elevated compared to those discharged alive (**Figure 3, left panel**). No apparent patterns were observed for critical care as an outcome (**Figure 3, right panel**).

Next, DM's association with gene expression 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 X).

Out of all genes found to be differentially expressed in COVID/non-COVID and DM/non-DM patients, there were five 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 4**.

## Inflammatory signature analysis

Cytokines and chemokines were measured using a multiplex ELISA assay. In total, 21 plasma protein concentrations were measured in 54 COVID and 68 non-COVID patients (**Table 5, Figure 5**). IFN alpha and IL-10 were increased in individuals with COVID-19 without DM versus those without either disease. IL-4, IL-5, and IP-10 in all individuals with COVID versus those without infection, regardless of DM status. There was a numerical increase in TNF alpha and IL-6 in individuals with COVID and DM versus those without either disease; however, the difference failed to reach significance.

## DPP-IV and ACE2 signature analysis

Plasma ACE2 and DPP-IV were measured using ELISA.

23.1 and 1,221.254.9, respectivelyWilcoxon test In patients with COVID-19, **those with DM had lower DPP-IV** concentrations compared to those without DM but the difference was not statistically significant (282.3 ± 44.1 pg/mL and 340.8 ± 27.4 pg/mL, respectively;Wilcoxon test p-value = 0.071).

Plasma ACE2 was not statistically significantly different in individuals with COVID-19 versus those without (8.6± 2.5 pg/mL and 3.3±0.9 pg/mL, respectively; Wilcoxon test p=0.411). In patients with COVID-19, those with DM had higher plasma ACE2 concentrations versus those without DM but the difference was not statistically significant (12.5 ± 6.3 pg/mL and 6.6 ± 1.9 pg/mL, respectively;Wilcoxon test p-value = 0.537).

## Clinical outcomes

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

There was no significant association between DM and COVID-19 patients’ in-hospital death rate (**Table 6**), with 6 DM (17.6%) and six non-DM (7.9%) COVID-19 patients dying in hospital (Chi-square test p-value = 0.236). Similarly, obesity and BMI were not 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 the 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). 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 the World Health Organization Original Scale for Clinical Improvement (WHO OSCI) scale (**Table 7**). 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 a WHO OSCI score of 5 or higher. Notable, 5 out of the 13 COVID-19 patients admitted to the ICU had a score of 5 and another 8 score of 6. At the same time, 64 out of 97 non-ICU patients (66.0%) scored 5 or above at admission. Additionally, all 12 COVID-19 patients who died in the hospital had WHO OSCI scores of 5 or 6 at admission, and their scores did not decrease until their death except for a single patient whose score declined from 5 to 4 on Day 3, just before death (Table 2).

On average, COVID-19 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 the 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 the hospital, the LOS were 11.0+/-3.8 and 12.8+/-2.9 for non-COVID vs. COVID patients, respectively.

# Discussion

Our study compares gene expression, protein expression, and clinical outcomes in patients hospitalized with COVID-19 stratified by diabetes. First, we evaluated the differential expression of several genes between COVID and non-COVID groups. The relevance of these gene pathways in the pathophysiology of COVID-19 has been documented in the literature or is biologically plausible. Several gene pathways we identified as differentially expressed in this study contribute to disease progression, such as AXL, BAMBI, CLEC6A, IFI27, Krt8, Nectin-2, PRKG1, and PDE2A, as previously reported.21-24 We provide a summary of the potential role of these genes in COVID-19 below. The altered regulation of several other genes was also identified, and their role in COVID-19 progression remains poorly elucidated. Further studies are required to understand their role in the pathogenesis of disease.

AXL functions as a tyrosine receptor kinase within the TAM subfamily of receptor tyrosine kinases and functions to control mechanisms of inflammation and coagulation. Like other TAM receptors (Tyro3 and Mer), AXL has important effects on hemostasis and inflammation.25 TAM subfamily of receptor tyrosine kinases, when activated, have also been demonstrated to reduce the production of cytokines, including type I IFNs, IL-6, and TNF, following activation of various TLRs, including TLR-3, 4, and 9.26 TLR-9 is associated with cellular defense against viral infections and is hypothesized to function similarly against COVID-19; thus, TAM activation may downregulate important cytokine functions in the immune and inflammasome response.27 Our study demonstrated a differential increase in AXL expression, which may stunt immune responses to COVID-19 and increase the risk of disease complications. In addition to AXLs role in cytokine production, previous research suggests that AXL may play a role in the entry of SARS-CoV-2 virus into human cells along with ACE2, especially given the elevated expression of AXL in comparison to ACE2 in human pulmonary and bronchial tissue.28 Increased expression of AXL thus may predispose specific patients to be more susceptible to COVID-19 infection.

Another potential mechanism of AXL in the pathogenesis of SARS-CoV-2 is described by its role in platelet activation. Mouse models have demonstrated that the binding of growth arrest-specific gene 6 (Gas6) to AXL receptors contributes to platelet thrombus formation. Similarly, the inhibition of such an interaction inhibits platelet aggregation and degranulation.29 Thrombosis is commonly seen in SARS-CoV-2, and several mechanisms, including inflammation and spike protein-ACE2 binding, have been cited as platelet activation pathways.30 Although thrombosis outcomes were not assessed in the patients of this study, the increased AXL expression and activation may similarly contribute towards the formation of platelet-derived thrombosis. Understanding the role of AXL may improve the selection of anticoagulant strategies in this patient population. For example, warfarin blocks Gas6-mediated AXL activation, while direct oral antithrombotics and heparins do not.31,32 Nonetheless, full-dose anticoagulation in hospitalized patients with COVID-19 who are not critically ill is recommended by several treatment guidelines without specific recommendations for a particular agent.33 In critically ill patients, standard prophylaxis is recommended unless the patient’s presentation is consistent with thrombosis.

BAMBI, also known as BMP and activin membrane-bound inhibitor, has been demonstrated to modulate the expression of ACE2 at the mRNA level.24 When upregulated in cells, BAMBI increases the proportion of COVID-19-infected cells.24 SARS-CoV-2 viral entry into human cells has been observed by binding the spike protein to the ACE2, promoting attachment and fusion. SARS-CoV-2 has a significantly higher affinity for ACE2 than SARS-CoV, contributing towards the greater degree of pathogenicity of the newer disease.34 Increased expression of BAMBI in COVID-19 patients may indicate underlying susceptibility to viral invasion and infection. BAMBI is also highly expressed in platelets and endothelial cells and has a role in thrombus formation.35

CLEC6A, also known as dectin-2, is a member of the C-type lectins typically expressed on macrophages and dendritic cells as part of C-type lectin receptors (CLRs). The activation of these receptors is responsible for a myriad of cellular functions, such as cell adhesion, stimulation of endocytosis, tissue repair, and the activation of platelets in the natural immune system.36 Activating CLRs via CLEC6A (and other pathways) stimulated the recruitment of tyrosine kinases and beta cell lymphoid tissue 10 to form complexes triggering the NF‐kB and MAPKs pathways.37 Although the role of CLEC6A is not well elucidated in COVID-19, some data suggest its relevance in the pathogenesis of MERS-CoV, a very closely related virus. In MERS-CoV, the increased activation of CLRs has been shown to contribute towards a more robust immune response and promote viral recognition, triggering a proinflammatory response.38 It is possible that CLEC6A plays a similar role in COVID-19 and may contribute to the “cytokine storm” that is often cited as the catalyst for COVID-19 mortality.

The protein coded by IFI27 was previously shown to be associated with other viral infections, including Hepatitis C, respiratory syncytial virus (RSV) infection, and Enterovirus 71 (EV71) hand, foot, and mouth disease. IFI27 has also been proposed as a biomarker to differentiate between influenza and bacterial respiratory infection, although its ability to distinguish between different viruses is limited.39 More recently, IFI27 has been proposed as a biomarker for an early prediction of COVID-19 outcomes.23 IFI27 counteracts innate immune responses and has a positive effect on SARS-CoV-2 replication.40 Therefore, mechanistic evidence suggests that elevated IFI27 leads to elevated SARS-CoV-2 viral load. While there is conflicting evidence on viral load and COVID-19 outcomes, older individuals with higher viral loads had worse outcomes, and overall transmissibility increases as viral load increases.41

KRT8 is a gene expressed by transitional alveolar epithelial cells during lung injury. Type 1 and type 2 alveolar epithelial cells constitute the functioning lung parenchyma. Following an injury event, the lung will undergo a recovery process that involves the proliferation of type 2 alveolar epithelial cells, which will then undergo a transitional state and fully differentiate into type 1 alveolar epithelial cells. This transitional state is characterized by the expression of specific gene signatures, including Krt8.42 Several studies have shown that Krt8+ transitional cells are abundant in patients with COVID-19. In lethal cases, however, it has been shown that the increase in Krt8+ is not matched by an increase of type 1 alveolar epithelial cells, indicating a disrupting differentiation process and suggesting that regenerative processes are impaired in the COVID-19 disease state.42,43 Chronically, Krt8 may also be implicated in the development of fibrotic patterns following COVID-19 infection. In bleomycin-induced models of pulmonary fibrosis, Krt8+ cells showed increased expression of pro-fibrotic proteins such as Areg and Hbegf and the presence of myofibroblasts.44 Examining gene profiles in non-resolvable COVID-19 revealed increases in fibrotic gene and Krt8 expression similar to those in idiopathic pulmonary fibrosis.45 Severe COVID-19 has been characterized by the development of fibrosis with increased collagen deposition, supporting the proposed fibrotic pathological process.46,47

NECTIN-2 is a member of the nectin family involved in cellular adhesion molecules (CAMs), which regulate important cell-cell interactions. One of these interactions is with the DNAX accessory molecule 1 (DNAM-1), which mediates the activation of natural killer cells and cytotoxic T cells.48 A study investigating the expression of natural killer cell ligands and receptors in COVID-19 found an increased proportion of activated natural killer cells in moderate to severe COVID-19. Paradoxically, however, there was a decrease in the amount of the activating DNAM-1 receptor despite an increase in nectin-2 expression.49 This response is hypothesized to be a stress-induced down-regulation in which high-stress environments promote receptor endocytosis and subsequent lysosome degradation. The loss of DNAM-1 receptors eventually impairs the function of natural killer cells.50 Thus, the high activity of nectin-2 in patients with COVID-19 is primarily a response to the initial infection, but overactivity may ultimately stunt long-term natural killer cell effectiveness.

PRKG1, also known as cGMP-dependent protein kinase 1, phorphoryles many targets, regulating functions such as platelet activation and adhesion and cardiomyocyte cGMP.51,52 Within PRKG1, however, is a ELDKY gene motif that demonstrates strong molecular mimicry to the COVID-19 spike protein.53 ELDKY has been seen to elicit antibody responses following COVID-19 immunization with the spike protein mRNA vaccine, and increased antibody response to ELDKY has been observed in patients with severe COVID-19.54,55 It is hypothesized that the cross-reactivity of the ELDKY motif and the spike protein may be responsible for antibody-mediated effects on both platelet and cardiac function seen in COVID-19.30,53 Another study examining genomic data of COVID-19 patients found that PRKG1 alleles are also linked with increased mortality in the middle-aged European-American population (ages 45-54). However, the definitive mechanism of this risk factor remains unknown.56

Phosphodiesterases (PDEs) play important roles in hydrolyzing and inactivating cAMP and cGMP in cellular processes. PDE2A, however, is stimulated explicitly by cGMP to hydrolyze cAMP preferentially.57 These intracellular cyclic nucleotides have been known to play a role in maintaining the endothelial cell barrier.58 Although little data specifically connecting PDE2A to COVID-19 exists, this gene has been previously implicated in the development of lung injury. In mouse studies, PDE2A has downregulated lung nitric oxide synthase (NOS) in early-stage injury, thus promoting alveolar inflammation and lung injury.59 In late-stage injury, however, PDE2A inhibits macrophage NOS expression, which has been suggested to promote lung injury resolution following initial insult.57 The mechanism of this pathological process is hypothesized to involve pulmonary endothelial barrier dysfunction caused by decreases in cAMP catalyzed by increased PDE2A expression.60 A separate study evaluating the effects of tumor necrosis factor-α (TNF-α) found an up-regulation in PDE2A downstream and increased membrane permeability. PDE2 inhibition in mice lungs reduced the wet-to-dry ratio and albumin movement, demonstrating minimized fluid translocation.61 PDE2A’s role in the development of lung injury through disrupted endothelial membrane barriers likely reflects the upregulation of this gene in COVID-19 patients and the increased risk for pulmonary complications such as acute respiratory distress syndrome (ARDS).

In addition to gene expression, we measured a panel of cytokines, chemokines, circulating ACE2, and circulating DPP-IV. While we did not find significant differences in TNF and IL-6 between groups, individuals with COVID-19 and DM did have numerically higher values than those without either disease. Previous literature has highlighted elevated cytokines and chemokines leading to a “cytokine storm” in individuals with severe COVID-19.62 Notably, nearly 80% of patients in the current study received dexamethasone, while others received alternative corticosteroids. These drugs are frequently administered at the earliest sign of severe illness to prevent cytokine storm.63 As such, drug therapy may have influenced the relatively small changes in cytokines and chemokines. In addition, individuals with DM were more likely to receive tocilizumab, an IL-6-directed monoclonal antibody.

Serum ACE2 is associated with more severe COVID-19 infection.64,65 Similarly, circulating DPP-IV has been reported to be lower in individuals with severe COVID-19.66,67 Our findings affirm these previous observations.

Finally, clinical outcomes were assessed, and we found an increased mortality in patients with COVID-19 and concomitant diabetes versus COVID-19 alone (17.6% vs 7.9%); however, the difference did not reach statistical significance. The study was not designed to evaluate clinical outcomes and likely had insufficient power to detect significant differences.

The strengths of this study include the sample size and the quality of sample collection. Furthermore, the study utilized whole blood, which likely reflects a more accurate representation of gene expression relative to samples from other sources, such as nasal swabs. In addition, stratified analysis by individuals with and without diabetes allowed focus on a high-risk population. Limitations include using leftover clinical specimens, which may have been available for only some subjects in the study. Nonetheless, this strategy allowed for the performance of the study without placing unnecessary burdens on patients for additional blood collection. The retrospective nature of data collection limited the clinical laboratory values available for each subject. Regardless of these limitations, the study provides important insight into the altered gene expression patterns in individuals with COVID-19 with and without diabetes mellitus.

Conclusions

Gene expression is altered in individuals with and without DM who have COVID-19. Many differentially expressed genes are involved in the disease process and represent potential drug targets.

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| --- | --- | --- | --- | --- | --- | --- | --- |
| Table 1. Subject demographic and clinical characteristics stratified by the presence and absence of diabetes and COVID-19. | | | | | | | |
|  | **No Covid** | | **COVID** | | **Overall** | | **p-Value\*** |
| No DM  **(N=39)** | Any DM  **(N=33)** | No DM  **(N=76)** | Any DM  **(N=34)** | No DM  **(N=115)** | Any DM  **(N=67)** |
| Hospital disposition |  |  |  |  |  |  | 0.993 |
| Inpatient | 34 (87.2%) | 30 (90.9%) | 69 (90.8%) | 31 (91.2%) | 103 (89.6%) | 61 (91.0%) |  |
| ER | 4 (10.3%) | 2 (6.1%) | 5 (6.6%) | 2 (5.9%) | 9 (7.8%) | 4 (6.0%) |  |
| OP | 1 (2.6%) | 1 (3.0%) | 2 (2.6%) | 1 (2.9%) | 3 (2.6%) | 2 (3.0%) |  |
| Age |  |  |  |  |  |  | 0.388 |
| Mean (SD) | 67.5 (18.1) | 64.2 (16.8) | 61.4 (16.4) | 67.0 (13.7) | 63.5 (17.2) | 65.6 (15.2) |  |
| Median [Min, Max] | 74.0 [22.0, 92.0] | 66.0 [28.0, 94.0] | 61.5 [21.0, 93.0] | 68.5 [36.0, 89.0] | 64.0 [21.0, 93.0] | 68.0 [28.0, 94.0] |  |
| Sex |  |  |  |  |  |  | 0.544 |
| Male | 18 (46.2%) | 17 (51.5%) | 45 (59.2%) | 20 (58.8%) | 63 (54.8%) | 37 (55.2%) |  |
| Female | 21 (53.8%) | 16 (48.5%) | 31 (40.8%) | 14 (41.2%) | 52 (45.2%) | 30 (44.8%) |  |
| Race |  |  |  |  |  |  | 0.005 |
| White non-Hispanic | 27 (69.2%) | 18 (54.5%) | 48 (63.2%) | 24 (70.6%) | 75 (65.2%) | 42 (62.7%) |  |
| Black | 3 (7.7%) | 3 (9.1%) | 7 (9.2%) | 1 (2.9%) | 10 (8.7%) | 4 (6.0%) |  |
| Asian | 4 (10.3%) | 2 (6.1%) | 14 (18.4%) | 6 (17.6%) | 18 (15.7%) | 8 (11.9%) |  |
| Hispanic | 3 (7.7%) | 3 (9.1%) | 7 (9.2%) | 3 (8.8%) | 10 (8.7%) | 6 (9.0%) |  |
| Other | 2 (5.1%) | 7 (21.2%) | 0 (0%) | 0 (0%) | 2 (1.7%) | 7 (10.4%) |  |
| Weight (kg) |  |  |  |  |  |  | 0.407 |
| Mean (SD) | 80.5 (24.5) | 85.7 (22.6) | 90.6 (30.8) | 88.8 (22.0) | 87.2 (29.1) | 87.3 (22.2) |  |
| Median [Min, Max] | 73.1 [48.0, 134] | 89.4 [45.4, 141] | 86.0 [42.0, 217] | 84.8 [45.2, 154] | 82.3 [42.0, 217] | 84.9 [45.2, 154] |  |
| BMI |  |  |  |  |  |  | 0.368 |
| Mean (SD) | 27.8 (6.97) | 29.5 (6.49) | 31.5 (9.87) | 31.9 (7.35) | 30.3 (9.13) | 30.7 (6.99) |  |
| Median [Min, Max] | 25.8 [18.9, 47.7] | 29.1 [16.7, 43.9] | 29.0 [20.0, 71.6] | 31.6 [19.5, 50.7] | 28.0 [18.9, 71.6] | 29.8 [16.7, 50.7] |  |
| Obesity | 16 (41.0%) | 15 (45.5%) | 33 (43.4%) | 19 (55.9%) | 49 (42.6%) | 34 (50.7%) | 0.588 |
| Obesity Class |  |  |  |  |  |  | 0.629 |
| I | 11 (28.2%) | 8 (24.2%) | 13 (17.1%) | 11 (32.4%) | 24 (20.9%) | 19 (28.4%) |  |
| II | 3 (7.7%) | 4 (12.1%) | 10 (13.2%) | 5 (14.7%) | 13 (11.3%) | 9 (13.4%) |  |
| III | 2 (5.1%) | 2 (6.1%) | 9 (11.8%) | 3 (8.8%) | 11 (9.6%) | 5 (7.5%) |  |
| Charlson Comorbidity Index |  |  |  |  |  |  | 0.096 |
| Mean (SD) | 5.26 (3.82) | 6.39 (3.79) | 2.22 (1.87) | 4.24 (2.43) | 3.25 (3.04) | 5.30 (3.33) |  |
| Median [Min, Max] | 5.00 [0, 14.0] | 6.00 [1.00, 18.0] | 2.00 [0, 7.00] | 3.50 [1.00, 9.00] | 3.00 [0, 14.0] | 5.00 [1.00, 18.0] |  |
| Myocardial Infarction | 6 (15.4%) | 5 (15.2%) | 0 (0%) | 1 (2.9%) | 6 (5.2%) | 6 (9.0%) | 0.002 |
| Heart Failure | 12 (30.8%) | 6 (18.2%) | 1 (1.3%) | 5 (14.7%) | 13 (11.3%) | 11 (16.4%) | <0.001 |
| Peripheral Vascular Disease | 2 (5.1%) | 2 (6.1%) | 1 (1.3%) | 1 (2.9%) | 3 (2.6%) | 3 (4.5%) | 0.542 |
| Cerebrovascular Disease | 9 (23.1%) | 10 (30.3%) | 2 (2.6%) | 1 (2.9%) | 11 (9.6%) | 11 (16.4%) | <0.001 |
| Dementia | 2 (5.1%) | 2 (6.1%) | 6 (7.9%) | 5 (14.7%) | 8 (7.0%) | 7 (10.4%) | 0.458 |
| COPD | 5 (12.8%) | 6 (18.2%) | 9 (11.8%) | 5 (14.7%) | 14 (12.2%) | 11 (16.4%) | 0.841 |
| Rheum/Connective Tissue Disease | 2 (5.1%) | 1 (3.0%) | 1 (1.3%) | 0 (0%) | 3 (2.6%) | 1 (1.5%) | 0.440 |
| Peptic Ulcer Disease | 2 (5.1%) | 1 (3.0%) | 0 (0%) | 0 (0%) | 2 (1.7%) | 1 (1.5%) | 0.161 |
| Mild Liver Disease | 2 (5.1%) | 0 (0%) | 0 (0%) | 2 (5.9%) | 2 (1.7%) | 2 (3.0%) | 0.104 |
| Hemoplegia | 1 (2.6%) | 3 (9.1%) | 0 (0%) | 0 (0%) | 1 (0.9%) | 3 (4.5%) | 0.020 |
| Renal Disease | 5 (12.8%) | 11 (33.3%) | 4 (5.3%) | 5 (14.7%) | 9 (7.8%) | 16 (23.9%) | 0.002 |
| Cancer\*\*\* | 5 (12.8%) | 4 (12.1%) | 0 (0%) | 0 (0%) | 5 (4.3%) | 4 (6.0%) | <0.001 |
| Moderate-to-severe Liver (?) | 2 (5.1%) | 1 (3.0%) | 0 (0%) | 0 (0%) | 2 (1.7%) | 1 (1.5%) | 0.161 |
| Metastatic Cancer\*\* | 6 (15.4%) | 5 (15.2%) | 0 (0%) | 0 (0%) | 6 (5.2%) | 5 (7.5%) | <0.001 |
| AIDS | 0 (0%) | 1 (3.0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (1.5%) | 0.300 |
| Hypertension | 26 (66.7%) | 26 (78.8%) | 39 (51.3%) | 30 (88.2%) | 65 (56.5%) | 56 (83.6%) | 0.001 |
| Hyperlipidemia | 24 (61.5%) | 23 (69.7%) | 23 (30.3%) | 26 (76.5%) | 47 (40.9%) | 49 (73.1%) | <0.001 |
| Metabolic Syndrome Criteria |  |  |  |  |  |  | <0.001 |
| 0 | 11 (28.2%) | 0 (0%) | 22 (28.9%) | 0 (0%) | 33 (28.7%) | 0 (0%) |  |
| 1 | 6 (15.4%) | 3 (9.1%) | 19 (25.0%) | 2 (5.9%) | 25 (21.7%) | 5 (7.5%) |  |
| 2 | 22 (56.4%) | 10 (30.3%) | 23 (30.3%) | 3 (8.8%) | 45 (39.1%) | 13 (19.4%) |  |
| 3 | 0 (0%) | 19 (57.6%) | 9 (11.8%) | 15 (44.1%) | 9 (7.8%) | 34 (50.7%) |  |
| 4 | 0 (0%) | 1 (3.0%) | 3 (3.9%) | 14 (41.2%) | 3 (2.6%) | 15 (22.4%) |  |

\* p-values are from analysis of variance (ANOVA) tests for continuous data and Chi-squared test for categorical data, with each condition tested across four groups: No COVID/no DM, No COVID/Any DM, COVID/No DM and COVID/Any DM.

\*\*Solid cancers only

\*\*\*Cancer =1 or 2 mean Yes

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Table 2. Available patient clinical laboratory values upon hospital presentation | | | | | | | p-Value |
|  | **No Covid** | | **COVID** | | **Overall** | |
| No DM  **(N=39)** | Any DM  **(N=33)** | No DM  **(N=76)** | Any DM  **(N=34)** | No DM  **(N=115)** | Any DM  **(N=67)** |  |
| HbA1c |  |  |  |  |  |  | <0.001 |
| Mean (SD) | 5.98 (0.911) | 8.85 (2.57) | 6.21 (0.586) | 8.48 (2.48) | 6.08 (0.779) | 8.66 (2.50) |  |
| Median [Min, Max] | 5.60 [4.80, 8.80] | 8.50 [5.40, 13.1] | 6.30 [5.50, 7.50] | 7.80 [6.00, 14.3] | 5.90 [4.80, 8.80] | 7.95 [5.40, 14.3] |  |
| Missing | 22 (56.4%) | 14 (42.4%) | 62 (81.6%) | 15 (44.1%) | 84 (73.0%) | 29 (43.3%) |  |
| Serum vitamin D |  |  |  |  |  |  | 0.154 |
| Mean (SD) | NA (NA) | 21.6 (6.94) | 83.1 (13.5) | 66.2 (73.5) | 83.1 (13.5) | 48.3 (57.5) |  |
| Median [Min, Max] | NA [NA, NA] | 21.6 [16.7, 26.5] | 88.0 [63.5, 93.0] | 36.0 [12.6, 150] | 88.0 [63.5, 93.0] | 26.5 [12.6, 150] |  |
| Missing | 39 (100%) | 31 (93.9%) | 72 (94.7%) | 31 (91.2%) | 111 (96.5%) | 62 (92.5%) |  |
| ALT |  |  |  |  |  |  | 0.969 |
| Mean (SD) | 22.6 (10.6) | 23.3 (31.7) | 46.7 (49.6) | 73.7 (165) | 38.8 (42.5) | 48.9 (121) |  |
| Median [Min, Max] | 20.0 [8.00, 49.0] | 15.0 [7.00, 186] | 31.0 [8.00, 286] | 29.0 [8.00, 915] | 27.0 [8.00, 286] | 22.0 [7.00, 915] |  |
| Missing | 3 (7.7%) | 1 (3.0%) | 3 (3.9%) | 1 (2.9%) | 6 (5.2%) | 2 (3.0%) |  |
| AST |  |  |  |  |  |  | 0.888 |
| Mean (SD) | 26.3 (20.8) | 22.5 (22.0) | 55.0 (53.7) | 96.6 (241) | 45.3 (47.2) | 60.1 (175) |  |
| Median [Min, Max] | 19.5 [11.0, 118] | 16.5 [9.00, 134] | 39.0 [11.0, 325] | 36.0 [14.0, 1350] | 32.5 [11.0, 325] | 26.0 [9.00, 1350] |  |
| Missing | 1 (2.6%) | 1 (3.0%) | 2 (2.6%) | 1 (2.9%) | 3 (2.6%) | 2 (3.0%) |  |
| hs-CRP |  |  |  |  |  |  | 0.257 |
| Mean (SD) | 0.0400 (NA) | NA (NA) | 9.00 (7.31) | 9.14 (7.68) | 8.44 (7.41) | 9.14 (7.68) |  |
| Median [Min, Max] | 0.0400 [0.0400, 0.0400] | NA [NA, NA] | 10.1 [0.118, 23.3] | 7.75 [1.55, 25.0] | 8.23 [0.0400, 23.3] | 7.75 [1.55, 25.0] |  |
| Missing | 38 (97.4%) | 33 (100%) | 61 (80.3%) | 26 (76.5%) | 99 (86.1%) | 59 (88.1%) |  |
| CRP |  |  |  |  |  |  | 0.054 |
| Mean (SD) | 2.45 (2.82) | 14.5 (9.31) | 9.49 (10.2) | 5.15 (3.27) | 7.73 (9.35) | 9.30 (7.88) |  |
| Median [Min, Max] | 1.63 [0.0300, 6.52] | 14.8 [2.79, 25.5] | 5.73 [0.300, 33.1] | 5.53 [0.590, 8.74] | 3.87 [0.0300, 33.1] | 7.54 [0.590, 25.5] |  |
| Missing | 35 (89.7%) | 29 (87.9%) | 64 (84.2%) | 29 (85.3%) | 99 (86.1%) | 58 (86.6%) |  |
| Fibrinogen |  |  |  |  |  |  | 0.001 |
| Mean (SD) | 357 (267) | 986 (NA) | 539 (156) | 539 (113) | 532 (161) | 561 (147) |  |
| Median [Min, Max] | 357 [168, 546] | 986 [986, 986] | 509 [269, 887] | 567 [218, 697] | 509 [168, 887] | 574 [218, 986] |  |
| Missing | 37 (94.9%) | 32 (97.0%) | 29 (38.2%) | 14 (41.2%) | 66 (57.4%) | 46 (68.7%) |  |
| D-Dimer |  |  |  |  |  |  | 0.981 |
| Mean (SD) | 1260 (1380) | 1270 (1160) | 436 (962) | 722 (959) | 529 (1040) | 777 (973) |  |
| Median [Min, Max] | 662 [322, 4350] | 1010 [267, 2540] | 17.6 [0.320, 7290] | 559 [0.210, 4330] | 322 [0.320, 7290] | 577 [0.210, 4330] |  |
| Missing | 31 (79.5%) | 30 (90.9%) | 13 (17.1%) | 7 (20.6%) | 44 (38.3%) | 37 (55.2%) |  |
| ESR |  |  |  |  |  |  | 0.171 |
| Mean (SD) | 25.5 (27.4) | 50.3 (29.4) | 27.7 (21.3) | 43.6 (34.8) | 27.3 (21.8) | 45.8 (32.4) |  |
| Median [Min, Max] | 19.5 [3.00, 60.0] | 44.5 [22.0, 101] | 24.0 [1.00, 80.0] | 31.5 [1.00, 115] | 24.0 [1.00, 80.0] | 37.0 [1.00, 115] |  |
| Missing | 35 (89.7%) | 27 (81.8%) | 57 (75.0%) | 22 (64.7%) | 92 (80.0%) | 49 (73.1%) |  |
| Ferritin |  |  |  |  |  |  | 0.636 |
| Mean (SD) | 214 (234) | 691 (928) | 1020 (1190) | 1490 (2890) | 967 (1170) | 1310 (2600) |  |
| Median [Min, Max] | 169 [27.0, 607] | 283 [16.1, 2880] | 541 [18.7, 5750] | 305 [116, 12700] | 516 [18.7, 5750] | 297 [16.1, 12700] |  |
| Missing | 34 (87.2%) | 24 (72.7%) | 6 (7.9%) | 2 (5.9%) | 40 (34.8%) | 26 (38.8%) |  |
| LDH |  |  |  |  |  |  | 0.139 |
| Mean (SD) | 485 (404) | 298 (100) | 340 (170) | 326 (160) | 348 (187) | 323 (153) |  |
| Median [Min, Max] | 325 [209, 1080] | 268 [219, 438] | 293 [148, 890] | 286 [142, 824] | 293 [148, 1080] | 286 [142, 824] |  |
| Missing | 35 (89.7%) | 29 (87.9%) | 6 (7.9%) | 3 (8.8%) | 41 (35.7%) | 32 (47.8%) |  |
| Lactic Acid |  |  |  |  |  |  | 0.969 |
| Mean (SD) | 1.99 (1.76) | 2.26 (1.77) | 5.86 (21.4) | 9.33 (30.8) | 4.79 (18.2) | 6.30 (23.4) |  |
| Median [Min, Max] | 1.70 [0.700, 8.50] | 1.65 [1.00, 7.50] | 1.30 [0.700, 118] | 1.60 [0.800, 150] | 1.40 [0.700, 118] | 1.60 [0.800, 150] |  |
| Missing | 21 (53.8%) | 15 (45.5%) | 29 (38.2%) | 10 (29.4%) | 50 (43.5%) | 25 (37.3%) |  |
| Procalcitonin |  |  |  |  |  |  | 0.254 |
| Mean (SD) | 0.944 (2.00) | 3.04 (5.76) | 0.932 (4.02) | 0.430 (0.953) | 0.933 (3.84) | 1.25 (3.46) |  |
| Median [Min, Max] | 0.157 [0.0340, 5.02] | 0.474 [0.0510, 18.9] | 0.130 [0.0400, 27.7] | 0.160 [0.0300, 4.65] | 0.130 [0.0340, 27.7] | 0.190 [0.0300, 18.9] |  |
| Missing | 33 (84.6%) | 21 (63.6%) | 27 (35.5%) | 8 (23.5%) | 60 (52.2%) | 29 (43.3%) |  |
| Plasma glucose |  |  |  |  |  |  | <0.001 |
| Mean (SD) | 122 (34.9) | 226 (107) | 118 (26.1) | 204 (96.2) | 120 (29.3) | 215 (101) |  |
| Median [Min, Max] | 110 [82.0, 215] | 198 [97.0, 515] | 112 [84.0, 257] | 173 [111, 498] | 112 [82.0, 257] | 189 [97.0, 515] |  |
| Missing | 0 (0%) | 1 (3.0%) | 1 (1.3%) | 1 (2.9%) | 1 (0.9%) | 2 (3.0%) |  |
| Serum creatinine |  |  |  |  |  |  | 0.866 |
| Mean (SD) | 1.15 (0.892) | 1.41 (0.945) | 2.39 (9.78) | 1.96 (3.22) | 1.96 (7.95) | 1.69 (2.39) |  |
| Median [Min, Max] | 0.843 [0.429, 5.06] | 1.07 [0.484, 3.84] | 0.850 [0.290, 82.0] | 0.860 [0.500, 18.0] | 0.850 [0.290, 82.0] | 0.960 [0.484, 18.0] |  |
| Missing | 0 (0%) | 1 (3.0%) | 1 (1.3%) | 1 (2.9%) | 1 (0.9%) | 2 (3.0%) |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Table 3. Comparison of medication usage between patient with and without COVID-19 and diabetes | | | | | | | |
|  | **No Covid** | | **COVID** | | **Overall** | | p-Value |
| No DM  **(N=39)** | Any DM  **(N=33)** | No DM  **(N=76)** | Any DM  **(N=34)** | No DM  **(N=115)** | Any DM  **(N=67)** |
| Tocilizumab | 0 (0%) | 0 (0%) | 6 (7.9%) | 6 (17.6%) | 6 (5.2%) | 6 (9.0%) | 0.007 |
| Remdesivir | 0 (0%) | 0 (0%) | 55 (72.4%) | 26 (76.5%) | 55 (47.8%) | 26 (38.8%) | <0.001 |
| Dexamethasone | 6 (15.4%) | 4 (12.1%) | 58 (76.3%) | 27 (79.4%) | 64 (55.7%) | 31 (46.3%) | <0.001 |
| Methylprednisolone | 5 (12.8%) | 3 (9.1%) | 3 (3.9%) | 2 (5.9%) | 8 (7.0%) | 5 (7.5%) | 0.343 |
| Prednisone | 8 (20.5%) | 1 (3.0%) | 1 (1.3%) | 0 (0%) | 9 (7.8%) | 1 (1.5%) | <0.001 |
| Hydrocortisone | 0 (0%) | 0 (0%) | 1 (1.3%) | 3 (8.8%) | 1 (0.9%) | 3 (4.5%) | 0.032 |
| Azithromycin | 3 (7.7%) | 1 (3.0%) | 5 (6.6%) | 0 (0%) | 8 (7.0%) | 1 (1.5%) | 0.379 |
| Hydroxychloroquine | 1 (2.6%) | 1 (3.0%) | 7 (9.2%) | 1 (2.9%) | 8 (7.0%) | 2 (3.0%) | 0.324 |
| ACEi | 2 (5.1%) | 8 (24.2%) | 6 (7.9%) | 7 (20.6%) | 8 (7.0%) | 15 (22.4%) | 0.023 |
| ARBs | 7 (17.9%) | 6 (18.2%) | 9 (11.8%) | 5 (14.7%) | 16 (13.9%) | 11 (16.4%) | 0.77 |
| ARNI | 2 (5.1%) | 0 (0%) | 0 (0%) | 2 (5.9%) | 2 (1.7%) | 2 (3.0%) | 0.104 |
| Insulin | 11 (28.2%) | 30 (90.9%) | 12 (15.8%) | 28 (82.4%) | 23 (20.0%) | 58 (86.6%) | <0.001 |
| Metformin | 0 (0%) | 5 (15.2%) | 1 (1.3%) | 6 (17.6%) | 1 (0.9%) | 11 (16.4%) | 0.001 |
| Glimepiride | 1 (2.6%) | 3 (9.1%) | 0 (0%) | 3 (8.8%) | 1 (0.9%) | 6 (9.0%) | 0.047 |
| Glipizide | 0 (0%) | 2 (6.1%) | 0 (0%) | 3 (8.8%) | 0 (0%) | 5 (7.5%) | 0.026 |
| Sitagliptin | 0 (0%) | 3 (9.1%) | 0 (0%) | 4 (11.8%) | 0 (0%) | 7 (10.4%) | 0.005 |
| Full Dose Anticoagulation | 25 (64.1%) | 27 (81.8%) | 61 (80.3%) | 29 (85.3%) | 86 (74.8%) | 56 (83.6%) | 0.114 |
| Prophylactic Anticoagulation | 21 (53.8%) | 16 (48.5%) | 66 (86.8%) | 28 (82.4%) | 87 (75.7%) | 44 (65.7%) | <0.001 |

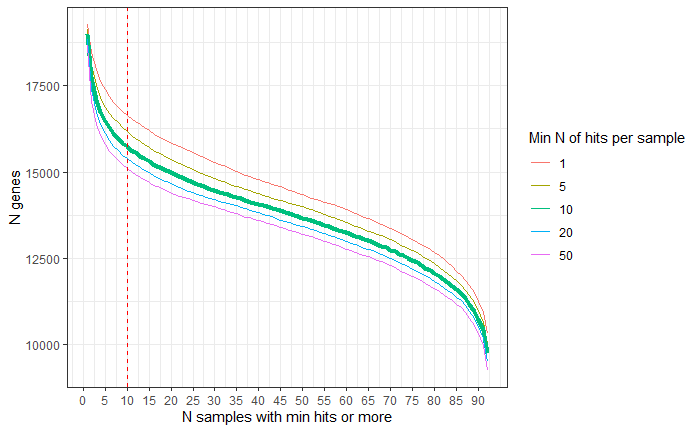
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Table 4. Differentially expressed genes in hospitalized patients with COVID-19 versus hospitalized patient admitted for other acute illnesses | | | | |
| Gene | Median hits  in non-COVID | Median hits  in COVID | Log2 Fold Change (SE) | Adjusted  p-Value |
| AC233755.2 | 0 | 2 | 3.43 (0.79) | 0.016 |
| ALKAL2 | 1 | 9.5 | 1.91 (0.46) | 0.028 |
| AXL | 1 | 8.5 | 2.36 (0.52) | 0.015 |
| BAMBI | 10 | 51 | 1.42 (0.33) | 0.016 |
| BFSP2 | 1 | 9 | 1.81 (0.41) | 0.016 |
| BMP6 | 124 | 421 | 1.10 (0.25) | 0.016 |
| CLEC6A | 73.5 | 17 | -1.46 (0.35) | 0.028 |
| CRYM | 5 | 39 | 1.82 (0.43) | 0.024 |
| GRASP | 112 | 312 | 1.57 (0.33) | 0.010 |
| IFI27 | 310 | 22806 | 2.37 (0.49) | 0.007 |
| KRT8 | 5 | 17 | 1.24 (0.31) | 0.040 |
| LIPN | 294 | 95.5 | -1.36 (0.32) | 0.019 |
| MYZAP | 7.5 | 62.5 | 1.42 (0.35) | 0.040 |
| NECTIN2 | 191 | 499 | 1.18 (0.28) | 0.024 |
| PDE2A | 42.5 | 149.5 | 1.41 (0.29) | 0.007 |
| PRKG1 | 4.5 | 18 | 1.53 (0.38) | 0.041 |
| SMIM24 | 122 | 315 | 1.58 (0.39) | 0.040 |
| TXNDC5 | 25.5 | 207.5 | 1.63 (0.37) | 0.016 |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Table 5. Plasma inflammatory cytokine and chemokine comparison between subjects with and without COVID-19 and diabetes mellitus | | | | | | | |
|  | **No Covid** | | **COVID** | | **Overall** | | **p-Value** |
| No DM  **(N=39)** | Any DM  **(N=33)** | No DM  **(N=76)** | Any DM  **(N=34)** | No DM  **(N=115)** | Any DM  **(N=67)** |
| G-CSF (CSF-3) |  |  |  |  |  |  | 0.203 |
| Mean (SD) | 1.83 (2.65) | 1.10 (1.28) | 2.17 (2.85) | 1.97 (1.77) | 1.99 (2.73) | 1.44 (1.53) |  |
| Median [Min, Max] | 1.04 [0, 11.6] | 0.880 [0, 5.04] | 1.19 [0, 10.5] | 1.83 [0, 6.11] | 1.19 [0, 11.6] | 1.04 [0, 6.11] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| GM-CSF |  |  |  |  |  |  | 0.196 |
| Mean (SD) | 4.38 (15.6) | 1.30 (4.26) | 2.54 (6.67) | 1.12 (2.25) | 3.50 (12.1) | 1.23 (3.59) |  |
| Median [Min, Max] | 0 [0, 77.1] | 0 [0, 19.1] | 0 [0, 25.7] | 0 [0, 6.59] | 0 [0, 77.1] | 0 [0, 19.1] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| IFN alpha |  |  |  |  |  |  | 0.975 |
| Mean (SD) | 0.0754 (0.236) | 0.0648 (0.156) | 1.28 (2.57) | 0.242 (0.565) | 0.652 (1.88) | 0.133 (0.377) |  |
| Median [Min, Max] | 0 [0, 1.30] | 0 [0, 0.743] | 0.390 [0, 11.7] | 0.0650 [0, 2.42] | 0 [0, 11.7] | 0 [0, 2.42] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| IFN gamma |  |  |  |  |  |  | 0.068 |
| Mean (SD) | 3.12 (3.63) | 1.98 (1.92) | 1.92 (1.09) | 2.41 (2.55) | 2.54 (2.77) | 2.15 (2.17) |  |
| Median [Min, Max] | 1.34 [0.290, 14.4] | 1.26 [0.145, 8.51] | 1.89 [0, 4.50] | 1.49 [0.415, 11.6] | 1.71 [0, 14.4] | 1.37 [0.145, 11.6] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| IL-1 beta |  |  |  |  |  |  | 0.373 |
| Mean (SD) | 0.859 (1.58) | 0.544 (0.935) | 1.18 (1.78) | 0.988 (1.05) | 1.01 (1.67) | 0.716 (0.996) |  |
| Median [Min, Max] | 0.418 [0, 6.67] | 0 [0, 3.74] | 0.435 [0, 7.66] | 0.720 [0, 3.73] | 0.435 [0, 7.66] | 0.290 [0, 3.74] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| IL-2 |  |  |  |  |  |  | 0.778 |
| Mean (SD) | 1.13 (4.41) | 1.52 (4.12) | 3.18 (8.47) | 1.90 (2.81) | 2.11 (6.70) | 1.67 (3.64) |  |
| Median [Min, Max] | 0 [0, 26.0] | 0 [0, 19.5] | 0 [0, 35.1] | 0.725 [0, 9.05] | 0 [0, 35.1] | 0 [0, 19.5] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| IL-4 |  |  |  |  |  |  | 0.829 |
| Mean (SD) | 7.61 (9.95) | 7.10 (9.53) | 13.1 (9.06) | 13.5 (10.1) | 10.3 (9.87) | 9.58 (10.1) |  |
| Median [Min, Max] | 4.40 [0, 54.2] | 4.24 [0.415, 48.5] | 9.64 [0, 41.0] | 9.82 [0.365, 34.1] | 7.54 [0, 54.2] | 6.24 [0.365, 48.5] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| IL-5 |  |  |  |  |  |  | 0.604 |
| Mean (SD) | 0.599 (0.850) | 0.962 (1.69) | 2.54 (4.85) | 1.99 (1.79) | 1.53 (3.52) | 1.36 (1.78) |  |
| Median [Min, Max] | 0.113 [0, 2.81] | 0 [0, 6.64] | 0.680 [0, 18.1] | 1.50 [0, 6.88] | 0.460 [0, 18.1] | 0.490 [0, 6.88] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| IL-6 |  |  |  |  |  |  | 0.910 |
| Mean (SD) | 9.27 (14.8) | 11.9 (19.4) | 9.02 (9.85) | 72.4 (245) | 9.15 (12.6) | 35.4 (154) |  |
| Median [Min, Max] | 1.75 [0, 59.9] | 3.61 [0, 86.2] | 5.28 [0, 27.5] | 3.32 [0, 1070] | 2.88 [0, 59.9] | 3.52 [0, 1070] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| IL-8 (CXCL8) |  |  |  |  |  |  | 0.512 |
| Mean (SD) | 3.16 (6.64) | 2.26 (2.24) | 1.33 (1.17) | 4.08 (10.3) | 2.28 (4.92) | 2.96 (6.61) |  |
| Median [Min, Max] | 0.600 [0, 30.9] | 1.47 [0, 9.57] | 1.04 [0, 4.23] | 0.940 [0, 45.8] | 0.930 [0, 30.9] | 1.31 [0, 45.8] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| IL-10 |  |  |  |  |  |  | 0.164 |
| Mean (SD) | 0.204 (0.495) | 0.985 (4.02) | 0.751 (1.32) | 0.768 (2.07) | 0.466 (1.01) | 0.901 (3.37) |  |
| Median [Min, Max] | 0 [0, 2.38] | 0 [0, 22.0] | 0.420 [0, 7.04] | 0.0750 [0, 9.13] | 0 [0, 7.04] | 0 [0, 22.0] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| IL-12p70 |  |  |  |  |  |  | 0.122 |
| Mean (SD) | 1.97 (4.09) | 0.952 (1.41) | 1.32 (1.98) | 1.15 (1.28) | 1.66 (3.25) | 1.03 (1.35) |  |
| Median [Min, Max] | 0.738 [0, 17.5] | 0.233 [0, 5.84] | 0.485 [0, 8.14] | 0.695 [0, 4.69] | 0.730 [0, 17.5] | 0.540 [0, 5.84] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| IL-13 |  |  |  |  |  |  | 0.067 |
| Mean (SD) | 4.33 (10.5) | 1.44 (3.89) | 1.45 (3.06) | 0.538 (0.951) | 2.95 (7.96) | 1.09 (3.11) |  |
| Median [Min, Max] | 0 [0, 48.9] | 0 [0, 19.7] | 0 [0, 12.0] | 0.150 [0, 3.58] | 0 [0, 48.9] | 0 [0, 19.7] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| IL-17A (CTLA-8) |  |  |  |  |  |  | 0.546 |
| Mean (SD) | 0.913 (2.84) | 0.597 (0.916) | 1.23 (2.42) | 0.871 (1.01) | 1.06 (2.63) | 0.703 (0.951) |  |
| Median [Min, Max] | 0.135 [0, 17.3] | 0.190 [0, 3.90] | 0.350 [0, 13.2] | 0.520 [0, 3.51] | 0.345 [0, 17.3] | 0.390 [0, 3.90] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| IL-18 |  |  |  |  |  |  | 0.425 |
| Mean (SD) | 103 (118) | 83.3 (110) | 58.8 (30.8) | 83.1 (122) | 81.7 (89.9) | 83.2 (114) |  |
| Median [Min, Max] | 51.4 [11.9, 533] | 54.8 [14.1, 619] | 55.8 [0, 128] | 42.5 [18.8, 551] | 54.0 [0, 533] | 49.6 [14.1, 619] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| IP-10 (CXCL10) |  |  |  |  |  |  | 0.608 |
| Mean (SD) | 16.4 (20.6) | 18.9 (21.7) | 27.9 (16.5) | 25.6 (19.6) | 21.9 (19.5) | 21.5 (21.0) |  |
| Median [Min, Max] | 8.80 [1.58, 114] | 13.5 [2.80, 93.0] | 26.1 [0, 66.9] | 19.8 [6.72, 85.6] | 15.4 [0, 114] | 14.4 [2.80, 93.0] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| MCP-1 (CCL2) |  |  |  |  |  |  | 0.418 |
| Mean (SD) | 35.6 (30.1) | 29.5 (21.8) | 36.3 (27.0) | 43.8 (46.8) | 36.0 (28.5) | 35.1 (34.0) |  |
| Median [Min, Max] | 25.2 [1.72, 110] | 20.2 [5.00, 106] | 22.0 [0, 90.8] | 33.4 [4.71, 200] | 25.1 [0, 110] | 22.0 [4.71, 200] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| MIP-1 alpha (CCL3) |  |  |  |  |  |  | 0.794 |
| Mean (SD) | 0.912 (1.45) | 0.792 (1.35) | 0.881 (1.79) | 1.19 (3.12) | 0.897 (1.61) | 0.948 (2.19) |  |
| Median [Min, Max] | 0.0225 [0, 4.96] | 0.205 [0, 5.67] | 0.0150 [0, 6.68] | 0.0200 [0, 11.2] | 0.0150 [0, 6.68] | 0.0800 [0, 11.2] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| MIP-1 beta (CCL4) |  |  |  |  |  |  | 0.527 |
| Mean (SD) | 10.8 (16.4) | 8.05 (13.7) | 18.3 (21.5) | 10.6 (16.8) | 14.4 (19.2) | 9.05 (14.9) |  |
| Median [Min, Max] | 3.83 [0, 70.1] | 2.59 [0, 67.1] | 6.56 [0, 69.0] | 2.51 [0, 51.6] | 4.56 [0, 70.1] | 2.51 [0, 67.1] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| TNF alpha |  |  |  |  |  |  | 0.370 |
| Mean (SD) | 1.34 (1.51) | 1.03 (0.945) | 1.40 (1.30) | 2.00 (2.04) | 1.37 (1.40) | 1.40 (1.52) |  |
| Median [Min, Max] | 0.865 [0, 8.60] | 0.680 [0, 3.61] | 0.945 [0, 4.99] | 1.18 [0.470, 9.01] | 0.890 [0, 8.60] | 1.06 [0, 9.01] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| TNF beta |  |  |  |  |  |  | 0.335 |
| Mean (SD) | 0.617 (1.81) | 0.242 (0.729) | 0.900 (2.16) | 0.200 (0.404) | 0.753 (1.98) | 0.225 (0.618) |  |
| Median [Min, Max] | 0 [0, 9.98] | 0 [0, 3.34] | 0 [0, 10.0] | 0 [0, 1.40] | 0 [0, 10.0] | 0 [0, 3.34] |  |
| Missing | 1 (2.6%) | 3 (9.1%) | 41 (53.9%) | 15 (44.1%) | 42 (36.5%) | 18 (26.9%) |  |
| **DPPIV (pg/ml)** |  |  |  |  |  |  | 0.204 |
| Mean (SD) | 1270 (551) | 1160 (337) | 341 (169) | 288 (189) | 812 (621) | 832 (515) |  |
| Median [Min, Max] | 1250 [65.4, 3040] | 1180 [567, 1920] | 284 [127, 736] | 211 [121, 890] | 621 [65.4, 3040] | 863 [121, 1920] |  |
| Missing | 0 (0%) | 0 (0%) | 38 (50.0%) | 14 (41.2%) | 38 (33.0%) | 14 (20.9%) |  |
| **ACE2 (ng/ml)** |  |  |  |  |  |  | 0.928 |
| Mean (SD) | 3.16 (4.67) | 3.58 (7.32) | 6.61 (11.9) | 12.5 (27.6) | 5.28 (9.86) | 8.16 (20.6) |  |
| Median [Min, Max] | 0.918 [0.0160, 17.5] | 0.803 [0.0490, 25.5] | 1.01 [0.0600, 50.3] | 1.18 [0.0210, 104] | 0.918 [0.0160, 50.3] | 0.984 [0.0210, 104] |  |
| Missing | 15 (38.5%) | 15 (45.5%) | 38 (50.0%) | 15 (44.1%) | 53 (46.1%) | 30 (44.8%) |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Table 6. Patient hospital disposition after admission | | | | | | | |
|  | **No Covid** | | **COVID** | | **Overall** | | p-Value |
| No DM  **(N=39)** | Any DM  **(N=33)** | No DM  **(N=76)** | Any DM  **(N=34)** | No DM  **(N=115)** | Any DM  **(N=67)** |
| Critical Care | 14 (35.9%) | 6 (18.2%) | 6 (7.9%) | 7 (20.6%) | 20 (17.4%) | 13 (19.4%) | 0.003 |
| ICU LOS |  |  |  |  |  |  | 0.309 |
| Mean (SD) | 2.90 (7.36) | 1.55 (4.40) | 0.553 (2.50) | 2.91 (8.60) | 1.35 (4.84) | 2.24 (6.84) |  |
| Median [Min, Max] | 0 [0, 43.0] | 0 [0, 20.0] | 0 [0, 19.0] | 0 [0, 47.0] | 0 [0, 43.0] | 0 [0, 47.0] |  |
| LOS |  |  |  |  |  |  | 0.553 |
| Mean (SD) | 8.21 (9.80) | 9.48 (8.61) | 6.70 (7.91) | 8.71 (11.1) | 7.21 (8.58) | 9.09 (9.89) |  |
| Median [Min, Max] | 4.00 [0, 46.0] | 6.00 [0, 34.0] | 4.00 [0, 41.0] | 4.00 [0, 53.0] | 4.00 [0, 46.0] | 6.00 [0, 53.0] |  |
| Expired | 2 (5.1%) | 1 (3.0%) | 6 (7.9%) | 6 (17.6%) | 8 (7.0%) | 7 (10.4%) | 0.129 |
|  | | | | | | |  |

|  |  |  |  |
| --- | --- | --- | --- |
| Table 7. Comparison of disease progression with the WHO Ordinal Scale for Clinical Severity | | | |
|  | COVID/No DM  (N=76) | COVID/Any DM  (N=34) | p-Value |
| WHO-OSCI D1 |  |  | 0.951 |
| 0 | 2 (2.6%) | 1 (2.9%) |  |
| 3 | 5 (6.6%) | 3 (8.8%) |  |
| 4 | 16 (21.1%) | 6 (17.6%) |  |
| 5 | 45 (59.2%) | 19 (55.9%) |  |
| 6 | 8 (10.5%) | 5 (14.7%) |  |
| 7 | 0 (0%) | 0 (0%) |  |
| WHO-OSCI D3 |  |  | 0.680 |
| 0 | 1 (1.3%) | 1 (2.9%) |  |
| 4 | 18 (23.7%) | 7 (20.6%) |  |
| 5 | 31 (40.8%) | 12 (35.3%) |  |
| 6 | 8 (10.5%) | 7 (20.6%) |  |
| 7 | 2 (2.6%) | 1 (2.9%) |  |
| Missing | 16 (21.1%) | 6 (17.6%) |  |
| WHO-OSCI D7 |  |  | 0.315 |
| 0 | 0 (0%) | 1 (2.9%) |  |
| 4 | 4 (5.3%) | 0 (0%) |  |
| 5 | 5 (6.6%) | 2 (5.9%) |  |
| 6 | 9 (11.8%) | 7 (20.6%) |  |
| 7 | 1 (1.3%) | 1 (2.9%) |  |
| Missing | 57 (75.0%) | 23 (67.6%) |  |
| WHO-OSCI D14 |  |  | 0.055 |
| 5 | 3 (3.9%) | 0 (0%) |  |
| 6 | 1 (1.3%) | 4 (11.8%) |  |
| 7 | 2 (2.6%) | 1 (2.9%) |  |
| Missing | 67 (88.2%) | 29 (85.3%) |  |
| WHO-OSCI D21 |  |  | 0.487 |
| 4 | 1 (1.3%) | 0 (0%) |  |
| 5 | 0 (0%) | 0 (0%) |  |
| 6 | 2 (2.6%) | 3 (8.8%) |  |
| 7 | 2 (2.6%) | 1 (2.9%) |  |
| Missing | 71 (93.4%) | 30 (88.2%) |  |
| WHO-OSCI D28 |  |  | 0.525 |
| 4 | 0 (0%) | 1 (2.9%) |  |
| 5 | 1 (1.3%) | 0 (0%) |  |
| 6 | 1 (1.3%) | 1 (2.9%) |  |
| 7 | 1 (1.3%) | 2 (5.9%) |  |
| Missing | 73 (96.1%) | 30 (88.2%) |  |
| WHO-OSCI DISCHARGE |  |  | 0.213 |
| 1 | 50 (65.8%) | 18 (52.9%) |  |
| 2 | 19 (25.0%) | 8 (23.5%) |  |
| 4 | 1 (1.3%) | 2 (5.9%) |  |
| 8 | 6 (7.9%) | 6 (17.6%) |  |

# Figures



**Figure 1.** Number of genes evaluated versus number of hits per gene in the total sample.



**Figure 2.** Differentially expressed genes in individuals hospitalized with and without COVID-19. Patients with diabetes are delineated with light blue coloring.



**Figure 3:** IFI27 expression by COVID and DM, color-coded for in-hospital deaths (left) and critical care (right).



**Figure 4.** Number of hits for specific differentially expressed genes in samples, grouped by COVID and DM diagnoses.

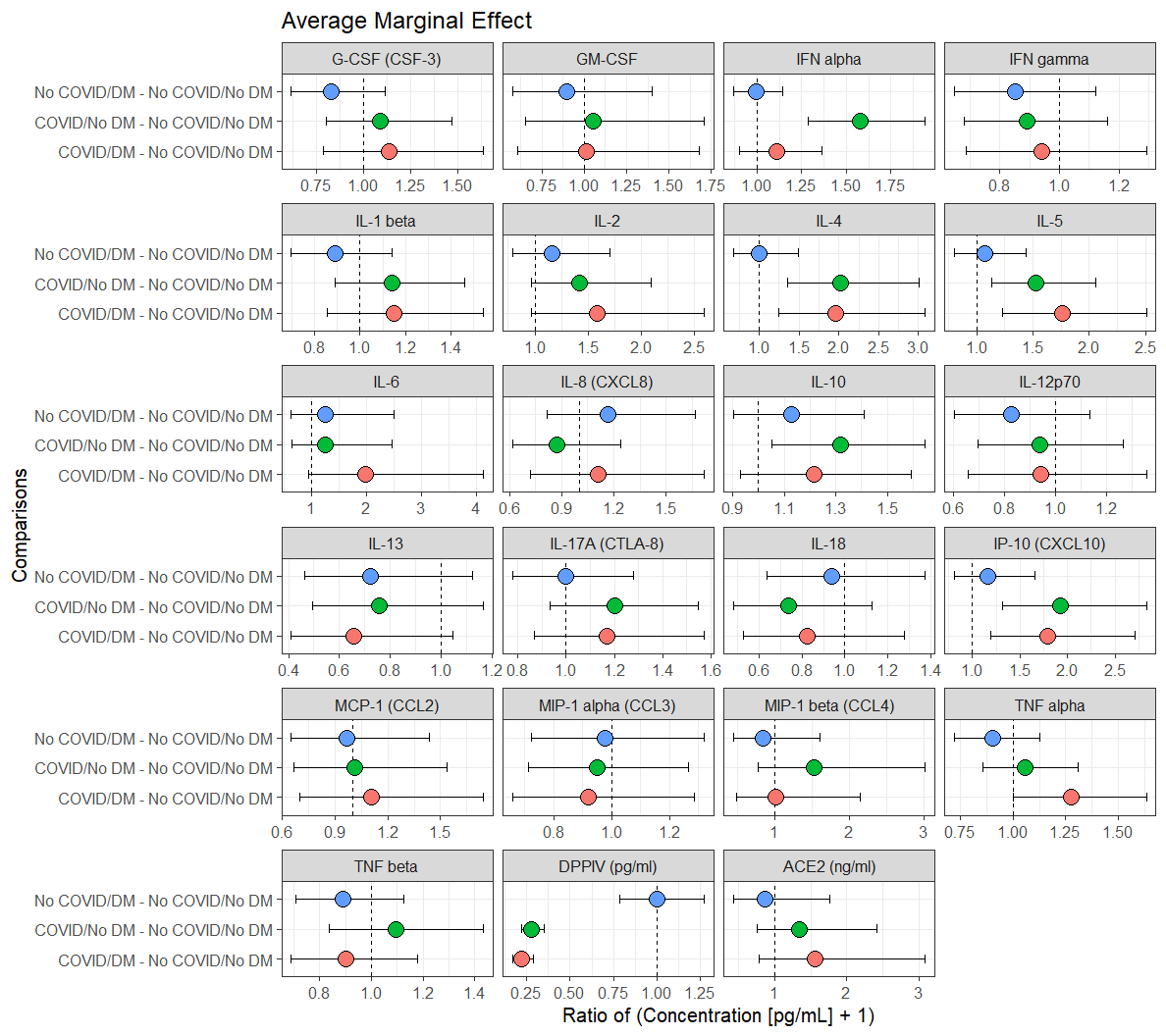


Figure 5. Comparison of select cytokine and chemokine differences between individuals with and without COVID-19 and diabetes mellitus (DM)

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For supplement?



