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Research article

COVID-19 immunopathological features for the prediction and prevention of future emerging respiratory viral infections

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ABSTRACT

Background & objective: Emerging viral infections can initiate a global pandemic with high mortality. Understanding the immunopathogenesis of these viruses is critical to developing effective strategies for managing/preventing such outbreaks.
Methods: Transcriptomics analysis was performed in a UAE cohort with respective COVID-19 severities. The findings were correlated with published studies of COVID-19 severity/progression GWAS, and transcriptomics data of patients infected with different respiratory viruses.
Results: The transcriptional profiling distinguished significantly between the infected COVID-19 groups and identified the interferon-induced protein, *GBP2,* as a common significantly up-regulated gene among the different COVID-19 infection severities (nominal $p = 0.0019$). Key inflammatory pathways were enriched in the higher-severity groups, including Interleukin-1 family signaling. A remarkable immune signature resulted in a trend of cytokine expression changes between all severities, including *CCL19, CCL21, IL-19, IL-20, IL-36RN,* and members of the IFNA family. The deconvolution of immune cells showed a trend of an uncontrolled pro-inflammatory state and poor immune function in higher disease severities. A systematic analysis of the transcriptomic and GWAS findings identified common signature genes between COVID-19 infection severities including *ALCAM, DKK3, EFNA5, FN1, GABRA5, LPAR1, METTL8, MTHFD1L, SPOCK1, TPM4, VTI1A,* and *WWC2.* Differential regulation in potential genes associated with the Interferon signaling pathway including *HERC5, IFFI44L, IFI6, RSAD2* and *SP100* was identified as a common feature in transcriptomes of patients afflicted with different virulent respiratory viruses.
Conclusion: Our findings highlight a direction where changes in immune response and specific biomarker panels could be considered as a strategy for the prediction/prevention of new emerging respiratory virus outbreaks.

1. Introduction

Throughout history, infectious diseases have posed significant threats to humanity, driven by the constant interplay between hosts and pathogens. This dynamic has led to periodic outbreaks, epidemics, and potential pandemics across different eras. Among various infectious agents, viruses have emerged as particularly dominant due to their high mutation rates, which contribute to zoonotic risks, adaptability, and

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increased virulence [1]. Despite significant technological and scientific advancements in understanding these viruses, effectively managing their rapid evolution remains challenging, often ending in pandemics. The ongoing coronavirus pandemic, represented by SARS-CoV-2, highlights this challenge.

SARS-CoV-2, the virus responsible for COVID-19, was first identified in Wuhan, China, in December 2019 and has since been declared a global pandemic. Presently, over 700 million people have been infected, and over 7 million have died due to this virus (https://www.worldometers.info/coronavirus/). The COVID-19 pandemic has initiated a healthcare crisis and caused a devastating economic burden [2,3]. COVID-19 manifests in a range of symptoms, from asymptomatic cases to severe respiratory issues and systemic complications, including cardiovascular disorders, thrombotic events, and organ injuries [4]. The primary pathophysiological mechanism of COVID-19 is attributed to the disruption of both innate and adaptive immune responses (IRs). This disruption results in prolonged viral presence, inflammation, and tissue damage, extending beyond the lungs to affect multiple organs and potentially leading to multiorgan failure. A distinctive feature of COVID-19 infection is the presence of lymphopenia, which is marked by a deficit in the blood count of B cells, T cells, and innate lymphoid cells [5]. Conversely, there is an amplified abnormal stimulation and recruitment of myeloid cells [6]. Particularly, patients with severe COVID-19 infection exhibit elevated levels of circulatory inflammatory cytokines, significantly linked to serious lung injury [7]. Additionally, inflammatory cytokines and chemokines are notably higher in the bronchoalveolar lavage fluid in comparison to blood in patients with severe symptoms, accordingly intensifying local inflammation [8]. This exaggerated IR communally progresses to pneumonia with vascular leakage, arising in respiratory failure manifested as acute respiratory distress syndrome [9].

In response to this universal threat, huge efforts from the scientific community have been mobilized to understand the complexity of the SARS-CoV-2 infection and the variation of its spread between individuals. Accordingly, using multi-omics analyses has been highly recommended to better understand the numerous difficulties and challenges resulting from the COVID-19 pandemic. It has been reported that to identify the genomic pathways that the virus has significantly affected, it is crucial to study the transcriptomic signatures of COVID-19 patients [10]. Additionally, due to the complexity of genomics and transcriptomics data, using integrative analysis is recommended to aid in simplifying features and helping the making of the best choices for designing innovative and successful preventive/treatment approaches [11].

In the current study, our aim, on one hand, is to understand and identify the genetic and molecular mechanisms of COVID-19-infected patients with respective severities by comparing individual severity levels and between severity categories using transcriptomics in Middle Eastern cohort, and on the other hand to compare our SARS-CoV-2 transcriptomics findings with published studies of COVID-19 severity/progression GWAS, as well as other respiratory virus infections transcriptomics data which can critically support in predicting/preventing future emerging infectious diseases.

2. Material and methods

2.1. Study design and sample collection

Following the approval of the ethical committee at the Department of Health Authority (DOH/DQD/2020/538), nasal swab samples were collected from COVID-19 patients with respective severities, including asymptomatic, mild, moderate, and severe patients, and healthy controls. The samples were collected from Shaikh Khalifa Medical City in Abu Dhabi, United Arab Emirates (UAE), between February and March 2020, during which the original SARS-CoV-2 strain (lineage B.1/Wuhan strain) was the predominant circulating variant. The selection criteria were: (i) an adult aged $\geq$ 18 years, (ii) a resident of the UAE, (iii) able to provide informed consent and complete the survey, and (iv) a positive COVID-19 test for a single individual. Consenting participants were provided with a questionnaire that included details on the demographic characterization, risk factors, and symptomatology, which were cross-checked with the electronic health record. The participants in the cohort were divided into five COVID-19 phenotypic groups, including healthy controls, asymptomatic, mild, moderate, and severe, based on the CO-RADS classification [12]. Some demographic and clinical information were incompletely recorded by the participants. SARS-CoV-2 diagnosis was confirmed in the patients by a positive nasopharyngeal swab at the time of hospital admission, while healthy controls were defined as not having COVID-19 with a negative nasopharyngeal swab. All samples were collected at the time of hospital admission and before initiating any immunomodulatory treatment. At the time of sample collection, patients had only received supportive care, including Paracetamol and Vitamin C. This timing ensured that the immune profiles captured in our analysis reflected the patients' baseline immune response to SARS-CoV-2 infection, without confounding effects from immunomodulating therapies. All participants were unvaccinated.

2.2. Sample size calculation

The sample size calculation was performed to determine the minimum number of samples needed to identify significantly differentially expressed genes between respective COVID-19 severities and healthy controls. The calculation was carried out using a library called "power.t.test" in the R statistical language according to Wei et al. [13]. The parameters were set as follows: the effect size was set to 3.5 and the standard deviation set to 2 based on previous OMIC studies, the significance was chosen at a value of 0.05 and the power at 0.9. The calculation is based on a two-sided hypothesis test. Based on these parameters, the sample size calculation showed that we need a minimum of 8 clinical samples from each subgroup of COVID-19 for the transcriptomic study.

2.3. Next-generation RNA sequencing

For the mentioned samples, the RNA was extracted using the TRIzol protocol [14]. The extracted RNA was treated with Turbo DNase (ThermoFisher Scientific, Cambridge, MA, USA) and subjected to Nanodrop 2000 (ThermoFisher Scientific, Cambridge, MA, USA) as a quality control measure. RNA sequencing was performed as previously described [15]. Next-generation sequencing was performed on the Ion S5 XL System with a targeted AmpliSeqTranscriptome panel, which covers more than 20,000 RefSeq genes (ThermoFisher Scientific, Cambridge, MA, USA). Briefly, cDNA from 10 ng of Turbo DNase-treated RNA was created using ThermoFisher Scientific's SuperScript VILO cDNA Synthesis reagent (ThermoFisher Scientific), and then it was amplified using primers from the Ion AmpliSeq gene expression core panel. To produce amplicons of less than 200 bp, enzymatic shearing was carried out using the FuPa reagent, and the amplicons were then ligated with an adapter and unique barcodes. Agencourt AMPure XP Beads (Beckman Coulter, Indianapolis, IN, USA) were used to purify the prepared library. Ion Library TaqManTM Quantitation Kit (Applied Biosystems, Waltham, MA, USA) was used to quantify the purified library. Following the manufacturer's directions, the libraries were pooled and amplified using emulsion PCR on an Ion OneTouchTM 2 instrument (OT2), and the enrichment was carried out on an Ion OneTouchTM ES. The produced template libraries were sequenced using the Ion 540TM Chip and the Ion S5 XL Semiconductor sequencer (ThermoFisher Scientific).

2.4. Transcriptomics analyses

The RNA-seq data were processed and analyzed using an in-house

pipeline [16]. Briefly, Ion Torrent Software Suite version 5.4 was initially used for processing the RNA-seq data, and the Torrent Mapping Alignment Program (TMAP) was used for alignment against the reference sequence obtained from the hg19 (GRCh37) assembly. Using the raw read counts from the RNA sequencing data, the differentially expressed genes (DEGs) analysis among groups of COVID-19-infected patients with respective severities and healthy controls was carried out using the R/Bioconductor DESeq2 package (Version 1.38.3). The input RNA-Seq data were processed with count normalization using DESeq2's median of ratios, which relies on making the counts comparable among different samples despite the possible depth sequencing variations. The transcriptomics analysis was modeled using the standard negative binomial generalized linear model with the design formula ~ condition, where 'condition' represented the COVID-19-infected vs the healthy groups comparison. The statistically significant threshold for identifying differentially expressed genes was fixed at $p < 0.05$. Next, absolute Gene Set Enrichment Analysis (GSEA), previously described [17], was performed to distinguish differentially regulated pathways between COVID-19 patients of respective severities. GSEA was carried out for all DEGs obtained upon comparing gene expression data from the transcriptomes of "Asymptomatic", "Mild", "Moderate", and "Severe" patients versus "Healthy controls". Absolute GSEA was employed to find significantly enriched pathways among around 21,237 gene sets obtained from the Human Molecular Signatures Database (MSigDB) (https://www.gsea-msigdb.org), including C2: curated gene sets; C5: ontology gene sets (including molecular function (MF) and biological process (BP)); and C7: immunologic signature gene sets. The GSEA results were ranked according to the p-values, considered statistically significant when $p \leq 0.05$.

2.5. In silico prediction of the immune cells' subsets

To profile the dynamics of COVID-19 immunopathology across respective disease severities, CIBERSORT, a digital cytometry tool that can categorize cell type composition and expression from a mixed cell population [18], was applied to the gene expression data of the DEGs gene lists of respective COVID-19 severities. For characterizing immune cell composition, CIBERSORT assumes that the used reference gene expression matrix reflects the cell type composition of the evaluated samples' gene expression in a mixed sample and a linear combination of the cell type composition [19]. To identify the immune cell profiles among each COVID-19 severity group, the *in-silico* immune cell fraction prediction was carried out using reference gene lists for the immune cells [20] and a relative mode analysis with at least 100 permutations at a significance level of $p < 0.05$.

2.6. Integration of genomic and transcriptomic data from SARS-CoV-2-infected patients

To uncover the most crucial genes/single-nucleotide polymorphisms (SNPs) that may drive the immunopathology of SARS-CoV-2 infection, DEGs lists from the current transcriptomic data were integrated with genomic data procured from preselected publicly available large-scale GWAS data. A list of genetic loci and SNPs associated with disease severity and/or susceptibility was selected based on a comprehensive search of the NHGRI-EBI GWAS Catalog (https://www.ebi.ac.uk/gwas/) using the search terms "SARS-CoV-2", "COVID-19", or "COVID-19 severity". All cataloged studies published between 2020 and 2023 were included. The exclusion criteria were defined as genes/genetic loci reported in association with (i) Other diseases or phenotypes (like autoimmune diseases, obesity, asthma, cardiometabolic risks, multiple deprivation index, macular degeneration, etc.) or (ii) Remission or (iii) Resistance to infection. Thus, from the remaining selected studies, SNP associations with traits were retrieved and classified according to (i) Susceptibility to SARS-CoV-2 infection, (ii) Severe SARS-CoV-2 infection, (iii) Hospitalization, (iv) Critical care necessitation, and (v) Mortality. Only SNPs that were significantly reported ($p \leq 0.05$) in the corresponding GWAS were retrieved from the GWAS catalog. The SNP–gene relationships were taken directly from the annotated mapped genes provided in the NHGRI–EBI GWAS Catalog for each included COVID-19 GWAS study. These curated mappings rely on annotation strategies employed in the original publications; we did not apply further SNP-to-gene mapping based on LD expansion, genomic windows, chromatin interactions, or positional scoring. All mapped genes were collapsed into a trait-specific nonredundant gene list. Next, to identify genetic loci possibly unique and associated with respective COVID-19 severities, the corresponding mapped genes of all significant SNP association studies were subsequently overlapped with our transcriptomic findings from each of the respective disease severities as described in the study workflow (Fig. 1). Lastly, to situate our findings in terms of immunopathology across different COVID-19 severities, a gene network diagram was generated using GeneMANIA (www.genemania.org).

2.7. Comparative transcriptomic analysis of different infectious respiratory diseases

To address whether there is any commonality in the immune signature activated by various infectious respiratory diseases in the host transcriptome, a comparative analysis of transcriptomic studies enrolling patients infected with different viruses causing acute infectious respiratory diseases was performed. Viruses that have previously caused pandemics and/or seasonal outbreaks, and are capable of producing severe infections, were selected in this subseries, including SARS-CoV-1, Influenza A subtype H1N1, Influenza A subtype H3N2, Respiratory syncytial virus (RSV) and SARS-CoV-2. Host transcriptomic datasets for the abovementioned virus-mediated infections were obtained from publicly available datasets from the Gene Expression Omnibus (GEO) database, except for the SARS-CoV-2 dataset, which was used in the current study. The inclusion criteria for host transcriptomic datasets were defined as (1) Availability of "Healthy", "Pre-challenge baseline" or "Asymptomatic" controls in the same cohort, (2) The study population was human adults and (3) The cohorts were not being administered vaccinations or new treatments. Hence, the following independent datasets were selected for the comparative transcriptomics analysis, including GSE5972 (SARS-CoV-1), GSE17156 (RSV), and GSE52428 (H1N1 and H3N2), along with the transcriptomic data from the current cohort of patients infected with SARS-CoV-2. Transcriptomic data from multiple time points were available for GSE52428; the time point at which symptoms were reported to peak was chosen (i.e., 101 h post-infection for H1N1 and 69.5 h post-infection for H3N2). For GSE5972, convalescing SARS-CoV-1 patients were excluded. To identify the shared immune signature between all respective host transcriptomics, DEGs from each independent dataset were studied for common trends in gene expression. For each included dataset, the raw expression files were processed uniformly using an analytical workflow of GEO2R, applying the same pre-processing steps and statistical pipeline across all studies. Differential expression was calculated by comparing diseased patients to healthy controls within each transcriptomic dataset. The threshold for significance was considered at $p < 0.05$ and the threshold for Fold Change (FC) was set at 1. The DEGs shared between at least four groups of comparison were chosen to be studied for any commonality in gene signatures and additionally annotated for functional pathway analysis.

3. Results

3.1. Subject characteristics

A total of 61 participants were included in this study, stratified into 10 asymptomatic, 11 mild, 13 moderate, and 16 severe patients and 11 healthy controls. The average age was 27.91 ± 7.34, 36.9 ± 6.64,

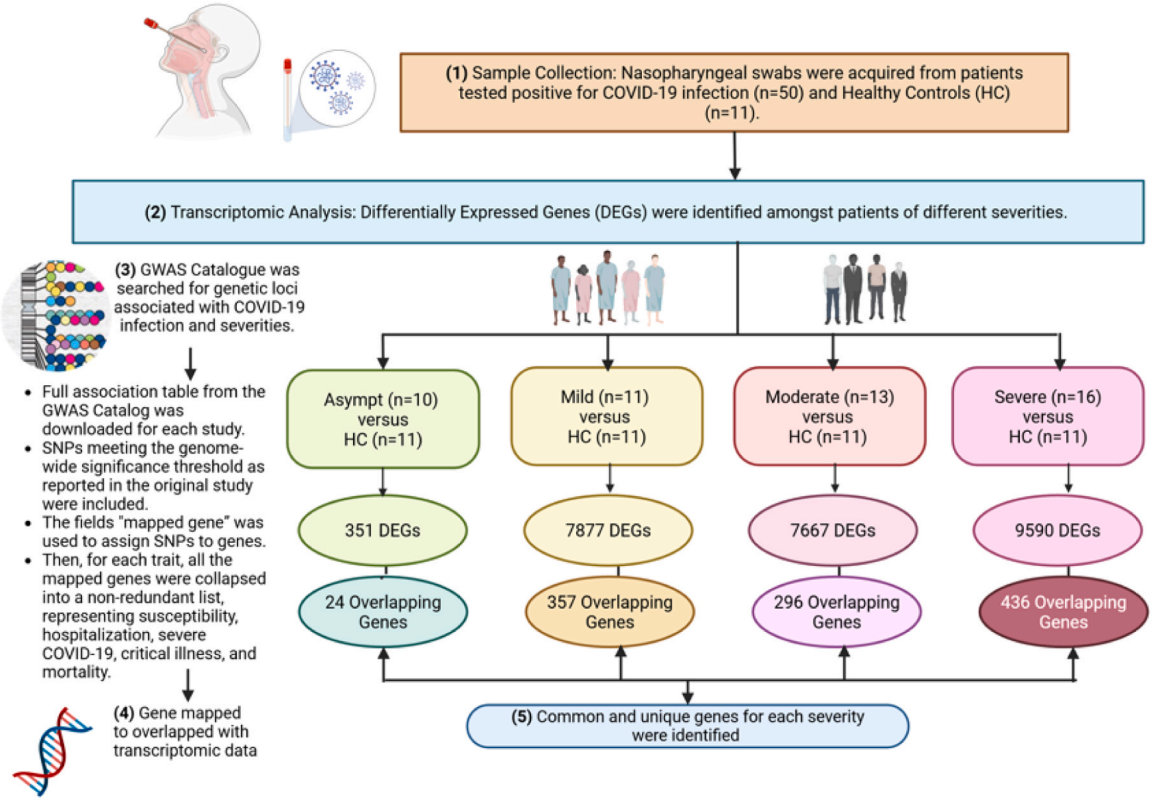

Fig. 1. Workflow of genomic and transcriptomic data integration from SARS-CoV-2 infected patients at various severities. HC: Healthy Controls; Asympt: Asymptomatic; DEGs: Differentially Expressed Genes.

34.18 ± 6.24, 48.92 ± 14.38 and 60.31 ± 16.52 years for the groups healthy, asymptomatic, mild, moderate, and severe, respectively. The average BMI was 25.18 ± 3.17, 27.88 ± 1.58, 23.26 ± 3.97, 28.24 ± 2.33, and 29.5 ± 5.3 kg/m^2 for the groups healthy, asymptomatic, mild, moderate, and severe, respectively. The majority of the COVID-19 patients were male (84 %) distributed as follows: 100 % in the asymptomatic, 73 % in the mild, 77 % in the moderate, and 88 % in the severe groups. The demographic data and medical history of the cohort are presented in Supplementary Table S1.

3.2. Transcriptional profiling distinguishes between the different infected COVID-19 groups

The transcriptomics data showed an average coverage depth of 106.3X and a mapping rate of 98.5 %. To illustrate sample clustering and variability based on the gene expression profiles, principal component analysis (PCA) was performed among the different studied groups, including healthy controls, asymptomatic, mild, moderate, and severe (Supplementary Figure 1). The PCA showed that PC1 and PC2 represent 37.3 % and 22.7 % of the variation between subjects, respectively, indicating differences in the transcriptomic profiles between groups. Additionally, the differential gene expression analysis resulted in distinct gene expression profiles among COVID-19 patients with different severities compared to healthy controls. The DEGs were identified in each comparison of asymptomatic, mild, moderate, and severe COVID-19 patients at a significance level of $p < 0.05$ (Table 1). The DEGs lists of the different studied severities compared to healthy controls are described in Supplementary Table S2. The volcano plots as shown in Fig. 2. A-D disclosed that the number of DEGs in the severe group was markedly higher than that in the asymptomatic, mild, and moderate groups, suggesting that the SARS-CoV-2 infection vigorously disturbed the general transcriptome homeostasis in patients with severe symptoms. A comparison of differentially expressed gene lists identified

Table 1
Number of differentially expressed genes (DEGs) among the COVID-19 patient groups versus healthy controls.

	#Comparison	Total DEGs	Up expressed genes	Down expressed genes
1	**Asymptomatic vs Controls**	351	131	220
2	**Mild vs Controls**	7877	2865	5012
3	**Moderate vs Controls**	7667	1591	6076
4	**Severe vs Controls**	9590	3506	6084

The statistically significant threshold for identifying differentially expressed genes was fixed at a p-value (p) < 0.05.

the interferon-induced protein, *GBP2,* as the only common significantly up-regulated gene among the different COVID-19 infection severities, with particularly increased expression in the moderate and severe patient groups compared to the asymptomatic and mild patient groups, with a significant gene expression difference between all groups at $p = 0.0019$ (Fig. 2. E-F).

3.3. Aberrant immune features in COVID-19 patients diagnosed with different severities

To better understand the potential variation in molecular profiles and biological processes among the COVID-19 patients affected with different severities, GSEA was carried out (Table 2). The GSEA-identified pathways for all severity groups were listed in Supplementary Table S3. The key pathways to ascertain the immunopathology of lower severities were about the negative regulation of biosynthesis and the negative regulation of RNA metabolic processes, possibly suggesting a preemptive response against virus replication and dissemination. For the mild subgroup, the pathways of negative regulation of chemotaxis, NK cell activation, and Interferon alpha-beta signaling were predominantly

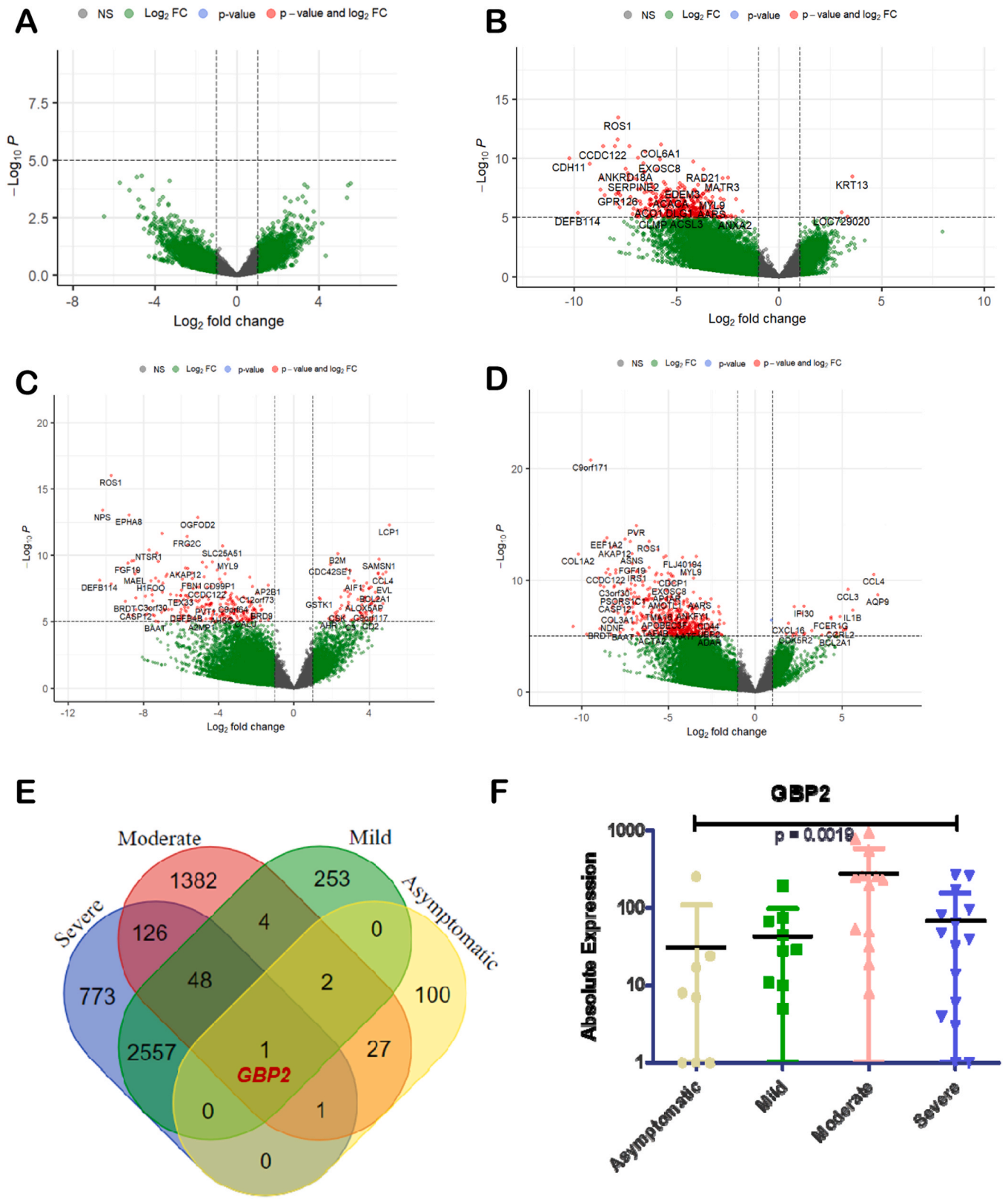

Fig. 2. Comparison of the significant differentially expressed genes amongst the COVID-19 patient groups with respective severities. Volcano plots of differentially expressed genes comparing (**A**) Asymptomatic COVID-19 patients versus HC, (**B**) Mild COVID-19 patients versus HC, (**C**) Moderate COVID-19 patients versus HC and (**D**) Severe COVID-19 patients versus HC. DEGs presented in red dots showed significantly different Log2 FC ($p < 0.05$). (E) Venn Diagram depicting unique and common up-expressed genes among COVID-19 patient groups with respective severities asymptomatic, mild, moderate and severe. (F) Gene expression profile of the unique common significant ($p < 0.05$) up-expressed gene *GBP2* among the COVID-19 patient groups with respective severities asymptomatic, mild, moderate and severe.

Table 2
Number of significant pathways identified by the absolute GSEA among COVID-19 patients with respective severities.

Comparison Groups	Number of Significant pathways			
	Curated gene sets	Ontology gene sets	Immunologic signature gene sets	Total
Asymptomatic vs Controls	21	91	1	113
Mild vs Controls	127	150	195	472
Moderate vs Controls	1231	2916	3042	7189
Severe vs Controls	229	385	419	1033

Cut-off P-value ≤ 0.05.

enriched, suggesting effective modulation of IR and anti-viral activity. Key inflammatory pathways were enriched in the higher severity subgroups, such as Interleukin 1 family signaling, MyD88 independent TLR4 cascade, Jak-Stat signaling pathways, cytokine receptor binding, and innate IR.

3.4. Dynamics of the COVID-19 immunopathology as an immune signature

Given the predominance changes in the immune response to the viral infection among the COVID-19 patients affected with different severities, we were further interested in singling out distinct cytokine expression patterns among the different phenotypes of COVID-19 infection. The changes of differentially expressed cytokines and their receptors, common between all severities, were particularly studied (Fig. 3). Of note, there was a remarkable trend of cytokine upregulation in the mild group compared to the healthy controls, as seen in the case of chemokines *CCL19* (FC = 1.79) and *CCL21* (FC = 1.57), which are both known to be responsible for T-cell and DC homing to lymph nodes and *CCL26* (FC = 1.83), which is known to facilitate eosinophil and basophil migration. Moreover, *IL-19* (FC = 1.61), *IL-20* (FC = 1.60), and *IL36RN* (FC = 1.58), upregulated in mild COVID-19 cases, are mainly described as capable of exerting pro-inflammatory action on epithelial cells/lung tissues. Another group of cytokines from the IFNA family, known to be responsible for orchestrating adaptive IRs to viral infection, showed remarkable patterns of expression. Noteworthy, at least eight out of thirteen members from the IFNA family (FC = [1.59–1.86]) were upregulated in the mild group, which distinctly contrasted with their reduced expression in the moderate patients (FC = [-2.30 to −2.04]). However, the gene expression of these IFNA family members was restored to an upregulated status in the severe patients (FC = [1.39–1.66]). This expression pattern seen in IFNA family members was consistent with all the previously mentioned chemokines and interleukins/receptors. For example the expression-changing levels of *CCL19, CCL21, CCL26, IL19, IL20* and *IL36RN* in the mild group were about 1.79, 1.57, 1.83, 1,61, 1.60, 1.58, respectively, they were reduced in the moderate disease state with FC = -2.26, −2.27, −2.46, −2.14, −1.80, −2.09, but significantly increased in the severe disease state with FC = 1.38, 1.33, 1.37, 1.16, 1.57, 1.52, respectively. This suggests an efficient and well-modulated IR in the mild infection state, while in the moderate infection state, immune cell exhaustion starts to appear. This IR pattern is further supported by the upregulation of immune checkpoints, including *TIGIT, HAVCR2/TIM-3,* and *PDCD1,* with FC = 2.35, 2.19, and 2.18, respectively, in moderate patients, which are known as markers for T-cell and/or NK cell exhaustion. Lastly, the upregulation of these same cytokines, *CCL19, CCL21, CCL26, IL19, IL20,* and *IL36RN* in

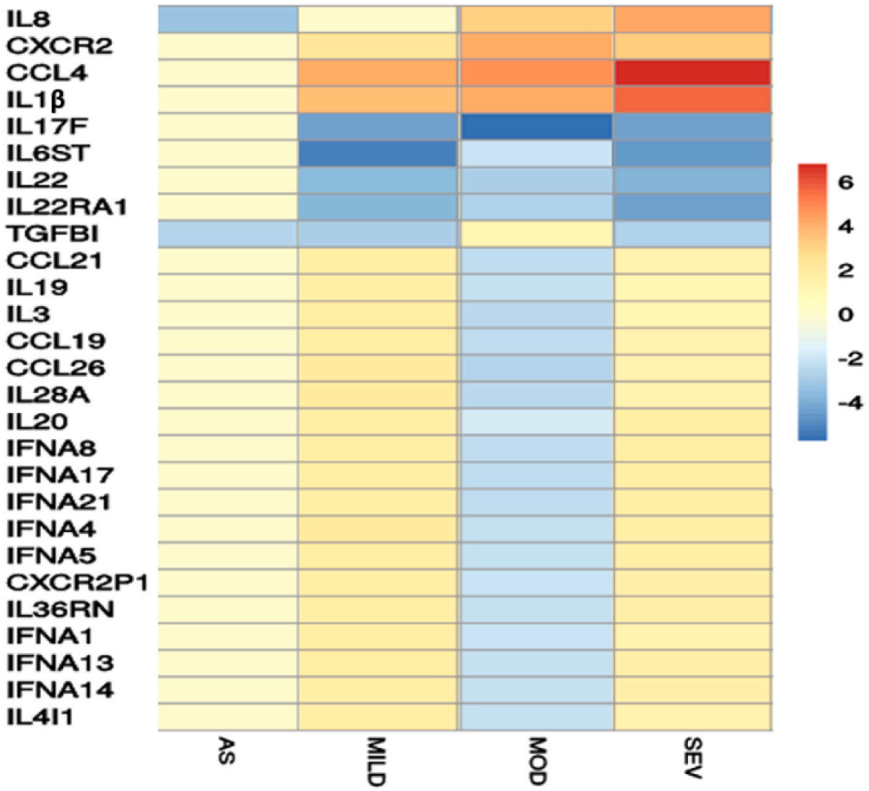

Fig. 3. Heatmap demonstrating the distinct gene expression profiles of cytokines and chemokines in respective severities of COVID-19 infection.
Columns indicate the respective severities of COVID-19 infection groups including AS: Asymptomatic; MOD: Moderate; SEV: Severe. Rows indicate the cytokines and chemokines differentially expressed genes. The color scale shows normalized expression values (log 10) of the cytokine and chemokine genes. Color intensity reflects direction and magnitude of differential expression: red signifies increased expression, blue denotes reduced expression, and yellow represents baseline or non-differential expression relative to healthy controls.

severe cases may contribute to heightened inflammation and immune overactivation due to a lack of inflammatory controls, although their FCs are comparable to the mild cases. This could be explained by the reduced relative immune cell population of anti-inflammatory T-regs in the severe cases when compared to the mild patients. It must also be noted that the presence of an obvious trend of cytokine overexpression seen in severe patients may reflect the Cytokine Release Syndrome (CRS) or cytokine storm. For instance, *IL-1β*, a pro-inflammatory cytokine essential for innate immune cell activation and acute tissue injury during infection, reported lesser expression in the mild (FC = 3.66) and moderate (FC = 4.10) groups, when compared to the severe group (FC = 5.6). Similarly, the level of pro-inflammatory chemokine *CCL4*, which is known to promote NK-cell and macrophage migration and support interactions between DCs and T-cells, was found to be considerably lower in the mild (FC = 4.16) and moderate (FC = 4.73) groups when compared to the severe (FC = 6.84) group. Another crucial pro-inflammatory cytokine *IL8*, which is known to order neutrophil trafficking, was reported with much lower expression in the asymptomatic (FC = −3.19) and moderate (FC = 3.11) groups compared to the severe (FC = 4.24) group. Furthermore, to complement the mRNA expression of these cytokine/chemokine signatures with COVID-19 immunopathology, we also compiled evidence showing protein levels of the same from previous reports in the Supplementary Table S4. The protein levels were consistent with the mRNA expression findings of the cytokines, chemokines, and their receptors from the current study.

3.5. The deconvolution of immune cells showed an uncontrolled pro-inflammatory state and poor immune function in higher COVID-19 infection severities

To take a closer look at the deconvolution of immune cells in the COVID-19 infection, the CIBERSORT computational method was employed to identify the relative fractions of immune cell subsets in each disease severity (Fig. 4). In the severe group, pro-inflammatory M1 cells (~36 %) emerge as the most predominant fraction among the entire immune cell population. These levels are rivaled by anti-inflammatory M2 cells (~29 %), memory CD8 + T-cells (~22 %), and T-regs (~9 %). While NK cells and CD4 + T cells make up to ~4 % of the entire immune cell population. Taken together, this particular immune cell pattern defines a hyper-inflammatory state that is simultaneously impaired in its ability to combat viral infection on account of underwhelming numbers of cytotoxic NK cells and adaptive IR-activating-CD4 + T cell populations. Similarly, the pro-inflammatory M1 cells (~23 %) remained the dominant immune cell subset in the moderate group, while the proportion of anti-inflammatory T-regs (~14 %) was higher in the moderate group compared to the severe group. Noteworthy, in the moderate group, the numbers of NK cells came up to (~10 %), CD4 + T-cells (~17 %), and memory CD8 + T-cells (~24 %). While in the asymptomatic and mild groups, the anti-inflammatory T-regs and M2 occupy nearly half of the immune cell population, seemingly balancing out the pro-inflammatory subsets of M1 and CD8 + T-cells/memory CD8 + T-cells to possibly favor cellular homeostasis. Taken together, these findings provided further evidence about the trend of an uncontrolled pro-inflammatory state and poor immune function in higher disease severities.

3.6. Well-modulated immune responses are crucial to lower COVID-19 disease severities

An integrative genomic-transcriptomic approach was adopted to pinpoint genetic loci, with reported single-nucleotide polymorphisms (SNPs) associated with gene expression changes in SARS-CoV-2 infection and related traits. A total of eighteen GWAS were included (Supplementary Table S5). We identified SNPs in genes that were unique and common to the four different COVID-19 infection severities (Supplementary Figure 2). Importantly, we identified 12 genes common to all four groups, including *ALCAM, DKK3, EFNA5, FN1, GABRA5, LPAR1, METTL8, MTHFD1L, SPOCK1, TPM4, VTI1A,* and *WWC2* (Supplementary Table S6). The gene annotation analysis showed that *EFNA5* (rs25999-?; rs152577-?), *FN1* (rs55739979-C; rs13014602-G), *LPAR1* (rs12115212-G) and *SPOCK1* (rs1229704-A) were the most notable for their role in the neuron projection development pathway ($p < 0.001$). While other genes in this series, including *ALCAM* (rs7620022-?; rs791942-C), *GABRA5* (rs9330524-C), *LPAR1* (rs12115212-G), and *VTI1A* (rs11196149-G) were mainly enriched in the chemotaxis pathways ($p < 0.001$). Noteworthy, genetic loci that are common to all subgroups suggest their potential risk for COVID-19 infection and its progression. The unique genes identified provide

A

Group	Severe	Moderate	Mild	Asymptomatic
CD4 T cell	2%	17%	5%	4%
NK cell	2%	10%	0%	13%
M2	29%	12%	24%	18%
M1	36%	23%	33%	13%
DC cell	0%	0%	0%	0%
T helper	0%	0%	1%	0%
Treg	9%	14%	13%	20%
CD8 T cell	0%	0%	0%	32%
Memory CD8 T cell	22%	24%	25%	0%

B

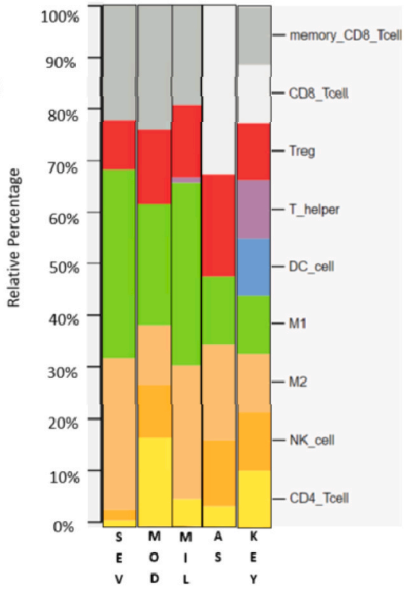

Fig. 4. Fractions of immune cell subsets among patients infected with SARS-CoV-2 with varying severities. A) The relative percentage of the immune cells' subsets distribution in COVID-19 patients vs Healthy controls among the respective severities. The data were extracted from the CIBERSORT *in silico* flow cytometry analysis. B) Stacked bar chart showing the proportions of immune subpopulations among the respective severities of COVID-19 infection groups including AS: Asymptomatic; MIL: Mild; MOD: Moderate; SEV: Severe.

strong insight into the distinct features of immunopathology in each state of COVID-19 infection severity. For instance, SNPs appearing in unique genetic loci in the asymptomatic or mild group (Supplementary Tables S7 and S8, respectively) may be relevant in a well-controlled immune response to the viral infection. Particularly, the *ZNF879* gene encoding a Kruppel-associated box-containing zinc finger protein (KRAB-ZFP), which can partner with *KAP1* to interrupt innate IRs, showed the association of the rs4700775-C SNP with the COVID-19 infection and was unique to the asymptomatic group with an upregulation status (FC = 3.8), suggesting a preventative response against the hyper-inflammatory state. Indeed, *IL10RB*, a subunit of the *IL10* cytokine receptor, which is related to the suppression of cytotoxic and innate immune cells upon activation, contains the rs8178521-T SNP associated with the COVID-19 infection and is uniquely presented in the mild group with a downregulation status (FC = −2.6). Taken together, these observations suggest that the immune defense was well-balanced against hyper-inflammation in asymptomatic and mild COVID-19 infection groups. The evidence from these findings points towards the idea that appropriate modulation of immune response and pre-emption of hyper-inflammation may prevent the development of severe respiratory viral infection symptoms.

3.7. A systems interaction network reveals coordinated regulation of IFN signaling, immune exhaustion and cytokine storm

To provide better context for how these differentially expressed immune regulators mechanistically interact across COVID-19 severities, an interaction network was generated based on shared co-expression, pathway activity, and protein domain similarity (Fig. 5). The network delineated three functionally distinct yet interconnected regulatory modules: a Type I interferon module driven by IFNA family members, *GBP2,* and *IFI44L*; an immune-exhaustion module dominated by *TIGIT, PDCD1, HAVCR2, LAG3,* and *CD96*; and a cytokine storm module featuring *IL1B, IL8, CCL4*, and reduced *DPP9*-linked control of *NLRP1* inflammasome activity. Of note, the IFN-related nodes were connected to exhaustion markers through shared signaling intermediates, and cytokine storm genes clustered with inflammasome regulators, suggesting escalating positive-feedback inflammation in severe cases. These mechanistic interactions support the progressive transition observed in our expression data-from early, balanced anti-viral signaling (asymptomatic/mild groups) to immune silencing (moderate group) and to uncontrolled hyperinflammation (severe group). Collectively, this systems-level representation reinforces the distinct yet interdependent axes defining COVID-19 immunopathology.

3.8. Genetic alterations and/or gene expression changes contribute to severe symptoms of COVID-19 disease

This section describes an overall exaggeration of the innate IR to SARS-CoV-2 infection in patients of the moderate and severe groups (Supplementary Tables S9 and S10, respectively). For instance, SNPs in the genes *CARD11* (rs73043603-A and rs883831-?), and *TRAF3* (rs7151954-G), were associated with COVID-19 infection. Both genes, known to be involved in the activation of the inflammatory NF-kβ pathway, were upregulated with FC = 3.01, and FC = 1.02, respectively, in the moderate group, suggesting a key contribution to the increased transcription of inflammatory cytokines. The *DPP9* gene, a regulator of the *NLRP1* inflammasome assembly, showed significant SNPs (rs2109069-A and rs12610495-G) and a downregulation (FC = −1.5) in the severe group of COVID-19 patients. Noteworthy, ablated inhibition of the *NLRP1* inflammasome activation leads to increased cleavage of *pro-IL-1β* and subsequent release from mononuclear cells [21], [22]. This correlates well with the ~ six-fold increase in the expression of *IL-1β* transcripts in patients from the severe group. Indeed, *IFITM3*, known to be involved in the IR to prevent viral entry and endosomal fusion into the host cell cytoplasm [23], showed significant SNPs

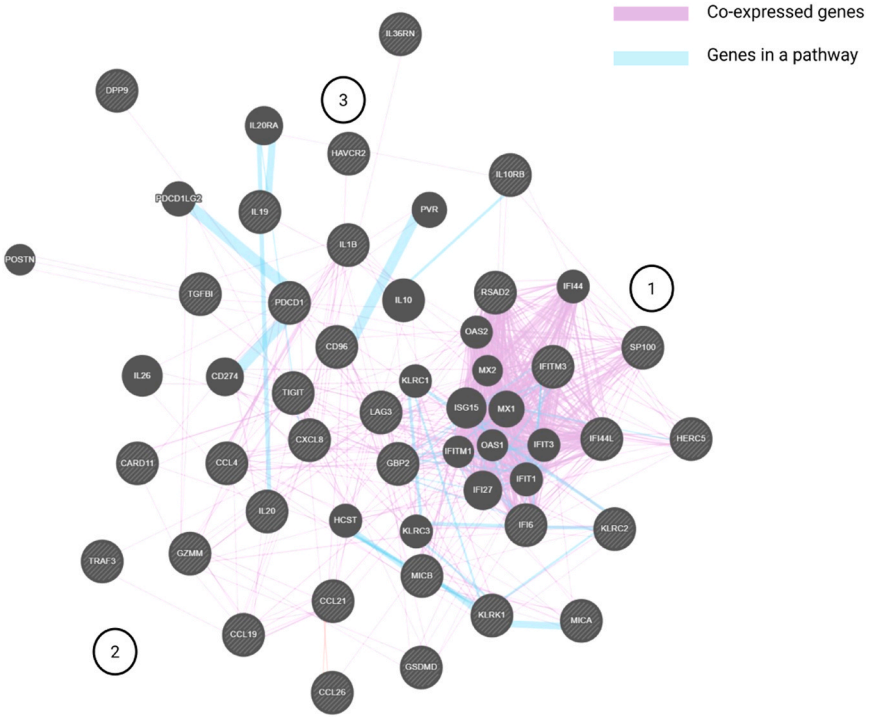

Fig. 5. Systems Network Model of Immune Dysregulation Across COVID-19 Severities. A gene–gene interaction network showing three interconnected regulatory modules that drive COVID-19 immunopathology (1) IFN signaling module, including the *IFNA* family members, *IFI44L*, and *GBP2*. (2) The cytokine storm/inflammasome module acts through converging pro-inflammatory cytokines and regulators, such as *IL1B, IL8,* and *CCL4*. (3) The immune exhaustion module contains checkpoint molecules such as *TIGIT, HAVCR2/TIM-3, PDCD1, CD96,* and *LAG-3*. Functional clustering underscores how early IFN responses, progressive immune exhaustion, and late hyperinflammation are mechanistically linked and provides a systems-level model of COVID-19 severity progression.

(rs7122797-G, rs67437716-T, and rs61876261-C), while its gene expression was found to be altered (FC = 0.8) in the severe group.

3.9. Differential regulation in the Interferon signaling pathway is a commonality in the immune profiles of patients infected with acute respiratory syndrome-causing viruses

Independent transcriptomic datasets of patients infected with pandemic-causing viruses or acute infectious respiratory disease-causing viruses, including SARS-CoV-1, H1N1, RSV, and H3N2, along with the current SARS-CoV-2 data were compared (Supplementary Table S11). 16 common DEGs were derived based on a criterion that significant DEGs must be shared between at least 4 groups of comparisons (Table 3). We additionally performed a STRING protein–protein interaction analysis on the common DEGs shared across SARS-CoV-2 and the other respiratory virus infections. The PPI network showed interconnected modules and suggested the possible functional relationships among these genes (Supplementary Figure 3). Importantly, differential regulation in potential genes associated with the Interferon signaling pathway, including *HERC5, IFFI44L, IFI6, RSAD2,* and *SP100,* was identified as a common feature in patients infected with different viruses (Fig. 6). Intriguingly, it is known that the interferon signaling pathway is a crucial biological mechanism for host defense against viral infection. Consequently, its activation induces changes in interferons and effector genes (ISGs) with an antiviral activity, which hence could explain the expression changes in such biomarkers *HERC5*, *IFI44L*, *IFI6*, *RSAD2* and *SP100.* Considering that chronic activation of the interferon signaling pathway in response to viral infection may be detrimental, resulting in immunosuppression and induction of apoptosis in immune cells [24], these findings indicate the importance of interferon signaling in the immunopathology of virus-causing pandemics and/or rapidly spreading infectious diseases despite the variable symptom severity of patients. Therefore, such insights into potential biomarkers and immune signatures may help in the prediction of eventual future pandemics or rapidly spreading acute respiratory infectious diseases.

4. Discussion

Enormous efforts have been employed by researchers all around the world to understand and fight the global COVID-19 pandemic. These efforts concluded with the importance of developing prevention, treatment, and vaccination strategies. Particularly, enhancing our knowledge of COVID-19's immunopathology is crucial to the continued fight against the global future pandemics that are assumed to cause millions of deaths and casualties worldwide.

Previous studies have mainly focused on blood-based signatures or single-severity cohorts; the present work integrates transcriptomic profiles obtained from nasopharyngeal swabs from a Middle Eastern COVID-19 cohort, stratified across a spectrum of severities, with large-scale GWAS data and comparative transcriptomes from other virulent respiratory viruses. This design enables us to (i) resolve severity-specific immune signatures in the upper respiratory tract, (ii) link genetic susceptibility loci to distinct immunopathological phenotypes, and (iii) identify both shared and SARS-CoV-2–specific interferon-inducible genes that may serve as candidates for pathogenesis-driven therapeutic targeting and pandemic preparedness.

Our study included COVID-19 patients collected from the UAE population with respective severities, including asymptomatic, mild, moderate, and severe patients, in addition to healthy controls. The majority of the COVID-19 patients in our cohort were male (84 %). This male predominance in our COVID-19 patients could be affected by the higher prevalence of severity, worse outcomes and mortality reported in men than women [25], [26]. This unequal gender COVID-19 infection effect could result from multiple factors including genetic factors, behavioral factors, comorbidities, sexual hormones, and immune system response [27]. Thus, a balanced gender distribution in a larger study cohort should be considered in the study design of future COVID-19 infection research.

The current patient cohort was recruited during the early 2020 pandemic (Wuhan strain). The findings are expected to vary with later variants (e.g., Delta and Omicron). The variants differ in their antigenicity (mainly due to the spike sequence and mutations) [28], the degree of fusion and the entry mechanism [29], and the ability to escape neutralizing antibodies [30]. Fortunately, there are still some shared features (e.g., the conserved T-cell immunity) that maintain the efficacy of most vaccines, as they induce T-cell responses and still recognize Delta/Omicron variants of the virus [28].

Nasopharyngeal swab samples were collected from the COVID-19 patients (positive nasopharyngeal swabs) and healthy controls (negative nasopharyngeal swabs) at the time of hospital admission for comparative transcriptomics analysis. The nasopharyngeal swabs are easy to obtain, less invasive, and routinely used for respiratory tract infections [31]. Although these samples primarily reflect the upper respiratory tract conditions and may not represent the full lower respiratory tract conditions, the nasopharyngeal swabs have been considered critical for accurate diagnosis and surveillance of COVID-19 [32]. Interestingly, many previous studies showed that transcriptomic landscape from nasopharyngeal samples can reflect viral activity and immune responses in a host, such as in the COVID-19 conditions [33], [34]. However, in severe COVID-19 infections where the lungs are prominently affected the nasopharyngeal swab samples may not precisely represent the whole spectrum of host-pathogen disturbances, and thus using lower respiratory tract specimens such as broncho-alveolar lavage (BAL), or lung biopsy would be of higher precision in expanding and validating the findings resulting from nasopharyngeal swab samples.

In the present work, we explored transcriptomic data of a COVID-19 cohort to provide further insight into the dynamic immunopathology of COVID-19 at different disease severities. We have used the Ion AmpliSeq Transcriptome panel, which captures more than 20,000 RefSeq targets of annotated human genes. While this approach may not capture newly described, poorly annotated transcripts, or non-canonical interferon-stimulated genes, it has been widely used to profile host immune responses in infection and immune-cell studies [35], [36]. Our transcriptomics findings showed that the interferon-induced protein, *GBP2*, guanylate-binding protein 2, was the only common significantly up-regulated gene among the different COVID-19 infection severities. Guanylate-binding proteins (GBP) are type-I and type-II interferon--stimulated genes (ISGs) and a subfamily of guanosine triphosphatases (GTPases) that can act as intracellular antiviral restriction factors [37].

Table 3
Expression Profile of shared DEGs in acute respiratory infection-causing viruses.

Genes	Fold Change ($p < 0.05$)				
	RSV	**H1N1**	**H3N2**	**SARS1**	**COVID19**
ATP5C1	0.629	0	0.611	0.42	-1.726
ELL2	0.456	0	0.977	0.333	-1.388
GGA1	-0.224	0	-0.275	-0.255	-1.558
HERC5	1.515	0	2.054	0.788	-1.808
HGSNAT	-0.245	-0.366	-0.377	-0.306	0
IFI44L	1.866	0	2.452	2.217	1.978
IFI6	1.076	1.451	1.8	-0.294	0
MECP2	-0.304	-0.349	-0.484	0	-1.582
RNF44	-0.311	0	-0.388	-0.542	-3.302
RSAD2	2.288	0	2.64	0.348	2.35
SNX2	0.479	0.443	0.487	-0.338	0
SP100	0.421	0.564	0.778	0	-1.719
SUPT6H	-0.255	0	-0.529	-1.021	0.509
TCF25	-0.241	-0.27	-0.267	-1.147	0
VRK2	0.445	0.505	0.565	-0.397	-2.253
WSB1	0.409	0	0.521	0.457	-1.921

Fold Change expression was calculated by comparing diseased patients to healthy controls from the same dataset. The null expression denotes a differential expression that was not found significant ($p > 0.05$).

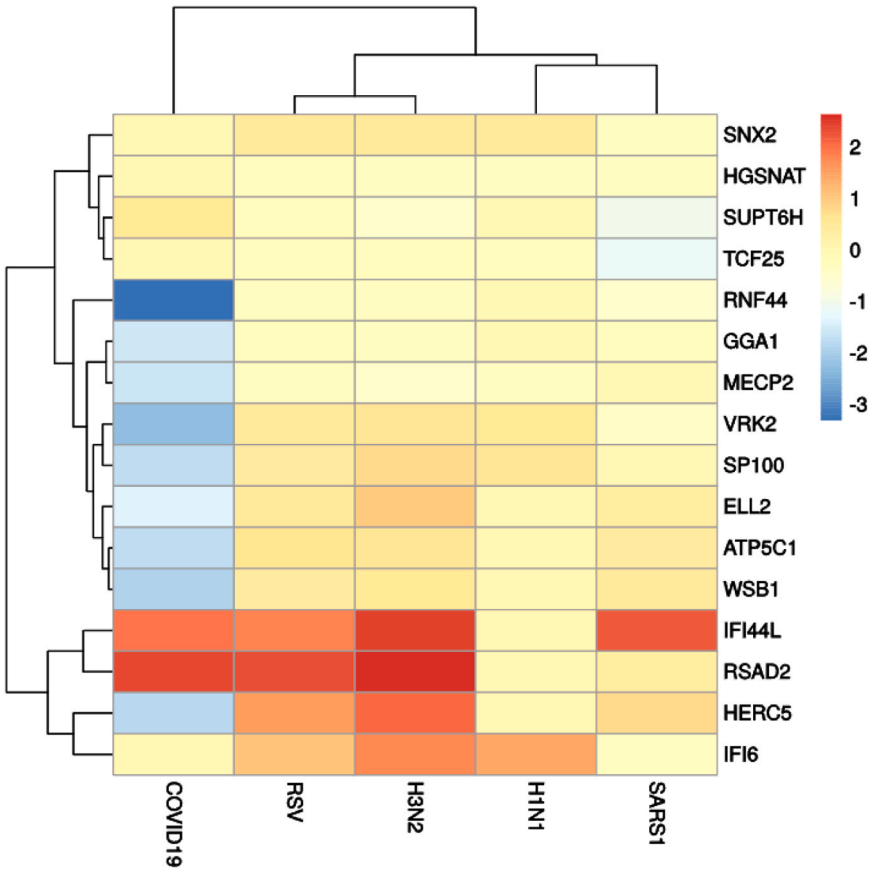

Fig. 6. Heatmap illustrates the expression of shared significant differentially expressed genes between virus-infected transcriptomes of patients across different pandemics. Columns indicate the different studied viruses causing acute infectious respiratory, including SARS-CoV-1 (SARS1), Influenza A subtype H1N1(H1N1), Influenza A subtype H3N2 (H3N2), Respiratory syncytial virus (RSV) and SARS-CoV-2 (COVID19). Rows indicate the common differentially expressed genes. Color intensity reflects direction and magnitude of differential expression: red signifies increased expression, blue denotes reduced expression, and yellow represents baseline or non-differential expression relative to healthy controls.

GBP2 plays an important role in host immunomodulation in viral infections, malignancies, and autoimmune diseases. It exerts anti-influenzal activity, promoting oxidative killing, and transfers antimicrobial peptides to autophagolysosomes, resulting in broad host protection against different pathogen types. A previous study showed that *GBP2* inhibits the cleavage of the SARS-CoV-2 spike, thus reducing viral infection [38]. This suggests that in our cohort, the *GBP2* was probably overexpressed as a compensatory mechanism to combat the SARS-CoV-2 infection.

Consistent with the literature, our transcriptomics analysis showed that the immunological profile of patients from each severity revealed distinct immunopathological features (Fig. 7). For instance, the asymptomatic phenotype was defined by the negative regulation of a pro-inflammatory innate IR, partly governed by *IFI44L*, an ISG that has been reported to inhibit IFN production and signaling by binding to *FKBP5*. This decreases the phosphorylation of IRF-3 and IκBα mediated by kinases IKKε and IKKβ, thereby lowering the innate IR [39]. These findings are further supported by the down-expression of pro-inflammatory cytokines, *IL-8*, responsible for the recruitment and trafficking of neutrophils [40]. Additionally, in asymptomatic patients, the innate IR may be regulated by the upregulation of *ZNF879,* which is a KRAB-ZFP gene that can recruit KAP1 to perform epigenetic changes at the histone or promoter site of genes such as ISGs [41], [42]. While, the mild phenotype exhibits a well-modulated IR, where the pro-inflammatory cytokines and chemokines, along with appropriate type 1 interferon responses, are proposed to fight SARS-CoV-2 infection. Furthermore, there was a downregulation of immunosuppressive molecules such as *IL-10RB* and *TGFβ1* that would otherwise probably inactivate effector functions of cytotoxic T-cells [43]. Yet, homeostasis is maintained by certain mechanisms, such as lowered expression of *GSDMD*, which is a molecule known to cleave the immune cell membrane during pyroptosis to enable the release of highly inflammatory cytokines such as *IL-1β* and *IL-18*. Interestingly, these findings were further highlighted by the lower levels of *IL-1β* expression in the mild group, compared to the moderate and severe groups. Taken together, these results further showed the immunopathology dynamics of COVID-19 in lower-severity infected patients.

Furthermore, our findings revealed that the COVID-19 moderate phenotype demonstrates strict control of the immune system. Remarkably, we have found signs of controlled IR, reflected in the upregulation of potential biomarkers such as *LAG-3, TIGIT, TIM-3,* and *PDCD1*, which are all immune checkpoints that are known to help reduce the effector functions of T-cells, NK cells and/or innate immune cells [44], [45]. Indeed, an immunosuppressive environment is presented by *IL-10RA* and *TGFβ1* upregulation, which further defines the fine-tuning of the IR [46]. However, the exact causes of the controlled immune cell response in specific contexts remain largely vague and may be attributed to the viral load and virulence [47]. Another interesting immune profile was deduced by the downregulation of specific cytokines and chemokines, including several members of the *IFNA* family. These results have further strengthened our conviction about the complex role and phased function of the DCs, which are reported as the prime producers of Type 1 interferons [48]. Simultaneously, there may be an increase in the *NLRP3* inflammasome assembly, which promotes the cleaving of dormant *IL-1β* into its active form, along with other tissue-damaging pro-inflammatory molecules such as *IL-18*. A further important implication is that NK-cell

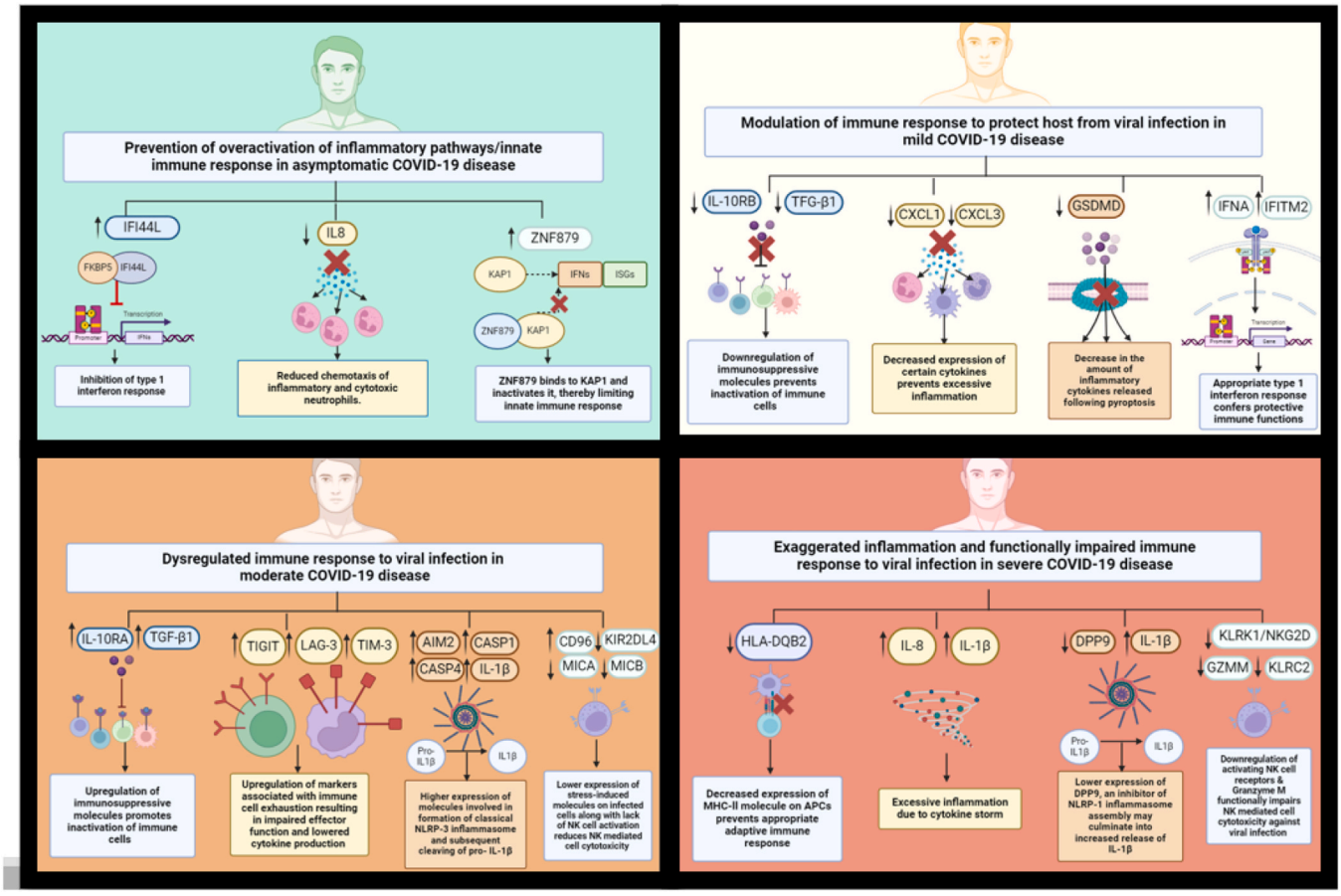

Fig. 7. Summary of the dynamic immunopathology of COVID-19 infection among the respective severities of SARS-CoV-2 infection groups.

cytotoxicity is possibly impaired due to an increased expression of inhibitory receptors such as *CD96* and *KIR2DL* [49,50]. Additionally, the downregulation of stress molecules such as *MICA* and *MICB* in virally infected cells may result in mitigating the exaggerated IR [51,52].

Lastly, in the severe phenotype, our transcriptomic findings showed an exaggeration of innate IR with a lack of immunosuppression from an underwhelming population of T-regs, and therefore low levels of *TGFβI*. Most prominently, there was a loss in negative regulation of the *NLRP1* inflammasome, which is probably regulated by *DPP9* which correlates well with the high levels of *IL-1β* [22]. Again, there was a functional impairment of NK cell cytotoxicity, which weakens the anti-viral defense system, due to decreased expression of activating receptors such as *NKG2D, KLRC2,* and *GZMM*. Moreover, the upregulation of inflammatory cytokines such as *IL-8, CCL4,* and *IL-1β* is potentially indicative of tissue damage accompanying the cytokine storm. For instance, *IL-8*, one of the key chemokines responsible for neutrophil recruitment, can trigger neutrophil activity such as NETosis to fight pathogenic invasion [53]. However, excessive neutrophil infiltration can damage host tissues, potentially leading to ARDS [54]. Furthermore, considering that *IL-1β* activates the NFκB pathway, thereby culminating in the release of several inflammatory cytokines and chemokines including *CCL4*, it consequently creates a hyperinflammatory environment associated with severe disease outcomes [55]. Indeed, the downregulation of MHC class 2 molecules such as *HLA-DQB2*, could be proposed as a critical strategy used to suppress adaptive IR in severe COVID-19 infection. These findings are consistent with previous results showing that the non-structural protein 5 of SARS-CoV-2 interacts with histone deacetylase *HDAC2* to dysregulate the transcription of MHC2 molecules [56]. Our findings exploring the IR pattern outlined a mechanistic explanation for the symptomatology and complexity of the SARS-CoV-2 infection which could be applied to future pandemics.

Particularly regarding the *IFNA* family genes, there was a biphasic expression with an upregulation in mild, suppression in moderate, and re-induction in severe COVID-19 patients. It has been reported that robust type I interferon responses are typically associated with viral control and lower severity [57]. In contrast, delayed or reduced IFN responses can impair initial viral clearance, contributing to prolonged viral replication and hyperinflammation. The suppressed *IFNA* expression in moderate COVID-19 patients, paired with increased expression of exhaustion markers such as *TIGIT*, and *PDCD1*, suggests immune silencing at this stage. In severe COVID-19 patients, the re-elevation of *IFNA* levels may be compensatory, driven by uncontrolled viral replication and systemic inflammation, but occurring too late to be protective, and instead it probably fuels cytokine storm and tissue damage. Additionally, raised serum cytokines/receptors and chemokines/receptors levels (e.g.*: IL-8, CCR1, IL-19)* were correlated to increased severities. In general, our findings extend and cross-validate already published literature on transcriptomic dynamics in the immune signature of various SARS-CoV-2 severities.

Noteworthy, the immune response to viral infections could pave the way for the identification of therapeutic targets. For instance, the hyperinflammatory environments *(IL-1β, DPP9)* and immune exhaustion *(TIM-3, TIGIT, CD96)* were all hallmarks of increased disease severity [58–60]. Subsequently, the cytokines of major significance can be modulated, particularly those involved in the cytokine storm or, on the contrary, immune exhaustion. In severe COVID-19 patients, upregulated pathways linked to specific cytokine modulation represent potential therapeutic targets. For instance, monoclonal antibodies designed to inhibit or block specific cytokines have been developed and some served as an effective therapeutic approach in the context of severe

COVID-19, such as the IL-6 inhibitor; Tocilizumab [61]. Hence, several pathways that were identified warrant further investigation as potential immunopathogenesis features for therapeutic targets.

The CIBERSORT was used to computationally profile an overview of the immune response and related immune cell type composition during COVID-19 infection across respective severities. This *in-silico* immune cell fraction helped us to understand the immune response dynamics among COVID-19 severities. The bulk gene expression signatures showed an elevated pro-inflammatory profile along with impaired adaptive immune responses in higher COVID-19 infection severities. These findings are in line with previously published single-cell RNA sequencing studies showing similar immune dysregulation patterns in severe COVID-19 patients. For instance, it has been reported that in severe COVID-19 cases, intense T cell activation is associated with macrophages exhibiting M2 polarization and upregulation of anti-inflammatory cytokines such as *IL-10* and *CCL18* [62,63]. The concordance between the *in-silico* and single-cell sequencing at the deconvolution of immune cells in the COVID-19 infection relies on the relevance of the COVID-19 immune signatures.

Furthermore, we performed an integrative analysis of our transcriptomic findings with publicly available GWAS data from patients infected with SARS-CoV-2. This systematic analysis helped in identifying genetic variations that are correlated with gene expression changes among the infection severities to understand the crucial players of the immune complexes responding to viral infections. Our findings showed that *ZNF879*, a *KRAB-ZFP* gene as aforementioned for its regulatory role by partnering with transcription repressor *KAP1* in silencing important IRs such as the expression of *ISGs* [64], was the most prominent altered genetic locus with an over-expression in the asymptomatic group. These results along with the upregulation of the *IFI44L* gene further support the precisely adjusted innate IRs in the asymptomatic state of viral infection. Whereas, regarding the mild group, we identified *IL-10RB* as the most prominent gene encompassing altered SNPs associated with the SARS-CoV-2 infection, along with a downregulation in the transcriptome profiles. These findings are in agreement with previous reports showing *IL-10RB* as amongst the top candidates for SARS-CoV-2 susceptibility, associated with high viral load and consequent activation of several molecular pathways [65]. In the moderate group, the loci of *CARD11* and *TRAF3,* encompassing SNPs significantly associated with the SARS-CoV-2 infection and reported increased expression, are indicative of overactivation of the NF-κB pathway that culminates in the release of inflammatory cytokines such *as IL-1β, IL-2, IL-6, IL-12, TNF-α*, and various chemokines [66]. This proposes cytokine storm and is therefore associated with more severe outcomes, which correlates well with the fourfold increased expression of *IL-1β* in the moderate group. Finally, in the severe group, we importantly found a downregulation in the *DPP9* and *IFITM3* genes along with the presence of altered SNPs. *IFITM3* is an inhibitor of the *NLRP1* sensor, thereby preventing the formation of the *NLRP1*-inflammasome as previously discussed [22]. Additionally, it has been reported that dysregulation of the *DPP9* gene can cause host-driven inflammatory lung injury as among the complex molecular mechanisms of critical infection in COVID-19 patients [67]. Indeed, it is believed that the *IFITM3* protein physically inhibits the endocytosis of a variety of viruses by producing a chain-like structure on the cell membrane between *IFITM3* protein monomers. In this line, a previous study reported an association between single-nucleotide variation in the *IFITM3* gene and high mortality rates [68]. This suggests that polymorphisms in the *IFITM3* gene may disable or lower its protective anti-viral function. Overall, this integrative approach applies a two-layer stratification of patients into phenotypes. Future prospects can focus on panels designed on the data presented at both genomic and transcriptomic levels, which can therefore open an avenue to precise medical solutions, and help in future pandemics even with other coronavirus variants or similar viral infections. The current findings can impact clinical practice after validation on a larger cohort of patient samples. A predictive model can exploit those biomarkers as an input, together with clinical parameters and/or laboratory markers. In a previous study, we employed a stochastic non-linear modeling approach to reduce multi-dimensional datasets. The predictive model of COVID-19 outcome showed that combining basic biochemical and radiological investigations with a limited number of curated cytokines will likely attain accurate predictive value in COVID-19 [69]. Such an approach can be used on various datasets to forecast the course of novel COVID-19 cases, as well as to pinpoint basic immune-mediated pathways and possible treatment targets for potential new variants. Identifying particular gene variants that contribute to disease occurrence and/or severity could play a role in patient triaging based on their expected needs for intense healthcare and follow-up.

Indeed, another aim of our study was to distinguish any commonality in the immune signatures of pandemics of past virulent respiratory viral infections. The comparative transcriptomics analysis of patients infected with SARS-CoV-1, H1N1, RSV, H3N2, and SARS-CoV-2 identified a significant differential regulation ($p < 0.05$) of five potential interferon-inducible genes, including *HERC5, IFI44L, IFI6, RSAD2,* and *SP100,* which are importantly annotated under the interferon signaling pathway. Particularly, *RSAD2* showed an overexpression pattern in the patients' transcriptome from all datasets except the H1N1 dataset. This gene is responsible for decreasing the release of viruses from infected cells by manipulating the activity of certain enzymes that can alter the fluidity of the infected host cell's plasma membrane [70]. Moreover, *SP100* was upregulated in all diseased patients except those infected with SARS-CoV-1. Conversely, a downregulation was reported in COVID-19 patients. Of note, the *Sp100A* isoform is ubiquitously situated in the cytosol of several cell types and is an abrupt responder to various extracellular stimuli, including virus infection, and IFN signaling through the phosphatidylinositol 3-kinase (PI3K) pathway. It has been reported that SP100A accumulates in the nucleus on the promoter regions of vital antiviral ISGs like those coding for *IFI16, RIG-I,* and *OAS2,* and mediates their transcriptional activation [71]. On the other hand, *HERC5* was upregulated in all infected patients except those with H1N1, and remarkably, it was decreased in COVID-19 patients. *HERC5* is known to be the chief cellular E3 ligase that forms a crucial part of the ISGylation machinery in the cell. Numerous studies have suggested that HERC5 inhibits the replication of evolutionary viruses by ligating ISG15 to viral proteins, thus, delaying viral replication in the host [72]. Additionally, our findings showed that *IFI44L* was upregulated except for H1N1 patients, while *IFI6* was upregulated in both Influenza A subtypes and RSV, but showed a slight downregulation in the SARS-CoV-1 infected patients. Both of these interferon-inducible genes are well known to negatively regulate innate IRs. The molecular mechanism underlying *IFI44L*-mediated prevention of innate IR was discussed earlier. Whereas *IFI6* was reported to bind with RIG-I RNA and thereby inactivate its function, knowing that RIG-I is an important sensor of virus infections inducing the transcription of IFNs and inflammatory proteins [73]. These distinct immune signatures emerge due to an overactivation of the interferon signaling pathway as a result of excessive interferon production during viral infections. While this mechanism can be protective in earlier stages of the viral infection disease, excessive and chronic stimulation of this particular pathway can lead to detrimental effects, such as the induction of immunosuppressive molecules *IL-10* and *PDL1* in immune cells. Interferons, during a viral infection, can induce immune cell apoptosis by upregulating the TRAIL death receptor and its ligand [24]. Additionally, the commonality between these viruses is that they can negatively modulate the innate IRs to their advantage by upregulating key genes such as *IFFI44L* and *IFI6*, therefore, these could be mechanisms by which viruses push their survival. Taken as a whole, we observed an expression trend that was not always common amongst all transcriptomics datasets of the studied patients infected with SARS-CoV-1, H1N1, RSV, H3N2, and SARS-CoV-2, which can be mainly explained by the lack of alignment between the sample collection time point and peak symptoms manifestation. Importantly, these findings were in agreement with the existing

literature. In a cohort of 133 European Americans, Influenza infection yielded significant upregulation of the Interferon pathway/ISGs including *IFI44L, IFI6, HERC5* and *RSAD2* in the acute phase of the disease [74]. Analysis of gene expression changes in human peripheral blood infected with avian influenza A virus (H7N9) showed that *RSAD2* emerged in the top 10 upregulated genes and other ISGs are among the significantly differentially expressed genes [75]. *In-vitro* comparative transcriptomics of Influenza A, Influenza B and Human Rhinovirus reported the upregulation of the *RSAD2* gene as a shared immune signature [76]. Another comparative transcriptomics study of Influenza, HIV and COVID-19 in a human cohort identified *IFI44L, IFI44* and *RASD2* as a common immune signature amongst the three infection patterns [77]. Our findings highlight the importance of the Interferon signaling pathway as a shared immunopathogenic pathway in the host defense response of patients infected with SARS-CoV-2 and other acute respiratory-causing viruses. The genes identified are biomarkers falling under potential signatures of antiviral host defense. Therefore be treated with considerable, these findings require validation in larger and more diverse cohorts to be generalized.

After validating our results on a larger cohort, a panel of specific predictive biomarkers can be created to help triage the patients on admission to different care strategies, according to the expected severity of individual cases infected with SARS-CoV-2 and other acute respiratory-causing viruses. During the pandemic, several medications were repurposed to combat the SARS-CoV-2 infection. Some were based on general tools (e.g., LINCS / CMap) that suggest repurposing of medication according to the gene expression changes in a specific condition using integrated digital resources to link cellular perturbation to predict the therapeutic response [78]. Eventually, more specific tools for COVID-19 treatment were developed. A good example is "the COVID-19 Drug and Gene Set Library," linking drug sets and gene sets related to the disease experiments, utilizing different digital tools [79]. It contains *in-vitro* drug screen hits based on cell culture and gene expression changes related to SARS-CoV-2 infection, CRISPR screens, and phosphorylated proteins. In future studies, we can suggest potential therapeutic targets and prophylactic medications by applying such tools to our genetic biomarker findings in the SARS-CoV-2 infection.

In a translational context, pandemic preparedness can be gauged in different populations by screening for polymorphisms in the aforementioned panel of genes. Detection of polymorphisms that impair the early Interferon response in patients may warrant the use of therapeutics such as ISG mimetics or vaccines that can enhance the host Interferon response. The comparative analysis of transcriptional patterns generated from various pandemics of virulent respiratory viral infections allowed us to define a common comprehensive signature of virulent viral infections and assume that changes in the particular resulting IR patterns and panels of biomarkers can be a candidate approach for predicting/preventing future emerging respiratory virus infections. Given that, these datasets were collected from diverse studies, multiple variables could result in heterogeneity among their datasets including the patients' characteristics (e.g. ethnicity, age and sex distributions), the protocol of samples' collection (e.g. the sample type, the timing post-infection of sample collection, the protocol used for RNA extraction), and the experimental methods used for transcriptome data generation (protocol of samples preparation, different platforms). Moreover, the non-uniform reported data across the different datasets could further add to the heterogeneity of the input transcriptome data and the complexity of variable adjustments. Together, these factors of data heterogeneity can reduce the reproducibility and generalization of the findings which need to be interpreted with caution. To minimize bias, in our approach, we applied strict inclusion criteria in the selection of the dataset encompassing adequate clinical annotation and detailed disease severity description which contributed to improving the robustness of our comparative analysis. Hence, identifying the shared upregulated ISGs as key immune signatures within the transcriptional profiling of the different studied respiratory infections underscores a core host antiviral transcriptional response despite inter-datasets variability.

Noteworthy, the potential variations in population genetics and environmental factors may significantly affect the immune response, hence the generalizability of our findings. Allele frequency of different genes affecting immune traits (e.g., cytokine genes and innate immune loci) varies across populations [80]. Moreover, the same variant can have different effect sizes in different genetic backgrounds due to the gene–gene interactions and the specific structure of linkage disequilibrium. To reveal shared vs population-specific loci, trans-ancestry GWAS can be employed [81]. On the other hand, environmental factors, including air pollution and exposure to chemicals, may modulate the innate and adaptive immunity and vaccine response. Such factors widely vary geographically and even by urbanization [82]. Furthermore, the microbiome that varies across populations can affect the immune response and vaccine immunogenicity. Such a microbiome is mainly determined by the diet, antimicrobial use, pollution, and sanitation [83]. To improve generalizability, trans-ancestry, meta-analysis and fine-mapping approaches could be used [81]. In addition, to generate generalizable models, gene $\times$ environment and polygenic $\times$ environment interactions should be considered to reflect measured exposures (e.g., pollution indices, microbiome features) [84].

The current study was limited by the relatively small sample size of 61 cases including 50 COVID-19 patients and 11 healthy controls. This limitation may reduce the statistical power and affect the individual differences in the results, particularly when comparing the transcriptomics differences between severity groups. However, to mitigate these limitations, on the one hand, appropriate statistical tests were used on the transcriptomics data. On the other hand, our key findings identified on the transcriptomic signatures and immune pathways were additionally correlated with large-scale cohorts from worldwide populations and ancestries through integration with 18 selected GWAS studies and external transcriptomics datasets infected with different viruses causing acute respiratory illness that are retrieved from three studies encompassing 165 infected patients with 82 controls samples from two different populations (UK, and Toronto). Both correlation analyses identified common genetic signatures between our study and the external studies, thereby enhancing the reliability of our findings. However, future explorations with larger sample cohorts should be guaranteed.

5. Conclusion

The current study reported potential immune signatures and panels of biomarkers resulting from the SARS-COV-2 infection at different severities including asymptomatic, mild, moderate, and severe symptoms by applying variable bioinformatics analyses on large transcriptomics and genomics datasets. Indeed, a common comprehensive signature of virulent viral infection was identified from the comparison of independent transcriptomics data of patients infected with SARS-CoV-1, H1N1, RSV, H3N2, and SARS-CoV-2. Together, these findings provide further insight into the dynamic immunopathology of COVID-19 at different severities and can serve in developing effective strategies for managing and/or preventing future emerging infectious diseases.

Author statement

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CRediT authorship contribution statement

Habiba Alsafar: Writing – review & editing, Project administration, Funding acquisition. **Saber Maha:** Writing – review & editing, Investigation. **Nawal Alkaabi:** Resources. **Maimunah Uddin:** Resources. **Guan Tay:** Resources. **Thenmozhi Venkatachalam:** Resources,

Methodology. **Mira Mousa:** Resources, Investigation. **Ayesha M. Yusuf:** Writing – original draft, Visualization, Validation, Formal analysis, Data curation. **Amal Bouzid:** Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. **Rifat Hamoudi:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2025.12.024.

Data Availability

All the relevant data are available in this paper and the related Supplementary files. The transcriptomics data used in this study from the sets GSE5972, GSE17156 and GSE52428 are available from Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo).

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