Distinct patterns of blood cytokines beyond a cytokine storm predict mortality in COVID-19

Christian Herr ¹, Sebastian Mang¹, Bahareh Mozafari ¹, Katharina Günther ¹, Thimoteus Speer ², Martina Seibert ¹, Sanjay Kumar Srikakulam ¹, Christoph Beisswenger ¹, Felix Ritzmann ¹, Andreas Keller ³, Rolf Müller ⁴, Sigrun Smola ⁵, Dominic Eisinger ⁶, Michael Zemlin ⁷, Guy Danziger ¹, Thomas Volk ⁸, Sabrina Hörsch ⁸, Marcin Krawczyk ⁹, Frank Lammert ⁹, Thomas Adams ⁹, Gudrun Wagenpfeil ¹⁰, Michael Kindermann ¹¹, Constantin Marcu ¹¹, Zuhair Wolf Dietrich Ataya ¹², Marc Mittag¹³, Konrad Schwarzkopf¹³, Florian Custodis¹³, Daniel Grandt¹³, Harald Schäfer¹⁴, Kai Eltges¹⁴, Philipp M. Lepper ¹, Robert Bals ¹ for the CORSAAR study group.

Affiliations

¹ Department of Internal Medicine V – Pulmonology, Allergology and Critical Care Medicine, Saarland University, D-66421 Homburg, Germany
² Department of Internal Medicine IV, Nephrology and Hypertension & Translational Cardio-Renal Medicine, Saarland University, Homburg/Saar, Germany Saarland
³ Clinical Bioinformatics, Saarland University, 66421 Homburg, Germany
⁴ Helmholtz-Institut für Pharmazeutische Forschung Saarland, 66123 Saarbrücken, Germany
⁵ Institute for Virology, Saarland University, 66421 Homburg, Germany
⁶ Myriad RBM Inc. 3300 Duval Rd. Austin, Texas 78759, USA

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
7 Department for General Pediatrics and Neonatology, Saarland University, 66421 Homburg, Germany

8 Clinic for Anesthesiology, Intensive Care Medicine and Pain Therapy, Saarland University, D-66421 Homburg, Germany

9 Department of Internal Medicine II, Saarland University, D-66421 Homburg, Germany.

10 Institute for Medical Biometry, Epidemiology and Medical Informatics, Saarland University, Campus Homburg/Saar, Germany.

11 Caritas Klinikum Saarbrücken St. Theresia, Saarbrücken, Saarland, Germany.

12 Innere Medizin und Internistische Intensivmedizin, Caritas Klinikum, Saarbrücken St. Josef, Dudweiler, Saarland, Germany.

13 Klinikum Saarbrücken, 66119 Saarbrücken, Germany.

14 Department of Internal Medicine and Pulmonology, SHG-Kliniken Völklingen, Völklingen, Saarland, Germany.
Corresponding author
Robert Bals, MD, PhD
Saarland University Hospital
Department of Internal Medicine V, Pulmonology, Allergology and Critical Care Medicine
Kirrberger Strasse, Building 41
D-66421 Homburg/Saar
Germany
Phone: +49 6841 16 15051
Mail: robert.bals@uks.eu

Word count: 3011

Key words: biomarker, inflammation, SARS-CoV2
Abstract

Background

COVID-19 comprises several severity stages ranging from oligosymptomatic disease to multi-organ failure and fatal outcomes. The mechanisms why COVID-19 is a mild disease in some patients and progresses to a severe multi-organ and often fatal disease with respiratory failure are not known. Biomarkers that predict the course of disease are urgently needed. The aim of this study was to evaluate a large spectrum of established laboratory measurements.

Patients and methods

Patients from the prospective PULMPOHOM and CORSAAR studies were recruited and comprised 35 patients with COVID-19, 23 with conventional pneumonia, and 28 control patients undergoing elective non-pulmonary surgery. Venous blood was used to measure the serum concentrations of 79 proteins by Luminex multiplex immunoassay technology. Distribution of biomarkers between groups and association with disease severity and outcomes were analyzed.

Findings

The biomarker profiles between the three groups differed significantly with elevation of specific proteins specific for the respective conditions. Several biomarkers correlated significantly with disease severity and death. Uniform manifold approximation and projection (UMAP) analysis revealed a significant separation of the three disease groups and separated between survivors and deceased patients. Different models were developed to predict mortality based on the baseline measurements of several protein markers.

Interpretation
Several newly identified blood markers were increased in patients with severe COVID-19 (AAT, EN-RAGE, ICAM-1, myoglobin, SAP, TIMP-1, vWF, decorin, HGF, MMP7, PECAM-1) or in patients that died (FRTN, SCF, TIMP-1, CA-9, CEA, decorin, HGF). The use of established assay technologies allows for rapid translation into clinical practice.

**Funding**

No role of the funding source.
Introduction
Coronavirus disease 2019 (COVID-19) is a novel illness and a rapidly evolving pandemic with large impact on global health. COVID-19 is caused by “severe acute respiratory syndrome coronavirus 2” (SARS-CoV-2), a member of the Coronaviridae family that is regarded as a spill-over of an animal coronavirus to humans [1]. At the time of submission of this work, SARS-CoV-2 has caused 120 million diagnosed infections and 6 million known deaths. The disease course is complex, increasing disease severity is associated with respiratory and extra-pulmonary organ manifestations. Most of the infected individuals have mild flu-like symptoms, nevertheless, about 20% of individuals develop more severe illness due to viral pneumonia, often resulting in the need for hospitalization. Often, the disease progresses, and patients need invasive life support. The mechanisms why COVID-19 is a mild disease in some patients and progresses to a severe multi-organ and often fatal disease with respiratory failure are currently unknown.

The natural history and the pathomechanisms of COVID-19 involve complex mechanisms including infection and viral replication in target cells and a broad dysregulation of host defense and immune mechanisms. Several studies have evaluated blood transcriptional data and highlighted the importance of dysregulation of innate [2] or adaptive immunity including activation of type 1 (t-bet, interferon-γ), type-2 (GATA) or type 3 (RORγt, IL-17) immune pathways [3, 4].

Elevation of routine inflammation markers, such as erythrocyte sedimentation rate, C-reactive protein (CRP) or procalcitonin (PCT), is frequently but not exclusively observed in the critically ill COVID-19 patient [5]. The term “cytokine storm” has been used to describe the elevation of several acute-phase proteins such as CRP, interleukin-6 (IL-6) or ferritin [6, 7]. Individual markers or patterns have been associated with disease severity or lethality [8-10].
There is a clinical need to identify markers that indicate the risk of disease progression or fatal outcome. The aim of the present study was to identify clinically relevant serum biomarkers from a large spectrum of established laboratory measurements. The marker panel applied in the present study consists of mediators of inflammation and immunity, which are not yet part of clinical routine testing but have been shown to be involved in cellular or humoral immune responses. We measured serum levels of a large panel of cytokines, chemokines, acute phase reactants, growth factors, tissue remodeling enzymes and clinical laboratory parameters in blood samples of hospitalized COVID-19 patients and compared them to control individuals undergoing non-thoracic surgery as well as patients with community-acquired pneumonia.
Methods

Study population

The present analysis used the baseline data and follow up data of the COVID-19 cohort CORSAAR (n = 35) and its control cohort PULMOHOM, which are multi-center studies focusing on pathomechanisms and the role of risk factors in COVID-19 and other inflammatory lung diseases. Within the PULMOHOM cohort study, 23 patients with pneumonia and 28 control patients undergoing elective non-pulmonary surgery have been included. Patients were recruited at the Saarland University Hospital, the Caritas Klinikum Saarbrücken, the Klinikum Saarbrücken Winterberg, and the SHG-Kliniken Völklingen. The studies have been approved by the ethics committee of the Ärztekammer des Saarlandes, and all patients or their legal representatives gave their informed consent. Basic and anthropomorphic characteristics, the prevalence of comorbidities, and vital parameters were assessed based on measurements, questionnaires, and standardized interviews.

Biosamples

Blood samples were drawn after obtaining informed consent from the patient and processed within 4 hours (time-to-centrifugation). Blood was centrifuged for 20 minutes at 2500 g at 4°C in a swing out rotor with break. The serum supernatant was separated immediately after centrifugation under aseptical conditions and transferred into cryo-vials. The vials were integrated into our local biobank (-80°C storage and Biobank Information System (BIMS)) for long term storage. The freezers of the biobank are temperature monitored and access controlled. Upon selection for analysis, the samples were sent on dry ice to the corresponding labs.
Multiplex immunoassay analysis

All samples were stored at less than -70°C until tested. Samples were thawed at room temperature, vortexed, spun at 3700 x g for 5 min for clarification and transferred to a master microtiter plate. Using automated pipetting, an aliquot of each sample was added to individual microsphere multiplexes of the selected Multi Analyte Profile (MAP) and blocker. This mixture was thoroughly mixed and incubated at room temperature for 1 hour. Multiplexed cocktails of biotinylated reporter antibodies were added robotically and after thorough mixing, incubated for an additional hour at room temperature. Multiplexes were labelled using an excess of streptavidin-phycoerythrin solution, thoroughly mixed and incubated for 1 hour at room temperature. The volume of each multiplexed reaction was reduced by vacuum filtration and washed thrice. After the final wash, the volume was increased by addition of buffer for analysis using a Luminex instrument and the resulting data interpreted using proprietary software developed by Myriad RBM. For each multiplex, both calibrators and controls were included on each microtiter plate. Eight-point calibrators to form a standard curve were run in the first and last column of each plate and controls at three concentration levels were run in duplicate. Standard curve, control, and sample QC were performed to ensure proper assay performance. Study sample values for each of the analytes were determined using 4 and 5 parameter logistics, weighted and non-weighted curve fitting algorithms included in the data analysis package.

The following assays were used: adiponectin, alpha-1-antitrypsin (AAT), alpha-2-macroglobulin (A2Macro), apolipoprotein(a) (Lp(a)), beta-2-microglobulin (B2M), brain-derived neurotrophic factor (BDNF), c-reactive protein (CRP), complement C3 (C3), EN-RAGE, eotaxin-1, factor VII, ferritin (FRTN), fibrinogen, granulocyte-macrophage colony-stimulating factor (GM-CSF), haptoglobin, immunoglobulin a (IgA), immunoglobulin m (IgM), intercellular adhesion molecule 1 (ICAM-1), interferon
gamma (IFN-gamma), interleukin-1 alpha (IL-1 alpha), interleukin-1 beta (IL-1 beta),
interleukin-1 receptor antagonist (IL-1ra), interleukin-2 (IL-2), interleukin-3 (IL-3),
interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7),
interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-12 Subunit p40 (IL-12p40),
interleukin-12 subunit p70 (IL-12p70), interleukin-17 (IL-17), interleukin-18 (IL-18),
macrophage inflammatory protein-1 alpha (MIP-1 alpha), macrophage inflammatory
protein-1 beta (MIP-1 beta), matrix metalloproteinase-3 (MMP-3), matrix
metalloproteinase-9 (MMP-9), monocyte chemotactic protein 1 (MCP-1), myoglobin,
plasminogen activator inhibitor 1 (PAI-1), pulmonary and activation-regulated
chemokine (PARC), serum amyloid p-component (SAP), stem cell factor (SCF), t-
cell-specific protein RANTES (RANTES), thyroxine-binding globulin (TBG), tissue
inhibitor of metalloproteinases 1 (TIMP-1), tumor necrosis factor alpha (TNF-alpha),
tumor necrosis factor beta (TNF-beta), tumor necrosis factor receptor 2 (TNFR2),
vascular cell adhesion molecule-1 (VCAM-1), vascular endothelial growth factor
(VEGF), vitamin d-binding protein (VDBP), von Willebrand factor (vWF)), AXL
receptor tyrosine kinase (AXL), chemokine CC-4 (HCC-4), FASLG receptor (FAS),
hepatocyte growth factor (HGF), TNF-related apoptosis-inducing ligand receptor 3
(TRAIL-R3)), alpha-fetoprotein (AFP), cancer antigen 125 (CA-125), cancer antigen
19-9 (CA-19-9), carcinoembryonic antigen (CEA), human chorionic gonadotropin
beta (hCG), neuron-specific enolase (NSE)), matrix metalloproteinase-1 (MMP-1),
matrix metalloproteinase-7 (MMP-7), matrix metalloproteinase-9, total (MMP-9, total),
angiopoietin-1 (ANG-1), carbonic anhydrase 9 (CA-9), decorin, interleukin-18-binding
protein (IL-18bp), platelet endothelial cell adhesion molecule (PECAM-1), pulmonary
surfactant-associated protein D (SP-D).
Statistical analyses

Continuous variables are presented as mean ± standard deviation when normally distributed or as median (standard deviation). Qualitative variables are presented as absolute and relative frequencies. Statistical differences between three groups considering continuous or categorical variables have been established using one-way Analysis of Variance (ANOVA, assuming normality) with Bonferroni correction as post-hoc test, Kruskal-Wallis test or chi-squared test, respectively. Differences between two groups were analyzed by two-sided T-test. For bivariate correlations, the Pearson’s R is reported. A correlation matrix was generated by using R language (version 3.6.1) and plotted using ggplot2 library. For plotting, only the absolute correlation values >0.3 were retained. A linear discriminant analysis was performed to test whether the serum markers identified above can be used to build a model that indicates increased risk of mortality. Neural network analysis (SPPS module multilayer perceptron, MLP, one hidden layer) was performed using “death” as dependent variable and the biomarkers with different serum concentrations in survivors vs. non-survivors as covariates. A two-sided $P$ value of less than 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 27.0. Dimension reduction and visualization of high dimensional profiles has additionally been carried out using the Uniform Manifold Approximation and Projection (UMAP) by using the R umap package. Input data were z-scaled before computing the UMAP. The number of components was set to 2, and the Euclidean distance was used. The computations for the UMAP used R 4.0.3 GUI 1.73 Catalina build (7892).
Role of the funding source

The funders of the study had no role in study design, data collection, data interpretation, or writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.
Results
The blood inflammatory profile differs between COVID-19 and conventional pneumonia

As a first step we compared the concentrations of blood mediators between patients with pneumonia, COVID-19 and a control group of patients undergoing non-pulmonary surgery. Table 1 summarizes the main characteristics of the patients in these groups and shows that the three groups did not differ with respect to age or body mass index (BMI). Table 1S summarizes the clinical characteristics of all patients. Only male patients were recruited for the control and pneumonia groups to avoid sex-related changes of metabolic parameters. We analyzed the data from the measurements and found that the concentrations of IL-1-alpha, IL-3, IL-4, IL-7, IL-12p70, TNF-beta, and TNF-alpha were below the detection threshold. These markers were excluded from further analysis.

A direct comparison of results from the three groups showed distinct patterns of elevated venous markers when analyzed by ANOVA with Bonferroni correction as post-hoc test. The three groups differ with regard to the concentrations of various cytokines: CRP, AAT, B2M, BDNF, EN-RAGE, fibrinogen, haptoglobin, IgA, IgM, ICAM-1, IL-1b, IL-8, MIP-1alpha, MMP-3, MMP-9, MCP-1, myoglobin, PAI-1, PARC, SAP, SCF, RANTES, TIMP-1, VCAM-1, vWF, AXL, HCC-4, HGF, MMP-7, MMP-9, NSE, PECAM-1, SP-D, A2Macro, IL-18, CA-125, CA-9, decorin, IL-18bp and TRAIL-R3. The data are summarized in Table 2. Figure 1A displays a selection of markers with significant differences.

Comparing COVID-19 to conventional pneumonia revealed significant differences and concentrations of BDNF, MMP-9, and NSE were increased, while A2Macro, B2M, IgA, MIP1alpha, MMP-3, myoglobin, SCF, TNFR2, vWF, AXL, CA-125, CA-9,
HCC-4, decorin, MMP-7, SPD, TRAIL-R3 were decreased in COVID-19 as compared to pneumonia patients. No significant differences were found for IL-6, or FRTN. As compared to the control group, COVID-19 patients showed increased concentrations of CRP, fibrinogen, IgM, PECAM-1, NSE, AAT, N-RAGE, IL-18, IL-18bp, MCP-1, PAI-1, and SAP. Using a UMAP approach, we classified individuals of all three groups based on their biomarker levels. This procedure resulted in significant segregation of the three groups (Fig. 1B).

Factors associated with disease severity in COVID-19 patients

COVID-19 is a dynamic disease that often progresses towards respiratory failure and systemic inflammation and shock. The differences of blood concentrations between the disease severity stages (normal care vs. intensive care treatment) were analyzed (Table 3A). Figure 2A displays the significantly altered biomarkers. More severely sick patients requiring ICU treatment had increased levels of AAT, CRP, EN-RAGE, IL-1ra, IL-8, IL-10, IL-18, MCP-1, myoglobin, SAP, TIMP-1, vWF, decorin, and HGF, but decreased blood concentrations of albumin, total protein, adiponectin, A2Macro, BDNF, IL-17, and RANTES.

To further evaluate the association between serum markers and disease severity, we measured the ICU scores SAPS II (Simplified Acute Physiology Score) and TISS (Therapeutic intervention scoring system) in COVID-19 patients. Table S2 presents results of bivariate correlation analysis, Figure 2B displays a summary correlation plot.

Factors associated with death

One important goal of the use of biomarkers is to identify patients with increased risk of dying. As no death occurred in the control group and few in the pneumonia group,
we identified factors associated with mortality only in the COVID-19 group (Table 3B). Figure 3A displays the significantly changed biomarkers between the survivors and the deceased patients. T-test identified increased levels of eotaxin, FRTN, IL-1beta, IL-1ra, IL-8, IL-10, MCP-1, SCF, TIMP-1, CA-9, CEA, IL-18p. Decreased concentrations were found for albumin, adiponectin, BDNF, and ANG-1. No significant differences were found for BMI or age. A UMAP analysis (Fig. 3B) shows a significant separation between these two groups.

A linear discriminant analysis was performed to test whether the serum markers identified above can be used to build a model that predicts mortality. The canonical discriminant functions revealed an Eigenvalue of 2.964 and a canonical correlation of 0.865 (Wilks-Lambda 0.252, Chi-quadrat P = 0.014) indicated a valid model with high standardized canonical discriminant function coefficients for IL-6 (0.532), IL-8 (0.467), MCP-1 (0.625), SCF (0.567), TIMP-1 (-0.920), decorin (-0.737), and HGF (0.614). This model resulted in a correct classification of 97.1 % of the original cases and 73.5 % of the cross-validation cases.

While the number of cases is low, we used several rounds neural network analysis (SPPS module multilayer perceptron, MLP, one hidden layer) using “death” as dependent variable and the biomarkers with different serum concentrations in survivors vs. non-survivors as covariates. The analysis was performed four times using different random training sets and resulted in: 1. Analysis: correct prediction in the training / test set of 88.5 / 87.5 % (with the 5 most relatively important variables: IL-6, Eotaxin, IL-8, IL-1RA, MCP-); 2. Analysis 100 / 100 % correct prediction (MCP-1, SCF, BDNF, Eotaxin, Decorin); 3. Analysis 95.5 / 91.7 % correct prediction (BDNF, IL-10, CA9, MCP-1, Adiponectin); 4. Analysis 95.8 / 100 % correct prediction (SCF, BDNF, IL18bp, IL-8, Age).
Finally, we performed a ROC analysis and found that IL-1ra, IL-8, IL-10, MCP-1, SCF and CA-9 have an AUC > 0.8. When these variables are subdivided in tertials and a score is established from these data, the AUC is 0.929.
Discussion

The main findings of this study are that severe COVID-19 differs from conventional pneumonia by a specific blood cytokine profile. Several serum mediators are correlated with disease severity and outcome. While some blood biomarkers are significantly elevated in severe COVID-19, a classical “cytokine storm” as seen in other conditions was not observed. Several biomarkers are associated with disease severity and risk of death.

Blood biomarkers have been used to determine disease severity or risk for deleterious outcome in conventional pneumonia [11-13]. Biomarkers such as CRP, procalcitonin, and others are important tools for decision making in pneumonia patients. The identification of diagnostic, predictive or prognostic biomarkers is even more relevant in COVID-19.

There are two approaches to study the inflammatory processes and to identify biomarkers in COVID-19. One is the application of OMICs technologies to array multiple layers of gene expression, protein and metabolome distribution. A multi-OMICs approach identified patterns that separate mild from severe disease [9]. The combination of transcriptomics, proteomics and metabolomics revealed 219 molecular features related to COVID-19 and linked to complement activation, dysregulated lipid metabolism, and neutrophil activation [4]. A dysregulation of the myeloid cell compartment was found by transcriptomics and single-cell-analysis [2].

The other approach focuses on the measurement of selected biomarkers in clinically relevant cohorts. This approach does not allow a deeper insight into mechanisms but aids to quickly develop clinically relevant information. IL-1RA, IL-10, and RANTES were found to associate with disease severity in a longitudinal investigation [14]. IFN-γ and type I IFN production were found to be relatively deceased, while TNF-α, IL-6, and IL-8 were increased for a prolonged time [15]. The serial analysis of several
cytokines in 113 patients showed the early rise in markers to be associated with worse outcome and an activation of type 2 cytokines in severe COVID-19 [3]. Measurement of IL-6, IL-8, TNF-α, and IL-1β were found to be predictive for severity and survival [8]. The measurement of a selected cytokine panel showed that COVID-19 is distinct from conventional pneumonia [7].

The present work applied a large panel of biomarkers to COVID-19 patients and control groups. The marker panel applied in the present study consists of mediators of inflammation and immunity, most of which are not yet part of clinical routine testing but have been shown to partake in cellular or humoral immune responses. The present study confirmed several of these findings and in addition shows a more comprehensive illustration of COVID-19 systemic inflammation. The blood marker profile of COVID-19 patients differs significantly from control patients with elective non-pulmonary surgery or severe pneumonia. The data show that COVID-19 is associated with a broad and complex activation of innate and adaptive immune responses.

In addition to several studies [10, 16], the present data showed increased serum concentrations of NSE, AAT, B2M, EN-RAGE, IL-18, MCP-1, PAI-1, SAP, IL-18bp, and MMP-9. Several newly identified blood markers were increased in patients with severe disease (AAT, EN-RAGE, ICAM-1, myoglobin, SAP, TIMP-1, vWF, decorin, HGF, MMP7, PECAM-1) or in patients that died during the disease course (FRTN, SCF, TIMP-1, CA-9, CEA, decorin, HGF).

The term “cytokine storm” in general describes the finding of an exaggerated immune response with excessively elevated cytokine levels and deleterious side effects [6] and it was often highlighted that this phenomenon would be a hallmark of COVID-19. Other reports questioned the role of a cytokine storm in COVID-19 in comparison to other inflammatory conditions [5]. Also, the preset results support this view with
COVID-19 patients having a distinct pattern of increased inflammatory mediators but not an out-of-proportion increase.

The present study has limitations and strengths: The use of established, rapid multiplex assays allows to translate these findings to validation studies and clinical practice. One important aspect is the direct comparison to non-COVID-19 pneumonia and a control group. The present study has included relatively few COVID-patients and does not cover very mild or asymptomatic individuals nor sequential sampling. Overfitting might be a result of low patient numbers and validation in other cohorts is mandatory.

In conclusion, COVID-19 shows a distinct inflammatory blood marker profile separate from conventional pneumonia and extrapulmonary disease. Several markers were associated with disease severity and lethal outcome. We recommend a thorough investigation which of these biomarkers might be causative for immune dysregulation in severe COVID-19 or just a by-product of immune activation. Ideally, proteins strongly connected to COVID-19 pathophysiology could reveal potential targets for immunomodulating therapies for patients at risk.
Acknowledgments

This work was supported by grants of the Rolf M. Schwiete Stiftung (2020-013), the Saarland University, and The State of Saarland. Protein biomarker assays have been performed in collaboration with Myriad RBM.
References

1. Coronaviridae Study Group of the International Committee on Taxonomy of V. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol* 2020: 5(4): 536-544.

2. Schulte-Schrepping J, Reusch N, Paclik D, Bassler K, Schlickeiser S, Zhang B, Kramer B, Krammer T, Brumhard S, Bonaguro L, De Domenico E, Wendisch D, Grasshoff M, Kapellos TS, Beckstette M, Pecht T, Saglam A, Dietrich O, Mei HE, Schulz AR, Conrad C, Kunkel D, Vafadarannejad E, Xu CJ, Horne A, Herbert M, Drews A, Thibeault C, Pfeiffer M, Hippienstiel S, Hocke A, Muller-Redetzky H, Heim KM, Machleidt F, Uhrig A, Bosquillon de Jarcy L, Jurgens L, Stegemann M, Glosenkamp CR, Volk HD, Goffinet C, Landthaler M, Wyler E, Georg P, Schneider M, Bang-Heine C, Neuwinger N, Kappert T, Tauber R, Corman V, Raabe J, Kaiser KM, Vihna MT, Rieke G, Meisel C, Ulas T, Becker M, Geffers R, Witzenrath M, Drosten C, Suttrop N, von Kalle C, Kurth F, Handler K, Schulte JL, Aschenbrenner AC, Li Y, Nattermann J, Sawitzki B, Saliba AE, Sander LC, Deutsche C-OI. Severe COVID-19 Is Marked by a Dysregulated Myeloid Cell Compartment. *Cell* 2020: 182(6): 1419-1440 e1423.

3. Lucas C, Wong P, Klein J, Castro TBR, Silva J, Sundaram M, Ellingson MK, Mao T, Oh JE, Israelow B, Takahashi T, Tokuyama M, Lu P, Venkataraman A, Park A, Mohanty S, Wang H, Wylie AL, Vogels CBF, Earnest R, Lapidus S, Ott IM, Moore AJ, Muenker MC, Fournier JB, Campbell M, Odone CD, Casanovas-Massana A, Yale IT, Herbst R, Shaw AC, Medzhitov R, Schulz WL, Grubaugh ND, Dela Cruz M, Farhadian S, Ko AI, Omer SB, Iwasaki A. Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature* 2020: 584(7821): 463-469.

4. Overmyer KA, Shishkova E, Miller J, Balnis J, Bernstein MN, Peters-Clarke TM, Meyer JG, Quan Q, Muehlbauer LK, Trujillo EA, He Y, Chopra A, Chien HC, Tiwari A, Judson MA, Paulson B, Brademan DR, Zhu Y, Serrano LR, Linke V, Drake LA, Adam AP, Schwartz BS, Singer HA, Swanson S, Mosher DF, Stewart R, Coon JJ, Jaitovich A. Large-Scale Multi-omic Analysis of COVID-19 Severity. *Cell Syst* 2020.

5. Leisman DE, Ronner L, Pinotti R, Taylor MD, Sinha P, Calfee CS, Hirayama A, Mastroiani F, Turtle CJ, Harhay MO, Legrand M, Deutschman CS. Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes. *Lancet Respir Med* 2020: 8(12): 1233-1244.

6. Fajgenbaum DC, June CH. Cytokine Storm. *N Engl J Med* 2020: 383(23): 2255-2273.

7. McElvaney OJ, McElvoy NL, McElvaney OF, Carroll TP, Murphy MP, Dunlea DM, Ni Choileain O, Clarke J, O'Connor E, O'Sullivan J, Gunaratnam C, Branagan P, O'Brien ME, Morgan RK, Costello RW, Hurley K, Walsh S, de Barra E, McNally C, McConkey S, Boland F, Galvin S, Kiernan F, O'Rourke J, Dwyer R, Power M, Geoghegan P, Larkin C, O'Leary RA, Freeman J, Gaffney A, Marsh B, Curley GF, McElvaney NG. Characterization of the Inflammatory Response to Severe COVID-19 Illness. *Am J Respir Crit Care Med* 2020; 202(6): 812-821.

8. Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, Lavin Y, Swartz TH, Madduri D, Stock A, Marron TU, Xie H, Patel M, Tubaltes K, Van Oekelen O, Rahman A, Kovatch P, Aberg JA, Schadt E, Jagannath S, Mazumdar M, Charney AW, Firpo-Betancourt A, Mendu DR, Jhang J, Reich D, Sigel K, Cordon-Cardo C, Feldmann M, Parekh S, Merad M, Gnajotic S. An inflammatory cytokine signature predicts COVID-19 severity and survival. *Nat Med* 2020: 26(10): 1636-1643.

9. Su Y, Chen D, Yuan D, Lausted C, Choi J, Dai CL, Voilet V, Duvvuri VR, Scherker K, Troisch P, Baloni P, Qin G, Smith B, Kornilov SA, Rostomily C, Xu A, Li J, Dong S, Rothchild A, Zhou J, Murray K, Edmark R, Hong S, Heath JE, Earls J, Zhang R, Xie J, Li S, Roper R, Jones L, Zhou Y, Rowen L, Liu R, Mackay S, O'Mahony DS, Dale CR, Wallico JA, Algren HA, Zager MA, Unit IS-SCB, Wei W, Price ND, Huang S, Subramanian N, Wang K, Magis AT, Hadlock JJ, Hood L, Aderem A, Bluestone JA, Lanier LL, Greenberg PD, Gottardo R.
Davis MM, Goldman JD, Heath JR. Multi-Omics Resolves a Sharp Disease-State Shift between Mild and Moderate COVID-19. *Cell* 2020: 183(6): 1479-1495 e1420.

10. Wang J, Jiang M, Chen X, Montaner LJ. 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* 2020: 108(1): 17-41.

11. Lepper PM, Ott S, Nuesch E, von Eynatten M, Schumann C, Pletz MW, Mealing NM, Welte T, Bauer TT, Suttorp N, Juni P, Bals R, Rohde G, German Community Acquired Pneumonia Competence N. Serum glucose levels for predicting death in patients admitted to hospital for community acquired pneumonia: prospective cohort study. *Bmj* 2012: 344: e3397.

12. Kruger S, Papassotiriou J, Marre R, Richter K, Schumann C, von Baum H, Morgenthaler NG, Suttorp N, Welte T. Pro-atrial natriuretic peptide and pro-vasopressin to predict severity and prognosis in community-acquired pneumonia: results from the German competence network CAPNETZ. *Intensive care medicine* 2007: 33(12): 2069-2078.

13. Christ-Crain M, Stolz D, Bingisser R, Muller C, Miedinger D, Huber PR, Zimmerli W, Harbarth S, Tamm M, Muller B. Procalcitonin guidance of antibiotic therapy in community-acquired pneumonia: a randomized trial. *Am J Respir Crit Care Med* 2006: 174(1): 84-93.

14. Zhao Y, Qin L, Zhang P, Li K, Liang L, Sun J, Xu X, Dai Y, Li X, Zhang C, Peng Y, Feng Y, Li A, Hu Z, Xiang H, Ogg G, Ho LP, McMichael A, Jin R, Knight JC, Dong T, Zhang Y. Longitudinal COVID-19 profiling associates IL-1RA and IL-10 with disease severity and RANTES with mild disease. *JCI Insight* 2020: 5(13).

15. Galani IE, Rovina N, Lampropoulou V, Triantafyllia V, Manioudaki M, Pavlos E, Koukaki E, Fragkou PC, Panou V, Rapti V, Koltsida O, Mentis A, Koulouris N, Tsiodras S, Koutsoukou A, Andreakos E. Untuned antiviral immunity in COVID-19 revealed by temporal type I/III interferon patterns and flu comparison. *Nat Immunol* 2021: 22(1): 32-40.

16. Hu B, Huang S, Yin L. The cytokine storm and COVID-19. *J Med Virol* 2021: 93(1): 250-256.
Table 1: Distribution of age, sex, and BMI between the study groups. Data are median (SD) and n (%). ** indicates p < 0.01 as calculated by Kruskal-Wallis test.

| Item          | Control | Pneumonia | COVID-19 | Stat. |
|---------------|---------|-----------|----------|-------|
| Patient number| 28      | 23        | 35       | -     |
| Sex, % male   | 100     | 100       | 74       | **    |
| Age           | 62.42 (2.14) | 64.61 (3.11) | 63.86 (3.18) | -     |
| BMI           | 26.30 (0.99) | 27.27 (2.22) | 27.08 (1.31) | -     |
Table 2: Serum concentrations of blood proteins in patients with pneumonia, COVID-19 and control patients without lung disease undergoing elective surgery. Data are median ± SD. * indicates p < 0.05, ** p < 0.005, *** p < 0.001 as calculated using one-way Analysis of Variance (ANOVA, assuming normality) with Bonferroni correction as post-hoc test. The column on the right indicated differences between groups.

| Analyte            | Control (A) | Pneumonia (B) | COVID-19 (C) | Statistical significance¹ |
|--------------------|-------------|---------------|--------------|---------------------------|
| Albumin [g/L]      | 33,16 ± 0,97| 27,96 ± 1,76  | 32,49 ± 1,69 | -                         |
| Total protein [g/L]| 51,36 ± 1,63| 50,83 ± 2,8   | 57,86 ± 1,88 | -                         |
| Adiponectin [µg/mL]| 5,31 ± 0,44 | 6,4 ± 0,78    | 6,77 ± 0,79  | -                         |
| AAT [mg/mL]        | 1,85 ± 0,11 | 3,41 ± 0,31   | 3,03 ± 0,19  | B > A ***, C > A ***      |
| A2Macro [mg/mL]    | 2,5 ± 0,14  | 2,77 ± 0,28   | 2,11 ± 0,1   | B > C *                   |
| Lp(a) [µg/mL]      | 257,55 ± 68,59 | 295,7 ± 66,69 | 153,74 ± 38,47 | -                         |
| B2M [µg/mL]        | 2,91 ± 0,29 | 9,57 ± 1,4    | 5,45 ± 0,86  | B > A ***, B > C **       |
| BDNF [ng/mL]       | 11,78 ± 0,89| 4,76 ± 0,6    | 10,41 ± 1,29 | A > B ***, C > B **       |
|                   |        |        |                  |         |            |
|-------------------|--------|--------|------------------|---------|------------|
| **CRP [µg/mL]**   | 43,1 ± 13,47 | 242,22 ± 37,04 | 189,81 ± 27,09 | **B > A *****<br>**C > A ** |
| **C3 [mg/mL]**    | 1,21 ± 0,08    | 1,38 ± 0,12    | 1,36 ± 0,07    | **-**    |
| **EN-RAGE [ng/mL]** | 250,77 ± 46,84 | 950,57 ± 153,36 | 871,77 ± 125,45 | **B > A ** **C > A ** |
| **Eotaxin-1 [pg/mL]** | 154,58 ± 19,09 | 177,61 ± 32,18 | 169,2 ± 24,35 | **-** |
| **Factor VII [ng/mL]** | 290,96 ± 22,32 | 355,09 ± 32,06 | 338,37 ± 20,82 | **-** |
| **FRTN [ng/mL]**  | 1439,23 ± 428,85 | 2236,35 ± 484,33 | 2904,2 ± 641,14 | **-** |
| **Fibrinogen [mg/mL]** | 0,01 ± 0 | 0,04 ± 0,01 | 0,03 ± 0 | **B > A ** **C > A ** |
| **GM-CSF [pg/mL]** | 9,38 ± 0,38 | 9,52 ± 0,52 | 9,77 ± 0,77 | **-** |
| **Haptoglobin [mg/mL]** | 2,29 ± 0,35 | 5,46 ± 0,93 | 4,03 ± 0,46 | **B > A ** |
| **IgA [mg/mL]**   | 3,33 ± 0,32 | 4,76 ± 0,62 | 3,31 ± 0,32 | **B > C ** |
| **IgM [mg/mL]**   | 2,22 ± 0,2 | 3,46 ± 0,43 | 3,49 ± 0,33 | **B > A ** **C > A ** |
| **ICAM-1 [ng/mL]** | 92,15 ± 9,43 | 199,61 ± 19,62 | 145,6 ± 22,86 | **B > A ** |
| **IFN-gamma [pg/mL]** | 2,62 ± 0,12 | 2,5 ± 0 | 3,92 ± 0,96 | **-** |
| **IL-1 beta [pg/mL]** | 8,19 ± 0,7 | 11,01 ± 0,87 | 10,44 ± 0,56 | **B > A ** |
|                |            |            |            |          |
|----------------|------------|------------|------------|----------|
| **IL-1ra [pg/mL]** | 490,46 ± 212,31 | 599,91 ± 172,38 | 600,11 ± 84,6 | -        |
| **IL-2 [pg/mL]**   | 26,6 ± 1,1 | 27,26 ± 1,76 | 25,5 ± 0   | -        |
| **IL-5 [pg/mL]**   | 5,83 ± 0,33 | 7,15 ± 1,32 | 5,5 ± 0    | -        |
| **IL-6 [pg/mL]**   | 22 ± 8,27  | 46,05 ± 13,28 | 108,16 ± 46,33 | -        |
| **IL-8 [pg/mL]**   | 42,79 ± 9,48 | 99,22 ± 18,82 | 63,1 ± 10,08 | B > A *  |
| **IL-10 [pg/mL]**  | 25,19 ± 7,34 | 20,65 ± 3,22 | 20,23 ± 2,96 | -        |
| **IL-12p40 [ng/mL]** | 0,59 ± 0,06 | 0,75 ± 0,06 | 0,69 ± 0,04 | -        |
| **IL-17 [pg/mL]**  | 1,58 ± 0,21 | 2 ± 0,31    | 1,81 ± 0,21 | -        |
| **IL-18 [pg/mL]**  | 332,48 ± 58,24 | 936,5 ± 187,53 | 855,4 ± 144,83 | B > A *  |
| **MIP-1 alpha [pg/mL]** | 26,81 ± 2,99 | 60 ± 9,45  | 29,09 ± 2,94 | B > A ***|
| **MIP-1 beta [pg/mL]** | 401,35 ± 56,26 | 686,78 ± 201,81 | 573,54 ± 63,52 | B > C ***|
| **MMP-3 [ng/mL]**  | 7,37 ± 1,34 | 20,2 ± 3,43 | 8,66 ± 1,11 | B > A ***|
| **MMP-9 [ng/mL]**  | 35,5 ± 2,78 | 46,57 ± 3,49 | 38,74 ± 2,58 | B > A *  |
| **MCP-1 [pg/mL]**  | 473,83 ± 60,27 | 957,74 ± 175,78 | 1274,91 ± 283,05 | C > A *  |
| **Myoglobin [ng/mL]** | 108,69 ± 17,2 | 179,96 ± 15,58 | 109,31 ± 15,84 | B > A *  |
| **PAI-1 [ng/mL]**  | 217,85 ± 253,41 ± 26,81 | 353,6 ± 33,72 | C > A ** |
|                  | Mean 1 ± SD 1 | Mean 2 ± SD 2 | Mean 3 ± SD 3 | Comparison       |
|------------------|--------------|--------------|--------------|------------------|
| PARC [ng/mL]     | 187,85 ± 31,35 | 319,52 ± 52,11 | 229,69 ± 24,21 | B > A *          |
| SAP [µg/mL]      | 15,38 ± 0,93  | 24,17 ± 2,23  | 20,63 ± 1,02  | B > A ***        |
|                  |              |              | C > A *       |                  |
| SCF [pg/mL]      | 438,98 ± 35,14 | 966,04 ± 98,35 | 563,67 ± 41,53 | B > A ***        |
| SCF [pg/mL]      |              |              | B > C ***     |                  |
| RANTES [ng/mL]   | 26,43 ± 3,01  | 16,88 ± 2,35  | 20,62 ± 1,71  | A > B *          |
| TBG [µg/mL]      | 44,19 ± 1,83  | 48,52 ± 2,83  | 45,29 ± 2,34  | -                |
| TIMP-1 [ng/mL]   | 298,27 ± 38,77 | 474,87 ± 30,13 | 404,03 ± 39,84 | B > A **         |
| TNFR2 [ng/mL]    | 110,88 ± 93,99 | 64,48 ± 8,43  | 34,87 ± 7,07  | -                |
| VCAM-1 [ng/mL]   | 947,04 ± 68,15 | 1568,09 ± 142,19 | 1291,77 ± 95,44 | B > A ***       |
| VEGF [pg/mL]     | 313,23 ± 44,04 | 421,7 ± 62,61  | 486,4 ± 49,45 | -                |
| VDBP [µg/mL]     | 250,31 ± 16,54 | 283,7 ± 26,06  | 281,37 ± 16,26 | -                |
| vWF [µg/mL]      | 343,92 ± 28,95 | 575,35 ± 45,67 | 440,06 ± 40,27 | B > A **        |
| AFP [ng/mL]      | 14,28 ± 13,63 | 0,83 ± 0,16   | 0,56 ± 0,04   | -                |
| ANG-1 [ng/mL]    | 36,12 ± 2,83  | 32,79 ± 3,87  | 41,96 ± 3,39  | -                |
| AXL [ng/mL]      | 7,36 ± 0,62   | 10,66 ± 1,01  | 7,31 ± 0,73   | B > A *          |
| Protein | Reference Value | Sample Value 1 | Sample Value 2 | Sample Value 3 | Significance |
|---------|-----------------|----------------|----------------|----------------|--------------|
| CA-125  | 10.53 ± 3.8     | 35.70 ± 8.71   | 7.07 ± 1.26    | B > C *        |
|         |                 |                |                | B > A **       |
|         |                 |                |                | B > C ***      |
| CA-19-9 | 47.28 ± 20.17   | 33.14 ± 11.35  | 22.22 ± 5.97   | -              |
| CA-9    | 0.35 ± 0.09     | 0.53 ± 0.08    | 0.34 ± 0.05    | B > C *        |
| CEA     | 1.99 ± 0.95     | 3.84 ± 0.95    | 2.20 ± 0.33    | -              |
| HCC-4   | 4.26 ± 0.32     | 5.93 ± 0.39    | 3.04 ± 0.32    | A > C *        |
|         |                 |                |                | B > A **       |
|         |                 |                |                | B > C ***      |
| Decorin | 2.26 ± 0.19     | 3.21 ± 0.22    | 2.58 ± 0.22    | B > A *        |
| FAS     | 29.82 ± 3.84    | 66.26 ± 6.4    | 43.7 ± 17.63   | -              |
| HGF     | 9.99 ± 0.94     | 36.75 ± 8.03   | 31.04 ± 8.28   | B > A *        |
| hCG     | 3.19 ± 0.89     | 2.9 ± 0.37     | 3.63 ± 0.78    | -              |
| IL-18bp | 11.92 ± 0.89    | 23.61 ± 2.18   | 20.19 ± 1.84   | B > A ***      |
|         |                 |                |                | C > A **       |
| MMP-1   | 13.96 ± 1.92    | 24.58 ± 4.51   | 25.55 ± 3.82   | -              |
| MMP-7   | 7.17 ± 1.55     | 11.81 ± 1.4    | 7.97 ± 1.04    | B > C *        |
| MMP-9,  | 869.77 ± 93.18  | 899.91 ± 87.2  | 1247.29 ± 107.05 | C > A *      |
| total   |                 |                |                | C > B *        |
| NSE     | 0.59 ± 0.06     | 1.03 ± 0.12    | 2.11 ± 0.22    | C > A ***      |
|         |                 |                |                | C > B ***      |
| PECAM-1 | 54.85 ± 2.84    | 92.43 ± 9.08   | 77.23 ± 5.94   | B > A ***      |
|         |                 |                |                | C > A *        |
| SP-D    | 15.71 ± 2.16    | 73.97 ± 14.57  | 34.19 ± 4.73   | B > A ***      |

The significance levels are indicated as follows: * indicates a p-value < 0.05, ** indicates a p-value < 0.01, and *** indicates a p-value < 0.001.
| TRAIL-R3 [ng/mL] | 6,82 ± 0,6 | 16,24 ± 2,39 | 8,61 ± 0,76 | B > A ***  
|-----------------|-----------|--------------|------------|----------------|
|                 | B > C *** |              |            |
Table 3: Biomarkers significantly increased in patients with severe COVID-19 disease (A) or in patients who died during the course of disease (B). Data are median ± SD. P values were calculated using two-sided T-test.

| Analyte         | Severe COVID-19 (A) | Survivor vs. Non-survivor (B) |       |       |
|-----------------|----------------------|--------------------------------|-------|-------|
|                 | complicated         | critical                       |       |       |
| Albumin [g/L]   | 35.13 ± 2.01         | 24.90 ± 2.06                   | 35.13 ± 1.89 | 27 ± 3.14 |
|                 | p = 0.005            | p = 0.026                      |       |       |
| Total protein [g/L] | 61.65 ± 1.71         | 48.60 ± 3.80                   | 60.39 ± 2.10 | 52.73 ± 3.72 |
|                 | p = 0.001            | p = 0.063                      |       |       |
| Adiponectin [µg/mL] | 7.41 ± 0.93         | 4.13 ± 0.51                    | 7.86 ± 1.12 | 4.77 ± 0.55 |
|                 | p = 0.032            | p = 0.019                      |       |       |
| AAT [mg/mL]     | 2.7 ± 0.20           | 3.87 ± 0.32                    | 3.01 ± 0.24 | 3.1 ± 0.34 |
|                 | p = 0.004            | p = 0.831                      |       |       |
| A2Macro [mg/mL] | 2.24 ± 0.11          | 1.77 ± 0.24                    | 2.20 ± 0.10 | 1.93 ± 0.24 |
|                 | p = 0.041            | p = 0.323                      |       |       |
| Lp(a) [µg/mL]   | 184.96 ± 56.54       | 99.40 ± 28.09                  | 192.30 ± 56.36 | 80.55 ± 22.05 |
|                 | p = 0.341            | p = 0.190                      |       |       |
| B2M [µg/mL]     | 5.52 ± 1.27          | 5.61 ± 0.84                    | 4.79 ± 1.17 | 7.11 ± 1.17 |
|                 | p = 0.964            | p = 0.226                      |       |       |
| BDNF [ng/mL]    | 12.48 ± 1.60         | 4.22 ± 0.75                    | 12.35 ± 1.73 | 6.02 ± 1.19 |
|                 | p = 0.00006          | p = 0.005                      |       |       |
| CRP [µg/mL]     | 132.5 ± 28.92        | 333.30 ± 37.68                 | 178.10 ± 33.76 | 226.09 ± 48.77 |
|                 | p = 0.0004           | p = 0.425                      |       |       |
| C3 [mg/mL]      | 1.36 ± 0.07          | 1.27 ± 0.14                    | 1.39.00 ± 0.08 | 1.27 ± 0.12 |
|                 | p = 0.515            | p = 0.403                      |       |       |
| EN-RAGE [ng/mL] | 606.30 ± 123.08      | 1484.00 ± 250.40               | 812.00 ± 144.24 | 1048.73 ± 260.16 |
|                 | p = 0.001            | p = 0.395                      |       |       |
| Protein          | Mean 1 ± SD 1 | Mean 2 ± SD 2 | Mean 3 ± SD 3 | Mean 4 ± SD 4 |
|------------------|---------------|---------------|---------------|---------------|
| Eotaxin-1 [pg/mL]| 160.09 ± 30.63| 198.20 ± 48.95| 122.04 ± 17.62| 269 ± 59.35   |
| p                | 0.505         |               | 0.036         |               |
| Factor VII [ng/mL] | 348.43 ± 27.71| 304.40 ± 34.48| 320.91 ± 27.97| 365.82 ± 29.33|
| p                | 0.365         |               | 0.330         |               |
| FRTN [ng/mL]     | 2014.57 ± 709.46| 4825.30 ± 1332.19| 2037.35 ± 498.16| 4964.09 ± 1629.9|
| p                | 0.051         |               | 0.112         |               |
| Fibrinogen [mg/mL]| 0.04 ± 0.005  | 0.03 ± 0.003  | 0.03 ± 0.01   | 0.03 ± 0.00   |
| p                | 0.325         |               | 0.198         |               |
| GM-CSF [pg/mL]   | 10.17 ± 1.17  | 9.00 ± 0.00   | 10.17 ± 1.17  | 9.00 ± 0.00   |
| p                | 0.518         |               | 0.498         |               |
| Haptoglobin [mg/mL]| 3.74 ± 0.60  | 4.32 ± 0.7    | 4.09 ± 0.65   | 4.26 ± 0.50   |
| p                | 0.575         |               | 0.860         |               |
| IgA [mg/mL]      | 3.00 ± 0.27   | 4.21 ± 0.87   | 3.40 ± 0.37   | 2.96 ± 0.64   |
| p                | 0.210         |               | 0.535         |               |
| IgM [mg/mL]      | 3.60 ± 0.41   | 3.45 ± 0.65   | 3.85 ± 0.45   | 2.68 ± 0.38   |
| p                | 0.849         |               | 0.104         |               |
| ICAM-1 [ng/mL]   | 108.70 ± 13.61| 239.70 ± 66.94| 119.74 ± 18.81| 206 ± 58.97   |
| p                | 0.085         |               | 0.189         |               |
| IL-1 beta [pg/mL]| 10.44 ± 0.73  | 10.61 ± 1.06  | 9.62 ± 0.67   | 12.03 ± 0.98  |
| p                | 0.899         |               | 0.049         |               |
| IL-1RA [pg/mL]   | 418.48 ± 74.94| 1062.20 ± 172.41| 441.39 ± 85.33| 969.09 ± 153.41|
| p                | 0.005         |               | 0.003         |               |
| IL-6 [pg/mL]     | 71.95 ± 56.84 | 211.40 ± 92.09| 39.94 ± 17.32 | 260.38 ± 135.85|
| p                | 0.195         |               | 0.138         |               |
| IL-8 [pg/mL]     | 35.76 ± 6.71  | 122.60 ± 21.63| 39.28 ± 6.36  | 116.82 ± 22.12|
|                  | p = 0.003         | p = 0.006         |
|------------------|-------------------|-------------------|
| **IL-10 [pg/mL]**| \(16.57 \pm 3.67\) | \(30.60 \pm 4.72\) |
|                  | \(14.57 \pm 2.72\) | \(33.45 \pm 5.89\) |
| **IL-12p40 [ng/mL]**| 0.73 ± 0.05 | 0.60 ± 0.10 | 0.65 ± 0.05 | 0.77 ± 0.09 |
|                  | p = 0.188         | p = 0.180         |
| **IL-17 [pg/mL]**| \(1.98 \pm 0.30\) | \(1.30 \pm 0.00\) | 1.84 ± 0.28 | 1.60 ± 0.30 |
|                  | p = 0.033         | p = 0.600         |
| **IL-18 [pg/mL]**| \(556.17 \pm 83.49\) | \(1505.90 \pm 395.08\) | 634.09 ± 96.95 | 1373.00 ± 378.61 |
|                  | p = 0.041         | p = 0.085         |
| **MIP-1 alpha [pg/mL]**| 27.13 ± 3.04 | 32.1 ± 7.18 | 26.04 ± 2.98 | 36.45 ± 6.66 |
|                  | p = 0.535         | p = 0.175         |
| **MIP-1 beta [pg/mL]**| 502.39 ± 71.82 | 690.5 ± 130.62 | 547.96 ± 74.92 | 660.36 ± 125.37 |
|                  | p = 0.184         | p = 0.423         |
| **MMP-3 [ng/mL]**| 9.28 ± 1.59 | 7.95 ± 1.32 | 8.07 ± 1.19 | 10.17 ± 2.53 |
|                  | p = 0.610         | p = 0.396         |
| **MMP-9 [ng/mL]**| 39.48 ± 3.40 | 36.1 ± 4.67 | 36.83 ± 3.39 | 44.82 ± 3.20 |
|                  | p = 0.578         | p = 0.149         |
| **MCP-1 [pg/mL]**| \(732.96 \pm 292.33\) | \(2694.90 \pm 512.90\) | \(576.35 \pm 114.48\) | \(2836.09 \pm 670.49\) |
|                  | p = 0.001         | p = 0.007         |
| **Myoglobin [ng/mL]**| 77.91 ± 15.42 | 195.4 ± 28.03 | 93.35 ± 18.98 | 150.91 ± 27.54 |
|                  | p = 0.0004        | p = 0.094         |
| **PAI-1 [ng/mL]**| 305.83 ± 29.42 | 417.4 ± 77.93 | 354.39 ± 45.47 | 368.73 ± 49.57 |
|                  | p = 0.108         | p = 0.848         |
| **PARC [ng/mL]**| 226.39 ± 32.54 | 253.20 ± 38.83 | 225.78 ± 29.96 | 228.73 ± 46.01 |
|                  | p = 0.634         | p = 0.957         |
| **SAP [µg/mL]**| \(18.43 \pm 1.19\) | \(25.00 \pm 1.12\) | 20.13 ± 1.40 | 21.82 ± 1.44 |
|                  | p = 0.002         | p = 0.462         |
|                      | SCF [pg/mL]          | RANTES [ng/mL]    | TBG [µg/mL]     | TIMP-1 [ng/mL]          | TNFR2 [ng/mL]        | VCAM-1 [ng/mL]       | VEGF [pg/mL]         | VDBP [µg/mL]         | vWF [µg/mL]          | AFP [ng/mL]          | ANG-1 [ng/mL]        | AXL [ng/mL]         | CA-125 [U/mL]       | CA-19-9 [U/mL]       | CA-9 [ng/mL]         | CEA [ng/mL]         |
|----------------------|----------------------|-------------------|----------------|------------------------|---------------------|-------------------|---------------------|---------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|-------------------|
|                      | 556.43 ± 43.23        | 22.78 ± 2.02      | 46.17 ± 2.99   | 324.04 ± 33.80         | 25.77 ± 5.56        | 1198.26 ± 119.11  | 456.65 ± 65.61      | 278.13 ± 21.49      | 361.39 ± 33.61     | 0.60 ± 0.06       | 41.26 ± 2.72       | 6.99 ± 0.67        | 8.02 ± 1.83        | 23.17 ± 8.6        | 0.34 ± 0.07       | 1.92 ± 0.38        |
|                      | 585.95 ± 108.10       | 15.27 ± 3.03      | 42.9 ± 4.39    | 598.4 ± 87.38          | 55.2 ± 19.99        | 1496.7 ± 165.26   | 576.1 ± 81.39       | 288.90 ± 29.08      | 632.5 ± 89.67      | 0.49 ± 0.00       | 34.55 ± 6.37       | 7.75 ± 2.06        | 5.67 ± 1.22        | 24.14 ± 6.81      | 0.38 ± 0.09       | 3.1 ± 0.65         |
|                      |                      |                   |                |                        |                     |                   |                     |                     |                   |                  |                   |                   |                   |                   |                   |                   | 1.58 ± 0.3         |
|                      | 473.15 ± 38.62        | 20.05 ± 2.15      | 44.78 ± 3.10   | 354.3 ± 37.08          | 28.15 ± 5.86        | 1961.65 ± 167.04  | 450.26 ± 63.22      | 273.26 ± 23.78      | 429.61 ± 54.04     | 0.52 ± 0.03       | 117.04 ± 11.15    | 6.63 ± 0.68        | 7.69 ± 1.86        | 22.4 ± 8.54       | 0.26 ± 0.06       |
|                      | 760.18 ± 78.54        | 20.86 ± 3.04      | 45.45 ± 3.71   | 526.82 ± 91.84         | 51.27 ± 18.45       | 1523.09 ± 166.03  | 581.55 ± 80.43      | 294.27 ± 14.49      | 481.36 ± 59.19     | 0.54 ± 0.05       | 166.03 ± 11.15    | 8.84 ± 1.82        | 5.69 ± 1.07        | 23.54 ± 6.85      | 0.48 ± 0.08       |
|                      |                      |                   |                |                        |                     |                   |                     |                     |                   |                  |                   |                   |                   |                   |                   |                   | 3.58 ± 0.68        |
| Protein   | Control | Sample 1 | Sample 2 | Sample 3 |
|-----------|---------|----------|----------|----------|
| HCC-4 [ng/mL] | 3.05 ± 0.38 | 3.03 ± 0.71 | 2.69 ± 0.34 | 3.56 ± 0.69 |
| Decorin [ng/mL] | 2.28 ± 0.24 | 3.42 ± 0.42 | 2.27 ± 0.18 | 3.26 ± 0.53 |
| FAS [ng/mL] | 25.49 ± 4.08 | 91.43 ± 60.48 | 21.76 ± 3.14 | 91.64 ± 54.6 |
| HGF [ng/mL] | 15.88 ± 3.91 | 70.64 ± 23.99 | 16.47 ± 3.05 | 63.14 ± 23.43 |
| hCG [miU/mL] | 3.74 ± 1.13 | 3.67 ± 0.86 | 3.87 ± 1.13 | 3.26 ± 0.78 |
| IL-18bp [ng/mL] | 18.18 ± 2.38 | 25.60 ± 2.67 | 17.52 ± 2.13 | 26.91 ± 2.89 |
| MMP-1 [ng/mL] | 24.98 ± 4.46 | 21.68 ± 4.7 | 24.27 ± 5.11 | 24.99 ± 5.09 |
| MMP-7 [ng/mL] | 6.47 ± 0.7 | 12.21 ± 2.91 | 6.85 ± 1.10 | 10.51 ± 2.26 |
| MMP-9, total [ng/mL] | 1152.48 ± 126.91 | 1389.8 ± 225.57 | 1372.09 ± 130.57 | 1080.09 ± 172.68 |
| NSE [ng/mL] | 1.85 ± 0.27 | 2.79 ± 0.41 | 1.99 ± 0.28 | 2.37 ± 0.40 |
| PECAM-1 [ng/mL] | 69.52 ± 5.63 | 96.7 ± 15.09 | 74.04 ± 5.51 | 87.27 ± 14.74 |
| SP-D [ng/mL] | 31.47 ± 5.76 | 42.4 ± 9.47 | 34.64 ± 5.29 | 35.67 ± 10.33 |
| TRAIL-R3 [ng/mL] | 8.34 ± 0.92 | 9.94 ± 1.55 | 8.17 ± 0.88 | 9.04 ± 1.54 |
Figure legends

Figure 1: Serum markers discriminate between control, pneumonia, or COVID-19. (A) Serum concentrations of biomarkers that showed differences between control, pneumonia, or COVID-19 groups. p < 0.05, ** p < 0.005, *** p < 0.001 as calculated using one-way Analysis of Variance (ANOVA, assuming normality) with Bonferroni correction as post-hoc test. (B) UMAP dimension reduction integrating all detectable biomarkers shows segregation the three groups. We clustered the individuals by 3-means clustering and computed for each cluster how many individuals from the three groups were contained. Following this clustering we computed Fisher’s Exact test on the 3x3 contingency table, validating a significant clustering (p < 0.0001).

Figure 2. Biomarkers levels are associated with disease severity. (A) Biomarkers with significantly altered serum concentration in severe patients. p < 0.05, ** p < 0.005, *** p < 0.001 as calculated by two-sided T-test. (B) Biomarkers are associated with SAPS and TISS measurements; the magnitude of Pearson’s R is indicated by the color scheme.

Figure 3. Biomarkers patterns are associated with mortality risk. (A) Serum markers with significantly altered serum concentration in patients who died during the course of disease. p < 0.05, ** p < 0.005, *** p < 0.001 as calculated by two-sided T-test. (B) UMAP analysis showed a significant separation of these two groups. We clustered the individuals by 2-means clustering and computed for each cluster how many individuals from the survival and non-survival group were contained in each
cluster. Following this clustering we computed Fisher’s Exact test on the 2x2 contingency table, validating a significant clustering (p < 0.01).
Herr et al., Figure 2
