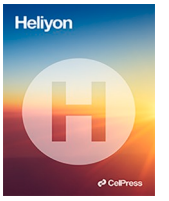
Heliyon 10 (2024) e23320

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Proteomic profiling identifies biomarkers of COVID-19 severity

Noa C. Harriott [a,b,c], Amy L. Ryan [a,b,c,*]

[a] Hastings Center for Pulmonary Research, Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, University of Southern California, Los Angeles CA 90033, USA
[b] Department of Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles CA 90033, USA
[c] Department of Anatomy and Cell Biology, Carver College of Medicine, University of Iowa, Iowa City IA 52240, USA

A B S T R A C T

SARS-CoV-2 infection remains a major public health concern, particularly for the aged and those individuals with co-morbidities at risk for developing severe COVID-19. Understanding the pathogenesis and biomarkers associated with responses to SARS-CoV-2 infection remain critical components in developing effective therapeutic approaches, especially in cases of severe and long-COVID-19. In this study blood plasma protein expression was compared in subjects with mild, moderate, and severe COVID-19 disease. Evaluation of an inflammatory protein panel confirms upregulation of proteins including TNFβ, IL-6, IL-8, IL-12, already associated with severe cytokine storm and progression to severe COVID-19. Importantly, we identify several proteins not yet associated with COVID-19 disease, including mesothelin (MSLN), that are expressed at significantly higher levels in severe COVID-19 subjects. In addition, we find a subset of markers associated with T-cell and dendritic cell responses to viral infection that are significantly higher in mild cases and decrease in expression as severity of COVID-19 increases, suggesting that an immediate and effective activation of T-cells is critical in modulating disease progression. Together, our findings identify new targets for further investigation as therapeutic approaches for the treatment of SARS-CoV-2 infection and prevention of complications of severe COVID-19.

1. Introduction

Beginning in late 2019 in Wuhan, China and spreading to the United States in early 2020, the global coronavirus disease (COVID-19) pandemic has presented a large-scale public health challenge, with the death toll in the United States exceeding 1 million (https://covid.cdc.gov/covid-data-tracker/#datatracker-home), and the global death count over 6 million as of July 2022 (https://covid19.who.int/). COVID-19, which results from infection by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus [1], is a disease primarily characterized by dry cough, fever, and fatigue. However, symptoms can also include sore throat, shortness of breath, loss of smell and/or taste, headache, chills, nausea, vomiting, and diarrhea [2–4]. Symptoms can also persist long after resolution of the initial infection, in some cases more than 14 months [5–7]. In addition to this wide variety of symptoms, COVID-19 is associated with significant variation in disease severity. While the majority of cases are mild or asymptomatic (>85 %), ~14 % of cases require hospitalization, and <2 % of all cases are lethal [8].

Clinical observations rapidly identified age as a primary risk factor for hospitalization and mortality [9,10]. Age-related risk for severe COVID-19 has been a core focus of scientific investigation, and a variety of plausible explanations have been presented in the literature. Expression of angiotensin converting enzyme 2 (ACE2), the primary cell surface receptor for SARS-CoV-2 [11,12], in addition to other SARS-CoV-2 entry factors, has been proposed as an explanation for age-related differences, with higher expression levels of these entry factors being detected in the nasal epithelium of older patients [13,14]. In addition to the airway epithelium, ACE2

* Corresponding author. Anatomy and Cell Biology Associate Director: Center for Gene Therapy BSB, 1-400 Core University of Iowa 51 Newton Road Iowa City, Iowa, 52242, USA.
E-mail address: amy-l-ryan@uiowa.edu (A.L. Ryan).

https://doi.org/10.1016/j.heliyon.2023.e23320
Received 24 February 2023; Received in revised form 27 November 2023; Accepted 30 November 2023
Available online 4 December 2023

Table 1
Summary of study subject demographics. Data represents summarized data from a total of 70 subjects used in the current study. All data is expressed as a percentage of the total of subjects.

	Demographic	COVID-19 Positive			COVID-19 Negative (N = 16)	All Cohorts (N = 70)
		Severe (N = 22)	Moderate (N = 22)	Mild (N = 10)		
Age group	<18 years	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	19–35 years	0 (0 %)	2 (3 %)	2 (3 %)	0 (0 %)	4 (6 %)
	36–50 years	3 (4 %)	5 (7 %)	4 (6 %)	5 (7 %)	17 (24 %)
	51–65 years	9 (13 %)	10 (14 %)	4 (6 %)	8 (11 %)	31 (44 %)
	>65 years	10 (14 %)	5 (7 %)	0 (0 %)	3 (4 %)	18 (26 %)
Sex	Female	8 (11 %)	7 (10 %)	4 (6 %)	8 (11 %)	27 (39 %)
	Male	14 (20 %)	15 (21 %)	6 (9 %)	8 (11 %)	43 (61 %)
Race	White or Caucasian	19 (27 %)	21 (30 %)	9 (13 %)	13 (19 %)	62 (89 %)
	Black or African American	0 (0 %)	0 (0 %)	0 (0 %)	1 (1 %)	1 (1 %)
	Asian	1 (1 %)	0 (0 %)	1 (1 %)	1 (1 %)	3 (4 %)
	American Indian or Alaska Native	0 (0 %)	1 (1 %)	0 (0 %)	0 (0 %)	1 (1 %)
	Native Hawaiian or other Pacific Islander	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Other race	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Unknown race	2 (3 %)	0 (0 %)	0 (0 %)	1 (1 %)	3 (4 %)
Ethnicity	Hispanic or Latinx	18 (26 %)	17 (24 %)	5 (7 %)	8 (11 %)	48 (69 %)
	Not Hispanic or Latinx	4 (6 %)	5 (7 %)	5 (7 %)	7 (10 %)	21 (30 %)
	Unknown ethnicity	0 (0 %)	0 (0 %)	0 (0 %)	1 (1 %)	1 (1 %)

is also expressed on endothelial cells [15], where infection of the endothelial lining has been proposed to contribute to endotheliitis observed in severe COVID-19 [16]. In general, children have comparatively healthier endothelium, potentially contributing to age-related differences in COVID-19 severity [17]. Other studies, however, have found no correlation between proportion of ACE2+ cells and disease severity [18].

Building on these hypotheses, a more robust innate immune response in response to infection with SARS-CoV-2 is observed in children compared to adults [19,20], and a dysregulation of innate immune function, similar to the "inflammaging" reported in aged populations, is observed in severe COVID-19 [21]. Increased efficiency of the adaptive immune system is associated with children, and while adults may mount a more activated response, children are known to maintain higher levels of regulatory cells and memory T-cells [19,22,23]. Increased activation of the adaptive immune system in this context appears to be detrimental and results in increased T-cell exhaustion. In this case, chronic T-cell activation leads to the impaired function commonly observed in older patients, and correlates to COVID-19 disease severity [24–27]. This aberrant regulation of the immune response can also be maintained over time [28], a finding with implications for the investigation of "long-haul COVID-19". This study was designed to investigate the disparity in clinical severity of COVID-19, also considering the impact of aging and ethnicity, through proteomic profiling of patient-derived plasma samples collected in the Keck School of Medicine (KSoM) Biospecimen Repository for COVID-19 at the University of Southern California (USC), representing the spectrum of COVID-19 disease severity.

2. Materials and methods

2.1. Study approval

The study was approved by the institutional review board (IRB) of the University of Southern California (Protocol#: HS-20-00519).

2.1.1. Patient recruitment

Patient plasma samples were collected between May 1, 2020 and June 9, 2021 from patients seen at the Keck Hospital, Verdugo Hills, and Los Angeles (LA) County Hospital and stored in the University of Southern California (USC) COVID-19 Biospecimen Repository. None of the subjects were vaccinated. Samples were not analyzed for SARS-CoV-2 variant. For this study, patients were assigned anonymized, coded IDs and were grouped according to the following cohort definitions: severe, indicating subjects who were admitted to the ICU for COVID-19 treatment; moderate, indicating subjects who were hospitalized for COVID-19 treatment but who were not admitted to the ICU; mild, indicating subjects who tested positive for SARS-CoV-2, but did not require hospitalization; and control, indicating subjects who tested negative for SARS-CoV-2 upon admission to the ICU for treatment of other severe diseases. Population demographics for these cohorts are summarized in Table 1. Participants were predominantly Hispanic/Latinx (69 %), reflecting the demographics of donors available from the source biorepository (57.4 % Hispanic/Latinx, https://sc-ctsi.org/about/covid-19-biorepository). The mean age of participants in this study was 56.1 ± 1.58 years (Supplemental Table S1).

2.1.2. Immunophenotyping

Plasma samples were analyzed for protein expression by Olink proximity extension assays (PEA) for quantification of 184 secreted markers. Olink's Target 96 Inflammation and Target 96 Oncology II panels were chosen for the spread of proteins related to immune response. Each panel consisted of 92 proteins each, of which 6 proteins were included on both panels, resulting in 184 proteins in total and 178 unique proteins. In total, 144 samples were analyzed. Absolute values for protein expression were normalized to internal extension controls per sample, and to median interplate controls and analyte-specific correction factors per assay. All normalization was performed by Olink. Data was returned to researchers as Normalized Protein eXpression (NPX) values, which represent signal of a given protein on a log2 scale, relative to expression of the same protein in other samples. NPX values are not comparable between different proteins.

2.2. Quality control

Olink proprietary controls consisted of internal controls, included in every sample to monitor individual sample quality and data normalization, and external controls, included on every plate for plate quality control and data normalization. Internal controls included 2 non-human antigens as incubation controls for quality control, oligo pair-conjugated IgGs as extension controls to monitor qPCR and for data normalization, and synthetic double-stranded DNA as detection controls to monitor qPCR quality. External controls included a pooled EDTA plasma sample for intra and inter CV calculations , buffer as a negative control and to measure limit of detection (LOD) for each protein and sample plate, and a pool of 92 antibodies conjugated with each oligo pair for data normalization of each assay.

Runs were determined to fail quality control if standard deviations for incubation controls and detection controls were outside the pre-determined quality threshold of <0.2, or if more than 16.6 % of samples failed individual quality control. Samples were determined to fail quality control if incubation controls and/or detection controls deviated ± 0.3 NPX value from the median value across all samples. Four samples from the severe cohort, failed both panels and was excluded. Eight samples, one from the control cohort, four from the moderate cohort, and three from the severe cohort, failed the Oncology II panel and were excluded from analysis of that panel but were included in the analysis of the Inflammation panel. Seven proteins had NPX values under the protein-specific limit of detection (LOD) in >50 % of samples in all cohorts, and were excluded from statistical analysis, leaving 171 unique proteins. The proteins excluded from analysis were IL-2RB, IL-1α, IL-2, β-NGF, IL-13, IL-33, and IL-4.

2.3. Statistics

Post-processing of data and all statistics, including principal component analysis (PCA) and clustering analysis, were performed using the Olink Statistical Analysis app (https://olinkproteomics.shinyapps.io/OlinkStatisticalAnalysis/, version 1.0). Pairwise comparisons between cohorts were conducted using unpaired Student's *t*-tests, performed for each individual protein included in the analysis panels. P-values resulting from this analysis were also adjusted for multiple testing using the Benjamini-Hochberg method. For PCA plotting only, samples with missing NPX values were substituted with the median NPX value from all samples for that assay, then centered at 0 and scaled to a standard deviation of 1 before performing PCA. For heatmap plotting, samples with missing NPX values were substituted with the median NPX value from all samples for that assay, then centered at 0 and scaled to a standard deviation of 1. Hierarchical clustering based on centered and scaled NPX values was performed on both samples and assays to generate heatmaps. Network analysis was performed using the STRING database (STRING Consortium, version 11.5). Protein-protein connections were assigned a combined "score" by evaluating probabilities of interaction derived from literature and database mining, then mapped according to these scores; full description of STRING analysis is described in [29].

3. Results

3.1. Subject demographics and assay quality control

Blood plasma samples were obtained from the USC COVID-19 Biorepository and were collected from subjects seen at the Keck Hospital (52.9 %), Verdugo Hills (12.9 %) and Los Angeles County Hospital (34.3 %) between January 5, 2020 and June 21, 2021. Table 1 provides the core demographics of the subject population that provided samples for this project. The population of biobank donors was predominantly Hispanic/Latinx (57.4 %, https://sc-ctsi.org/about/covid-19-biorepository), with samples unevenly distributed across all categories of COVID-19 severity. In cases requiring hospitalization, >75 % of samples were from Hispanic/Latinx subjects. Subjects were segregated into four independent cohorts based on the hospitalization status of the patient. Categories of severe, moderate, mild and control were based on the following cohorts: 1) severe were COVID-19 positive subjects in the intensive care unit (ICU) being treated for COVID-related illness, 2) moderate were COVID-19 positive subjects that were hospitalized but not requiring ICU treatment, 3) mild were COVID-19 positive subjects that did not require hospitalization and 4) control were COVID-19 negative subjects that were treated in the ICU for other severe illness. The mean age of participants in the study across all categories was 56.1 ± 1.58 years. 26 % of the subjects were over 65 years and 6 % were under 35 years. The mean age within each subject cohort is included in Supplemental Table S2. Overall, 61 % of the subjects were male, 89 % were White/Caucasian, and 69 % were Hispanic/Latinx. For hospitalized COVID-19 patients in the severe and moderate groups, samples were obtained on the day of admission (Day 1), Day 3, Day 5, and Day 7, where available. For the control cohort and mild cohort, the only sample evaluated was day of test/admission (Day 1).

Proteomic analysis of the plasma samples was completed using Olink® proximity extension assays (PEA) for quantification of 184 secreted immunoregulatory biomarkers. The Olink Target 96 Inflammation and Target 96 Oncology II panels were chosen for the spread of proteins related to immune response. Each panel consisted of 92 proteins each, of which 6 proteins were analyzed on both panels, resulting in 184 proteins in total and 178 unique proteins. Samples were determined to fail quality control if internal incubation and detection controls deviated ± 0.3 Normalized Protein eXpression (NPX) value from the median value across all samples. Four samples, from the severe cohort, failed both panels and was excluded. Eight samples, one from the control cohort, four from the moderate cohort, and three from the severe cohort, failed only the Oncology II panel and were excluded from analysis of that panel but were included in the analysis of the Inflammation panel. Seven proteins had NPX values under the protein-specific limit of detection (LOD) in >50 % of samples in all cohorts, and were excluded from downstream analysis, leaving 171 unique proteins.

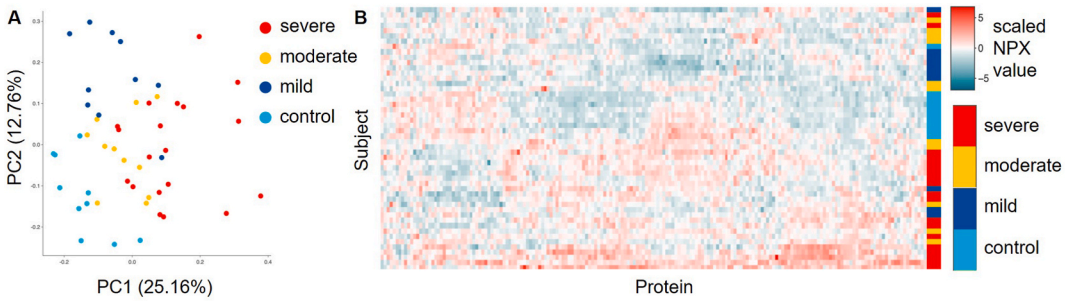


Fig. 1. Plasma protein expression signatures are associated with severity of COVID-19. A) Principal component analysis (PCA) plot comparing samples across all cohorts. B) Heatmap of relative protein expression. Proteins were evaluated through an unbiased hierarchical clustering according to scaled NPX value for each sample.

3.2. Plasma protein expression signatures are associated with severity of COVID-19

We first performed a principal component analysis (PCA) to highlight variation between proteomic signatures of plasma samples collected at Day 1 (Fig. 1A). Samples in the severe COVID-19 cohort (Fig. 1A, red) and moderate cohort (Fig. 1A, yellow) cluster together, but are distinct from mild (Fig. 1A, blue) and control cohorts (Fig. 1A, cyan). Unbiased clustering by protein NPX values is visualized in the heatmap featured in Fig. 1B. Again, the samples clustered by severity, indicating that protein expression signatures exist representing COVID-19 severity in our patient cohorts. To specifically evaluate changes in protein expression and their association with COVID-19 disease severity, pairwise comparisons of the mean NPX values for each protein were used to determine differentially expressed proteins (DEPs) between subject cohorts. Of the 171 unique proteins analyzed, 109 DEPs were observed between our study cohorts. DEPs between each study cohort are summarized in Table 2, and a comprehensive list of the significant DEPs across all study cohorts, as determined by unpaired Student's *t*-tests of NPX value by cohort, is available in the full data set.

3.2.1. Network analysis identifies markers of inflammation and cell proliferation in severe COVID-19

To establish the identity of the proteomic signatures associated with the severity of a subject's response to SARS-CoV-2 infection, we further compared DEPs between subject cohorts. To evaluate the connections within the biological processes associated with the DEPs in our targeted proteomics analysis, we performed STRING network analysis and Ingenuity Pathway Analysis (IPA) focusing specifically on the DEPs between severe COVID-19 and all other cohorts. As expected, two major "hubs" of protein interactions were observed centered around the core of the inflammation and oncology 2 proteomics panels. The inflammation hub is associated with the activation of both Th1 and pro-inflammatory cytokines and chemokines including tumor necrosis factor beta (TNFβ), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-12 (IL-12), CXC motif chemokine ligand 9 (CXCL9), and CC motif chemokine ligand 3 (CCL3), as well as Th2 and anti-inflammatory cytokines and chemokines including interleukin-10 (IL-10), thymic stromal lymphopoietin (TSLP), and CC motif chemokine ligand 20 (CCL20) (Fig. 2A, red cloud). Interleukin-17 (IL-17) signaling and Th1 and Th2 activation pathways were among the most significantly changed pathways specific to severe COVID-19 (Fig. 2B). The "oncology 2" hub clustered around growth factors and growth factor receptors including fibroblast growth factor 5 (FGF-5), colony stimulating factor (CSF), ephrin type-A receptor 2 (EPHA2), protransforming growth factor alpha (TGF-α), and beta-nerve growth factor (β-NGF), suggesting significant activation of tissue regeneration, differentiation and survival signaling pathways (Fig. 2A, green cloud). IPA also identified wound healing and airway pathologies associated with chronic lung disease as some of the most significantly changed pathways specific to severe COVID-19 (Fig. 2B). In conjunction with tissue remodeling, we also identified connections between Fas-associated death domain protein (FADD), Fas ligand (FASLG), TNF-related apoptosis-inducing ligand (TRAIL), and caspase 8 (CASP-8), markers associated with apoptosis. IPA also validated the upregulation of a pathogenic response including granulocyte and agranulocyte adhesion, diapedesis, and pattern recognition receptor pathways (Fig. 2B).

3.3. Markers of inflammation and cell proliferation are expressed significantly higher in severe COVID-19

Volcano plots highlighting significant DEPs between the severe COVID-19 cohort and each of the other subject cohorts show 109 DEPs comparing the severe and mild COVID-19 cohorts (Fig. 3A), 43 DEPs comparing the severe and moderate COVID-19 cohorts (Fig. 3B), and 84 DEPs comparing the severe and control cohorts (Fig. 3C). To determine a protein signature specific to severe COVID-19, DEPs between all paired analyses represented in the volcano plots were overlaid in a Venn diagram (Fig. 3D). In total, 29 DEPs were significant in all comparisons (Table 3). Plots representing the samples included in each cohort highlight a severity-associated decline in the amount of plasma proteins detected, as shown for syndecan-1 (SYND1, Fig. 3E), EN-RAGE (S100A12, Fig. 3F), and mesothelin (MSLN, Fig. 3G).

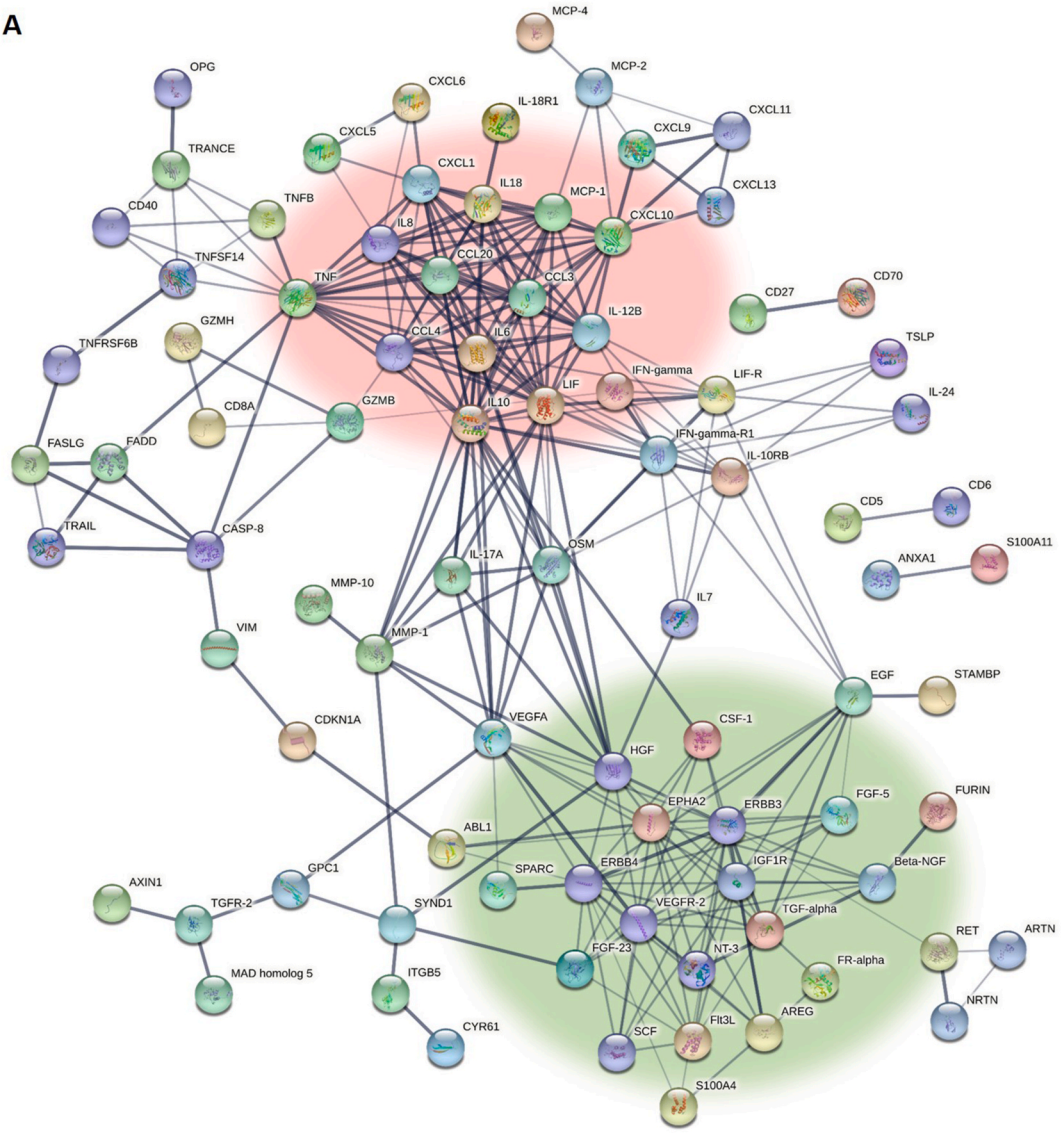
Interestingly, several proteins that were elevated in both severe and moderate COVID-19 on Day 1 differentially resolved over time in the moderate COVID-19 cohort, lowering to levels comparable to the mild cohort by Day 5 while remaining elevated in the severe COVID-19 cohort. These proteins included interleukin-18 receptor 1 (IL18-R1, Fig. 3H), hepatocyte growth factor (HGF, Fig. 3I), and CXC motif chemokine ligand 10 (CXCL10, Fig. 3J). Such changes in the signaling pathways associated with these proteins likely underly the timely resolution of COVID-19 disease.

Ethnicity was established as a risk factor for severe COVID-19 early during the pandemic, following the observation of higher levels of hospitalization and more severe disease outcomes for Hispanic compared to non-Hispanic subjects [30,31]. Given the ethnic

Table 2
Summary of top DEPs comparing all subject cohorts. For each pairwise comparison, total DE indicates the total number of differentially expressed proteins (DEPs); up indicates the number of DEPs in that comparison that were increased, down indicates the number of DEPs in that comparison that were decreased. "Most sig" indicates the most significant DEP in each comparison; % change, p-value, and adjusted (adj.) p-value are listed for each DEP.

Analysis	Total DE	Up	Down	Most sig.	% change	p-value	Adj. p-value
Severe vs mild	109	85	24	EN-RAGE	479.03	4.96E-11	3.86E-09
Severe vs moderate	43	37	6	TNFB	−40.16	1.57E-04	0.0213
Severe vs control	84	77	7	MSLN	258.83	3.69E-08	6.79E-06
Mild vs moderate	65	12	53	IL6	−85.88	7.46E-07	6.82E-05
Mild vs control	87	46	41	TRANCE	155.67	1.97E-07	3.63E-05
Moderate vs control	54	52	2	GZMB	178.31	7.17E-05	0.0049

A



B

Top Canonical Pathways	P-value	Direction
Wound Healing Signaling Pathway	3.69E-21	Up
Airway Pathology in Chronic Obstructive Pulmonary Disease	3.48E-19	N/A
Granulocyte Adhesion and Diapedesis	5.18E-19	N/A
HMGB1 Signaling	2.19E-18	Up
IL-17 Signaling	1.37E-17	Up
Hepatic Fibrosis / Hepatic Stellate Cell Activation	2.48E-17	N/A
Th1 and Th2 Activation Pathway	1.14E-16	N/A
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	8.51E-16	N/A
Th1 Pathway	1.00E-15	Up
Agranulocyte Adhesion and Diapedesis	3.02E-15	N/A

Fig. 2. Network analysis identifies markers of inflammation and cell proliferation in severe COVID-19. A) STRING network analysis map of all significantly differentially expressed proteins (DEPs) in severe vs any other cohort. Lines indicate known associations between proteins. Thickness of line indicates confidence score (minimum = 0.4). Networks of associated markers are highlighted by background clouds, inflammatory markers in red, proliferative markers in green. B) Top 10 most enriched pathways as determined by Ingenuity Pathway Analysis (IPA) of all significant DEPs in severe vs any other cohort. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

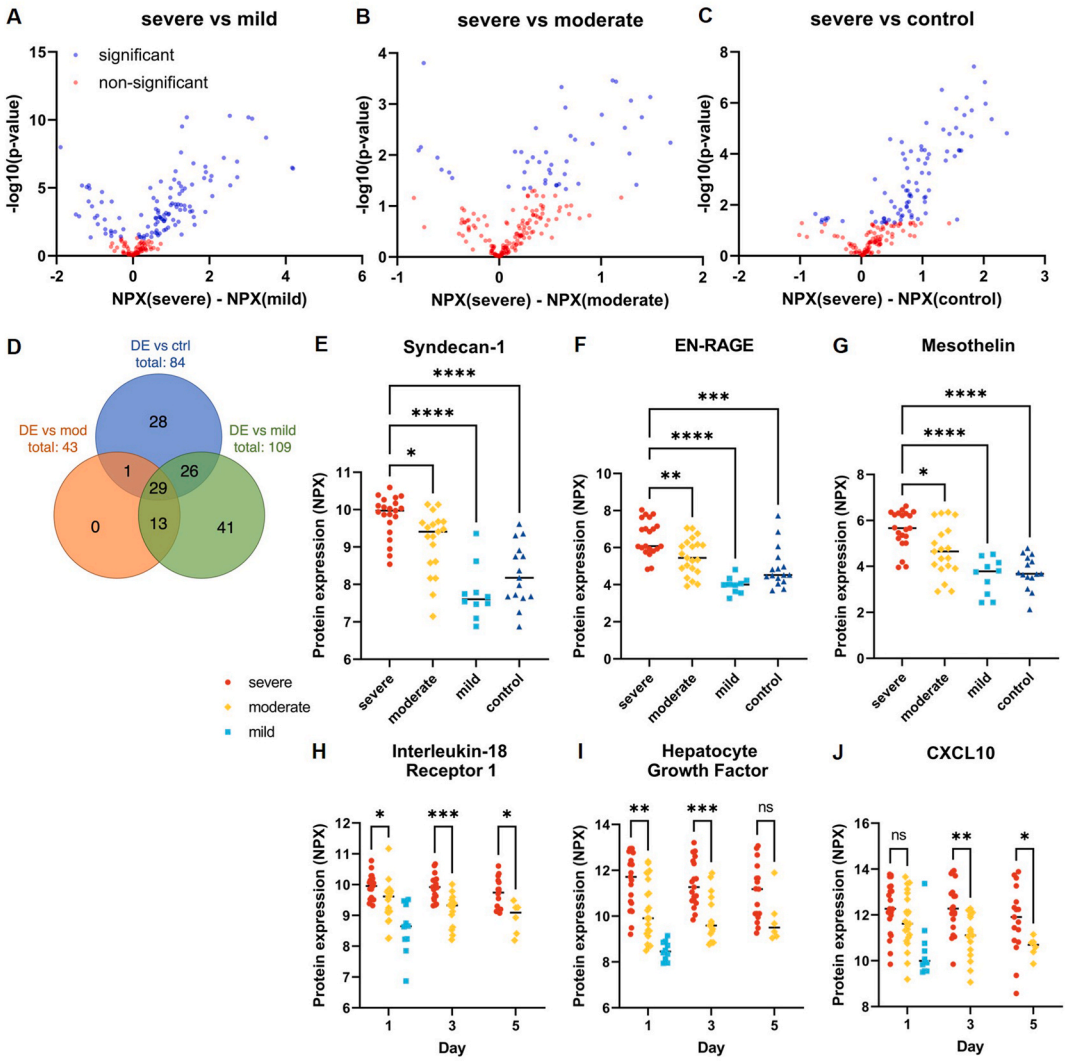


Fig. 3. Markers of inflammation and cell proliferation are expressed significantly higher in severe COVID-19. A-C) Volcano plots showing DEPs between A) severe and mild, B) severe and moderate, and C) severe and control cohorts. Significance was determined by $p < 0.05$. D) Venn diagram overlaying DEPs between severe and mild (green), severe and moderate (orange), and severe and control cohorts (blue). E-G) Cluster plots of normalized protein expression (NPX) values by cohort. E) Syndecan-1, F) EN-RAGE, and G) Mesothelin. H-J) Cluster plots of NPX values in severe and moderate cohorts over collection days 1, 3, and 5. Mild values at day 1 included for reference. H) Interleukin-18 Receptor 1, I) Hepatocyte Growth Factor, and J) CXCL10. For all cluster plots data is presented as: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Black horizontal line indicates the median. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

disparity in the Los Angeles community, the samples in our cohort were majority Hispanic/LatinX (69 %, Table 1), providing an opportunity to evaluate the intersection of ethnicity and severity as characterized by proteomic profiling. 22 significant DEPs were identified between Hispanic and non-Hispanic subjects (Supplemental Table S2). All 22 of these DEPs were also significantly differentially expressed between the severe cohort and at least one other cohort. 21 were significant between severe and mild cohorts, 18 between severe and control cohorts and 14 between severe and moderate cohorts. Disparity in disease outcome has also been associated with sex as a biological variable with males overrepresented in cases having severe complications from COVID-19 [32]. Our subject population comprised of 61 % male and 39 % female subjects. While the control cohort was evenly split, with 50 % male and 50 % female subjects, all COVID-19 cohorts were predominantly male (severe 64 %, moderate 68 % and mild 60 %). Comparing NPX values between male and female subjects we observed no significant DEPs.

3.4. A subset of "protective" proteins are significantly higher in mild disease also correlate to younger age groups

In search of biomarkers potentially associated with an efficient and "protected" response to SARS-CoV-2 infection DEPs unique to the mild COVID-19 cohort were identified. In total, 109 significant DEPs were identified comparing the mild to severe COVID-19

Table 3
DEPs significant to severe COVID-19 versus all other cohorts. List of DEPs that were significant when comparing the severe cohort (N = 22) to mild (N = 10), moderate (N = 22), and control (N = 16) cohorts. % change, p-value, and adjusted (adj.) p-value are listed for each pairwise comparison of each DEP.

Assay	OlinkID	UniProt	severe vs mild			severe vs moderate			severe vs control		
			% change	p-value	adj. p-value	% change	p-value	adj. p-value	% change	p-value	adj. p-value
EN-RAGE	OID00541	P80511	479.03	4.96E-11	3.86E-09	100.95	0.0016	0.0371	202.63	7.21E-05	5.64E-04
WFDC2	OID00732	Q14508	165.74	6.95E-11	3.86E-09	52.97	4.64E-04	0.0213	60.28	9.84E-05	6.71E-04
MCP-3	OID00474	P80098	1021.80	2.06E-09	6.33E-08	142.61	0.0094	0.0823	419.78	1.55E-05	2.02E-04
TRANCE	OID00521	O14788	−73.14	1.03E-08	2.71E-07	−42.19	0.0081	0.0811	−31.33	0.0328	0.0875
AREG	OID00728	P15514	420.32	6.15E-08	1.26E-06	145.19	8.52E-04	0.0261	306.00	1.53E-07	1.41E-05
CEACAM5	OID00739	P06731	560.98	1.19E-07	2.18E-06	179.61	7.28E-04	0.0261	337.96	4.35E-06	8.88E-05
WISP-1	OID00724	O95388	200.20	1.58E-07	2.64E-06	116.47	3.43E-04	0.0213	111.75	1.48E-04	9.05E-04
TNFRSF6B	OID00663	O95407	272.55	2.84E-07	4.36E-06	121.42	3.62E-04	0.0213	72.47	0.0179	0.0547
IL6	OID00666	P05231	1703.12	3.30E-07	4.67E-06	154.57	0.0386	0.1779	196.22	0.0375	0.0932
S100A11	OID00727	P31949	116.92	6.00E-07	7.36E-06	36.77	0.0363	0.1779	109.30	6.03E-06	1.11E-04
SYND1	OID00664	P18827	316.31	1.34E-06	1.45E-05	62.76	0.0042	0.0645	193.66	1.68E-06	5.10E-05
MSLN	OID00660	Q13421	307.86	2.85E-06	2.50E-05	88.97	0.0060	0.0737	258.83	3.69E-08	6.79E-06
IL-24	OID00524	Q13007	130.69	4.37E-06	3.49E-05	58.20	0.0133	0.0974	73.50	0.0038	0.0150
hK8	OID00676	O60259	−55.16	6.62E-06	4.63E-05	−41.32	0.0070	0.0807	−35.80	0.0390	0.0943
EPHA2	OID00695	P29317	139.50	7.72E-06	5.07E-05	56.36	0.0238	0.1326	72.69	0.0043	0.0165
IFN-gamma-R1	OID00670	P15260	37.00	1.02E-05	6.16E-05	21.16	0.0136	0.0974	17.65	0.0462	0.1049
ANXA1	OID00745	P04083	156.17	1.09E-05	6.16E-05	71.61	0.0368	0.1779	115.47	0.0027	0.0112
CD27	OID00703	P26842	96.49	4.99E-05	2.42E-04	42.66	0.0155	0.1059	71.39	0.0012	0.0059
PD-L1	OID00518	Q9NZQ7	127.55	1.57E-04	6.42E-04	56.96	0.0012	0.0308	230.54	6.12E-07	2.81E-05
CXCL13	OID00733	O43927	138.42	1.82E-04	7.13E-04	50.37	0.0373	0.1779	76.95	0.0255	0.0721
IL-18R1	OID00517	Q13478	156.96	3.98E-04	0.0014	37.62	0.0088	0.0811	66.55	0.0027	0.0112
MK	OID00711	P21741	136.17	4.14E-04	0.0014	41.71	0.0228	0.1312	45.59	0.0492	0.1067
GPC1	OID00674	P35052	58.08	6.75E-04	0.0021	40.52	0.0196	0.1245	36.83	0.0388	0.0943
FR-alpha	OID00746	P15328	92.01	8.17E-04	0.0024	47.58	0.0389	0.1779	153.81	1.11E-05	1.70E-04
hK14	OID00690	Q9P0G3	−49.96	0.0016	0.0040	−32.46	0.0194	0.1245	−36.25	0.0474	0.1052
CD8A	OID05124	P01732	−39.17	0.0233	0.0421	−34.26	0.0112	0.0898	−40.45	0.0227	0.0653
CEACAM1	OID00659	P13688	11.27	0.0270	0.0473	6.57	0.0420	0.1839	16.83	9.88E-04	0.0048
S100A4	OID00680	P26447	13.05	0.0390	0.0646	28.37	0.0030	0.0494	58.30	3.45E-05	3.53E-04
Gal-1	OID00697	P09382	15.59	0.0438	0.0713	16.31	0.0087	0.0811	38.78	2.66E-05	2.88E-04

cohorts (Fig. 4A), 65 comparing the mild to moderate COVID-19 cohorts (Fig. 4B), and 87 comparing the mild to control cohorts (Fig. 4C). Overlaying these DEPs in the Venn diagram in Fig. 4D highlights 40 proteins specific to mild COVID-19 (Table 4). Most of these proteins were detected at significantly lower abundance in the mild cohort (80 %, Table 4), likely representative of a tempered immune response and decreased persistence of pro-inflammatory cytokines and chemokines. DEPs that were significantly augmented in the mild subject cohort and downregulated with severity of COVID-19 included TNF superfamily member 11 (TRANCE aka RANK-L, Fig. 4E), FASLG (Fig. 4F), XPNPEP2 (Fig. 4G), and CD207 (Fig. 4H), suggesting expression of these proteins may be associated with a

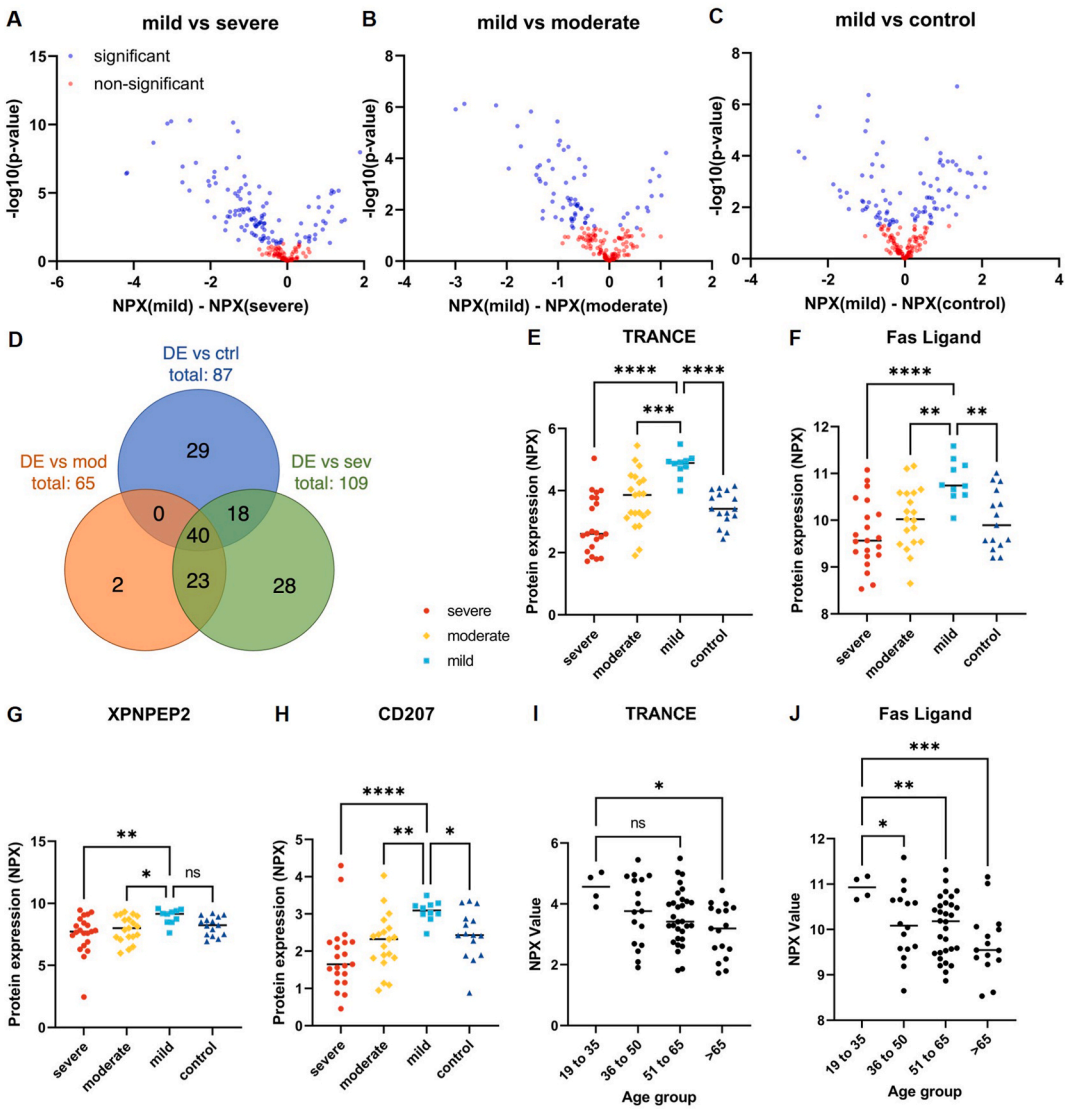


Fig. 4. A subset of "protective" proteins are significantly higher in mild disease also correlate to younger age groups. A-C) Volcano plots showing DEPs between A) mild and severe, B) mild and moderate, and C) mild and control cohorts. Significance was determined by $p < 0.05$. D) Venn diagram overlaying DEPs between mild and severe (green), mild and moderate (orange), and mild and control cohorts (blue). E-I) Cluster plots of normalized protein expression (NPX) values by cohort. E) TRANCE, F) Fas Ligand, G) XPNPEP2, and H) CD207. I-J) Cluster plots of NPX values by age group for I) TRANCE and J) Fas Ligand. For all cluster plots: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Black horizontal line indicates the median. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

stronger response to fighting infection with SARS-CoV-2. In addition, osteoprotegerin (OPG), a decoy receptor for TRANCE, was more highly expressed in the severe cohort (Supplemental Fig. S1). Interestingly, two of these proteins (TRANCE and FASLG) were also expressed at higher levels in the youngest patient cohort (19–35 years) decreasing in expression with age (Fig. 4I and J). Interestingly, while senescence-related proteins were correlated to increased severity of COVID-19, they did not correlate with the age of the subject. This suggests that COVID-19-induced expression of immune senescence-related markers that is un-related to age, supporting recently published data [33,34] (Supplemental Fig. S2). This finding is consistent with age being a predominant co-morbidity associated with COVID-19 [32,35] and suggests that circulating levels of TRANCE and FASLG may be associated with a more effective response to SARS-CoV-2 infection.

4. Discussion

One of the major challenges in managing the COVID-19 pandemic is the wide variation in disease severity, ranging from mild respiratory symptoms that resolve with minimal outpatient treatment to acute respiratory distress syndrome (ARDS) requiring ICU

Table 4
DEPs significant to mild COVID-19 versus every other cohort. List of DEPs that were significant when comparing the mild cohort (N = 10) to severe (N = 22), moderate (N = 22), and control (N = 16) cohorts. % change, p-value, and adjusted (adj.) p-value are listed for each pairwise comparison of each DEP.

Assay	OlinkID	UniProt	mild vs severe			mild vs moderate			mild vs control		
			% change	p-value	adj. p-value	% change	p-value	adj. p-value	% change	p-value	adj. p-value
EN-RAGE	OID00541	P80511	−82.73	4.96E-11	3.86E-09	−65.30	1.48E-06	6.82E-05	−47.74	0.0064	0.0227
WFDC2	OID00732	Q14508	−62.37	6.95E-11	3.86E-09	−42.44	3.52E-05	6.48E-04	−39.69	8.45E-05	0.0014
HGF	OID00706	P14210	−88.53	8.40E-11	3.86E-09	−69.72	3.32E-05	6.48E-04	−79.34	2.71E-06	1.25E-04
TGF-alpha	OID00687	P01135	−58.98	3.02E-10	1.11E-08	−48.61	2.00E-05	5.26E-04	−48.09	4.24E-07	3.90E-05
MCP-3	OID00474	P80098	−91.09	2.06E-09	6.33E-08	−78.37	8.46E-07	6.82E-05	−53.67	0.0056	0.0209
TRANCE	OID00521	O14788	272.35	1.03E-08	2.71E-07	115.24	6.09E-05	9.33E-04	155.67	1.97E-07	3.63E-05
WISP-1	OID00724	O95388	−66.69	1.58E-07	2.64E-06	−27.89	0.0312	0.0896	−29.46	0.0052	0.0201
TNFRSF6B	OID00663	O95407	−73.16	2.84E-07	4.36E-06	−40.57	0.0062	0.0285	−53.71	5.69E-04	0.0039
IL6	OID00666	P05231	−94.45	3.30E-07	4.67E-06	−85.88	7.46E-07	6.82E-05	−85.21	6.74E-05	0.0014
IL8	OID00471	P10145	−73.35	6.95E-07	7.99E-06	−62.73	4.34E-04	0.0036	−52.82	0.0061	0.0224
FGF-BP1	OID00713	Q14512	−84.93	1.68E-06	1.71E-05	−51.61	0.0193	0.0647	−72.46	0.0013	0.0075
MUC-16	OID00741	Q8WXI7	−72.57	1.89E-06	1.83E-05	−62.14	2.22E-04	0.0024	−69.02	0.0022	0.0107
OPG	OID00479	O00300	−52.82	2.44E-06	2.24E-05	−37.99	5.67E-04	0.0043	−44.38	2.13E-04	0.0021
TNFSF13	OID00661	O75888	−39.67	3.37E-06	2.81E-05	−28.53	4.33E-04	0.0036	−33.12	2.48E-04	0.0022
IL-24	OID00524	Q13007	−56.65	4.37E-06	3.49E-05	−31.42	0.0092	0.0361	−24.79	0.0481	0.0983
CDCP1	OID00476	Q9H5V8	−65.83	5.76E-06	4.42E-05	−55.91	1.22E-04	0.0016	−36.19	0.0265	0.0626
OSM	OID00494	P13725	−82.93	6.68E-06	4.63E-05	−74.33	2.44E-04	0.0024	−64.89	0.0027	0.0124
CTSV	OID00702	O60911	152.12	6.80E-06	4.63E-05	95.93	4.82E-04	0.0039	136.17	1.65E-04	0.0019
EPHA2	OID00695	P29317	−58.25	7.72E-06	5.07E-05	−34.71	0.0059	0.0283	−27.90	0.0168	0.0442
CD207	OID00730	Q9UJ71	131.27	8.62E-06	5.47E-05	79.74	2.56E-04	0.0024	54.40	0.0041	0.0165
ESM-1	OID00729	Q9NQ30	−49.64	1.04E-05	6.16E-05	−32.01	0.0083	0.0333	−49.00	4.22E-06	1.55E-04
FASLG	OID00694	P48023	120.35	1.10E-05	6.16E-05	73.62	7.64E-04	0.0053	78.87	7.82E-04	0.0050
CXCL9	OID00490	Q07325	−76.28	4.09E-05	2.03E-04	−61.26	6.19E-04	0.0046	−50.72	0.0113	0.0347
CSF-1	OID00562	P09603	−31.31	5.61E-05	2.65E-04	−27.86	2.17E-04	0.0024	−32.77	2.95E-05	6.79E-04
LIF-R	OID00511	P42702	−35.27	9.60E-05	4.42E-04	−33.54	1.06E-04	0.0015	−32.45	0.0023	0.0107
IL-17C	OID00483	Q9P0M4	−60.74	2.02E-04	7.60E-04	−53.51	2.53E-04	0.0024	−38.66	0.0197	0.0491
SCF	OID00684	P21583	110.67	2.31E-04	8.49E-04	66.19	0.0081	0.0333	55.96	0.0391	0.0838
TFPI-2	OID00675	P48307	−64.39	2.36E-04	8.53E-04	−47.41	0.0242	0.0719	−49.35	0.0097	0.0318
DLL1	OID00710	O00548	−34.07	3.67E-04	0.0013	−18.53	0.0425	0.1152	−23.53	0.0070	0.0243
MK	OID00711	P21741	−57.66	4.14E-04	0.0014	−39.99	0.0233	0.0703	−38.35	0.0492	0.0994
IL10	OID00528	P22301	−65.88	5.04E-04	0.0016	−45.98	4.64E-05	7.75E-04	−45.16	6.39E-04	0.0042

(*continued on next page*)

Table 4 (*continued*)

Assay	OlinkID	UniProt	mild vs severe			mild vs moderate			mild vs control		
			% change	p-value	adj. p-value	% change	p-value	adj. p-value	% change	p-value	adj. p-value
CCL20	OID00556	P78556	−64.67	5.51E-04	0.0017	−60.72	7.74E-04	0.0053	−68.52	0.0039	0.0164
LIF	OID00547	P15018	−43.38	9.81E-04	0.0028	−27.96	0.0317	0.0896	−29.31	0.0308	0.0701
XPNPEP2	OID00704	O43895	165.62	0.0012	0.0033	102.11	0.0027	0.0163	70.18	0.0108	0.0343
IL-17A	OID00485	Q16552	−38.97	0.0014	0.0036	−50.45	3.62E-06	1.33E-04	−36.69	0.0154	0.0412
ARTN	OID00526	Q5T4W7	−37.78	0.0014	0.0036	−25.38	0.0172	0.0586	−36.57	0.0423	0.0884
VEGFR-2	OID00677	P35968	28.21	0.0017	0.0041	16.02	0.0387	0.1080	47.79	2.15E-05	5.64E-04
FGF-23	OID00507	Q9GZV9	−67.37	0.0053	0.0111	−39.68	0.0426	0.1152	−63.49	0.0115	0.0348
FGF-5	OID00509	P12034	−23.75	0.0071	0.0146	−21.09	0.0099	0.0381	−18.92	0.0369	0.0799
EGF	OID00662	P01133	65.34	0.0109	0.0221	68.58	0.0112	0.0413	120.04	0.0022	0.0107

admission and mechanical ventilation. The phenomena underlying this disparity in disease progression are still poorly understood. In this study, we present data, utilizing the Olink immunoassay, which characterizes COVID-19 disease response by severity, evaluating protein expression beyond the typical analysis of inflammation-associated proteins. Previous studies utilizing the Olink immunoassay platform have focused on assessing markers of inflammation, organ damage, and cardiovascular and cardiometabolic health, see Supplemental Table S3 [36–39]. In addition, there are a few studies that have taken a quantitative proteomics approach to evaluating both COVID-19 severity and changes that are associated with post-COVID-19 sequalae [37,40–43]. One study took a similar approach to compare disease severity, trajectory and recovery [43] and highlighted protein biomarker candidates interesting findings that centered around perturbation of ECM related proteins and TNF signaling that was maintained in patients post-COVID-19 suggesting persistence of tissue remodeling after COVID-19 infection [43]. In agreement with our data, pro-inflammatory cytokines, CXCL10 and IL-6 have been frequently highlighted in these studies to be significantly associated with severe disease [44–47]. Several of these have ongoing clinical trials evaluating the effectiveness of regulating these proteins in preventing progression to severe COVID-19 and associated ARDS. Furthermore, our data correlates with previous findings showing a COVID-19-specific elevation of inflammatory signaling pathways [48]. SYND1 and EN-RAGE, markers associated with COVID-19 disease severity and mortality [36,49–51] were among the most significantly elevated markers in our severe cohort. The additional inclusion of a panel evaluating markers of tissue growth and repair, led to the identification of markers of cell proliferation in diverse tissue types including the endothelium, central nervous system, and mesothelium to be significantly associated with severe COVID-19, highlighting significant differences in the regulation of tissue damage and repair in patients with varied responses to SARS-CoV-2 infection. Additionally, while other studies focused on markers significantly enriched in severe disease, we find a subset of markers associated with T-cell activity, dendritic cells, and bradykinin signaling significantly enriched among those with mild disease that may be involved in protection from severe disease.

SYND1 is a heparan sulfate proteoglycan, involved in leukocyte recruitment, vascular repair, and tumor angiogenesis [52]. Increased expression of this marker is associated with acute endothelial glycocalyx degradation, as well as higher oxygen and mechanical life support requirements and mortality from COVID-19 [53–56]. In addition to its significance as a potent marker of endothelial damage during the pathogenesis of COVID-19 [57], both facilitation of viral entry via ACE2 co-localization [58] and transmission of virus to epithelial cells via binding with dendritic cells [59] have been proposed as mechanisms by which SYND1 actively modulates SARS-CoV-2 infection, making it an important candidate as both a biomarker and a potential target for therapeutic intervention.

EN-RAGE is a pro-inflammatory calcium-binding protein associated with a variety of inflammatory conditions across organ systems, including rheumatoid and psoriatic arthritis, coronary heart disease, cystic fibrosis, autoimmune hepatitis, and Kawasaki disease [60–64]. Elevated levels of EN-RAGE have been shown to correlate to increased inflammation in response to COVID-19 [65], including in asymptomatic cases up to 8 months after infection [66]. It has also been found elevated in the blood plasma of subjects with chronic obstructive pulmonary disease (COPD) versus healthy controls [67], a disease which is associated with higher risk of hospitalization, ICU admission, and mortality from COVID-19 [68]. EN-RAGE has been explored in the context of COVID-19 in connection with other major inflammatory mechanisms that have been implicated in COVID-19 disease severity, including the renin-angiotensin system [69,70], T-cell associated cytokines [71], and a dysregulated macrophage population [72]. The variety of mechanisms that EN-RAGE intersects with suggests that further experiments are needed to define its precise role in increasing COVID-19 severity.

In addition to inflammatory pathways which have been previously reported and an ongoing focus of research to target the "cytokine storm" associated with progression to ARDS [73–75], our analysis also identified a cluster of growth factors and associated proteins that are significantly and specifically elevated in severe COVID-19, including FGF-5, CSF, EPHA2, TGF-α, and β-NGF. These proteins have known roles in cell proliferation of multiple tissue types, including hair growth [76], macrophage and monocyte cell populations [77], lymphatic endothelial cells [78], and the central nervous system [79], as well as various cancers [80–84], suggesting a dysregulated attempt to repair tissues in response to damage caused by viral infection. Fatal COVID-19 is marked by severe pulmonary damage, diffuse alveolar damage, in conjunction with endotheliitis and microthrombosis [85,86]. This damage is compounded by a dysregulated and ineffective attempt at tissue repair, with loss of basal cell populations, squamous cell metaplasia, fibrogenesis,

red blood cell dysfunction and coagulation, and increased cellular senescence of epithelial and endothelial cells [33,87]. To overcome this ineffective tissue repair, several studies have suggested mesenchymal stem cell therapy as a treatment for COVID-19 [88,89]. The cell proliferation and growth markers identified in this study may have roles in regulating, and dysregulating, the process of tissue repair in COVID-19.

Of these growth markers, mesothelin (MSLN) is particularly interesting. Mesothelin is a differentiation antigen that has been almost exclusively studied in the context of malignant cancers such as mesothelioma, renal carcinoma, pancreatic cancer, ovarian cancer, and lung adenocarcinoma [90,91], and was one of the most significantly elevated proteins in our dataset. While it is an attractive target for cancer immunotherapies due to its overexpression on cancer cells versus low expression on normal human tissue [92], its function under normal physiological conditions is still largely unknown. It has been shown to bind mucin 16 (MUC16, aka CA125) [93], and this relationship has been suggested to contribute to metastasis [94]; however, mouse models have shown it is not required for normal development or reproduction [95], and its biological function remains ambiguous. More research is required to not only identify the mechanism by which mesothelin augments COVID-19 pathogenesis, but also the biological role of mesothelin in the lung.

Also particularly interesting are a subset of proteins significantly elevated in milder COVID-19 disease versus severe (TRANCE, FASLG, XPNPEP2, and CD207), these represent an "efficient" disease response and improved prognoses. TRANCE and FASLG were also significantly elevated in response to SARS-CoV-2 infection in subjects 18–35 years old compared to those over 65 years old. These two proteins are already known to have critical roles in regulation of the T-cell response to viral infection, and activation of the signaling pathways associated with these proteins would indicate that a robust induction of T-cell activity has taken place in response to viral infection. As mentioned above, TRANCE and FASLG are both markers T-cell activity and it is established that T-cell exhaustion is associated with more severe COVID-19 disease [48]. TRANCE is upregulated in T-cells following antigen receptor stimulation, and promotes dendritic cell-mediated stimulation of naïve T-cells [96]; furthermore, mutations in TRANCE have been found to associate with higher chronicity of other viral infections [97]. While upregulation has previously been reported to be associated with severe COVID-19, and chronically elevated FASLG is known to increase with aging [98,99], none of these studies have directly compared circulating protein levels in severe cases to mild and moderate disease and chronic elevation may correlate to an inadequate T-cell response to viral infection in older people. Additionally, while these studies suggest that elevated expression of FASLG is indicative of its role in T-cell- and NK-cell-mediated apoptosis, FASLG also functions as a modulator of T-cell differentiation through non-apoptotic signaling, and facilitates the clearance of activated T-cells and B-cells cells [100] as well as promoting the resolution of type 2 lung inflammation [101,102]. Therefore, a multifaceted role for elevated FASLG expression in COVID-19 is not necessarily contradictory, and this pathway has been previously proposed as a mechanism behind the abnormal T-cell activity and subsequent exhaustion observed in severe cases of the disease [98,103]. Additionally, other published studies have either only focused on mRNA concentrations and not protein [104], or compared "severe" ICU patients to "moderate" non-ICU hospitalized patients [105], and not to "mild" cases. The lower levels observed in severe and moderate cohorts in this study may reflect the conclusion of the process of T-cell exhaustion, while the mild cohort maintains T-cell activation and proliferation at the time of sample collection. Our data suggests that a significantly higher activation of FASLG in response to infection occurs in milder disease. Therefore, the significantly augmented levels of FASLG protein associated with severity and age suggest that circulating levels of these proteins may correlate to a more effective response to SARS-CoV-2 infection. Our data underlines the potential importance of these proteins as targets for stimulating an effective response to SARS-CoV-2 infection and require further investigation in this context.

XPNPEP2, a bradykinin-degrading hydrolase, is heavily associated with ACE activity [106], and variants in the coding gene are associated with higher risk for ACE-inhibitor induced angioedema [107,108]. Given that a "bradykinin storm" has been implicated in elevating disease severity during SARS-CoV-2 infection [109–111], and given XPNPEP2's function in degrading bradykinin, elevated XPNPEP2 expression may have a potential protective effect in decreasing the inflammatory effects of bradykinin signaling, an effect supported by the data presented in this study. Due to its association with ACE, XPNPEP2 has been previously identified as potentially involved in SARS-CoV-2 infection in exploratory analyses using protein-protein interaction network databases [112,113], and has been significantly associated with asymptomatic COVID-19 versus both symptomatic COVID-19 and healthy controls in pregnant women [114]. However, XPNPEP2 has not yet been thoroughly investigated as a modulator of COVID-19 disease severity.

CD207, also known as langerin, is involved in efficient antigen presentation in DCs [115] and has been shown to bind the SARS-CoV-2 spike protein [116], suggesting that it may play a role in viral entry, though this role has not been investigated further. However, studies showing that impairment of DC numbers and function is associated with severe COVID-19, and that this impairment can persist long after resolution of infection [117,118], along with observation of an increase in mature DCs in the bronchoalveolar lavage fluid of COVID-19 patients versus healthy controls [119], support an important role for DCs in mediating COVID-19 disease response. In addition, our data supports further investigation into CD207's interactions with SARS-CoV-2 and its role in efficient DC-mediated disease response.

It is important to note that diagnosis of COVID-19 for those patients with a positive SARS-CoV-2 test did not include determination of variant; therefore, variants were not assessed in this study. Indeed, samples were collected before many of the SARS-CoV-2 variants were in circulation (between 2020 and 2021), therefore more recent variants including omicron and delta are unlikely to be considered. As the pandemic progresses, new variants may result in nuanced differences in disease responses, which reduces the specificity of this study's results to current variants of concern. The patient cohort available for this study was small and therefore the impact of co-morbidities, treatment responses, nutritional status and physical activity, was not evaluated; the high variability across the patient cohort did not yield data that could be statistically evaluated. Finally, while correlations were made between individual proteins that have interactions previously well-established in literature, direct interactions were not investigated in the context of SARS-CoV-2 infection. Further investigation is required into the mechanisms by which the proteins highlighted in this study contribute to the pathogenesis of severe COVID-19.

In conclusion, analysis of protein concentration in plasma samples of patients infected with SARS-CoV-2 and presenting with differential severity of disease has identified proteins as biomarkers of both severe and effective responses to viral infection in mild cases. Our findings simultaneously assess a broad range of physiological responses to the disease, finding significant markers associated with multiple inflammatory pathways, viral entry and membrane fusion, endothelial damage, and cell proliferation and tissue repair, highlighting the complexity of COVID-19 pathogenesis. These proteins and associated signaling pathways are potential targets for therapeutic intervention to both stimulate an effective response to SARS-CoV-2 infection or to prevent progression to severe disease and warrant further investigation.

Data availability statement

As there is no applicable publicly available database to deposit our Olink proteomics dataset into we have made the Normalized Protein Expression (NPX) data for all samples available on Figshare at the following link. https://figshare.com/s/d136a74ef05c3dfa3a21.

CRediT authorship contribution statement

Noa C. Harriott: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Amy L. Ryan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was made possible by the USC COVID-19 Biospecimen Repository, which provided patient plasma samples. This study was supported by a Keck School of Medicine (KSoM) COVID-19 research fund and the Hastings Foundation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23320.

References

[1] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, et al., A novel coronavirus from patients with pneumonia in China, 2019, N. Engl. J. Med. 382 (2020) 727–733, https://doi.org/10.1056/NEJMoa2001017.
[2] W.J. Guan, Z.Y. Ni, Y. Hu, W.H. Liang, C.Q. Ou, J.X. He, L. Liu, H. Shan, C.L. Lei, D.S.C. Hui, et al., Clinical characteristics of coronavirus disease 2019 in China, N. Engl. J. Med. 382 (2020) 1708–1720, https://doi.org/10.1056/NEJMoa2002032.
[3] J. Song, Y.K. Deng, H. Wang, Z.C. Wang, B. Liao, J. Ma, C. He, L. Pan, Y. Liu, I. Alobid, et al., Self-reported taste and smell disorders in patients with COVID-19: distinct features in China, Curr Med Sci 41 (2021) 14–23, https://doi.org/10.1007/s11596-021-2312-7.
[4] D. Wang, B. Hu, C. Hu, F. Zhu, X. Liu, J. Zhang, B. Wang, H. Xiang, Z. Cheng, Y. Xiong, et al., Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in wuhan, China, JAMA 323 (2020) 1061–1069, https://doi.org/10.1001/jama.2020.1585.
[5] V. Rass, R. Beer, A.J. Schiefecker, A. Lindner, M. Kofler, B.A. Ianosi, P. Mahlknecht, B. Heim, M. Peball, F. Carbone, et al., Neurological outcomes 1 year after COVID-19 diagnosis: a prospective longitudinal cohort study, Eur. J. Neurol. 29 (2022) 1685–1696, https://doi.org/10.1111/ene.15307.
[6] D.A.P. de Miranda, S.V.C. Gomes, P.S. Filgueiras, C.A. Corsini, N.B.F. Almeida, R.A. Silva, M. Medeiros, R.V.R. Vilela, G.R. Fernandes, R.F.Q. Grenfell, Long COVID-19 syndrome: a 14-months longitudinal study during the two first epidemic peaks in Southeast Brazil, Trans. R. Soc. Trop. Med. Hyg. (2022), https://doi.org/10.1093/trstmh/trac030.
[7] A.D. Desai, M. Lavelle, B.C. Boursiquot, E.Y. Wan, Long-term complications of COVID-19, Am J Physiol Cell Physiol 322 (2022) C1–C11, https://doi.org/10.1152/ajpcell.00375.2021.
[8] Z. Wu, J.M. McGoogan, Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese center for disease control and prevention, JAMA 323 (2020) 1239–1242, https://doi.org/10.1001/jama.2020.2648.
[9] E. Schafer, C. Scheer, K. Salje, A. Fritz, T. Kohlmann, N.O. Hubner, M. Napp, L. Fiedler-Lacombe, D. Stahl, B. Rauch, et al., Course of disease and risk factors for hospitalization in outpatients with a SARS-CoV-2 infection, Sci. Rep. 12 (2022) 7249, https://doi.org/10.1038/s41598-022-11103-0.
[10] H.N. Buttenschon, V. Lynggaard, S.G. Sandbol, E.N. Glassou, A. Haagerup, Comparison of the clinical presentation across two waves of COVID-19: a retrospective cohort study, BMC Infect. Dis. 22 (2022) 423, https://doi.org/10.1186/s12879-022-07413-3.
[11] M. Letko, A. Marzi, V. Munster, Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses, Nat Microbiol 5 (2020) 562–569, https://doi.org/10.1038/s41564-020-0688-y.
[12] M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Kruger, T. Herrler, S. Erichsen, T.S. Schiergens, G. Herrler, N.H. Wu, A. Nitsche, et al., SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor, Cell 181 (2020) 271–280 e278, https://doi.org/10.1016/j.cell.2020.02.052.
[13] M. Singh, V. Bansal, C. Feschotte, A single-cell RNA expression map of human coronavirus entry factors, Cell Rep. 32 (2020), 108175, https://doi.org/10.1016/j.celrep.2020.108175.

[14] T. Abrehart, R. Suryadinata, C. McCafferty, J. Jacobson, V. Ignjatovic, P. Robinson, N.W. Crawford, P. Monagle, K. Subbarao, C. Satzke, D. Wurzel, Age-related differences in SARS-CoV-2 binding factors: an explanation for reduced susceptibility to severe COVID-19 among children? Paediatr. Respir. Rev. (2022) https://doi.org/10.1016/j.prrv.2022.01.008.

[15] C.M. Ferrario, J. Jessup, M.C. Chappell, D.B. Averill, K.B. Brosnihan, E.A. Tallant, D.I. Diz, P.E. Gallagher, Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2, Circulation 111 (2005) 2605–2610, https://doi.org/10.1161/CIRCULATIONAHA.104.510461.

[16] Z. Varga, A.J. Flammer, P. Steiger, M. Haberecker, R. Andermatt, A.S. Zinkernagel, M.R. Mehra, R.A. Schuepbach, F. Ruschitzka, H. Moch, Endothelial cell infection and endotheliitis in COVID-19, Lancet 395 (2020) 1417–1418, https://doi.org/10.1016/S0140-6736(20)30937-5.

[17] P. Zimmermann, N. Curtis, Why does the severity of COVID-19 differ with age?: understanding the mechanisms underlying the age gradient in outcome following SARS-CoV-2 infection, Pediatr. Infect. Dis. J. 41 (2022) e36–e45, https://doi.org/10.1097/INF.0000000000003413.

[18] D. Ma, S. Liu, L. Hu, Q. He, W. Shi, D. Yan, Y. Cao, G. Zhang, Z. Wang, J. Wu, C. Jiang, Single-cell RNA sequencing identify SDCBP in ACE2-positive bronchial epithelial cells negatively correlates with COVID-19 severity, J. Cell Mol. Med. 25 (2021) 7001–7012, https://doi.org/10.1111/jcmm.16714.

[19] J. Loske, J. Rohmel, S. Lukassen, S. Stricker, V.G. Magalhaes, J. Liebig, R.L. Chua, L. Thurmann, M. Messingschlager, A. Seegebarth, et al., Pre-activated antiviral innate immunity in the upper airways controls early SARS-CoV-2 infection in children, Nat. Biotechnol. 40 (2022) 319–324, https://doi.org/10.1038/s41587-021-01037-9.

[20] C.A. Pierce, S. Sy, B. Galen, D.Y. Goldstein, E. Orner, M.J. Keller, K.C. Herold, B.C. Herold, Natural mucosal barriers and COVID-19 in children, JCI Insight 6 (2021), https://doi.org/10.1172/jci.insight.148694.

[21] J.L. Schultze, A.C. Aschenbrenner, COVID-19 and the human innate immune system, Cell 184 (2021) 1671–1692, https://doi.org/10.1016/j.cell.2021.02.029.

[22] M.R. Petrara, F. Bonfante, P. Costenaro, A. Cantarutti, F. Carmona, E. Ruffoni, C. Di Chiara, M. Zanchetta, L. Barzon, D. Dona, et al., Asymptomatic and mild SARS-CoV-2 infections elicit lower immune activation and higher specific neutralizing antibodies in children than in adults, Front. Immunol. 12 (2021), 741796, https://doi.org/10.3389/fimmu.2021.741796.

[23] C.A. Cohen, A.P.Y. Li, A. Hachim, D.S.C. Hui, M.Y.W. Kwan, O.T.Y. Tsang, S.S. Chiu, W.H. Chan, Y.S. Yau, N. Kavian, et al., SARS-CoV-2 specific T cell responses are lower in children and increase with age and time after infection, Nat. Commun. 12 (2021) 4678, https://doi.org/10.1038/s41467-021-24938-4.

[24] C.U. Blank, W.N. Haining, W. Held, P.G. Hogan, A. Kallies, E. Lugli, R.C. Lynn, M. Philip, A. Rao, N.P. Restifo, et al., Defining 'T cell exhaustion, Nat. Rev. Immunol. 19 (2019) 665–674, https://doi.org/10.1038/s41577-019-0221-9.

[25] S. Palmer, N. Cunniffe, R. Donnelly, COVID-19 hospitalization rates rise exponentially with age, inversely proportional to thymic T-cell production, J R Soc Interface 18 (2021), 20200982, https://doi.org/10.1098/rsif.2020.0982.

[26] P.H. Yang, Y.B. Ding, Z. Xu, R. Pu, P. Li, J. Yan, J.L. Liu, F.P. Meng, L. Huang, L. Shi, et al., Increased circulating level of interleukin-6 and CD8(+) T cell exhaustion are associated with progression of COVID-19, Infect Dis Poverty 9 (2020) 161, https://doi.org/10.1186/s40249-020-00780-6.

[27] J. Westmeier, K. Paniskaki, Z. Karakose, T. Werner, K. Sutter, S. Dolff, M. Overbeck, A. Limmer, J. Liu, X. Zheng, et al., Impaired cytotoxic CD8(+) T cell response in elderly COVID-19 patients, mBio 11 (2020), https://doi.org/10.1128/mBio.02243-20.

[28] J.K. Files, S. Boppana, M.D. Perez, S. Sarkar, K.E. Lowman, K. Qin, S. Sterrett, E. Carlin, A. Bansal, S. Sabbaj, et al., Sustained cellular immune dysregulation in individuals recovering from SARS-CoV-2 infection, J. Clin. Invest. 131 (2021), https://doi.org/10.1172/JCI140491.

[29] C. von Mering, L.J. Jensen, B. Snel, S.D. Hooper, M. Krupp, M. Foglierini, N. Jouffre, M.A. Huynen, P. Bork, STRING: known and predicted protein-protein associations, integrated and transferred across organisms, Nucleic Acids Res 33 (Database issue) (2005), D433-7, https://doi.org/10.1093/nar/gki005.

[30] B.D. Renelus, N.C. Khoury, K. Chandrasekaran, E. Bekele, W.M. Briggs, A. Ivanov, S.R. Mohanty, D.S. Jamorabo, Racial disparities in COVID-19 hospitalization and in-hospital mortality at the height of the New York city pandemic, J Racial Ethn Health Disparities 8 (2021) 1161–1167, https://doi.org/10.1007/s40615-020-00872-x.

[31] G. Chiumento, K. Yousey-Hindes, A. Edmundson, J.L. Hadler, Persistence of racial/ethnic and socioeconomic status disparities among non-institutionalized patients hospitalized with COVID-19 in Connecticut, July to December 2020, Influenza Other Respir Viruses 16 (2022) 532–541, https://doi.org/10.1111/irv.12945.

[32] T. Mikami, H. Miyashita, T. Yamada, M. Harrington, D. Steinberg, A. Dunn, E. Siau, Risk factors for mortality in patients with COVID-19 in New York city, J. Gen. Intern. Med. 36 (2021) 17–26, https://doi.org/10.1007/s11606-020-05983-z.

[33] F. D'Agnillo, K.A. Walters, Y. Xiao, Z.M. Sheng, K. Scherler, J. Park, S. Gygli, L.A. Rosas, K. Sadtler, H. Kalish, et al., Lung epithelial and endothelial damage, loss of tissue repair, inhibition of fibrinolysis, and cellular senescence in fatal COVID-19, Sci. Transl. Med. 13 (2021), eabj7790, https://doi.org/10.1126/scitranslmed.abj7790.

[34] U. Tripathi, R. Nchioua, L. Prata, Y. Zhu, E.O.W. Gerdes, N. Giorgadze, T. Pirtskhalava, E. Parker, A. Xue, J.M. Espindola-Netto, et al., SARS-CoV-2 causes senescence in human cells and exacerbates the senescence-associated secretory phenotype through TLR-3, Aging (Albany NY) 13 (2021) 21838–21854, https://doi.org/10.18632/aging.203560.

[35] G. Suleyman, R. Fadel, I. Brar, R. Kassab, R. Khansa, N. Sturla, A. Alsaadi, K. Latack, J. Miller, R. Tibbetts, et al., Risk factors associated with hospitalization and death in COVID-19 breakthrough infections, Open Forum Infect. Dis. 9 (2022) ofac116, https://doi.org/10.1093/ofid/ofac116.

[36] C.D. Russell, A. Valanciute, N.N. Gachanja, J. Stephen, R. Penrice-Randal, S.D. Armstrong, S. Clohisey, B. Wang, W. Al Qsous, W.A. Wallace, et al., Tissue proteomic analysis identifies mechanisms and stages of immunopathology in fatal COVID-19, Am. J. Respir. Cell Mol. Biol. 66 (2022) 196–205, https://doi.org/10.1165/rcmb.2021-0358OC.

[37] M. Zoodsma, A.H. de Nooijer, I. Grondman, M.K. Gupta, A. Bonifacius, V. Koeken, E. Kooistra, G. Kilic, O. Bulut, N. Godecke, et al., Targeted proteomics identifies circulating biomarkers associated with active COVID-19 and post-COVID-19, Front. Immunol. 13 (2022), 1027122, https://doi.org/10.3389/fimmu.2022.1027122.

[38] A. Razaghi, A. Szakos, M. Alouda, B. Bozoky, M. Bjornstedt, L. Szekely, Proteomic analysis of pleural effusions from COVID-19 deceased patients: enhanced inflammatory markers, Diagnostics 12 (2022), https://doi.org/10.3390/diagnostics12112789.

[39] D. Feyaerts, J. Hedou, J. Gillard, H. Chen, E.S. Tsai, L.S. Peterson, K. Ando, M. Manohar, E. Do, G.K.R. Dhondalay, et al., Integrated plasma proteomic and single-cell immune signaling network signatures demarcate mild, moderate, and severe COVID-19, Cell Rep Med 3 (2022), 100680, https://doi.org/10.1016/j.xcrm.2022.100680.

[40] M.A.Y. Al-Nesf, H.B. Abdesselem, I. Bensmail, S. Ibrahim, W.A.H. Saeed, S.S.I. Mohammed, A. Razok, H. Alhussain, R.M.A. Aly, M. Al Maslamani, et al., Prognostic tools and candidate drugs based on plasma proteomics of patients with severe COVID-19 complications, Nat. Commun. 13 (2022) 946, https://doi.org/10.1038/s41467-022-28639-4.

[41] M. Grossegesse, D. Bourquain, M. Neumann, L. Schaade, J. Schulze, C. Mache, T. Wolff, A. Nitsche, J. Doellinger, Deep time course proteomics of SARS-CoV- and SARS-CoV-2-infected human lung epithelial cells (Calu-3) reveals strong induction of interferon-stimulated gene expression by SARS-CoV-2 in contrast to SARS-CoV, J. Proteome Res. 21 (2022) 459–469, https://doi.org/10.1021/acs.jproteome.1c00783.

[42] A. McArdle, K.E. Washington, B. Chazarin Orgel, A. Binek, D.M. Manalo, A. Rivas, M. Ayres, R. Pandey, C. Phebus, K. Raedschelders, et al., Discovery proteomics for COVID-19: where we are now, J. Proteome Res. 20 (2021) 4627–4639, https://doi.org/10.1021/acs.jproteome.1c00475.

[43] M. Villar, J.M. Urra, F.J. Rodriguez-Del-Rio, S. Artigas-Jeronimo, N. Jimenez-Collados, E. Ferreras-Colino, M. Contreras, I.G.F. de Mera, A. Estrada-Pena, C. Gortazar, J. de la Fuente, Characterization by quantitative serum proteomics of immune-related prognostic biomarkers for COVID-19 symptomatology, Front. Immunol. 12 (2021), 730710, https://doi.org/10.3389/fimmu.2021.730710.

[44] J. Zhao, M. Schank, L. Wang, X. Dang, D. Cao, S. Khanal, L.N.T. Nguyen, Y. Zhang, X.Y. Wu, J.L. Adkins, et al., Plasma biomarkers for systemic inflammation in COVID-19 survivors, Proteomics Clin Appl 16 (2022), e2200031, https://doi.org/10.1002/prca.202200031.

[45] K. Suhre, H. Sarwath, R. Engelke, M.U. Sohail, S.J. Cho, W. Whalen, S. Alvarez-Mulett, J. Krumsiek, A.M.K. Choi, F. Schmidt, Identification of robust protein associations with COVID-19 disease based on five clinical studies, Front. Immunol. 12 (2021), 781100, https://doi.org/10.3389/fimmu.2021.781100.

[46] J. Gisby, C.L. Clarke, N. Medjeral-Thomas, T.H. Malik, A. Papadaki, P.M. Mortimer, N.B. Buang, S. Lewis, M. Pereira, F. Toulza, et al., Longitudinal proteomic profiling of dialysis patients with COVID-19 reveals markers of severity and predictors of death, Elife 10 (2021), https://doi.org/10.7554/eLife.64827.

[47] H. Patel, N.J. Ashton, R.J.B. Dobson, L.M. Andersson, A. Yilmaz, K. Blennow, M. Gisslen, H. Zetterberg, Proteomic blood profiling in mild, severe and critical COVID-19 patients, Sci. Rep. 11 (2021) 6357, https://doi.org/10.1038/s41598-021-85877-0.

[48] J.W. Song, C. Zhang, X. Fan, F.P. Meng, Z. Xu, P. Xia, W.J. Cao, T. Yang, X.P. Dai, S.Y. Wang, et al., Immunological and inflammatory profiles in mild and severe cases of COVID-19, Nat. Commun. 11 (2020) 3410, https://doi.org/10.1038/s41467-020-17240-2.

[49] F. Maldonado, D. Morales, C. Diaz-Papapietro, C. Valdes, C. Fernandez, N. Valls, M. Lazo, C. Espinoza, R. Gonzalez, R. Gutierrez, et al., Relationship between endothelial and angiogenesis biomarkers envisage mortality in a prospective cohort of COVID-19 patients requiring respiratory support, Front. Med. 9 (2022), 826218, https://doi.org/10.3389/fmed.2022.826218.

[50] D. Zhang, L. Li, Y. Chen, J. Ma, Y. Yang, S. Aodeng, Q. Cui, K. Wen, M. Xiao, J. Xie, et al., Syndecan-1, an indicator of endothelial glycocalyx degradation, predicts outcome of patients admitted to an ICU with COVID-19, Mol Med 27 (2021) 151, https://doi.org/10.1186/s10020-021-00412-1.

[51] H.L. Zeng, D. Chen, J. Yan, Q. Yang, Q.Q. Han, S.S. Li, L. Cheng, Proteomic characteristics of bronchoalveolar lavage fluid in critical COVID-19 patients, FEBS J. 288 (2021) 5190–5200, https://doi.org/10.1111/febs.15609.

[52] Y.H. Teng, R.S. Aquino, P.W. Park, Molecular functions of syndecan-1 in disease, Matrix Biol. 31 (2012) 3–16, https://doi.org/10.1016/j.matbio.2011.10.001.

[53] F. Ogawa, Y. Oi, K. Nakajima, R. Matsumura, T. Nakagawa, T. Miyagawa, K. Sakai, R. Saji, H. Taniguchi, K. Takahashi, et al., Temporal change in Syndecan-1 as a therapeutic target and a biomarker for the severity classification of COVID-19, Thromb. J. 19 (2021) 55, https://doi.org/10.1186/s12959-021-00308-4.

[54] A. Dupont, A. Rauch, S. Staessens, M. Moussa, M. Rosa, D. Corseaux, E. Jeanpierre, J. Goutay, M. Caplan, P. Varlet, et al., Vascular endothelial damage in the pathogenesis of organ injury in severe COVID-19, Arterioscler. Thromb. Vasc. Biol. 41 (2021) 1760–1773, https://doi.org/10.1161/ATVBAHA.120.315595.

[55] S. Karampoor, H. Zahednasab, M. Farahmand, R. Mirzaei, F. Zamani, A. Tabibzadeh, B. Bouzari, H. Ajdarkosh, M. Nikkhah, M.R. Hashemi, et al., A possible pathogenic role of Syndecan-1 in the pathogenesis of coronavirus disease 2019 (COVID-19), Int. Immunopharm. 97 (2021), 107684, https://doi.org/10.1016/j.intimp.2021.107684.

[56] P.I. Johansson, J. Stensballe, L.S. Rasmussen, S.R. Ostrowski, A high admission syndecan-1 level, a marker of endothelial glycocalyx degradation, is associated with inflammation, protein C depletion, fibrinolysis, and increased mortality in trauma patients, Ann. Surg. 254 (2011) 194–200, https://doi.org/10.1097/SLA.0b013e3182261 13d.

[57] K. Stahl, P.A. Gronski, Y. Kiyan, B. Seeliger, A. Bertram, T. Pape, T. Welte, M.M. Hoeper, H. Haller, S. David, Injury to the endothelial glycocalyx in critically ill patients with COVID-19, Am. J. Respir. Crit. Care Med. 202 (2020) 1178–1181, https://doi.org/10.1164/rccm.202007-2676LE.

[58] A. Hudak, A. Letoha, L. Szilak, T. Letoha, Contribution of syndecans to the cellular entry of SARS-CoV-2, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms22105336.

[59] M. Bermejo-Jambrina, J. Eder, T.M. Kaptein, J.L. van Hamme, L.C. Helgers, K.E. Vlaming, P.J.M. Brouwer, A.C. van Nuenen, M. Spaargaren, G.J. de Bree, et al., Infection and transmission of SARS-CoV-2 depend on heparan sulfate proteoglycans, EMBO J. 40 (2021), e106765, https://doi.org/10.15252/embj.2020106765.

[60] D. Foell, D. Kane, B. Bresnihan, T. Vogl, W. Nacken, C. Sorg, O. Fitzgerald, J. Roth, Expression of the pro-inflammatory protein S100A12 (EN-RAGE) in rheumatoid and psoriatic arthritis, Rheumatology 42 (2003) 1383–1389, https://doi.org/10.1093/rheumatology/keg385.

[61] S. Ligthart, S. Sedaghat, M.A. Ikram, A. Hofman, O.H. Franco, A. Dehghan, EN-RAGE: a novel inflammatory marker for incident coronary heart disease, Arterioscler. Thromb. Vasc. Biol. 34 (2014) 2695–2699, https://doi.org/10.1161/ATVBAHA.114.304306.

[62] D. Foell, S. Seeliger, T. Vogl, H.G. Koch, H. Maschek, E. Harms, C. Sorg, J. Roth, Expression of S100A12 (EN-RAGE) in cystic fibrosis, Thorax 58 (2003) 613–617, https://doi.org/10.1136/thorax.58.7.613.

[63] R. Wu, Y. Liu, R. Yan, X. Liu, L. Duan, Assessment of EN-RAGE, sRAGE and EN-RAGE/sRAGE as potential biomarkers in patients with autoimmune hepatitis, J. Transl. Med. 18 (2020) 384, https://doi.org/10.1186/s12967-020-02556-w.

[64] D. Foell, F. Ichida, T. Vogl, X. Yu, R. Chen, T. Miyawaki, C. Sorg, J. Roth, S100A12 (EN-RAGE) in monitoring Kawasaki disease, Lancet 361 (2003) 1270–1272, https://doi.org/10.1016/S0140-6736(03)12986-8.

[65] R.S. Thwaites, A. Sanchez Sevilla Uruchurtu, M.K. Siggins, F. Liew, C.D. Russell, S.C. Moore, C. Fairfield, E. Carter, S. Abrams, C.E. Short, et al., Inflammatory profiles across the spectrum of disease reveal a distinct role for GM-CSF in severe COVID-19, Sci Immunol 6 (2021), https://doi.org/10.1126/sciimmunol.abg9873.

[66] L. Tserel, P. Jogi, P. Naaber, J. Maslovskaja, A. Haling, A. Salumets, E. Zusinaite, H. Soeorg, F. Lattekivi, D. Ingerainen, et al., Long-Term elevated inflammatory protein levels in asymptomatic SARS-CoV-2 infected individuals, Front. Immunol. 12 (2021), 709759, https://doi.org/10.3389/fimmu.2021.709759.

[67] N. Acevedo, J.M. Escamilla-Gil, H. Espinoza, R. Regino, J. Ramirez, L. Florez de Arco, R. Dennis, C.A. Torres-Duque, L. Caraballo, Chronic obstructive pulmonary disease patients have increased levels of plasma inflammatory mediators reported upregulated in severe COVID-19, Front. Immunol. 12 (2021), 678661, https://doi.org/10.3389/fimmu.2021.678661.

[68] F.V. Gerayeli, S. Milne, C. Cheung, X. Li, C.W.T. Yang, A. Tam, L.H. Choi, A. Bae, D.D. Sin, COPD and the risk of poor outcomes in COVID-19: a systematic review and meta-analysis, EClinicalMedicine 33 (2021), 100789, https://doi.org/10.1016/j.eclinm.2021.100789.

[69] S. Chiappalupi, L. Salvadori, A. Vukasinovic, R. Donato, G. Sorci, F. Riuzzi, Targeting RAGE to prevent SARS-CoV-2-mediated multiple organ failure: hypotheses and perspectives, Life Sci. 272 (2021), 119251, https://doi.org/10.1016/j.lfs.2021.119251.

[70] S. Chiappalupi, L. Salvadori, R. Donato, F. Riuzzi, G. Sorci, Hyperactivated RAGE in comorbidities as a risk factor for severe COVID-19-the role of RAGE-RAS crosstalk, Biomolecules 11 (2021), https://doi.org/10.3390/biom11060876.

[71] X.H. Luo, Y. Zhu, J. Mao, R.C. Du, T cell immunobiology and cytokine storm of COVID-19, Scand. J. Immunol. 93 (2021), e12989, https://doi.org/10.1111/sji.12989.

[72] S.T. Chen, M.D. Park, D.M. Del Valle, M. Buckup, A. Tabachnikova, N.W. Simons, K. Mouskas, B. Lee, D. Geanon, D. D'Souza, et al., Shift of lung macrophage composition is associated with COVID-19 disease severity and recovery, bioRxiv (2022), https://doi.org/10.1101/2022.01.11.475918.

[73] B. Hu, S. Huang, L. Yin, The cytokine storm and COVID-19, J. Med. Virol. 93 (2021) 250–256, https://doi.org/10.1002/jmv.26232.

[74] P. Song, W. Li, J. Xie, Y. Hou, C. You, Cytokine storm induced by SARS-CoV-2, Clin. Chim. Acta 509 (2020) 280–287, https://doi.org/10.1016/j.cca.2020.06.017.

[75] J. Wang, M. Jiang, X. Chen, L.J. Montaner, Cytokine storm and leukocyte changes in mild versus severe SARS-CoV-2 infection: review of 3939 COVID-19 patients in China and emerging pathogenesis and therapy concepts, J. Leukoc. Biol. 108 (2020) 17–41, https://doi.org/10.1002/JLB.3COVR0520-272R.

[76] C.A. Higgins, L. Petukhova, S. Harel, Y.Y. Ho, E. Drill, L. Shapiro, M. Wajid, A.M. Christiano, FGF5 is a crucial regulator of hair length in humans, Proc Natl Acad Sci U S A 111 (2014) 10648–10653, https://doi.org/10.1073/pnas.1402862111.

[77] I. Ushach, A. Zlotnik, Biological role of granulocyte macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) on cells of the myeloid lineage, J. Leukoc. Biol. 100 (2016) 481–489, https://doi.org/10.1189/jlb.3RU0316-144R.

[78] Y. Yoshimatsu, S. Kimuro, J. Pauty, K. Takagaki, S. Nomiyama, A. Inagawa, K. Maeda, K.A. Podyma-Inoue, K. Kajiya, Y.T. Matsunaga, T. Watabe, TGF-beta and TNF-alpha cooperatively induce mesenchymal transition of lymphatic endothelial cells via activation of Activin signals, PLoS One 15 (2020), e0232356, https://doi.org/10.1371/journal.pone.0232356.

[79] M.F. Iulita, A.C. Cuello, The NGF metabolic pathway in the CNS and its dysregulation in down syndrome and alzheimer's disease, Curr. Alzheimer Res. 13 (2016) 53–67, https://doi.org/10.2174/1567205012666150921100030.

[80] P. Bragado, P. Fernandez-Nogueira, N. Carbo, P. Gascon, Unraveling the role of fibroblasts, FGF5 and FGFR2 in HER2-targeted therapies resistance and tumor progression, Oncotarget 11 (2020) 4541–4543, https://doi.org/10.18632/oncotarget.27829.

[81] M.A. Cannarile, M. Weisser, W. Jacob, A.M. Jegg, C.H. Ries, D. Ruttinger, Colony-stimulating factor 1 receptor (CSF1R) inhibitors in cancer therapy, J Immunother Cancer 5 (2017) 53, https://doi.org/10.1186/s40425-017-0257-y.

[82] J. Feng, S.S. Lu, T. Xiao, W. Huang, H. Yi, W. Zhu, S. Fan, X.P. Feng, J.Y. Li, Z.Z. Yu, et al., ANXA1 binds and stabilizes EphA2 to promote nasopharyngeal carcinoma growth and metastasis, Cancer Res. 80 (2020) 4386–4398, https://doi.org/10.1158/0008-5472.CAN-20-0560.

[83] J. Li, R. Liang, C. Song, Y. Xiang, Y. Liu, Prognostic significance of epidermal growth factor receptor expression in glioma patients, OncoTargets Ther. 11 (2018) 731–742, https://doi.org/10.2147/OTT.S155160.

[84] B. Li, S. Cai, Y. Zhao, Q. He, X. Yu, L. Cheng, Y. Zhang, X. Hu, M. Ke, S. Chen, M. Zou, Nerve growth factor modulates the tumor cells migration in ovarian cancer through the WNT/beta-catenin pathway, Oncotarget 7 (2016) 81026–81048, https://doi.org/10.18632/oncotarget.13186.

[85] H. Bosmuller, M. Matter, F. Fend, A. Tzankov, The pulmonary pathology of COVID-19, Virchows Arch. 478 (2021) 137–150, https://doi.org/10.1007/s00428-021-03053-1.

[86] L. Ostergaard, SARS CoV-2 related microvascular damage and symptoms during and after COVID-19: consequences of capillary transit-time changes, tissue hypoxia and inflammation, Physiol Rep 9 (2021), e14726, https://doi.org/10.14814/phy2.14726.

[87] M. Laforge, C. Elbim, C. Frere, M. Hemadi, C. Massaad, P. Nuss, J.J. Benoliel, C. Becker, Tissue damage from neutrophil-induced oxidative stress in COVID-19, Nat. Rev. Immunol. 20 (2020) 515–516, https://doi.org/10.1038/s41577-020-0407-1.

[88] D. Esquivel, R. Mishra, P. Soni, R. Seetharaman, A. Mahmood, A. Srivastava, Stem cells therapy as a possible therapeutic option in treating COVID-19 patients, Stem Cell Rev Rep 17 (2021) 144–152, https://doi.org/10.1007/s12015-020-10017-6.

[89] K. Rajarshi, A. Chatterjee, S. Ray, Combating COVID-19 with mesenchymal stem cell therapy, Biotechnol Rep (Amst) 26 (2020), e00467, https://doi.org/10.1016/j.btre.2020.e00467.

[90] I. Pastan, R. Hassan, Discovery of mesothelin and exploiting it as a target for immunotherapy, Cancer Res. 74 (2014) 2907–2912, https://doi.org/10.1158/0008-5472.CAN-14-0337.

[91] R. Hassan, T. Bera, I. Pastan, Mesothelin: a new target for immunotherapy, Clin. Cancer Res. 10 (2004) 3937–3942, https://doi.org/10.1158/1078-0432.CCR-03-0801.

[92] R. Hassan, M. Ho, Mesothelin targeted cancer immunotherapy, Eur. J. Cancer 44 (2008) 46–53, https://doi.org/10.1016/j.ejca.2007.08.028.

[93] O. Kaneko, L. Gong, J. Zhang, J.K. Hansen, R. Hassan, B. Lee, M. Ho, A binding domain on mesothelin for CA125/MUC16, J. Biol. Chem. 284 (2009) 3739–3749, https://doi.org/10.1074/jbc.M806776200.

[94] S. Muniyan, D. Haridas, S. Chugh, S. Rachagani, I. Lakshmanan, S. Gupta, P. Seshacharyulu, L.M. Smith, M.P. Ponnusamy, S.K. Batra, MUC16 contributes to the metastasis of pancreatic ductal adenocarcinoma through focal adhesion mediated signaling mechanism, Genes Cancer 7 (2016) 110–124, https://doi.org/10.18632/genesandcancer.104.

[95] T.K. Bera, I. Pastan, Mesothelin is not required for normal mouse development or reproduction, Mol. Cell Biol. 20 (2000) 2902–2906, https://doi.org/10.1128/MCB.20.8.2902-2906.2000.

[96] A. Leibbrandt, J.M. Penninger, RANK/RANKL: regulators of immune responses and bone physiology, Ann. N. Y. Acad. Sci. 1143 (2008) 123–150, https://doi.org/10.1196/annals.1443.016.

[97] P. Huang, Y.Q. Hou, J.J. Wu, Y.D. Wang, X.Y. Ye, F. Zang, R.B. Yu, S. Yang, Genetic mutations in TNFSF11 were associated with the chronicity of hepatitis C among Chinese han population, Front. Med. 8 (2021), 743406, https://doi.org/10.3389/fmed.2021.743406.

[98] A.J. Leonardi, C.P. Argyropoulos, A. Hamdy, R.B. Proenca, Understanding the effects of age and T-cell differentiation on COVID-19 severity: implicating a fas/FasL-mediated feed-forward controller of T-cell differentiation, Front. Immunol. 13 (2022), 853606, https://doi.org/10.3389/fimmu.2022.853606.

[99] L.G. de Almeida Chuffa, P.P. Freire, J. Dos Santos Souza, M.C. de Mello, M. de Oliveira Neto, R.F. Carvalho, Aging whole blood transcriptome reveals candidate genes for SARS-CoV-2-related vascular and immune alterations, J. Mol. Med. (Berl.) 100 (2022) 285–301, https://doi.org/10.1007/s00109-021-02161-4.

[100] S. Nagata, Fas ligand-induced apoptosis, Annu. Rev. Genet. 33 (1999) 29–55, https://doi.org/10.1146/annurev.genet.33.1.29.

[101] J.W. Williams, C.M. Ferreira, K.M. Blaine, C. Rayon, F. Velazquez, J. Tong, M.E. Peter, A.I. Sperling, Non-apoptotic Fas (CD95) signaling on T cells regulates the resolution of Th2-mediated inflammation, Front. Immunol. 9 (2018) 2521, https://doi.org/10.3389/fimmu.2018.02521.

[102] S.K. Sharma, F.A. Almeida, S. Kierstein, L. Hortobagyi, T. Lin, A. Larkin, J. Peterson, H. Yagita, J.G. Zangrilli, A. Haczku, Systemic FasL neutralization increases eosinophilic inflammation in a mouse model of asthma, Allergy 67 (2012) 328–335, https://doi.org/10.1111/j.1398-9995.2011.02763.x.

[103] A.J. Leonardi, R.B. Proenca, Akt-fas to quell aberrant T cell differentiation and apoptosis in covid-19, Front. Immunol. 11 (2020), 600405, https://doi.org/10.3389/fimmu.2020.600405.

[104] D. Ramljak, M. Vukoja, M. Curlin, K. Vukojevic, M. Barbaric, U. Glamoclija, B. Purisevic, O. Peric, V. Soljic, Early response of CD8+ T cells in COVID-19 patients, J Pers Med 11 (2021), https://doi.org/10.3390/jpm11121291.

[105] S. Andre, M. Picard, R. Cezar, F. Roux-Dalvai, A. Alleaume-Butaux, C. Soundaramourty, A.S. Cruz, A. Mendes-Frias, C. Gotti, M. Leclercq, et al., T cell apoptosis characterizes severe Covid-19 disease, Cell Death Differ. (2022), https://doi.org/10.1038/s41418-022-00936-x.

[106] E.R. Rasmussen, P. Hallberg, E.V. Baranova, N. Eriksson, M. Karawajczyk, C. Johansson, M. Cavalli, C. Maroteau, A. Veluchamy, G. Islander, et al., Genome-wide association study of angioedema induced by angiotensin-converting enzyme inhibitor and angiotensin receptor blocker treatment, Pharmacogenomics J. 20 (2020) 770–783, https://doi.org/10.1038/s41397-020-0165-2.

[107] A.H. Pall, E.R. Rasmussen, M. Wadelius, Pharmacogenetics of angiotensin-converting enzyme inhibitor-induced angioedema, Pharmacogenomics 22 (2021) 319–321, https://doi.org/10.2217/pgs-2021-0036.

[108] V. Montinaro, M. Cicardi, ACE inhibitor-mediated angioedema, Int. Immunopharm. 78 (2020), 106081, https://doi.org/10.1016/j.intimp.2019.106081.

[109] M.R. Garvin, C. Alvarez, J.I. Miller, E.T. Prates, A.M. Walker, B.K. Amos, A.E. Mast, A. Justice, B. Aronow, D. Jacobson, A mechanistic model and therapeutic interventions for COVID-19 involving a RAS-mediated bradykinin storm, Elife 9 (2020), https://doi.org/10.7554/eLife.59177.

[110] F.L. van de Veerdonk, M.G. Netea, M. van Deuren, J.W. van der Meer, Q. de Mast, R.J. Bruggemann, H. van der Hoeven, Kallikrein-kinin blockade in patients with COVID-19 to prevent acute respiratory distress syndrome, Elife 9 (2020), https://doi.org/10.7554/eLife.57555.

[111] J.A. Roche, R. Roche, A hypothesized role for dysregulated bradykinin signaling in COVID-19 respiratory complications, Faseb. J. 34 (2020) 7265–7269, https://doi.org/10.1096/fj.202000967.

[112] M. Khodadoost, Z. Niknam, M. Farahani, M. Razzaghi, M. Norouzinia, Investigating the human protein-host protein interactome of SARS-CoV-2 infection in the small intestine, Gastroenterol Hepatol Bed Bench 13 (2020) 374–387.

[113] A. Saih, H. Baba, M. Bouqdayr, H. Ghazal, S. Hamdi, A. Kettani, L. Wakrim, In silico analysis of high-risk missense variants in human ACE2 gene and susceptibility to SARS-CoV-2 infection, BioMed Res. Int. 2021 (2021), 6685840, https://doi.org/10.1155/2021/6685840.

[114] S.S. Foo, M.C. Cambou, T. Mok, V.M. Fajardo, K.L. Jung, T. Fuller, W. Chen, T. Kerin, J. Mei, D. Bhattacharya, et al., The systemic inflammatory landscape of COVID-19 in pregnancy: extensive serum proteomic profiling of mother-infant dyads with in utero SARS-CoV-2, Cell Rep Med 2 (2021), 100453, https://doi.org/10.1016/j.xcrm.2021.100453.

[115] J. Idoyaga, C. Cheong, K. Suda, N. Suda, J.Y. Kim, H. Lee, C.G. Park, R.M. Steinman, Cutting edge: langerin/CD207 receptor on dendritic cells mediates efficient antigen presentation on MHC I and II products in vivo, J. Immunol. 180 (2008) 3647–3650, https://doi.org/10.4049/jimmunol.180.6.3647.

[116] Y. Gu, J. Cao, X. Zhang, H. Gao, Y. Wang, J. Wang, J. He, X. Jiang, J. Zhang, G. Shen, et al., Receptome profiling identifies KREMEN1 and ASGR1 as alternative functional receptors of SARS-CoV-2, Cell Res. 32 (2022) 24–37, https://doi.org/10.1038/s41422-021-00595-6.

[117] R. Zhou, K.K. To, Y.C. Wong, L. Liu, B. Zhou, X. Li, H. Huang, Y. Mo, T.Y. Luk, T.T. Lau, et al., Acute SARS-CoV-2 infection impairs dendritic cell and T cell responses, Immunity 53 (2020) 864–877 e865, https://doi.org/10.1016/j.immuni.2020.07.026.

[118] A. Perez-Gomez, J. Vitalle, C. Gasca-Capote, A. Gutierrez-Valencia, M. Trujillo-Rodriguez, A. Serna-Gallego, E. Munoz-Muela, M.L.R. Jimenez-Leon, M. Rafii-El-Idrissi Benhnia, I. Rivas-Jeremias, et al., Dendritic cell deficiencies persist seven months after SARS-CoV-2 infection, Cell. Mol. Immunol. 18 (2021) 2128–2139, https://doi.org/10.1038/s41423-021-00728-2.

[119] Y. Xiong, Y. Liu, L. Cao, D. Wang, M. Guo, A. Jiang, D. Guo, W. Hu, J. Yang, Z. Tang, et al., Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients, Emerg. Microb. Infect. 9 (2020) 761–770, https://doi.org/10.1080/22221751.2020.1747363.