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Urinary Peptides Significantly Associate with COVID-19 Severity: Pilot Proof-of-Principle Data and Design of a Multicentric Diagnostic Study

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SARS-CoV-2 infection results in a mild-to-moderate disease course in most patients, allowing outpatient self-care and quarantine. However, in ≈10% of cases a two- or three-phasic critical disease course with starting from day 7 to 10 is observed. To facilitate and plan outpatient care, biomarkers prognosing such worsening at an early stage appear of outmost importance. In this accelerated article, the identification of urinary peptides significantly associated with SARS-CoV-2 infection, and the development of a multi-marker urinary peptide based test, COVID20, that may enable prognosis of critical and fatal outcomes in COVID-19 patients is reported. COVID20 is composed of 20 endogenous peptides mainly derived from various collagen chains that enable differentiating moderate or severe disease from critical state or death with 83% sensitivity at 100% specificity. Based on the performance in this pilot study, testing in a prospective study on 1000 patients has been initiated.

Even though the understanding of Corona virus disease 2019 (COVID-19) pathophysiology is improving and first therapeutic options evolve,[1,2] clinical management of patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is challenging. A key issue is prognosis of critical or fatal disease courses, observed in 6–10 % of patients.[3,4] Identification of these patients is essential to apply preventive measures, expected to reduce severity of disease course.

Severe disease courses after SARS-CoV-2 infection are associated with endothelial damage and thrombosis.[5] Both these processes result in substantial changes in protein metabolism, and in the release of a large number of peptides in the bloodstream as a consequence of protein breakdown. Peptides are expected to be cleared via filtration in the kidney and should therefore also be present in urine. In addition, the kidney was reported as an organ substantially affected especially in severe cases of COVID-19,[6,7] as indicated by early albuminuria excretion.[8] Based on these observations, the hypothesis was generated that COVID-19 results in a significant change of not only albumin, but a disease-specific cluster of urinary peptides, especially collagen derived,[9,10] and that these changes are associated with disease severity. To test this hypothesis, we initiated a pilot study with the aim to identify peptides that undergo >50% change in disease. As the limitation was the availability of samples infected with SARS-CoV-2, we opted for a 3:1 ratio, which would allow detecting such peptides with 80% power, assuming an SD of 50%, by investigating 11 cases and 33 controls. The study was based on the following aims: 1) investigate if significant changes can be observed in urinary peptides in COVID-19 patients, in comparison to controls; 2) identify peptides associated with disease severity; 3) combine the peptides associated with disease severity in a high-dimensional classifier; 4) if this classifier can be generated and shows good performance in complete take-one-out cross-validation, its performance in a multi-center prospective study at several different time points after SARS-CoV-2 infection will be tested. In this manuscript we describe the results of this pilot study, and the design of the prospective study.

The capillary electrophoresis - mass sepectometry (CE-MS) technology was employed for the comparable analysis of currently >70 000 samples[11] and has previously been qualified for

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prognosis of progression and outcome in large-scale prospective and longitudinal clinical studies, for example, in the setting of diabetic nephropathy,[9] in the context of graft versus-host disease,[12] or in the context of predicting death after ICU stay.[10]

Samples from patients and matching controls were analyzed using CE-MS, and lists of peptides were generated, as described.[11] The proteomics data were deposited in PRoteomics IDEntifications database (PRIDE) under the dataset identifier PXD020444. In a first step, the data were investigated for significant changes between incident (March–April 2020) COVID-19 patients in one German tertiary infectious disease center and uninfected age-, gender-, and comorbidity-matched controls. Proteomic profiles of control individuals were recruited in silico from foregoing non-COVID-19 projects from the Human Urine Proteome database (Table S1, Supporting Information).[11] Applying a frequency threshold of 30%, a total of 1941 urinary peptides were investigated. The distribution of the peptides in COVID-19 patients and controls is depicted in the volcano plot in **Figure 1**. By statistically comparing the peptide distribution in these two groups, 166 peptides were identified that showed a significant difference between the case and control group after correction for multiple testing.[13] Sequence from 85 of these 166 peptides could be obtained. These peptides and their distribution in COVID-19 patients and uninfected controls are presented in Table S2, Supporting Information. When these 166 peptide markers were combined into a classifier, they allowed 100% correct separation of the case and control group in total take-one-out cross-validation. Detailed examination of results of this classifier indicated that disease severity may display in specific urinary peptides, as a yet not significant difference between moderate and severe cases was detected (**Figure 2**).

Significance Statement

The aim of the present study was to identify endogenous urinary peptides indicative for an unfavorable outcome in patients infected with SARS-CoV-2 and to integrate the peptide markers into a non-invasive and outpatient diagnostic test that enables prognosis of a critical disease outcome. Such an approach is of outmost importance because SARS-CoV-2 infection results in a mild-to-moderate disease course in the majority of patients, allowing outpatient self-care and quarantine with the goal to avoid hospital resources congestion. However, in up to 10% of patients severe worsening is observed, typically starting from day 7 to 10 after disease detection. To guide patient management, biomarkers that prognose severe or critical course of the disease would be highly beneficial, enabling early and personalized intervention.

Based on this observation, in a next step we aimed towards identifying peptides significantly changed in moderate and severe versus critical and fatal disease. 31 urinary peptides were identified that showed significant changes in the distribution between these two severity groups. These peptides are listed in **Table 1**, their distribution is shown in **Figure 4**. To assess potential specificity of these peptides for COVID-19, we have investigated the overlap between them and the peptides contained in the well described classifiers for CKD (CKD273),[14] heart

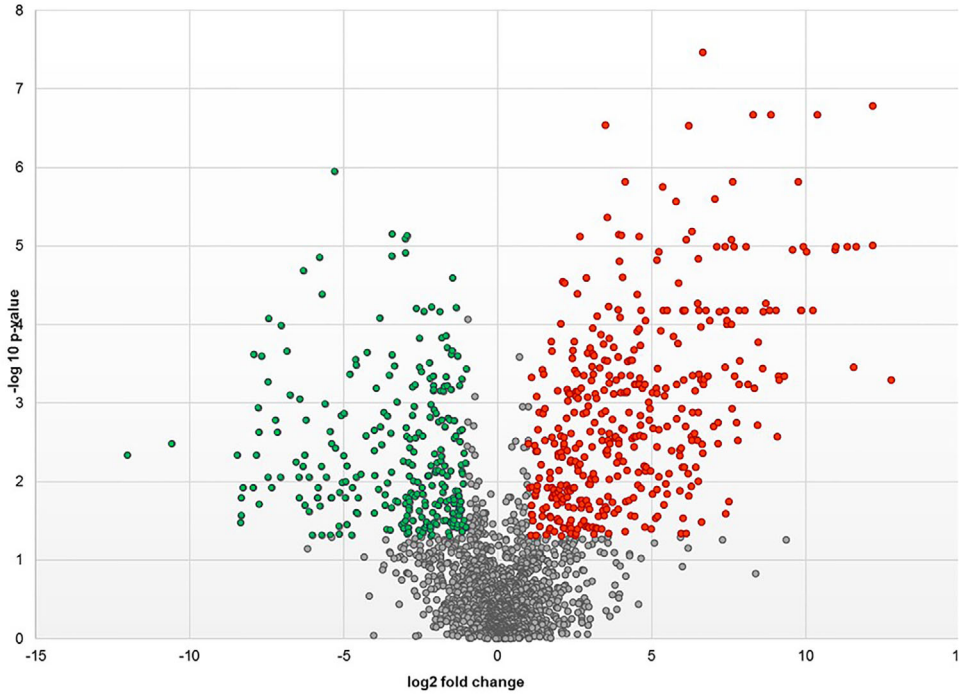

Figure 1. Volcano plot depicting the distribution of the 1941 peptides that were found in >30% of all subjects. As evident, a large number of peptides appear to differ in abundance between COVID-19 patients and matched controls.

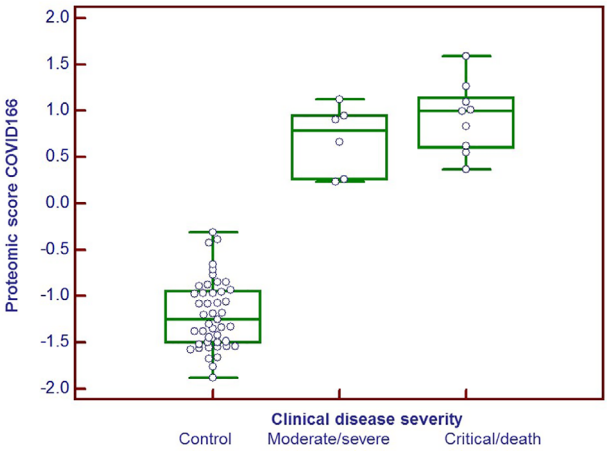

Figure 2. Distribution of the proteomics scoring of a classifier based on the 166 urinary peptides significantly different between SARS-CoV-2 patients and controls. Shown is the scoring in the complete take-one-out cross-validation.

failure (HF1),[15] and coronary artery disease (CAD238).[16] Of the 85 peptides contained in HF1, none was part of the 31 peptides described here. One peptide from CAD238 and 3 peptides from CKD273 were also present. A modest (≈10%) overlap with CKD biomarkers, but essentially no overlap with biomarkers for cardiovascular disease, is well within the expectation for COVID-19, where impact on the kidney has been reported.[8]

Almost all of the amino acid sequences identified ($N = 20$) are fragments of collagen. Besides major fibril-forming collagens (COL1A1, COL2A1, COL3A1, COL1A2) and minor fibril-forming cross-linking collagens (COL5A1, COL11A2), there are also peptides potentially derived from structural components of glomerular basement membranes (COL4A2), sub-endothelium (COL8A1), and saliva secreting glands (COL9A2, COL9A3). We investigated the expression levels of the different collagen proteins relevant in this study using the human protein atlas. The results, presented in Table S2, Supporting Information, indicate expression of all collagens in more than one organ or cell type investigated, especially also in lung and smooth muscle cells, indicating potential relevance in pulmonary and vascular damage.

The 20 sequence-identified COVID-19 peptides associated with severity were combined into a classifier, COVID20, using support vector machine (SVM) supervised learning. This classifier demonstrated an AUC of 0.91 ($p < 0.0001$) for differentiation of moderate and severe cases from critical cases and those subsequently dying. Clinical severity was classified according to the criteria of the World Health Organization.[17] The model resulted in correct classification of all (9 out of 9) moderate and severe cases and 5 out of 6 critical and fatal cases (see also **Figure 3**). Two elder and multi-morbid patients that were initially categorized as severe, but not critical, based on the clinical assessment, died on the low-care ward during the course of viral infection after having declined measures of intensive care medicine including mechanical ventilation. For these two patients COVID20 correctly prognosed a critical outcome, further indicating a prognostic value of COVID20. The relation of the classification values to the clinical features of the 15 patients is provided in **Table 2**. One patient with a critical disease course was classified negative. This might be explained by response to Remdesivir treatment. Severe and critical patients under Remdesivir therapy appear to have the tendency towards lower COVID20 scores compared to those without antiviral drug treatment or a combination of azithromycin and hydroxychloroquine (Table 1). The majority of age-, gender-, and comorbidity-matched control subjects (43 out of 45) scored negative, as demonstrated in the distribution plot of classification values between the different groups (Figure 2).

The results presented in this pilot study support the initial hypothesis that COVID-19 is associated with a significant change in urinary peptides and kidney involvement, and that several of these are further associated with disease severity and the organ-specific inclusion of specific compartments, that go further than albuminuria. The most prominent peptidomic change observed is an upregulation of multiple collagen fragments, indicating increased proteolysis of extracellular matrix, as expected in inflammation and endothelial damage.

In a study by Ruan et al.,[3] the authors reported that virus-activated cytokine release syndrome (CRS) and fulminant myocarditis are suggested to be the main causes of worse patient outcomes. Thus, the prevalence of exaggerated immune responses against the virus and cytokine-related adverse events in patients with a severe course of COVID-19 infection is expected to be high.

Since the test should be applicable to outpatient care it must rely on a non-invasive sample collection strategy, which is most ideally realized by a urinary test. This limits the number of suitable biomarkers to those that can be detected in urine and that are connected to host immune responses, acute phase reactions, and inflammation. Our research group was already successful in establishing urinary peptide marker tests for the diagnosis of systemic immune and inflammatory diseases.[18,19]

The involvement of collagens in COVID-19 was already reported on the transcriptomics level by Ackermann et al.[20] Moreover, increased excretion of collagen derived peptides in urine is consistent with the histological observations of alveolar epithelial cell injury, hyaline membrane formation, fibroblastic proliferation with extracellular matrix and fibrin forming clusters occurring in lungs, patchy necrosis in the liver, and mild focal fibrosis and myocardial hypertrophy in the heart secondary to SARS-CoV-2 infection[21] all of which are altering extracellular matrix turnover. Only one non-collagen peptide, a fragment of the endogenous thiol protease inhibitor Cystatin-B, was found decreased in the critical disease courses. Cystatin-B is involved in the inhibition of cathepsins[22] and transported upon autophagy by extracellular vesicles to the urine.[23,24]

Also due to the fact that this is a pilot study and owed to the specific situation in the context of COVID-19, the study has several shortcomings. First, the study design was cross sectional, with inclusion of additional follow-up information. It is not a well-controlled prospective study, and, as such, relevant information, for example, on disease onset, timing of sampling, maximal disease activity, etc. was not collected in a systematic way. However, the study has resulted in the detection of multiple potential biomarkers, which are now being tested in an appropriately powered prospective study. Second, not all the potential biomarkers described and included in the classifier could be identified via sequencing. As outlined previously in detail, sequencing of naturally occurring peptides represents a major challenge, likely due to unknown post-translational modifications (PTM).[25] As such,

Table 1. List of the 31 peptides significantly changed in moderate and severe versus critical and fatal disease. Given are the mass, migration time, average signal intensity, and the two groups, sequence, parental protein, and start as well as stop amino acid position, if available, and the observed regulation in critical/death versus moderate/severe cases of COVID-19.

Mass	CE-T	Moderate/severe	Critical/death	Sequence	Protein	Start AA	Stop AA	Fold regulation
875.48	21.9	119.17	0	—	—	—	—	0.000
883.41	23.3	130.25	0	PpGENGKpG	COL3A1	649	657	0.000
896.46	22.5	34.18	5.27	—	—	—	—	0.154
912.38	25.2	142.01	19.62	GDRGEpGpP	COL1A1	800	808	0.138
964.48	24.2	0	25.56	QGAPGERGPP	COL4A2	1250	1259	∞
988.52	22.4	302.12	107.7	YQTNKAKH	CSTB	85	92	0.356
1110.39	33.6	442.99	41.53	—	—	—	—	0.094
1115.55	26.3	0	94.04	YDGKGVGLGPGP	COL1A2	81	92	∞
1141.54	37.3	215.84	1.38	GPpGPpGPPGPpA	COL8A1	560	572	0.006
1383.59	27.6	317.17	282.01	GSpGGpGSDGKpGPpG	COL3A1	540	555	0.889
1405.67	28.1	170.63	157.62	GISGPpGPpGPAGKEG	COL1A2	802	817	0.924
1561.45	37	234.03	19.31	—	—	—	—	0.083
1593.75	39.6	0	150.16	PGTpGNPGpPGpPGPPGP	COL2A1	155	172	∞
1632.78	30.3	0	214.91	ERGpPGpPGpPGVpGSD	COL9A2	31	47	∞
1750.78	23.8	624.76	38.21	GPpGPpGKNGDDGEAGKpG	COL1A1	221	239	0.061
1848.78	23.7	0	15.23	—	—	—	—	∞
2101.96	27.7	0	9.87	DGQPGAKGEPGDAGAKGDAGPPGP	COL1A1	820	843	∞
2132.91	25.8	2784.88	26.57	GARGpEGAQGPRGEpGTPGSpGP	COL2A1	381	403	0.010
2234.01	34	490.18	221.63	pGpSGEKGETGDVGPMGpPGPpGP	COL11A2	1161	1184	0.452
2327	33.5	0	21.95	DRGETGPAGpPGApGApGApGPVGPAG	COL1A1	1035	1061	∞
2334.99	33.6	18.08	141.71	—	—	—	—	7.838
2642.21	27.7	1002.38	37.18	GNEGpSGPPGpAGSPGERGAAGSGGPIGpPG	COL11A2	1003	1033	0.037
3036.32	22.3	0	11.49	mPGFKGpTGYKGEQGEVGKDGEKGDpGpPG	COL9A3	151	180	∞
3247.55	25.7	0	59.63	—	—	—	—	∞
3326.44	25.2	4.08	383.05	—	—	—	—	93.885
3416.1	32	0	282.92	GPpGADGQPGAKGEpGDAGAKGDAGPPGpAGPAGPPGpIG	COL1A1	815	854	∞
3441.61	31.4	16 398.7	9131.52	pGpPGPPGVTGMDGQPGPKGNVGPQGEPGPPGQQGNP	COL5A1	690	726	0.557
3718.72	32.5	796.44	235.25	SGPPGRAGEPGLQGPAGPpGEKGEPGDDGpSGAEGPpGPQG	COL2A1	935	975	0.295
4349.58	23.5	0	64.31	—	—	—	—	∞
5724.44	28.1	395.21	32.84	—	—	—	—	0.083
10 753.3	19.7	160.72	1504.2	—	—	—	—	9.359

sequence could not be assigned to all peptides with the high confidence level applied in this study. However, these peptides are nevertheless well defined via their mass and migration time, and may be identified once additional information on PTMs present in urine peptides becomes available.

CE-MS based urine proteome analysis is registered as in in vitro diagnostics in Germany for a number of applications. Among others, it is applied for the early detection of diabetic kidney disease,[9] the detection of cholangiocarcinoma,[26] and the detection and assessment of clinical relevance of prostate cancer.[27] As such, a routine procedure has been established, including shipment of urine samples without the need for refrigeration. In the more acute setting, comparable to the application in COVID-19 patients, CE-MS analysis is used for the early detection and prognosis of graft versus host disease.[28] Potential application of a CE-MS based test to assess risk of adverse outcome (severe or critical course of COVID-19) would be based on this well-established approach, enabling reporting of results within 24 h after sample arrival. Based on the applicability of this approach and as the results of these pilot data, a prospective European multi-center observational study has been initiated. This study aims at including 1000 patients diagnosed with SARS-CoV-2 infection by respiratory PCR detection[3] of SARS-CoV-2. In close cooperation with the "Standing Working Group of Competence and Treatment Centers for Diseases Caused by Highly Pathogenic Pathogens" (STAKOB) at the Robert Koch Institute (RKI), clinical and demographic data, as well as data on the course of the disease, are registered in an electronic case report form (eCRF) developed specifically for quarantine and COVID-19 studies. This eCRF also enables the recruitment of presumably mildly ill, outpatient-managed patients in home quarantine. A validated (Good clinical praxis, GCP and Good automated manufacturing, GAMP5 adapted) electronic data capture (EDC) system (MARVIN) is used for the study, which also allows patient-reported outcome documentation online. The study aims at including on third outpatients and two thirds inpatients, half of

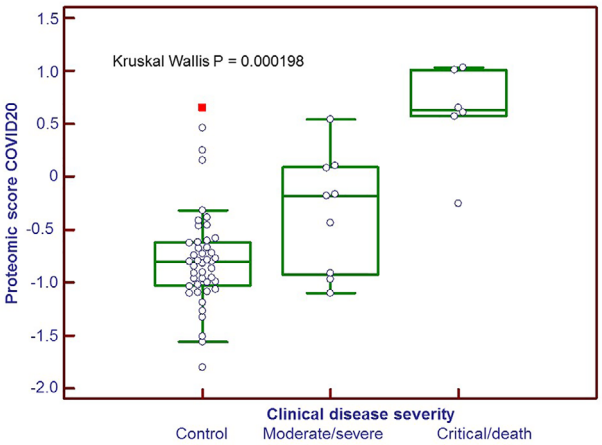

Figure 3. Distribution of classification scores of the COVID20 urinary peptide marker model for age-, gender-, and comorbidity-matched control subjects (left panel) and COVID-19 patients with either moderate/severe (N = 9, intermediate panel) or critical/death (N = 6, right panel) disease outcomes. Moderate disease: uncomplicated upper airway symptoms without requirement of supplemental oxygen, no respiratory symptoms (vomiting/diarrhea/fever); Severe disease: receiving supplemental oxygen, Sp02 ≤90%, PaFiO2 ratio <300 mmHg, respiratory rate >30/min; Critical disease: receiving ventilatory support (nasal high-flow canula, non-invasive ventilation, invasive ventilation), multiple organ failure, death.

which (≈330) each are with severe/critical disease. Considering a possible national testing strategy, this distribution should ensure that statements about the severity can be made in the outpatient group. Three urine samples will be collected from each patient, a first urine sample within 48 h of a positive test, a second around day 4, and a third around day 14. The three time points serve to determine the ideal time for sampling with respect to prognostic value, considering that severe illnesses usually appears 7 to 10 days after the first symptoms. The home delivery of samples corresponds to the procedure developed in an outpatient COVID-19 therapy study (COVIDVal; Eudract No. 2020-001431-27). Classification into moderate, severe, and critical disease courses is applied according to the classification criteria of the WHO,[17] which include intensive care, ventilation, and death as patient-relevant endpoints. For descriptive assessment, previous disease, comorbidities, any (antiviral) therapy, and routine laboratory parameters (liver and kidney function, oxygenation) will be recorded. Samples are collected in borate tubes (to prevent microbial growth) and sent to the central laboratory for CE-MS analysis, as in the recently completed *"Proteomic prediction and Renin angiotensin aldosterone system Inhibition prevention Of early diabetic nephRopathy In TYpe 2 diabetic patients with normoalbuminuria"* (PRIORITY) study.[29] The peptide profiles of the first 250 patients are used to verify the COVID20 peptide marker panel at a higher level of statistical significance than in this pilot study. Due to the increased statistical power, additional peptide markers are expected to be identified. Based on the results of the statistical analysis, the COVID20 classifier may be refined. The completion of the first phase for model optimization is documented before prospective validation in routine operation on additional 750 patients, using the predetermined limit value for a positive test result.

In conclusion, this report indicates that a urinary peptide-based biomarker panel may enable prognosis of COVID-19 disease course, and consequently implementation of proteomics guided personalized intervention.

Experimental Section

Study Population: Fifteen patients with COVID-19 and 45 matching controls were included in the study. Inclusion criteria were a positive SARS-CoV-2 test and consent to be enrolled in the study. Matching was performed from a pool of 1759 controls, based on age, gender, hypertension, and diabetes. The characteristics of the cohort are presented in Table S1, Supporting Information. The study complied with the Helsinki declaration for research in humans. Participants provided informed written consent for their data and biological samples to be stored and analyzed. The Ethics Committee of the Saxon State Medical Association approved the study (#EK-BR 14/20-1).

Urinary Proteomics: The urine samples were prepared as described in detail previously,[30] using ultrafiltration to remove proteins >20 kDa,

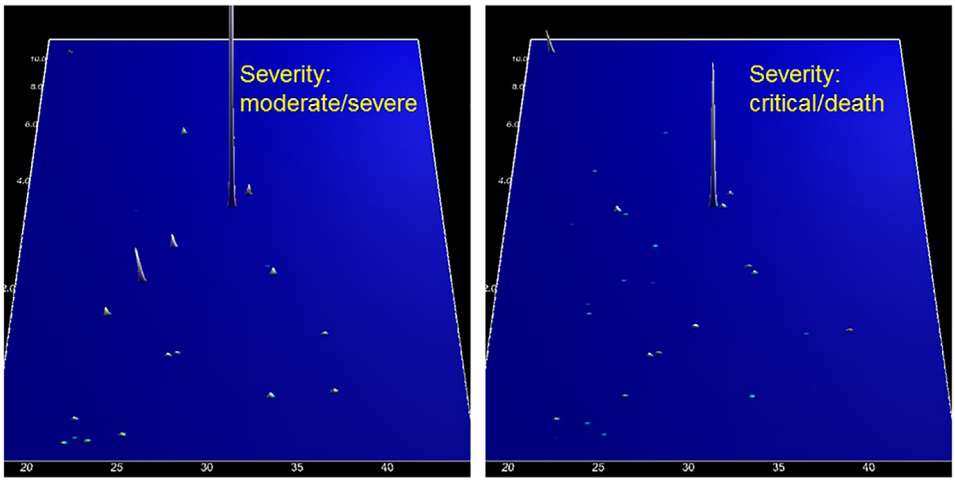

Figure 4. Graphic depiction of the distribution of the 31 peptides associated with COVID-19 disease severity. Left panel: distribution in the 9 moderate/severe cases, right panel: distribution in the 6 critical/death cases. The molecular mass (0.8–10 kDa, on a logarithmic scale) is plotted against normalized CE migration time (18–45 min). The signal intensity is encoded by the peak height.

Table 2. Classification values of the COVID20 peptide marker model and patient's clinical history for the analyzed cross-sectional COVID-19 patients. Patients are listed sequentially from the lowest to the highest COVID20 classification values. Of note, severe and critical patients under Remdesivir treatment (marked in bold) have the tendency towards lower COVID20 scores compared to those without or a combination of Azithromycin and Hydroxychloroquine.

Patient ID	Severity of disease	COVID20 score	Age	Gender	Symptoms	Complications	Comorbidities	Therapy
50154	Moderate	−0.97	88	Female	PNE, COU	None	CVA, AH, HHD, T2D, CKD, ATF	n.s.
50152	Moderate	−0.908	87	Female	GIS	None	AH, MI, TP	n.s.
50153	Moderate	−1.099	80	Male	DYS	None	COPD, CHD	n.s.
50141	Moderate	−0.435	77	Female	FEV, COU, RPI	None	CVA, AH, EL	n.s.
50146	**Critical**	**−0.257**	**36**	**Male**	**PNE, NAU, DYS**	**HFOV, PE**	**Obesity, ABA**	**RDV**
50150	Moderate	−0.182	55	Male	PNE, FEV, DIA, MYA, COU	None	AH	n.s.
50147	**Severe**	**−0.165**	**79**	**Female**	**PNE, GIS**	**None**	**Severe ASVD, SSS**	**RDV**
50143	Severe	0.085	75	Male	PNE, FEV, COU	None	Dementia, VHF	n.s.
50142	**Severe**	**0.101**	**80**	**Male**	**PNE, FEV**	**None**	**AH, PD, CVA**	**RDV**
50145	Death	0.608	94	Female	PNE, DYS	CRS, ESRD, Death	AKI, CHF, AH, CP	n.s.
50151	**Critical**	**0.575**	**46**	**Male**	**PNE, FEV, COU, Exertional DYS**	**HFOV**	**AH, T2D, OSAS**	**RDV**
50148	Critical	1.01	80	Male	PNE, DYS, COU	HFOV, BSI, TI, KI, HD, PE	VHF, CHF, CES paralysis	AZM/HCQ
50140	Moderate	0.544	90	Female	PNE, FEV, DYS	PE	AH, PUD, DU, OP	n.s.
50144	Death	0.656	79	Male	None	MID, Death	EL, MID, MI, AM, T2D, AH, NHL	n.s.
50149	Critical	1.032	83	Male	PNE, DYS	TI, ARDS, HD, DIC, CIP	SVT, AUF, Varikosis	AZM/HCQ

Abbreviations: ABA, allergic bronchial asthma; AH, arterial hypertension; AKI, acute kidney injury; AM, autoimmune myositis; ARDS, acute respiratory distress syndrome; ASVD, arteriosclerotic vascular disease; ATF, atrial fibrillation; AZM, Azithromycin; BSI, bacterial superinfection; CES, Cauda Equina syndrome; CHD, chronic heart disease; CHF, congestive heart failure; CIP, critical illness polyneuropathy; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; COU, coughing; CP, chronic pancreatitis; CRS, cardiorenal syndrome; CVA, cerebrovascular accident; DIA, diarrhea; DIC, disseminated intravascular coagulation; DU, decubitus ulcer; DYS, dyspnea; EL, epilepsy; ESRD, end-stage renal disease; FEV, fever; GIS, gastrointestinal symptoms; HCQ, Hydroxychloroquine; HD, hemodialysis; HFOV, high frequency oscillatory ventilation; HHD, hypertensive heart disease; KI, kidney injury; MI, myocardial infraction; MID, multi-infarct dementia; MYA, myalgia; NAU, nausea; NHL, Non-Hodgkin lymphoma; OP, osteoporosis; OSAS, obstructive sleep apnea syndrome; PD, Parkinson's disease; PE, pulmonary embolism; PNE, pneumonitis; PUD, peptic ulcer disease; RDV, Remdesivir; RPI, respiratory partial insufficiency; SSS, subclavian steal syndrome; SVT, supraventricular tachycardia; T2D, type 2 diabetes; TI, tracheal intubation; TP, tetraparesis.

desalted, and lyophilized. Average sample recovery of peptides and low molecular weight proteins were ≈85%. Shortly before CE-MS analysis, lyophilisates were resuspended in HPLC–grade water (Merck KGaA, Darmstadt, Germany). CE-MS analysis was performed with a P/ACE-MDQ CE electrophoresis system (Beckman Coulter, Brea, CA, USA) coupled in line with a micrOTOF (Bruker Daltonics, Bremen, Germany) as described.[30] Mass spectral ion signals representing identical molecules at different charge states were deconvoluted into single masses using MosaiquesVisu software.[31] To achieve high mass accuracy, mass signals were calibrated based on FTICR–derived accurate masses as described previously.[32] In parallel, CE migration time and signal intensities were calibrated and normalized based on internal standards as previously described[33] by applying local linear regression. All detected mass signals of peptides were deposited, matched, and annotated in a Microsoft SQL database.[11] For peptide sequencing, samples were analyzed using a Dionex Ultimate 3000 RSLS nanoflow system (Dionex, Camberly, UK) or a Beckman CE, coupled to an Orbitrap Q Exactive plus instrument (Thermo Scientific, Waltham, MA).[34] The data were analyzed using Proteome Discoverer 2.4 (precursor mass tolerance, 5 ppm; fragment mass tolerance, 0.05 Da) and were searched against the UniProt human non-redundant database without enzyme specificity. No fixed modifications were selected. Oxidation of methionine and proline were considered as variable modifications. The criteria for accepting sequences were high confidence (Xcorr ≥ 1.9) and the absence of unmodified cysteine. A strong correlation between peptide charge at pH 2 and CE migration time was used to avoid false sequence assignment to peptides.[35]

Statistical Analysis and Classifier Development: Proteomics data were compared by Wilcoxon test, proven to be of superior statistical power in proteomics datasets.[36] A p-value of <0.05 was considered statistically significant. Only peptides with a frequency of at least 30% in one group were considered for further analysis. To control for the false discovery rate, p-values were adjusted by the Benjamini and Yekutieli method.[13] Biomarkers were combined into a multi-dimensional classifier, using the SVM based MosaCluster software.[37] The cost parameter C and the functional parameter gamma of the Gaussian Radial Basis Function Kernel were optimized via leave-one-out cross-validation error estimation. The classifier returns a score for risk; a higher score indicates higher disease risk.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

H.M. is the co-founder and co-owner of Mosaiques Diagnostics. J.M. and A.L. are employed by Mosaiques Diagnostics. All other authors declare no conflict of interest.

Keywords

biomarker, COVID-19, proteome, SARS-CoV-2, urine

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