Sex-specific transcriptional profiles associate with severe SARS-CoV-2 infection

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BACKGROUND

Males have a higher rate of severe illness and death from SARS-CoV-2 infection[1,2] but the mechanisms of this difference are incompletely understood. Our prior study of hospitalized patients identified differences in the inflammatory clinical parameters in males and females at hospital presentation as modifiers of the sex difference in outcomes[2]. In this study, we sought to explore the immune transcriptional and plasma cytokine features associated with severe disease. We hypothesized that there would be sex-specific immune profiles linked to severe disease and to the impact of dexamethasone.

METHODS

Individuals enrolled in the Clinical Characterization Protocol for Severe Infectious Diseases (CCPSEI) protocol on admission to the Johns Hopkins Hospital were included. Participants had laboratory confirmed SARS-CoV-2 infection and did not have a concurrent diagnosis of HIV, hepatitis C, autoimmune disease or cancer. Male and female are defined as sex assigned at birth. Two cohorts were studied, individuals enrolled between April and July of 2020 (Cohort 1) and between July 2020 and January of 2021 (Cohort 2); all participants in Cohort 2 received dexamethasone. Participants were classified as mild-moderate or severe/death by the WHO scale. All participants had whole blood RNA extracted from cryopreserved Paxgene tubes from the earliest timepoint with subsequent library prep(Takara Bio) and sequencing (NovaSeq). Plasma N antigen level quantification (Simoa, Quanterix)[3]. Differentially expressed genes (DEGs) and pathways, N antigen levels, and cytokine expression were compared between mild-moderate and severe death groups in the full cohort and within sex, all adjusted for age and BMI. A publicly available bulk neutrophil sequencing dataset from COVID-19 patients was used to assess neutrophil phenotype by sex[4].

RESULTS

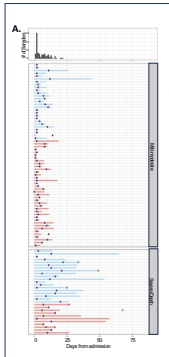


Figure 1. Characteristics of Cohort 1 (A) and Cohort 2 (B). Bar lengths indicate duration of inpatient hospitalization. Purple circles indicate timepoint of sampling. X indicates date of dexamethasone administration. Open diamond indicates time of death where applicable. Bar chart at the top summarizes the frequency of sampling done at each timepoint. Additional demographics are indicated in the Tables below.

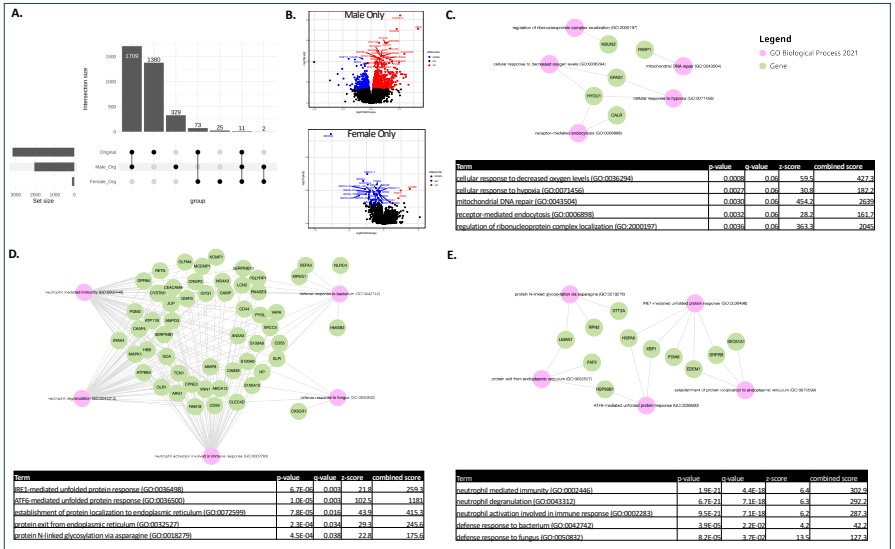
Table 1. Cohort 1 Demographics

	Original Cohort	
	Male	Female
Number (%)	46 (48%)	49 (52%)
Median age [IQR], years	54 [43 - 63]	48 [35 - 64]
Age>65, Number (%)	8 (17%)	10 (20%)
Race/ethnicity, Number (%)		
Black, Non-Hispanic	24 (52%)	23 (47%)
White, Non-Hispanic	5 (11%)	7 (14%)
Other, Non-Hispanic	1 (2.2%)	4 (8.2%)
Hispanic	16 (35%)	15 (31%)
Median BMI [IQR], kg/m2	31 [27 - 38]	32 [27 - 40]
BMI>=35 kg/m2, Number (%)	13 (28%)	18 (37%)
Sample timing, Median [IQR]		
Relative to test positive date, days	4.0 [2.0 - 11]	4.0 [2.0 - 8.0]
Relative to peak disease status, days	1.0 [0 - 4.0]	1.0 [0 - 3.0]
Relative to dexamethasone dose	NA	NA
Outcomes, Number (%)		
Severe/Death	19 (41%)	11 (22%)
Death	3 (6.5%)	3 (6.1%)
Comorbid conditions, Number (%)		
Diabetes	16 (35%)	16 (33%)
Coronary disease	4 (8.7%)	2 (4.1%)
Congestive heart failure	4 (8.7%)	7 (14%)

Table 2. Cohort 2 Demographics

	Dexamethasone Cohort	
	Male	Female
Number (%)	33 (36%)	26 (44%)
Median age [IQR], years	53 [45 - 65]	61.5 [47 - 70]
Age>65, Number (%)	8 (24%)	10 (38%)
Race/ethnicity, Number (%)		
Black, Non-Hispanic	7 (21%)	15 (58%)
White, Non-Hispanic	10 (30%)	7 (27%)
Other, Non-Hispanic	7 (21%)	0 (0%)
Hispanic	9 (27%)	4 (15%)
Median BMI [IQR], kg/m2	31 [27 - 34]	32 [27 - 38]
BMI>=35 kg/m2, Number (%)	8 (24%)	11 (42%)
Sample timing, Median [IQR]		
Relative to test positive date, days	3.2 [2.0 - 4.5]	4.1 [2.2 - 7.4]
Relative to peak disease status, days	1.1 [0.0 - 3.7]	2.5 [0.2 - 4.8]
Relative to dexamethasone dose	0.9 [0.1 - 2.3]	1.3 [0.1 - 3.7]
Outcomes, Number (%)		
Severe/Death	18 (54%)	12 (46%)
Death	2 (6.1%)	2 (7.7%)
Comorbid conditions, Number (%)		
Diabetes	10 (30%)	8 (31%)
Coronary disease	3 (9%)	2 (8%)
Congestive heart failure	4 (12%)	4 (15%)

Severe COVID-19 is associated with a much larger transcriptional shift in whole blood of males versus females and predominantly characterized by enrichment of neutrophil signatures. There is a sex differential transcriptional response to dexamethasone administration. These results suggest that different immune inflammatory pathways are linked to severe COVID-19 and may impact the efficacy of immunomodulatory therapies.



Term	p-value	q-value	z-score	combined score
cellular response to decreased oxygen levels (GO:0036294)	0.0008	0.06	59.5	427.3
cellular response to hypoxia (GO:0071456)	0.0027	0.06	30.8	182.2
mitochondrial DNA repair (GO:0043504)	0.0030	0.06	454.2	2628
receptor-mediated endocytosis (GO:0006898)	0.0032	0.06	28.2	161.7
regulation of ribonucleoprotein complex localization (GO:2000197)	0.0036	0.06	363.3	2045

Term	p-value	q-value	z-score	combined score
IRE1-mediated unfolded protein response (GO:0036498)	6.7E-06	0.003	21.8	259.3
ATF6-mediated unfolded protein response (GO:0036500)	1.0E-05	0.003	102.5	1181
establishment of protein localization to endoplasmic reticulum (GO:0072599)	7.8E-05	0.016	43.9	415.3
protein exit from endoplasmic reticulum (GO:0032527)	2.3E-04	0.036	29.3	245.6
protein N-linked glycosylation via asparagine (GO:0018279)	4.5E-04	0.038	22.8	175.6

Term	p-value	q-value	z-score	combined score
neutrophil mediated immunity (GO:0002446)	1.9E-21	4.4E-18	6.6	302.9
neutrophil degranulation (GO:0043312)	6.7E-21	7.1E-18	6.3	292.2
neutrophil activation involved in immune response (GO:0002283)	9.5E-21	7.1E-18	6.2	287.3
defense response to bacterium (GO:0042742)	3.9E-05	2.2E-02	4.2	42.2
defense response to fungus (GO:0050832)	8.2E-05	3.7E-02	13.5	127.9

Figure 2. Transcriptional patterns in mild-moderate versus severe disease. (A) Upset plot indicating the total number of differentially expressed genes(DEGs) in whole blood between mild-moderate and severe/death individuals in the overall cohort and specifically within males and females. (B) Volcano plots demonstrating DEGs in males and females demonstrated more upregulation of genes in severe disease in males and more downregulation in females. (C) Gene Ontology(GO) pathways enriched by the limited DEGs in severe disease shared in both males and females enrich hypoxia associated pathways. (D) In males with severe disease neutrophil activation and degranulation pathways showed marked enrichment. (E) DEGs in females enriched the unfolded protein response and endoplasmic reticulum pathways.

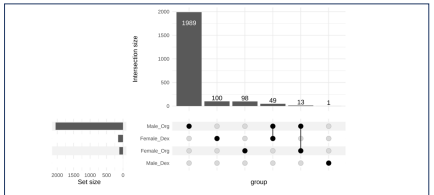


Figure 3. Transcriptional patterns in mild-moderate versus severe disease in Cohort 2. Dexamethasone treatment ablated the transcriptional differences between mild-moderate and severe disease in males and had little quantitative impact on DEGs in females.

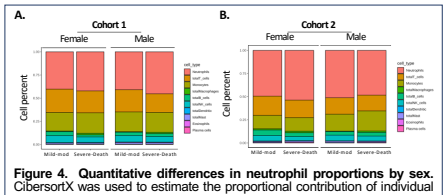


Figure 4. Quantitative differences in neutrophil proportions by sex. CibersortX was used to estimate the proportional contribution of individual immune cell subsets to the whole transcriptional profile. Severe disease is associated with increased neutrophil proportions in both males and females in Cohort 1. Dexamethasone treatment ablates the neutrophil enrichment in males with severe disease, but not in females.

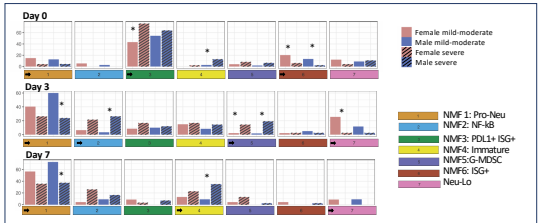


Figure 5. Bulk neutrophil sequencing demonstrates sex-specific enrichment of neutrophil sub-phenotypes. Data from a prior study of COVID-19[4] were accessed from GEO and sex assigned based on expression of Y chromosome genes. Non-negative matrix factorization (NMF) clustering as described in the original publication was used to identify neutrophil sub-phenotypes in each sex at each timepoint. * indicates significant enrichment severe versus mild, arrows indicate phenotypes that were statistically different in the male and female combined analysis in the original publication.

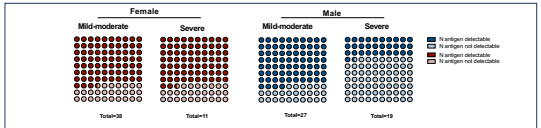


Figure 6. Plasma N antigen detection was less frequent in males with severe disease. Plasma samples from Cohort 1 were tested for SARS-CoV-2 nucleocapsid antigen.

CONCLUSIONS

- Whole blood transcriptional profiles show marked upregulation of neutrophil response genes in males with severe COVID-19.
- Dexamethasone treatment ablates the transcriptional differences of severe disease in males but with minimal impact in females.
- There are quantitative and qualitative differences in neutrophils in severe disease in males versus females.
- Males were less likely to have detectable N antigen levels in severe disease.
- These data suggest that severe COVID-19 is disproportionately driven by inflammation in males.

ADDITIONAL KEY INFORMATION

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