Test plan

Prospective validation (follow-up) of a urine test for early prediction of clinical course in patients with a SARS-CoV-2 infection

Test plan code: Crit-Cov-U 01

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1 Objectives

1.1 Overall objective of the project

As described in a study conducted in the PR China[1], as well as a technical paper from Germany[2], on average 6-10% of all patients with SARS-CoV-2 infection develop a critical disease progression with the need for intensive care and possibly controlled ventilation therapy. In the majority of cases, this unfavorable disease progression is triggered by the body’s excessive immune reaction to the viral infection with development of cytokine release syndrome, myocarditis, multiple organ failure, and even death. However, the development of such a critical progression cannot be predicted at the beginning of the disease. It would therefore be of great diagnostic benefit to have a non-invasive test available that could reliably detect the severity of the disease at an early stage. Such a test would enable the treating physician to apply therapy measures tailored to the individual patient. This is particularly critical with the quarantine measures required for COVID-19, as in an outpatient setting outside the hospital, the patient cannot be kept under constant observation and cannot be appropriately treated immediately in the event of a sudden deterioration in health.

This study will include 1000 patients diagnosed with a SARS-CoV-2 infection by respiratory PCR detection [3] of SARS-CoV-2 (pharyngeal lavage or nasopharyngeal swab) at disease onset. For patient recruitment, close cooperation with the clinical treatment centers of the "Standing Working Group of Competence and Treatment Centers for Diseases Caused by Highly Pathogenic Pathogens" (STAKOB) at the Robert Koch Institute (RKI) is planned. Clinical and demographic data, as well as data on disease progression, is registered in pseudonymized form via an electronic Case Report Form (eCRF) already developed specifically for quarantine and COVID-19 studies and agreed with the respective data protection officers. This specific eCRF also allows recruitment of presumably more mildly ill outpatients in home quarantine. For the large validation study now planned, a validated (GCP and GAMP5 adapted) EDC system (MARVIN) will be used, which also allows patient-reported outcome (PRO) documentation online.

The distribution of severity is expected to include 33% (approximately 330) outpatients and 66% inpatients, half of whom (approximately 330) with severe disease and the other half with critical disease. Appropriate patients will be recruited by block specifications to study sites and will be rechecked and redistributed as necessary during the inclusion process. With a view to a possible national testing strategy at disease onset, this distribution should ensure that statements can be made about the (unexpected and thus dangerous) severity especially in the outpatient group.

Three urine samples will be collected from each patient or sent from home; an initial urine sample at the time of first presentation (day 0-1), a second on day 4-5, and a third sample on day 10-14. The three time points will be used to determine the best time to collect the sample, in terms of prognostic information. The third sample time point was chosen taking into consideration that severe disease progression usually occurs 7 to 10 days after the first signs of severe symptomatology. The home sample shipment corresponds to the procedure recently developed in an outpatient COVID-19 therapy study already accepted by the BfArM (Covidval; Eudract No. 2020-001431-27). Classification into moderate, severe, and critical disease is performed according to the WHO classification criteria (found at: https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf), which also records intensive care, ventilation, and death as patient-relevant endpoints. For a descriptive assessment, pre-existing conditions, comorbidities, and
any antiviral therapy, e.g., with remdesivir, and routinely available laboratory data (liver and kidney function, oxygenation) for inpatients are included.

The samples are preserved in borate monovettes for easy sample transport and shipped to the central laboratory of the biotechnology company Mosaiques Diagnostics GmbH in Hanover, Germany, which specializes in proteomic in vitro diagnostic test systems. There, urine samples will be collected according to established SOP specifications and in compliance with ISO standard 13485/2016, prepared, measured by CE-MS, analyzed, and patient-specific peptide lists generated. The peptide profiles of the first 250 patients at all sample time points will be used to verify the 31 peptide markers associated with severity of the SARS-CoV-2 infection identified in the pilot study. The higher statistical power will also be used to search for additional peptide markers that show correlation to the severity of a SARS-CoV-2 infection. Based on the results of the statistical analysis, the COVID31 classification model established in a previous pilot study will be adapted. Classification of the first 250 patients at all sample time points will be used to determine the cut-off value for a positive test result with respect to the prediction of critical disease progression in total cross-validation. Completion of the first phase for model optimization will be documented before subsequently validating the model prospectively in routine operation on an additional 750 patients using the predetermined cut-off value for a positive test result. The final evaluation of the study will be performed using the established statistical procedures (Receiver Operating Characteristics and Precision Recall curves), as well as uni/multivariate Cox regression analyses.

1.2 Scientific and/or technical work objectives of the project

CRIT-COV-U aims to use patient-specific proteomic data analysis in order to validate and qualify for clinical use an in vitro diagnostic test (based on peptides present in the urine of COVID-19 patients) that can make early and highly accurate predictions about the development of critical disease progression. The study will use the first 250 patients to test the significance of the peptides included in the COVID31 model on a larger patient population. At the same time, the researchers will look for additional peptides that, when included in the peptide marker model, will significantly improve its predictive power for critical disease progression. As samples taken from each patient at three different time points will be analyzed, it will be possible to determine how early a peptide marker can indicate critical disease progression. In the final phase of CRIT-COV, the optimized peptide marker model will be applied to a validation cohort consisting of 750 COVID-19 patients and the prognostic significance of the urine peptide test will be determined after completion of all sample analyses and complete determination of the patient outcome. The ultimate goal of the project is to achieve the diagnostic predictive power of the urine peptide test determined in the sample calculation.

The research and development approach taken by the CRIT-COV-U project is novel and original in the sense that it has not been applied to COVID-19 disease before and, instead of merely comparing proteomic and clinical datasets, it will also attempt to integrate them into a higher-level diagnostic and prognostic testing system. The feasibility of such an approach is based on previous work carried out by the research and development department of Mosaiques Diagnostics GmbH, which was commissioned to develop the urine peptide test.

2 Detailed description of the work plan
2.1 Study type
This is a prospective, single-arm, international, multicenter diagnostic study. There will thus be no change in patient treatment as a result of participation in this study.

2.2 Detailed description of the study design
The CRIT-COV-U project aims at developing a non-invasive urine peptide test for early and accurate prediction of critical disease progression in COVID-19 infected patients for clinical use. Patients will be recruited in close cooperation with the "Standing Working Group of Competence and Treatment Centers for Diseases Caused by Highly Pathogenic Pathogens" (STAKOB) at the RKI and other study sites with a high COVID-19 recruitment frequency. This will therefore be an international, multicenter study. The study is divided into two clinical phases. In the first phase, the peptide markers identified in the pilot study will be tested in a larger patient population consisting of the first 250 patients and characterized in more detail with regard to the sequence of their occurrence in the patients’ urine. The 250 patients will also be screened for additional peptide markers that show a correlation to the severity of SARS-CoV-2 infection. The model will be fitted for diagnostic accuracy, earliness, and robustness, and the optimal cut-off value for a positive test result will be determined. The completion of the first phase for model optimization will be documented. The model will then be evaluated prospectively and the diagnostic parameters such as sensitivity, specificity, positive predictive value and negative predictive value will be determined using the predetermined cut-off value for a positive test result on the subsequent 750 patients.

In the prospective, single-arm, multicenter diagnostic study, a urine sample for the mass spectrometric index test will be obtained (at the three defined time points (Day 0 - 1, Day 4 - 5, and Day 10 - 14) after presentation at a competence center from patients who have given their informed consent to participation in the study. Concurrently, clinical data will be collected to establish the WHO viral pneumonia endpoint. For inpatients, this is done by study or clinic staff; outpatients enter their data into a validated eCRF system on their own. In addition, important surrogate laboratory parameters will be collected during the stay from inpatients and once only after de-isolation from outpatients. Based on the predefined peptide marker model and the determined proteome pattern, a diagnosis (critical complications expected/not expected) will be made for the urine sample. To maximize the value of the study, the investigators performing and evaluating the urine test will have no information about a given patient’s clinical situation and progression.

2.2.1 Patients and study sites
A total of 1000 patients will be included in the CRIT-COV-U study. To define the peptide marker model, 660 inpatients and 330 outpatients must be included from clinical centers within the EU [e.g. Vienna General and University Hospital (Prof. Dr. M. Hecking), Innsbruck University Hospital (PD Dr. M. Rudnicki), Skövde Hospital, Sweden (PD Dr. B. Peters)].

2.2.2 Benefit-risk assessment
The study is a diagnostic study. Patient utilization will be limited to providing urine samples and consent to make clinical data and laboratory findings from routine care available for scientific evaluation. Invasive procedures, e.g. venipuncture for the study, will be performed
once at the end of the study and only from outpatients. Molecular genetic testing will be prepared via separate patient information as a sub-study.

2.2.3 Duration of study
Recruitment of the planned 1000 patients is expected to take 9 months. This period is dependent on the current COVID-19 incidence at the time of the recruitment phase and may be extended until the recruitment target is reached. The individual observation period is 4 weeks.

2.2.4 Inclusion criteria
Patients will be included as soon as a SARS-CoV-2 infection is detected by the investigator, based on generally accepted criteria (PCR). Other inclusion criteria include the following: written informed consent to participate in the study, capacity to consent; the patient must be able to understand and follow the investigator's instructions, and be at least 18 years of age. Based on the ongoing recruitment in the study, the clinical monitoring center will ensure that the outpatient and inpatient inclusion numbers are achieved as planned.

2.2.5 Exclusion criteria
Patients younger than 18 years, patients without written informed consent, and anuric patients (e.g., dialysis patients without residual diuresis) will be excluded from the study.

2.2.6 Study objectives
Primary: Determining the association of urinary protein fragment pattern expression and severity of the COVID-19 illness according to the WHO 7-step endpoint.

Secondary: Establishing the time of highest association between urine protein fragment pattern and the COVID-19 illness.

2.2.7 Accompanying treatment
Therapy of patients included in this study will be continued as per the standard of care of the respective study site. The proteomic test results will not influence therapy in any way. Inclusion in other interventional studies or curative trials is possible. Patients already in intervention/therapy studies and curative trials may be included in the study described here.
2.2.8 Evaluation and statistical presentation

2.2.8.1 Estimation of the sample size

The primary objective of the study is to demonstrate that the urine test can predict a critical progression of SARS-CoV-2 infection with high prognostic accuracy. The cut-off value for sensitivity and specificity is assumed to be 80% in each case. Sample sizes were estimated separately for sensitivity and specificity. The prevalence for a critical/life-threatening SARS-CoV-2 course of infection on the study population is approximately 33%. In the previous pilot study of 15 COVID-19 patients, including 6 with a critical course infection or fatal outcome, there was an estimated sensitivity of 83% and an estimated specificity of 89%. A one-sided $\chi^2$-test for probability with a type 1 error of 2.5% was used for sample size estimation. A minimum sensitivity of 75%, assuming an estimated sensitivity of 83%, requires 212 patients with a critical/fatal SARS-CoV-2 course of infection. A minimum specificity of 80%, assuming an estimated specificity of 89%, requires 271 patients without a critical/fatal SARS-CoV-2 course of infection. This means that a total sample size of 643 patients should be sufficient, with patients with critical/fatal SARS-CoV-2 course of infection (33% of 643) defining the total number of patients needed. No correction for multiple testing is required to test sensitivity and specificity, as both hypotheses must be independently refuted.

Of the remaining 357 patients, 250 will be used to characterize and optimize the peptide marker model. The remaining 107 patients will be additionally included in the prospective validation and be used to compensate for patients with missing data (14% retained samples).

2.2.8.2 Statistical evaluation

Point estimates and two-sided 95% Wald confidence intervals for sensitivity and specificity will be calculated for the primary analysis. The null hypothesis for sensitivity and specificity will be rejected if the lower limit of the associated confidence interval is greater than 80%.

For the analysis of key secondary endpoints, predictive values for all patients, as well as sensitivity, specificity, and predictive values for subgroups will be analyzed descriptively. Subgroups include: patients with varying severity of SARS-CoV-2 infection, patients from any STAKOB reference center, patients on antiviral therapy, e.g., remdesivir.

There will also be a secondary investigation to determine whether clinical variables, laboratory values or pathological findings affect the result of the urine test (search for potential confounding variables, definition of exclusion criteria).

The urine test classification score obtained at the different sample time points is associated with the development of a critical SARS-CoV-2 course of infection (in a survival analysis using the Cox regression model under the proportional hazards assumption). This will be done both with and without consideration of possible covariate influencing variables. Demographic factors, such as age and sex, and laboratory parameters, such as glomerular filtration rate, liver enzymes, lymphocytopenia, or the serum markers CRP, D-dimer, LDH, and troponin, will be considered as possible influencing variables.
2.2.9 Clinical study procedure/work packages

2.2.9.1 WP1: Project organization, sample collection and acquisition of data relevant to the study

The objective of WP1 is to collect all the samples and clinical data needed in the CRIT-COV-U project. Furthermore, WP1 also contains all activities for the exploitation and dissemination of the study results. To achieve these objectives, WP1 includes the following activities:

(i) establishment an effective management structure for sample and data exchange,
(ii) organization of sample and data transfer,
(iii) management of legal obligations for data security,
(iv) establishment of a project-specific proteomic and clinical database,
(v) establishment of a project-specific website to disseminate results to the general public, relevant groups, and regulatory agencies,
(vi) preparation of a publication paper, as well as conference articles and test descriptions at the end of the project.

A urine sample will be obtained from each patient with COVID-19 on Day 0-1, Day 4-5, and Day 10-14. The COVID-19 patients in home quarantine can collect urine samples on their own. The necessary materials for sample collection will be provided by the study coordinator.

For the test, midstream urine, preferably from the second urination in the morning, will be collected in a urine cup. The urine is then transferred to the enclosed urine monovette (sample syringe) containing borate solution. For transport, the urine monovette is placed in protective packaging. Urine cup, urine monovette and protective packaging are components of the sample collection kit provided to patients in home quarantine. Outpatients can ship their samples to the laboratory by mail using appropriately tested and certified laboratory shipping bags and outer packaging.

Midstream urine samples will be collected and stored according to the general guidelines of the EuroKUP and HKUPP protocol (http://www.hkupp.org/Urine%20collectiion%20Documents.htm).

Medical history and clinical data will be recorded at the time of sample collection. Said data includes preexisting conditions, comorbidities, and any antiviral therapy, e.g., with remdesivir, as well as routinely available laboratory data (liver and kidney function, oxygenation) from inpatients. Clinical, demographic, and disease progression data will be documented online via the MARVIN web-based EDC system.

2.2.9.2 AP2: Proteomic analysis and proteomic data interpretation

In WP2, urine samples from patients included in the study will be analyzed using capillary electrophoresis-coupled mass spectrometry (CE-MS) and subsequently subjected to proteomic data analysis to generate patient-specific peptide profiles and peptide lists. The latter will be used for the definition, validation, and characterization of urinary peptide markers in
the initial testing and optimization phase of the COVID31 peptide marker model developed in the pilot study and for subsequent blinded classification with the urinary peptide test in the later prospective validation phase of the study.

Objectives: The main objective of AP2 is:

(i) A comprehensive proteomic analysis of all urine samples available in the CRIT-COV-U project using CE-MS, according to the developed SOPs and in an ISO13485 environment.

(ii) Generation of peptide profiles and peptide lists by proteomic data processing according to established procedures.

(iii) Transfer of proteomic data to the central CRIT-COV-U project database.

Since the measurement principle underlying the CE-MS method has already been described under "Own preliminary work", here we only explain the procedure used to perform CE-MS analyses.

The mass spectrometric analysis will be carried out according to a standard operating procedure (SOP) available in the laboratory. This includes storage and preparation of the samples, performance of the CE-MS analyses, processing of the raw data in all steps through to storage and backup of data. Measurement reproducibility will be checked and ensured according to defined peptide standards. Sample material not used for measurement will be archived to allow repeat measurements and sequencing of peptides.

The comparison of peptide profiles is based on minimization of analytical variance. For this purpose, CE-MS measurements were automated by Mosaiques, and a procedure was developed that allows normalization of migration times in capillary electrophoresis, molecular masses detected in mass spectrometry, and signal strengths of all detected peptides by means of internal calibrants. This patented method is the basis for the precise comparison of proteomic profiles of individual samples and the use of CE-MS technology in medical diagnostics.

All peptides identified in the urine samples will be stored in a Microsoft SQL database and can be retrieved for proteomic and statistical analyses by database queries and linked to medical data collected via the eCRF system.

2.2.9.3 AP3: Characterization and optimization of the peptide marker model

Unblinded analysis of the first 250 COVID-19 patients will be used to validate and optimize the peptide marker model. This step is necessary because, although the selection of the peptide markers summarized in the COVID31 classification model is based on the statistically valid comparison of 15 representative COVID-19 patients and 45 age-, sex-, and comorbidity-matched control patients in the pilot study, the validity of the markers needs to be transferred to a wide range of patient samples and also recorded over time before moving on to the final prospective clinical phase.

Objectives: WP3 includes the following objectives:
(i) Checking and characterizing the 31 peptide markers (identified in the pilot study) associated with adverse disease progression at different time points in the course of infection using statistical data analysis.

(ii) Looking for additional peptide markers indicative of a critical/fatal course of infection in the expanded set of 250 patients, including the patients in home quarantine.

(iii) Determination of the amino acid sequence of the identified peptide markers in tandem mass spectrometry.

(iv) Cross-validation of the multimarker model to establish a defined cut-off, as well as a confidence range for use in subsequent prospective analysis (WP4).

2.2.9.4 AP4: Prospective validation/testing of the peptide marker model

In the final clinical trial, the peptide marker model will be applied to 750 patients to assess the progression of the SARS-CoV-2 infection. This will be done in a single-blinded manner and under the coordination and control of data and quality management and monitoring by the Hannover Clinical Trial Center. In an interim analysis after 4 to 6 months at the latest and after final database cleaning after 12 months at the end of the project, the diagnostic and prognostic test characteristics will be evaluated using appropriate statistical methods, such as receiver operating characteristics and precision recall curves and uni/multivariate Cox regression analyses. Systems biology and mathematical models as well as approaches based on protein interaction, gene ontology and network analysis will also be applied to elucidate the pathophysiological context of the peptide markers included in the peptide marker model.

In summary, the following objectives are thus relevant in WP4:

(i) Regular completeness and plausibility checks, as well as final clean-up of the proteomic and clinical CRIT-COV-U project database.

(ii) Determining the diagnostic and prognostic potential of the urine peptide test and testing the study hypotheses.

(iii) Further statistical analysis regarding the influence of clinical and demographic variables on urine test results.

3 Study procedures

3.1 Study variable and endpoint

3 testing times are scheduled for urine sample storage.

Day 0-1 after detection of the SARS-CoV2 infection
Day 4-5
Day 10-14

On said days, urine samples from the second morning urination will be collected in a standard urine tube or a stabilizer-containing (for immediate shipment) urine tube and frozen. Outpatients ship their urine samples on their own. In addition, physical well-being endpoint data according to WHO coding will be established by clinical staff or outpatients on said days:
1. No hospitalization, normal physical activities - **OUTPATIENT**

2. No hospitalization, but unable to engage in normal physical activities - **OUTPATIENT**

3. Hospitalization, without supplemental oxygen

4. Hospitalization, supplemental oxygen required

5. Hospitalization, high-flow nasal oxygen administration, noninvasive mechanical ventilation, or both required

6. Hospitalization, ECMO, invasive mechanical ventilation, or both required

7. Death

Surrogate markers (laboratory, oxygenation) will be collected retrospectively after availability of routine data. The following markers will be considered above all: respiratory indices (Horovitz) [21], viral load or PCR results, ventilation frequency, length of hospital stay, and routine laboratory values.

### 3.2 Study flowchart

<table>
<thead>
<tr>
<th>Day</th>
<th>0 to 1</th>
<th>4-5</th>
<th>10-14</th>
<th>Last day of hospitalization or de-isolation (+5 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed consent</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>In-/exclusion criteria</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>SARS-CoV-2 PCR or standard COVID-19 confirming procedures</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demographic data</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>X-ray or low-dose chest CT</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Heart rate</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>ECG rhythm abnormalities</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>ECG ST abnormalities</td>
<td>X</td>
<td></td>
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<td>X</td>
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<tr>
<td>ACE-I</td>
<td>X</td>
<td></td>
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<tr>
<td>ARB</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRA</td>
<td>X</td>
<td></td>
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<tr>
<td>Steroids</td>
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</tr>
<tr>
<td>Remdesivir</td>
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<td></td>
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<tr>
<td>Statins</td>
<td>X</td>
<td></td>
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<tr>
<td>Aspirin</td>
<td>X</td>
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<tr>
<td>NOAK</td>
<td>X</td>
<td></td>
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<tr>
<td>Heparin</td>
<td>X</td>
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<tr>
<td>Warfarin</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>Tocilizumab or other biologicals</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Other anti SARS-CoV-2 medication</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine sample for proteomic test</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Oxygenation level</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Need for ventilation support / additional organ support?</td>
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<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Horovitz index if available</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>WHO R&amp;D Blueprint Ordinal Scale for Clinical Improvement</td>
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</tr>
<tr>
<td>Body temperature</td>
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<td></td>
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<td>X</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>X</td>
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<td>X</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>GFR, LDH, troponin I, CRP, IL-6, leukocytes, thrombocytes, K+,</td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

1 Only for inpatients
### 4 Data management and data protection

#### 4.1 Collection, storage (type, location, duration) and transfer of data

All data will be collected pseudonymously. The urine and blood samples will be pseudonymized with the clinic case number at the clinical study sites and shipped to the urine laboratory in Hanover. Demographic and medical data will be collected via a validated electronic case report form (eCRF) system. Data from hospitalized patients will be entered into the eCRF system by trained study site staff. Outpatients will be provided with an account by the supervising study site so that they can enter their data in the eCRF system on their own. For the evaluation, the clinical and anthropometric data recorded in the eCRF system will be transferred to Mosaiques Diagnostics GmbH after completion of the respective study phase, where it will be combined with the results of the urine proteome analysis and evaluated. Mosaiques assigns a separate ident number per record independent of the pseudonymized clinic case number. After completion of the study, the personal access data (data keys) will be destroyed at the clinical centers and the medical study data will be pertubated (obfuscated) by the principal investigator in order to render impossible any reference to the respective study participant [16]. Biological data from this study can thus be leveraged in other studies using the Mosaiques ident number without having recourse to the respective center number.
5 Measures to ensure data quality
The quality of the study will be assured at various levels:

a. All patients included in the study will be selected under the coordination of STAKOB reference centers of the RKI. An assessment of the severity of disease progression will be performed strictly according to the WHO classification criteria (found at: https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf) which also includes intensive care, ventilation and death as patient-relevant endpoints.

b. The mass spectrometric analysis will be carried out according to a standard operating procedure (SOP) available in the laboratory. This includes storage and preparation of the samples, the capillary electrophoresis-mass spectrometry procedure, processing of the raw data in all steps through to making a diagnosis as well as storage and backup of data. Measurement reproducibility will be checked and ensured according to defined peptide mixing standards. Sample material not used for measurement will be archived to allow repeat measurements and sequencing of peptides.

All study sites will be provided with a "standard operating procedure" for the collection, storage, and shipment of urine samples. Samples will be uniformly and uniquely marked with a bar code system. Essential sample collection, storage, and shipping information will be recorded on the eCRF.

c. Data will be documented using (commercially available) MARVIN study software established at HCTC-KKS ("electronic case report form; eCRF"). Appropriate programming will ensure that plausibility, completeness and correctness checks are already performed at the data entry level. The study monitor will check submissions centrally and in on-site reviews, and relevant inquiries can be made to the study site in a timely manner in case of any data that needs to be reviewed.

d. Before final evaluation of the data, the entire data set will reviewed using predefined algorithms to identify implausible data, implausibly missing data, and outliers. Such data will also be verified with the study sites through queries and during on-site visits.

6 Funding
A grant from the German Federal Ministry of Health was obtained (approx. 2.49 Mio €) for project coordination, recruitment of clinical study sites, collection of clinical data, urine sample collection, proteomic measurement, impact and statistics. A formal application was submitted to the FMH for detailed financial planning, and a written pledge for funding has been received. Coordinated by Partner 2 (HCTC-KKS), this amount will be disbursed to the patient recruitment and care partners, including data collection (per patient fee), laboratory urine protein measurement (Mosaiques), logistics, clinical monitoring, biometrics and statistics. Outpatients who enter their data and send their urine samples on their own will receive an expense allowance of €100.
7 Bibliography
