Differential Cytokine Signatures of SARS-CoV-2 and Influenza Infection Highlight Key Differences in Pathobiology

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Summary: Severe COVID-19 is marked by dysregulated inflammation and is associated with elevated BMI. By comparing cytokines and chemokines in patients with either COVID-19 or influenza, we identified distinct inflammatory pathways and a cytokine mediator of the effect of BMI.

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ABSTRACT

Background: Several inflammatory cytokines are upregulated in severe COVID-19. We compared cytokines in COVID-19 versus influenza in order to define differentiating features of the inflammatory response to these pathogens and their association with severe disease. Because elevated body mass index (BMI) is a known risk factor for severe COVID-19, we examined the relationship of BMI to cytokines associated with severe disease.

Methods:

Thirty-seven cytokines and chemokines were measured in plasma from 135 patients with COVID-19, 57 patients with influenza, and 30 healthy controls. Controlling for BMI, age, and sex, differences in cytokines between groups were determined by linear regression and random forest prediction was utilized to determine the cytokines most important in distinguishing severe COVID-19 and influenza. Mediation analysis was utilized to identify cytokines that mediate the effect of BMI and age on disease severity.

Results:

IL-18, IL-1ß, IL-6, and TNF-α were significantly increased in COVID-19 versus influenza patients while GM-CSF, IFN-γ, IFN-λ1, IL-10, IL-15, and MCP-2 were significantly elevated in the influenza group. In subgroup analysis based on disease severity, IL-18, IL-6, and TNF-α were elevated in severe COVID-19, but not severe influenza. Random forest analysis identified high IL-6 and low IFN-λ1 levels as the most distinct between severe COVID-19 and severe influenza. Finally, IL-1RA was identified as a potential mediator of the effects of BMI on COVID-19 severity.
Conclusions:

These findings point to activation of fundamentally different innate immune pathways in SARS-CoV-2 and influenza infection, and emphasize drivers of severe COVID-19 to focus both mechanistic and therapeutic investigations.

Key Words: COVID-19, Influenza, Cytokines, SARS-CoV-2, Obesity
INTRODUCTION:

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) led to more than 2 million deaths worldwide in 2020 [1]. COVID-19, the disease caused by SARS-CoV-2, spans mild disease to multiorgan failure and death [2,3]. One hallmark of severe disease is immune dysregulation characterized by elevated proinflammatory markers and cytokines [4–9] including interleukin (IL)-6, IL-10, IP-10, IL-1RA, and MCP-1 [8,10–15]. Studies have challenged the uniqueness of the inflammatory cytokine profile of COVID-19 by highlighting similarities to sepsis or acute respiratory distress syndrome due to other causes [16–18].

Influenza is another respiratory viral cause of severe pneumonia and pandemics [19]. The case fatality rate for influenza is lower than that of COVID-19, but many of the cytokines upregulated in COVID-19 are also increased in severe influenza infection [15,20,21]. Thus, it is unclear what unique cytokine upregulation in SARS-CoV-2 infection leads to more severe disease than influenza [22]. Several clinical factors correlate with severe COVID-19, including advanced age and elevated BMI [23,24]. Obese patients are at increased risk for hospitalization and death, particularly at younger ages [25,26]. Obesity leads to chronic inflammation, and elevated BMI is associated with increases in IL-10, IL-6, TNF-α, and IL-1RA [27–29]. Yet, few studies examining these cytokines in COVID-19 have incorporated this in their analyses.

To determine how cytokines produced during COVID-19 and influenza differ and to understand the increased pathogenicity of SARS-CoV-2, we measured thirty-seven cytokines and chemokines in patients hospitalized with either influenza or COVID-19 and compared cytokine levels based on disease severity. We also performed mediation analysis to identify cytokines that mediate the effect of BMI and age on disease severity. We found that severe COVID-19 induces a macrophage proinflammatory cytokine profile, while severe influenza leads to interferon induction. We found that while multiple cytokines mediate the effects of advanced age, IL-1RA is the primary mediator underlying the relationship between obesity and severe COVID-19. These findings highlight
that disparate immune pathways are activated in these potentially life-threatening respiratory viral infections.

METHODS:

Study Participants and Samples

All studies were approved by the Johns Hopkins (JH) Institutional Review Board. Hospitalized patients diagnosed with COVID-19 by positive SARS-CoV-2 RNA testing in the Johns Hopkins Healthcare System were enrolled in a prospective consented protocol to investigate research questions specific to the clinical course of COVID-19 (IRB 00245545). Demographic information, clinical laboratory test results, ICD-10 coded diagnoses (comorbidities), BMI, and other clinical parameters were linked to data for COVID-19 patients in the study. Those who received tocilizumab prior to cytokine measurement were excluded. Participants were categorized by maximum COVID-19 disease severity score based on the WHO severity scale [30]. Those with a score <4 were categorized as having mild/moderate disease and those with a ≥5 were considered severe. Blood was obtained as close to admission as feasible and centrifuged to separate cells from plasma in BSL2+ laboratory conditions.

Healthy control (HC) plasma was obtained from HIV/HCV-antibody seronegative participants enrolled before 2020 in the Baltimore Before and After Acute Study of Hepatitis (BBAASH) study (IRB NA_00046368), an ongoing prospective, community-recruited, observational cohort study of people who inject drugs, as previously described [31].

Plasma from hospitalized patients infected with influenza between 2017 and 2019 was obtained as previously described for comparison to hospitalized COVID-19 patients in this study [31,32](IRB 00091667). Patients hospitalized with influenza requiring no more than nasal cannula and those that required higher levels of oxygen support were classified as having mild/moderate disease or severe disease, respectively, which approximates the WHO COVID-19 severity score [30].
All plasma samples were frozen at -80°C until thawed for cytokine measurement as described below.

**Cytokine measurement**

Plasma cytokines and chemokines (IFN-α2a, IFN-β, IL-18, IL-1RA, IL-23, IFN-λ1, IL-2Ra, MCP-2, GM-CSF, IL-23p40, IL-15, IL-16, IL-17A, IL-1α, IL-5, IL-7, TNF-β, VEGF, Eotaxin, Eotaxin-3, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, TARC, IFN-γ, IL-10, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α) were measured using a custom multiplex kit from Meso Scale Diagnostics (MSD, Rockville, MD) according to the manufacturer’s protocol and data were acquired on a Meso QuickPlex SQ 120. Each sample was measured on first thaw and in duplicate. If an analyte signal was below background, it was set to 0 and if detectable, but below the manufacturer’s lower limit of quantification, to the lower limit of detection.

**Statistical Analysis**

Data were analyzed using the statistical computing software R version 3.6.3 [33]. The cytokine/chemokine (i.e., analyte) signals were first log2 transformed after adding a pseudocount of one. To compare the analytes between patient groups a linear regression analysis, which is equivalent to a two-tailed t-test after adjusting for covariates, was applied. For instance, a linear regression model was fitted for each analyte to test the difference between every pair of patient groups (e.g., COVID-19 vs. influenza) after adjusting for covariates (age, gender, and BMI). P-values of the coefficient for the patient group from the model were obtained and converted to false discovery rates (FDR) using the Benjamini-Hochberg (BH) procedure[34]. An FDR of 0.25 was considered significant. Random forest and mediation analysis were performed as described in the supplemental methods [35–37].
RESULTS:

Cohort Characteristics

A total of 135 SARS-CoV-2 infected participants, 57 influenza infected participants, and 30 HCs were studied. Based on final infection outcome, we categorized influenza and COVID-19 subgroups as mild/moderate or severe, as described in Materials and Methods. Thirteen out of 57 (23%) participants in the influenza cohort and 80 out of 135 (59%) COVID-19 patients had severe disease (Table 1). The influenza and COVID-19 cohorts were not significantly different in gender, non-white race, or BMI. The influenza cohort was younger than the COVID-19 cohort on average (mean age 48.2 versus 56.2 years). The interval between admission and cytokine measurement was not significantly different between the mild/moderate and severe disease subgroups (Supplemental Figure 1a). Principal component analysis (PCA) of the two cohorts revealed clustering of the severe subgroups together in the principal component space, suggesting a similar level of overall inflammation between the two subgroups (Supplemental Figure 1b).

Cytokine Elevations in COVID-19 and Influenza Compared to Healthy Controls

To determine which cytokines and chemokines are upregulated in influenza and COVID-19, we compared cytokines/chemokines in HCs to those with influenza and COVID-19. We selected potentially important markers of disease severity for our custom panel based on prior publications in SARS-CoV-1, SARS-CoV-2, and influenza [8,15,38–40]. We found that GM-CSF, IFN-β, IFN-γ, IFN-λ1, IL-10, IL-15, IL-18, IL-1RA, IL-6, IL-8, IP-10, MCP-1, MCP-2, and TNF-α were significantly increased in both influenza and COVID-19 compared to HCs (Figure 1). In contrast, IL-12p70, IL-13, eotaxin-3, MDC, and TARC were significantly decreased in COVID-19 and influenza compared to HCs. Seven analytes were not significantly elevated in either virus group: IL-1α, IL-23, IL-4, IL-5, MCP-4, MIP-1α, and MIP-1β (Supplemental Figure 1C). IL-1β, IL-2, IL-23p40, IL-2Ra, IL-7, and VEGF were elevated in
COVID-19 exclusively, not influenza, compared to HCs. Conversely, IFN-α2a, IL-16, and IL-17A were significantly elevated solely in the influenza cohort, with no difference between COVID-19 participants and HCs.

**Differences in Cytokines and Chemokines Between Influenza and COVID-19 Reveal a Proinflammatory Macrophage Signature in COVID-19.**

Focusing on analytes elevated in one or both viral cohorts, we found that IL-18, IL-1β, IL-6, IL-7, TNF-α, and VEGF were higher in COVID-19, while GM-CSF, IFN-α2a, IFN-γ, IFN-λ1, IL-10, IL-15, IL-16, IL-17A, and MCP-2 were higher in influenza (Figure 1). (Supplemental Table 1 and 2). The cytokines most elevated in the COVID-19 group are produced primarily by macrophages and characterize macrophage activation syndrome (MAS) [41,42]. Elevated levels of IL-18 and IL-1β suggests prominent inflammasome activation in COVID-19 relative to influenza [43]. Macrophages are major sources of inflammasome cytokines in other viral infections [44–46]. Conversely, IFN-λ1 was nearly 2-fold higher in influenza compared to COVID-19, consistent with a small study demonstrating lower interferon production in COVID-19 versus influenza [47] and an *in vitro* study demonstrating limited induction of IFNs-λ1, -α2a, and -β by SARS-CoV-2 [39]. Though IL-10 was implicated in COVID-19 pathogenesis [15,48,49], IL-10 levels were actually higher in moderate influenza compared to COVID-19.

Plotting the correlation of each analyte with every other analyte in correlation matrices by disease revealed that many of the cytokines/chemokines elevated in influenza relative to COVID-19 strongly correlate, including IL-10, IFN-λ1, MCP-2, and IFN-γ. Similarly, those increased in COVID-19 relative to influenza positively correlate including IL-18, TNF-α, and IL-6 (Supplemental Figure 2A and 2B). A similar pattern emerged after generating a heatmap grouped by disease subgroup
(Supplemental Figure 2C). These findings suggest that distinct inflammatory pathways are activated in these respiratory viral infections.

When we compared elevated analytes statistically by severity subgroups, we found minimal overlap in the cytokines/chemokines that distinguished severe from mild/moderate influenza and those that distinguished severe from mild/moderate COVID-19 (Figure 2A and 2B). IL-1RA, IL-1β, IL-2, IL-7, MCP-1, MCP-2, and VEGF were elevated in both severe diseases compared to their mild/moderate counterpart. Only IFN-β and IFN-λ1 were elevated in severe influenza, but not in severe COVID-19 (Figure 2 and Supplemental Table 1), consistent with low interferon responses in COVID-19. Cytokines elevated in severe COVID-19, but not severe influenza, include GM-CSF, IL-10, IL-15, IL-16, IL-17A, IL-18, IL-2Ra, IL-6, IL-8, and IP-10. Zero influenza and 22 COVID-19 participants received steroids prior to cytokine measurement. When excluding these, IFN-γ and TNF-α were also significantly elevated in severe COVID-19 (Figure 2A and 2B, Supplemental Figure 3 and Supplemental Table 3). Of the cytokines elevated in severe COVID-19, but not severe influenza, only IL-18, IL-6, and TNF-α were also elevated in the whole COVID-19 cohort compared to the whole influenza cohort (Figures 1 and 2). These three cytokines are highly associated with proinflammatory macrophages[50].

When comparing severe COVID-19 to severe influenza directly, only IL-6 was significantly higher in severe COVID-19 whether those who received steroids were included or not (Figure 2 and Supplemental Tables 1 and 3). When excluding steroid recipients, IL-18 narrowly missed our predetermined false discovery rate (FDR) cutoff for significance of 0.25 (FDR = 0.26) (Supplemental Table 1 and 2). Elevated analytes higher in severe influenza compared to severe COVID-19 included IFN-λ1, IFN-α2a, IFN-β, IL-10, and MCP-2 (Figure 2 and Supplemental Table 5).
IL-6 and IFN-λ1 Are the Most Important Cytokines in Distinguishing Severe COVID-19 from Severe Influenza

To further characterize differences in the inflammatory pathways activated, we performed a multivariate analysis based on random forest using all the analytes and basic demographic information to compare severe COVID-19 and severe influenza. IL-6 and IFN-λ1 emerged as the most important factors distinguishing these two diseases in this analysis (Figure 3), with the highest fold changes between the severe subgroups. The importance of IL-6 and IFN-λ1 were confirmed when removing participants treated with steroids and when using a univariate analysis (Supplemental Figure 4 and Supplemental Methods). These findings underscore differences in the innate immune programs activated by these viruses; inflammatory macrophage activation pathways in COVID-19 and interferon pathways in influenza.

IL-1RA is a Potential Mediator of the Effect of BMI on COVID-19 Severity

Previous studies demonstrated an association between BMI and elevation of multiple cytokines increased in severe COVID-19, including IL-6, IL-1β, and IL-1RA [27,29,51,52]. Plotting BMI vs. cytokine concentration demonstrated a positive association between IL-1RA, IL-23p40, MDC, IL-17A, and MCP-2 (Figure 4A and Supplemental Figure 5). With mediation analysis and after adjusting for multiple testing, diabetes, and heart disease, only IL-1RA had a significant mediation effect. While the total effect of BMI on COVID-19 severity was 0.0078 (FDR = 0.13), most of the effect was indirectly through IL-1RA. Indeed, the direct effect of BMI on COVID-19 severity was minimal (0.0007, FDR=0.88) compared to the indirect effect of BMI on severity through IL-1RA (0.0071, FDR < 0.05). This suggests that the effect of increased BMI on the likelihood of severe COVID-19 may be mediated by IL-1RA (Figure 4B, Supplemental Table 6). Cardiovascular disease and diabetes are associated with elevated BMI, but we compared the mild/moderate and severe COVID-19 subgroups while adjusting for these conditions and found no differences in the cytokines that were significant between severe and mild/moderate COVID-19 (Supplemental Table 4). Analysis of the influenza
cohort did not reveal any cytokines/chemokines mediating BMI and disease severity (Supplemental Table 7).

Given that advanced age is a risk factor for severe COVID-19, we also performed mediation analysis using age as the independent variable. Unlike BMI, numerous cytokines are potential mediators of the effect of age on severity including IL-10, IL-1RA, IL-2Ra, and MCP-1 (Supplemental Figure 6 and Supplemental Table 8).

Discussion:

Our analysis of cytokines and chemokines elevated in COVID-19 compared to influenza reveals distinct cytokine profiles of these respiratory diseases. We found that several cytokines previously reported to be elevated in COVID-19 were not different from or were higher in influenza than in COVID-19, including IFN-γ, IL-10, and IL-15 [15]. Some cytokines (GM-CSF, IL-10, IL-15, IL-16, and IL-17A) that were higher in influenza than COVID-19 overall and distinguished severe COVID-19 from moderate COVID-19 did not differ between severe and moderate influenza. Influenza infection generally induces high levels of these cytokines and severe influenza is not marked by additional elevations.

Another study also found that many cytokines were either not different or were significantly less elevated in COVID-19 versus influenza [53]. Consistent with their results, we found that IFN-γ and IL-17A were elevated in influenza. They found significant increases in IL-1RA, IL-2, and MIP-1α in their influenza cohort relative to COVID-19, while we did not. These differences may be due to our adjustment for BMI, our larger cohort size, different platforms used to measure cytokines, or the fact that a higher percentage of their influenza patients had severe disease. They observed a trend toward increased IL-6 in the COVID-19 group compared to influenza and we found high IL-6 to be one of the most distinguishing cytokines in COVID-19. They did not measure IL-18 or IFN-λ1,
important in distinguishing severe COVID-19 and severe influenza in our analysis. While the “cytokine storm” hypothesis was proposed to explain the pathology observed in COVID-19, our study and others demonstrate lower overall cytokine levels in COVID-19 than those observed in other inflammatory diseases [17,18,53,54].

Both influenza and SARS-CoV-2 are respiratory RNA viruses, but our study emphasizes that they induce distinct inflammatory pathways. We found that severe COVID-19 leads to upregulation of cytokines associated with a proinflammatory macrophage phenotype [55] characterized by high levels of IL-6, TNF-α, and IL-18, whereas interferons and cytokines involved in T cell activation (IL-15, IL-16 and IFN-γ) are upregulated in influenza. IL-18 and IL-1β, which is difficult to detect in blood, are released from macrophages upon activation of a component of the innate immune system called the inflammasome [43,45,56]. Emerging evidence suggests inflammasome activation is central to the SARS-CoV-2 pathogenesis and marks severe disease [8,12,57,58]. Our study provides additional evidence that the inflammasome is activated in SARS-CoV-2 infection. High IL-6 and low IFN-λ1 were the most distinct features of severe COVID-19 compared to severe influenza, consistent with results of another study of COVID-19 and influenza patients [47]. We do not know if these cytokine disparities mediate pathology differences, or are merely correlates of other distinct immune responses. While steroids have proven beneficial in later stages of the disease in COVID-19 patients requiring oxygen, early immunosuppression is not beneficial [59]. Given the association we observed with severe COVID-19 and a proinflammatory macrophage phenotype, targeting of the cytokines mediating MAS, singly or in combination, might provide a more specific approach to immune modulation. Both targeted anti-IL-6 therapy and IL-1 antagonists are associated with benefit in some studies. [60–71].

Another strength of our analysis is the focus on BMI as a contributor to severe disease. While this association has been widely described, few analyses of inflammatory cytokines have taken this variable into account [72]. We found that IL-1RA is a potential mediator of the effect of BMI on
COVID-19 disease severity. This novel observation points to a possible mechanism linking BMI to severe COVID-19. IL-1RA is an acute phase reactant produced by adipocytes, macrophages, and the liver in response to inflammatory cytokines and pathogens through pathways that upregulate IL-6 and TNF-α [73,74]. This was an unexpected finding given the anti-inflammatory nature of this cytokine and the role of IL-1RA in pathogenesis warrants additional investigation. Inflammation is an important component of aging [75]. In contrast to IL-1RA being the sole potential cytokine mediator of the effect of BMI on disease severity, we identified numerous potential cytokine mediators of the effect of age on disease severity, highlighting the specificity of our association between IL-1RA, BMI, and COVID-19 severity.

Limitations to our study include that outpatients with milder COVID-19 were not included. However, we would predict that differences between those with and without severe disease in our study would be more significant if milder disease were included. In addition, our study participants with influenza also required hospitalization, making them an appropriate comparator. An additional limitation is that we examined a single timepoint, and that the time from admission to sampling was not identical, but PCA revealed similar inflammatory states. It is possible that dynamic changes in these cytokines during the course of hospitalization would make the patterns more or less distinct from influenza. However, as patients remain hospitalized, complications from critical illness arise that could obfuscate this comparison. Finally, while the influenza cohort was, on average, younger than the COVID-19 cohort, we adjusted for age in our analysis.

This study provides insight into pathways activated by SARS-CoV-2 and influenza, demonstrating that some inflammatory cytokines elevated in COVID-19 likely reflect common pathways activated in respiratory tract inflammation, while others are more specific to COVID-19 pathogenesis. In summary, this study demonstrates activation of a proinflammatory cytokine macrophage pathway and a role for IL-1RA in the effect of BMI on severe COVID-19, highlighting potential therapeutic targets.
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Conflicts of Interest:

None of the authors have any relevant conflicts of interests to disclose.
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| Subject group      | COVID-19 | Influenza | Healthy Controls |
|--------------------|----------|-----------|------------------|
| **Demographics**   |          |           |                  |
| Male N (%)         | 70 (52)  | 26 (46)   | 19 (63)          |
| Female N (%)       | 65 (48)  | 31 (54)   | 11 (37)          |
| Mean age (range)   | 56.2 (20-90) | 48.2 (19-89) | 31.2 (20-45) |
| Mean BMI (range)   | 32.8 (12-70) | 30.2 (18-60) | NA              |
| **Race and Ethnicity** |        |           |                  |
| Race N (%)         |          |           |                  |
| Black              | 62 (46)  | 40 (70)   | 11 (37)          |
| White              | 26 (19)  | 10 (18)   | 19 (63)          |
| Other*             | 38 (28)  | 5 (9)     | 0 (0)            |
| Asian              | 9 (7)    | 2 (3)     | 0 (0)            |
| **Ethnicity**      |          |           |                  |
| Hispanic/Latinx    | N (%)    |           |                  |
| Yes                | 33 (24)  | NA        | 0 (0)            |
| No                 | 102 (76) | NA        | 30 (100)         |
| **Maximum Disease Severity** | N (%)   |           |                  |
| Mild/Moderate      | 55 (41)  | 44 (77)   | NA               |
| Severe             | 80 (59)  | 13 (23)   | NA               |
| **Comorbidities**  | N (%)    |           |                  |
| Diabetes mellitus  | 56 (41)  | NA        | 0 (0)            |
| HIV infection      | 4 (3)    | 7 (12)    | 0 (0)            |

*Most self-identified as Hispanic/Latino.

**Maximum disease severity indicates the most severe COVID-19 disease class for the patient while under observation: Mild/Moderate = no or low flow oxygen required, or high flow oxygen or noninvasive positive-pressure ventilation (NIPPV) required; Severe = patient required intubation or patient died (ventilated or not). NA indicates data not available. P-values were calculated as described in the Supplemental Methods Section.
FIGURE LEGENDS

Figure 1. Cytokines and Chemokines in Influenza and COVID-19 Compared to Healthy Controls.

Differences between the COVID-19 cohort (blue) or influenza cohort (orange) and healthy controls (grey) were determined by two-tailed t-test after adjusting for sex and age. Differences between the COVID-19 cohort and influenza cohort for each analyte were determined by two-tailed t-test after adjusting for sex, age, and BMI. FDR was obtained using the Benjamini-Hochberg procedure.

Statistical significance is indicated by NS, *, **, or *** above the brackets indicating FDR >0.25, <0.25, <0.1, or <0.05 respectively.

Figure 2. Cytokines and Chemokines Elevated in Severe Disease Compared to Mild/Moderate Disease and According to Infection.

A. Differences between disease severity subgroups were determined by two-tailed t-test after adjusting for sex, age, and BMI. Participants who received steroids prior to cytokine measurement were excluded. Differences between the severe COVID-19 cohort (green) and severe influenza cohort (orange) for each analyte were determined by two-tailed t-test after adjusting for sex, age, and BMI. False discovery rate (FDR) was obtained using the Benjamini-Hochberg procedure.

Statistical significance is indicated by NS, *, **, or *** above the brackets indicating FDR >0.25, <0.25, <0.1, or <0.05 respectively.

B. Top: Cytokines/chemokines higher in the COVID-19 cohort compared to influenza (left side), influenza compared to COVID-19 (right side) both COVID-19 and influenza compared to healthy controls, but not significantly different between COVID-19 and influenza (overlap center). Bottom: Cytokines/chemokines elevated in severe COVID-19 relative to mild/mod COVID-19 (left side), those
elevated in severe influenza relative to mild/mod influenza (right side), and those that are elevated in severe forms of both diseases (overlap center).

**Figure 3. Multivariable analysis based on random forest revealed the most important variables in distinguishing severe COVID-19 and severe influenza.**

Feature importance was obtained from the random forest model for predicting severe COVID-19 vs. severe influenza. The color indicates the log₂ fold change of the analyte signal between severe COVID-19 and severe influenza. Red color indicates a higher value in COVID-19 and blue color indicates a higher value in influenza.

**Figure 4. Mediation Analysis of BMI and IL-1RA on COVID-19 Severity**

A. BMI (X axis) was plotted vs. IL-1RA level (Y axis). The yellow line is a linear regression line for the COVID-19 cohort and the blue line is a linear regression line for the influenza cohort. The Pearson’s correlation coefficient between IL-1RA and BMI is 0.34 for the COVID-19 cohort and 0.15 for the influenza cohort.

B. Diagram of the mediation analysis of BMI and IL-1RA on COVID-19 severity while controlling for diabetes and heart disease. The indirect, direct, and total effects were showed in the diagram. Statistical significance is indicated by *, **, or *** representing FDR<0.25, <0.1, or <0.05 respectively.
Figure-2
Figure 3

Severe COVID vs. Severe Flu

Feature importance

- IFN-λ1
- IL-6
- IL-4
- IFN-α2a
- IL-16
- TNF-β
- IFN-γ
- MIP-1α
- IL-8
- MDC
- IL-13
- IL-10
- MCP-2
- IFN-β
- IL-2Ra
- IL-18
- IP-10
- TNF-α

Log2FC

Values:
- 1
- 0
- -1
- -2
Figure 4

A. IL-1RA
Pearson's r: COVID=0.34, Flu=0.15

B. Indirect Effect: 0.0071***

BMI (X)

COVID Severity (Y)

IL-1RA (M)

Direct Effect: 0.0007

Total Effect: 0.0088