Differentially-expressed immunological genes in mild and severe cases of COVID19

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INTRODUCTION

Coronavirus disease 2019 (COVID19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has varied clinical presentations from mild subclinical to severe disease with high mortality. Our aim was to determine whether examining immune-related gene expression early in the infection could predict progression to severe disease.

HYPOTHESIS

Differential expression of specific genes in the immune response at early stages of the infection can predict the severity to be reached as the clinical presentation progresses.

METHODS

Samples: 120 peripheral blood mononuclear cells (PBMC) samples from the *All Ireland Infectious Diseases* cohort study were chosen, collected between April and July of 2020 from positive patients starting the infection (~5 days) classified as mild (n=31) or moderate/severe (n=31), according to the WHO criteria [1], and 58 negative samples matched for age and gender. The presence of SARS-CoV-2 was confirmed with Real Time RT-PCR.

Age group→	above 60		below 60		Total
Severity↓	F	M	F	M	
Mild	7	7	3	14	31
Moderate/Severe	5	14	7	5	31
Negative Control	6	21	13	18	58
Total	18	42	23	37	120

NanoString gene expression profiling: the expression of 579 immune response related genes was measured with NanoString nCounter Immunology panels, *NS Immunology v2C2328* in the nCounter Analysis System (NanoString, Seattle, USA). The expression analysis was performed with nSolver Analysis Software 4.0.70, with SARS-CoV-2-negative samples as reference, significant changes in expression in the mild and moderate/severe groups were separately assessed.

Statistical analysis: Genes are tested for differential expression in response to the severity compared against negative samples, controlling for sex and age of the patients. For each gene, a single linear regression is fit using all selected covariates to predict expression. The statistical significance of gene expression differences was adjusted with the Benjamini-Yekutieli to reduce the false-positive rate.

RESULTS

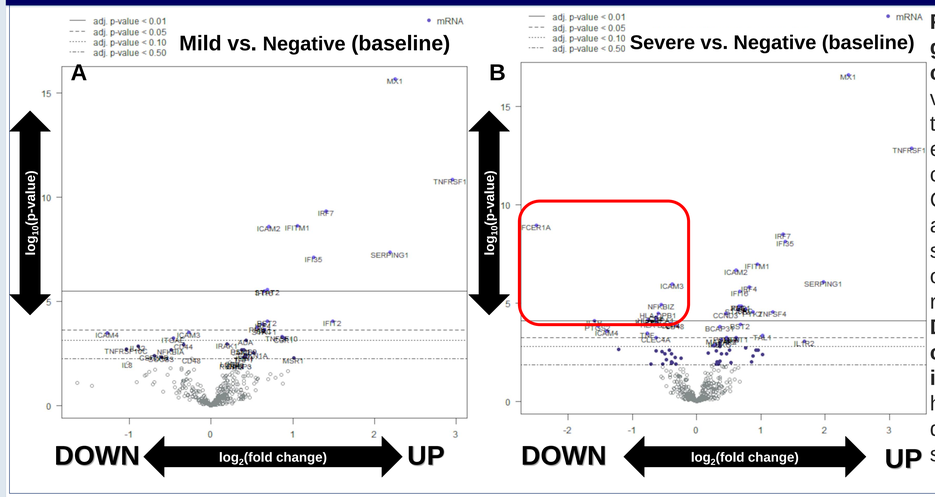
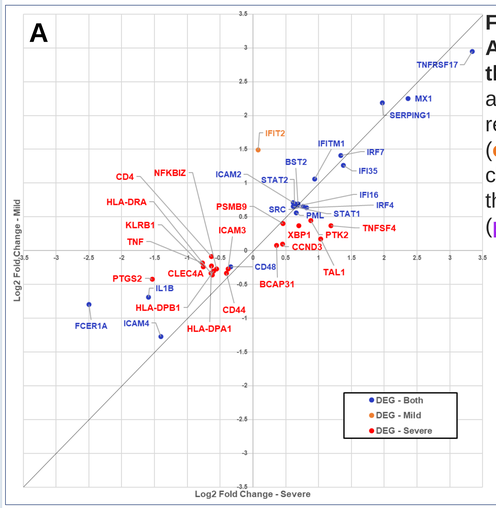


Figure 1. Deregulated genes in COVID19 cases. Horizontal and vertical axes represent the changes in the expression when compared against the COVID-negative samples and the statistical significance of the observed deregulation, respectively. **A) Deregulation in mild cases. B) Deregulation in severe cases.** Red highlight for genes downregulated only in severe cases (p<0.05).



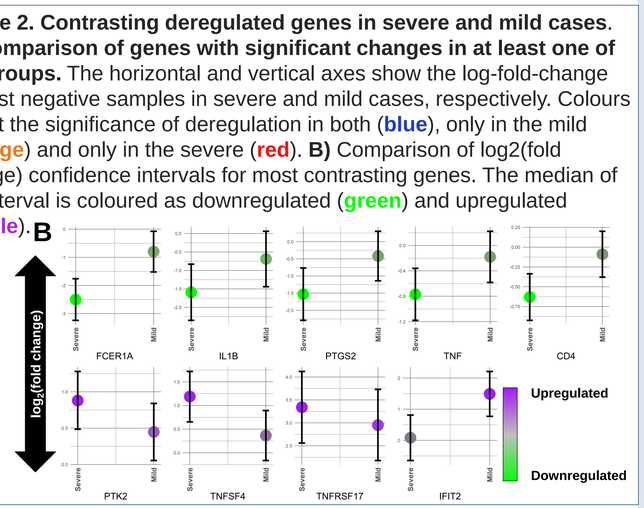
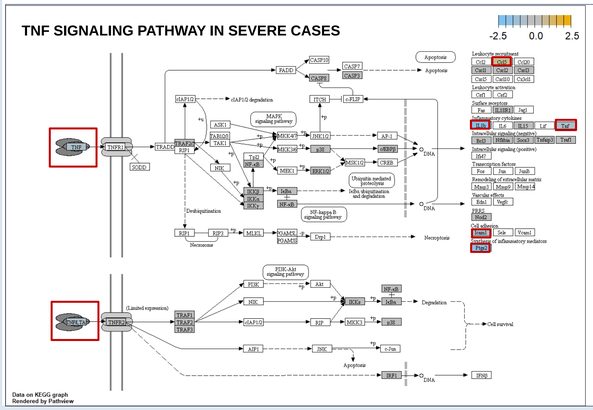
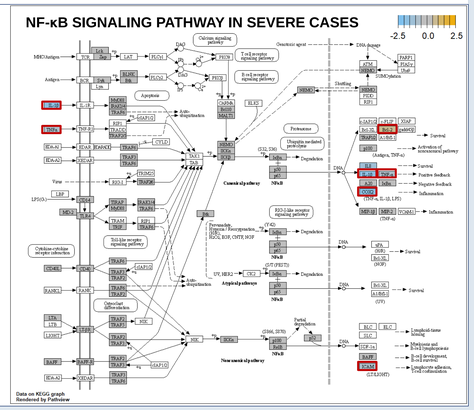


Figure 2. Contrasting deregulated genes in severe and mild cases. A) Comparison of genes with significant changes in at least one of the groups. The horizontal and vertical axes show the log-fold-change against negative samples in severe and mild cases, respectively. Colours reflect the significance of deregulation in both (**blue**), only in the mild (**orange**) and only in the severe (**red**). **B)** Comparison of log2(fold change) confidence intervals for most contrasting genes. The median of the interval is coloured as downregulated (**green**) and upregulated (**purple**).





RESULTS (CONTINUED)

Downregulation of inflammatory response in severe cases

Counterintuitively, severe cases were characterized by the ***downregulation*** of multiple genes associated with the inflammatory response in the early stages of the infection. Such an observation is reversed in later stages of the infection, when severe cases present complications such as acute respiratory disease syndrome (ARDS), shock or multiple organ dysfunction syndrome (MODS) due to cytokine storm [2].

Upregulation of IFIT2 in mild cases

IFIT2 provides antiviral activity by inhibiting the expression of viral RNA and has shown antiviral activity against Coronavirus and West Nile virus. Also, IFIT2 is an effector in the type I IFN response facilitating a positive feedback loop [3].
IFIT2 has been implicated as a positive regulator of IFN-α/β expression in infections by mouse hepatitis virus members of the coronavirus [4].

Downregulating TNF and NF-κB pathways in severe cases

Significant downregulation of multiple proteins in the pathways of TNF and NF-κB in severe cases reflects the overall destabilising effect hypothesised to lead to the cytokine storm.

CONCLUSIONS

Early downregulation of inflammation regulators and mediators in severe COVID19 patients, suggested inflammation dysregulation. IFIT2 upregulation in mild cases and FCER1A downregulation in severe cases, points to early differences in host responses centered on deregulation of the interferon and inflammation. Further research is required to clarify whether these patterns reflect delayed interferon involvement in pathways to control the infection or contribute to pathological inflammation and cytokine storms observed in severe COVID19.

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