STUDY PROTOCOL

Control of COVID-19 in hospitals (COCON-study)

Sero-epidemiology in healthcare workers

PROTOCOL TITLE 'Control of COVID-19 in hospitals (COCON study) – sero-epidemiology in healthcare workers'

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Enrolling sites	5.1.2e 5.1.2e

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Other sites	Utrecht University, Utrecht
Pharmacy	Not applicable.

PROTOCOL SIGNATURE SHEET

Version 2.3, 01-09-2020

Name	Signature	Date
Manager research: Prof. dr. 5.1.2e		10-09-2020
Coordinating investigator: Prof. dr. 5.1.2e		10-09-2020

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AE	Adverse Event
AVG	General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming
CA	Competent Authority
COVID-19	Coronavirus Disease 2019
Ct	Cycle Threshold
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Record Form
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)
GGD	Gemeentelijke Gezondheidsdienst
HCW	Healthcare Worker
METC	Medical research ethics committee (MREC); in Dutch: Medisch Ethische Toetsingscommissie
RIVM	National Institute for Public Health and the Environment; in Dutch: Rijksinstituut voor Volksgezondheid en Milieu
sqRT-PCR	Semi-Quantitative Real-Time Reverse Transcriptase Polymerase Chain Reaction
(S)AE	(Serious) Adverse Event
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
TCID50	Median Tissue Culture Infectious Dose
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG

WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Since December 2019, the world has been in the grip of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease it causes, coronavirus disease 2019 (COVID-19). Effective management of this pandemic requires estimation of the burden of disease. Currently available literature on COVID-19 mostly represents severe cases admitted to the hospital; data on mild and unsuspected clinical presentations and asymptomatic infections are largely unknown. Sero-epidemiologic studies are urgently needed to help uncover the burden of disease, in particular the rate of asymptomatic infections, and to get better estimates on the incidence of disease. Sero-epidemiologic studies can help identify the extent to which the virus has spread and whether this has led to protective immunity. Such information could help guide infection control policies. This study will evaluate the sero-epidemiology in healthcare workers (HCWs) in Dutch hospitals in regions with varying incidence of COVID-19.

Objective: Primary objective: to determine the seroprevalence of SARS-CoV-2 neutralising antibodies in healthcare workers in Dutch hospitals upon enrolment (baseline). Secondary objectives: to determine the seroprevalence of SARS-CoV-2 total antibodies in HCWs in Dutch hospitals upon enrolment (baseline); to determine if the seroprevalence of SARS-CoV-2 (neutralising) antibodies in HCWs is related to illness or (unplanned) absenteeism in the four months before enrolment; to determine if the seroprevalence is related to the risk of SARS-CoV-2 exposure; to describe the clinical presentation of documented SARS-CoV-2 infection in the four months before enrolment; to determine the duration of (unplanned) absenteeism in the four months before enrolment; to determine the seroprevalence of SARS-CoV-2 (neutralising) antibodies in HCWs in Dutch hospitals three months after enrolment; to determine the cumulative incidence of seroconversion for SARS-CoV-2 (neutralising) antibodies during follow-up; to determine the cumulative incidence of SARS-CoV-2 (re)infection, measured by semi-quantitative real-time reverse transcriptase PCR (sqRT-PCR) and quantitative virus culture in subjects with self-reported symptoms suspected for COVID-19 during follow-up; to determine the duration of (unplanned) absenteeism during follow-up; to determine the cumulative incidence and duration of hospital admission during follow-up; to determine the cumulative incidence of death during follow-up; to determine if seroconversion is related to (severity of) illness or (unplanned) absenteeism during follow-up; to determine if seroconversion is related to the risk of SARS-CoV-2 exposure; to quantify the serological response during and shortly after an infection with SARS-CoV-2; to determine if the serological response during and shortly after an infection with SARS-CoV-2 is related to the presence/viral load of SARS-CoV-2, measured by sqRT-PCR and quantitative virus culture; to determine the viral load of SARS-CoV-2, measured by sqRT-PCR and quantitative

virus culture, 1-2 days and 8-10 days after the resolution of symptoms; to describe the clinical presentation of documented SARS-CoV-2 infection in HCWs during follow-up.
Study design: Cross-sectional study with prospective follow-up.
Study population: HCWs employed in one of the participating hospitals.
Intervention: Not applicable.
Main study endpoints: Primary endpoint: seroprevalence of SARS-CoV-2 neutralising antibodies. Secondary endpoints: seroprevalence of SARS-CoV-2 total antibodies, cumulative incidence of seroconversion for SARS-CoV-2 (neutralising) antibodies, sqRT-PCR-confirmed SARS-CoV-2 infection, virus culture-confirmed infection, self-reported symptoms suspected for COVID-19, (unplanned) absenteeism, hospital admission and death.
Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Participation in this observational study poses a negligible risk and the burden is considered minimal. Subjects will have a 10-mL blood sample drawn on two occasions if no self-reported symptoms suspected for COVID-19. In case of self-reported symptoms suspected for COVID-19 during the follow-up, a 10-mL blood sample and a nasopharyngeal/throat swab will be obtained on three additional occasions. A retrospective questionnaire will be administered at enrolment and 6 two-weekly short questionnaires during follow-up. In case of self-reported symptoms suspected for COVID-19, a diary on symptoms will be kept until the resolution of symptoms. Participation in the study will not interfere with, or influence local infection control policies for HCWs. There is no direct benefit to subjects, except that individual test results will be made available to the subject during and after the end of the study.

1. INTRODUCTION AND RATIONALE

Since December 2019, the world has been in the grip of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease it causes, coronavirus disease 2019 (COVID-19) (WHO 2020). On February 27, 2020, the first patient with COVID-19 was detected in the Netherlands (Alderweireld 2020).

Effective management of this pandemic requires estimation of the burden of disease (incidence, severity and outcome). Currently available literature on COVID-19 mostly represents severe cases admitted to the hospital (Munster 2020, Wu 2020), and data on mild and unsuspected clinical presentations and asymptomatic infections are largely unknown. A recent study in Dutch hospitals reported a high frequency of mild and unsuspected clinical presentations in healthcare workers (HCWs) with SARS-CoV-2 infection, suggesting that many infections are undetected (Kluytmans 2020).

Sero-epidemiologic studies are urgently needed to help uncover the burden of disease, including the rate of asymptomatic infections, and to get better estimates on the incidence of disease (Okba 2020). Sero-epidemiologic studies can help identify the extent to which the virus has spread and whether this has led to protective immunity. Such information could help guide infection control policies.

This study will evaluate the sero-epidemiology of SARS-CoV-2 in HCWs in Dutch hospitals in regions with varying incidence of COVID-19.

2. OBJECTIVES

Primary objective

- To determine the seroprevalence of SARS-CoV-2 neutralising antibodies in HCWs in Dutch hospitals upon enrolment (baseline).

Secondary objectives

Baseline, all participants

- To determine the seroprevalence of SARS-CoV-2 total antibodies in HCWs in Dutch hospitals upon enrolment (baseline).
- To determine if the seroprevalence of SARS-CoV-2 (neutralising) antibodies in HCWs is related to illness or (unplanned) absenteeism in the four months before enrolment.
- To determine if the seroprevalence is related to SARS-CoV-2 exposure in the four months before enrolment
- To describe the clinical presentation of documented SARS-CoV-2 infection in the four months before enrolment.

- To determine the duration of (unplanned) absenteeism in the four months before enrolment.

3-month follow-up, all participants

- To determine the seroprevalence of SARS-CoV-2 (neutralising) antibodies in HCWs in Dutch hospitals three months after enrolment.
- To determine the cumulative incidence of seroconversion for SARS-CoV-2 (neutralising) antibodies during follow-up.
- To determine the cumulative incidence of SARS-CoV-2 (re)infection, measured by semi-quantitative real-time reverse transcriptase PCR (sqRT-PCR) and quantitative virus culture in subjects with self-reported symptoms suspected for COVID-19 during follow-up.
- To determine the duration of (unplanned) absenteeism during follow-up.
- To determine the cumulative incidence and duration of hospital admission during follow-up.
- To determine the cumulative incidence of death during follow-up.

3-month follow-up, participants who are seronegative at baseline

- To determine if seroconversion is related to (severity of) illness or (unplanned) absenteeism during follow-up.
- To determine if seroconversion is related to SARS-CoV-2 exposure during follow-up.

3-month follow-up, participants with documented SARS-CoV-2 infection during follow-up

- To quantify the serological response during and shortly after an infection with SARS-CoV-2.
- To determine if the serological response during and shortly after an infection with SARS-CoV-2 is related to the presence/viral load of SARS-CoV-2, measured by sqRT-PCR and quantitative virus culture.
- To determine the viral load of SARS-CoV-2, measured by sqRT-PCR and quantitative virus culture, 1-2 days and 8-10 days after the resolution of symptoms.
- To describe the clinical presentation of documented SARS-CoV-2 infection in HCWs during follow-up.

3. STUDY DESIGN

This observational study is designed as a cross-sectional study with prospective follow-up in HCWs of ten to twelve Dutch university and non-university hospitals with a representative

participation of hospitals from areas with a high incidence of COVID-19 (Figure 1). The duration of follow-up will be three months after enrolment. Follow-up of symptoms that started within these three months will be followed-up until symptom resolution; persisting symptoms will be followed until three months after enrolment, with a minimum of four weeks after start of symptoms. The study will end after the last follow-up period has ended for the last subject.

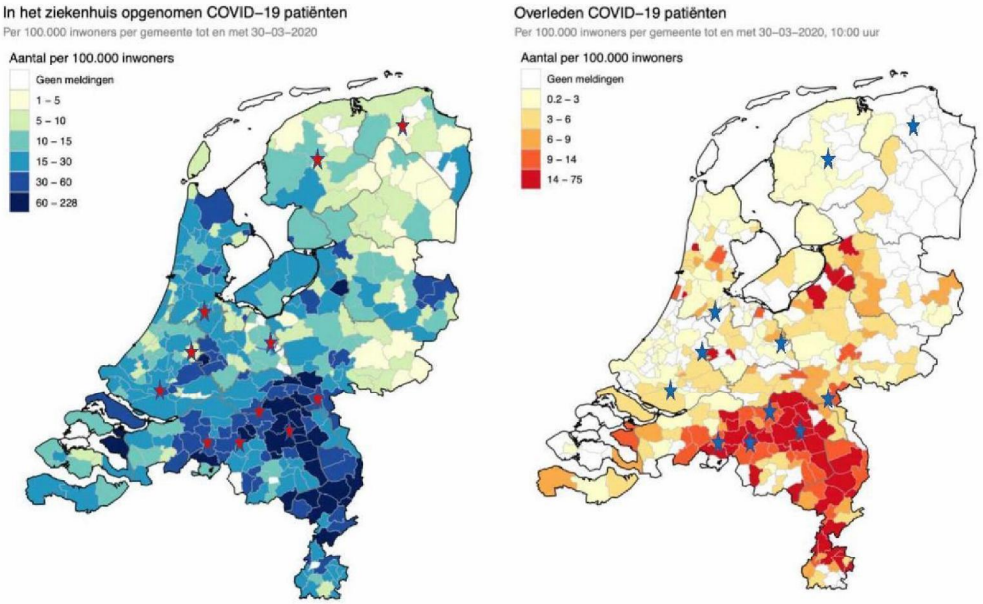

Figure 1. Geographical distribution of hospitalisation and mortality in patients with COVID-19 (in Dutch).

4. STUDY POPULATION

4.1 Population (base)

HCWs will be recruited from the population of employees working in the participating hospitals. The planned 150-200 HCWs to be enrolled per hospital represent up to 10% of HCWs employed in the participating hospitals and is aimed to include at least 100 HCWs with direct patient contact.

4.2 Inclusion criteria

A subject who meets all of the following criteria will be eligible to participate in this study:

- HCW employed in one of the participating hospitals

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Age below 18 years
- Direct involvement in the design or execution of this study
- Expected absence from work for more than four weeks during follow-up
- Legally incapacitated or unwilling to provide informed consent

Participation in other COVID-19 studies, e.g. BCG-vaccination studies is not an exclusion criterion.

4.4 Sample size calculation

The number of deaths due to COVID-19 in the population as reported by the National Institute for Public Health and the Environment (RIVM) on April 7, 2020 was 2,101. The true number of deaths is estimated to be at least two times higher than the number reported, since not all COVID-19 patients outside the hospitals are tested (https://www.rivm.nl/coronavirus-covid-19/actueel - RIVM Archive Corona updates April 3, 2020, Oversterfte). Case-fatality has so far been estimated to be 0.5% to 1.0%. Based on these estimates it is expected that at this point in time 400,000 to 800,000 inhabitants have been affected. The seroprevalence is therefore expected to be between 2.5% and 5.0%. Taking the highest estimate for seroprevalence (5.0%) and a 2% width for the two-sided 95% confidence interval, the required sample size is 1,825 (Daniel 1999). To account for 10% loss of blood samples available for analysis of the primary endpoint, the total number of subjects to be enrolled is 2,000.

5. TREATMENT OF SUBJECTS

Not applicable.

6. INVESTIGATIONAL PRODUCT

Not applicable.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study endpoint

- Seroprevalence of SARS-CoV-2 neutralising antibodies.

8.1.2 Secondary study endpoints

- Seroprevalence of SARS-CoV-2 total antibodies.
- SARS-CoV-2 serum neutralisation titre (serum).
- SARS-CoV-2 sqRT-PCR cycling threshold (Ct) value (nasopharyngeal/throat swab).
- Median tissue culture infectious dose (TCID50) (nasopharyngeal/throat swab).
- Cumulative incidence of seroconversion for SARS-CoV-2 (neutralising) antibodies.
- Cumulative incidence of sqRT-PCR- and/or virus culture-confirmed SARS-CoV-2 (re)infection.
- Cumulative incidence of self-reported symptoms suspected for COVID-19.
- Duration (number of days) of self-reported symptoms suspected for COVID-19.
- Duration (number of days) of (unplanned) absenteeism.
- Duration (number of days) of (unplanned) absenteeism because of documented SARS-CoV-2 infection.
- Duration (number of days) of (unplanned) absenteeism because of self-reported symptoms suspected for COVID-19.
- Duration (number of days) of (unplanned) absenteeism because of imposed quarantine for being exposed to SARS-CoV-2 infection.
- Cumulative incidence of hospital admission for any reason.
- Cumulative incidence of hospital admission for documented SARS-CoV-2 infection.
- Duration (number of days) of hospital admission for any reason.
- Duration (number of days) of hospital admission for documented SARS-CoV-2 infection.
- Cumulative incidence of all-cause death.
- Cumulative incidence of death due to documented SARS-CoV-2 infection.

8.1.3 Other study parameters

Other parameters that will be collected at enrolment:

- Date of enrolment
- Hospital
- Department (incl. SARS-CoV-2 designation and patient exposure)
- Profession
- Average number of workdays per week
- Percentage of workdays with direct patient contact
- Age
- Sex
- Residence
- Body weight
- Body length
- History of pulmonary disease (asthma, chronic obstructive pulmonary disease, other)
- History of cardiovascular disease
- History of immunodeficiency disease
- Use of antidiabetic drugs
- Use of antihypertensive drugs
- Use non-steroidal anti-inflammatory drugs
- Use of immunosuppressive drugs
- Recent BCG vaccination
- Influenza vaccination 2019-2020
- Household composition (number, children, pets)
- Recent foreign travel
- Smoking habits

8.2 Randomisation, blinding and treatment allocation

Not applicable.

8.3 Study procedures

All study procedures are part of this study. Participation in the study will not interfere with any other diagnostic procedure or medical treatment necessary during the course of the study. An overview of the study assessments is provided in Figure 2.

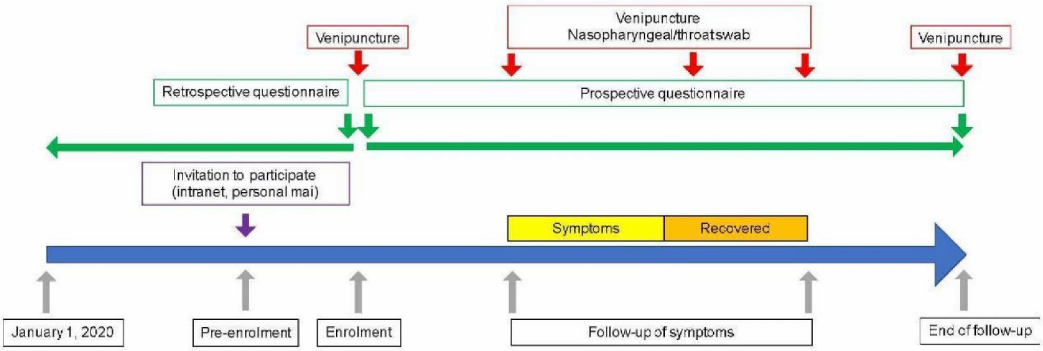

Figure 2. Schedule of assessments

Pre-enrolment

HCWs will be informed about the study by the head of their department, and through announcements on the hospital intranet and in-hospital personal e-mail. HCWs who are interested to participate are invited to contact the local research nurse, either in by telephone or e-mail. The research nurse will explain the study objectives and procedures and check the eligibility criteria. HCWs who are eligible and willing to participate will receive the written study information and informed consent form per e-mail and an enrolment visit will be planned. The HCW will be provided sufficient time to consider the study information before the enrolment visit.

Enrolment and consent

The research nurse will explain and discuss the study objectives and procedures and allow the HCW to ask questions. Informed consent will be signed and dated in duplicate by both the participant and the research nurse. After obtaining informed consent, a venipuncture will be performed to draw a 10-mL blood sample for the detection SARS-CoV-2 serum (neutralising) antibodies. Subjects are asked to fill-out a retrospective questionnaire on demographic characteristics and self-reported symptoms suspected for COVID-19, documented SARS-CoV-2 infection, (unplanned) absenteeism, hospitalisation, exposure to in-hospital patients with COVID-19 and foreign travel since January 1, 2020. In case of a previous documented SARS-CoV-2 infection, the laboratory that performed the test will be asked to provide the test results, and test results will be entered in the eCRF. Explicit permission for this procedure will be requested from the subject at informed consent.

At informed consent, subjects can indicate whether they want to be informed on their serological test results. Within a few weeks after the blood sample is taken, subjects will be informed about their individual serological test results.

During follow-up

During the 3-months follow-up, subjects are asked to fill-out a short (two-weekly) questionnaire on self-reported symptoms suspected for COVID-19, (unplanned) absenteeism, hospitalisation, exposure to in-hospital patients with COVID-19, and foreign travel. In case of not filling-out the questionnaire, subjects will receive a reminder. Subjects with incomplete follow-up despite notifications will be contacted by telephone with the request to complete the follow-up data.

In case of self-reported symptoms suspected for COVID-19

In case of the development of symptoms suspected for COVID-19*, subjects are asked to contact the local study team, and three follow-up visits will be planned: 1) at the day of onset of symptoms (or at the latest 1 day after the onset of symptoms); 2) 2-4 days after the last day of symptoms; and 3) 8-10 days after the last day of symptoms. Persisting symptoms will be followed until the 3-months visit, with a minimum of four weeks after the start of symptoms. This may extend the follow-up of persisting symptoms after the 3-months visit for a maximum of four weeks. In case of persisting symptoms, follow-up visit 2 and 3 will be omitted and a final (symptomatic) follow-up visit will be scheduled at the end of (extended) follow-up. During all three visits, a venipuncture will be performed to draw a 10-mL blood sample for the detection SARS-CoV-2 serum (neutralising) antibodies and a nasopharyngeal/throat swab will be obtained for SARS-CoV-2 sqRT-PCR (laboratory developed test (LDT) or COBAS6800, Roche Diagnostics), quantitative virus culture and amplicon-based whole-genome sequencing (WGS) (Okba 2020, 5.1.2e 2020, Oude Munnik *submitted*, Wölfel 2020). An additional sqRT-PCR for other respiratory viruses will be performed on the nasopharyngeal swab that is obtained at the start of symptoms. Serological tests, sqRT-PCR, quantitative virus culture and amplicon-based WGS will be performed in the two participating laboratory sites (Department of Virology, Erasmus MC, Rotterdam and Microvida Laboratory for Medical Microbiology, Roosendaal/Breda). All test results will be uploaded to the eCRF.

Subjects will keep a diary on symptoms until all symptoms have resolved. The data kept in the diaries will be entered in the eCRF by the local research nurse.

In case of multiple symptomatic episodes during the 3-months follow-up, the follow-up of symptoms will be identical for each episode with three diagnostic visits per episode.
Visits for blood- and nasopharyngeal/throat sampling will be scheduled in the hospital, unless the subject is not able or not allowed to come to the hospital due to local infection control policy. In that case, blood sampling will be cancelled and a nasopharyngeal/throat swab will be send to the subject per mail for self-sampling.
Within a few weeks after nasopharyngeal/throat sample is taken, subjects will be informed about their individual sqRT-PCR test results. To comply with the mandatory reporting of SARS-CoV-2 infection (Meldingsplicht groep A), positive test results will be reported to the clinical microbiologist of the respective hospital, who will notify the Gemeentelijke Gezondheidsdienst (GGD) and the hospital's occupational physician.

* Symptoms suspected for COVID-19 include: fever (>=38.0ºC), chills, coughing, shortness of breath, severe myalgia, general malaise, a sore throat, a runny nose, painful eyes, headache, chest pain (retrosternal and/or subscapular), abdominal pain, diarrhea, loss of smell and/or taste.

End of follow-up

Three months after enrolment, a venipuncture will be performed to draw a 10-mL blood sample for the detection of SARS-CoV-2 serum (neutralising) antibodies.
At informed consent, subjects can indicate whether they want to be informed on their serological test results. Within a few weeks after the blood sample is taken, subjects will be informed about their individual test results.

Post-study

Subjects are asked to indicate whether they are willing to be contacted for participation in future studies.

Note on serological tests used

Developments in the field of serological tests for SARS-CoV-2 are moving fast and are expected to continue during the course of this study. For initial analyses in this study, the best available test in terms of sensitivity and specificity at start of the study will be used, in accordance with available guidance from the Working Group Serology of the Netherlands Society for Medical Microbiology.
Based on current knowledge, an enzyme-linked immunosorbent assay (ELISA) will be used for screening of samples, followed by confirmatory testing using plaque reduction neutralisation assays and antibody profiling by use of protein microarray

for comparative testing of antibody reactivity to SARS-CoV-2 and seasonal human coronaviruses. As the market for serological assays is quite competitive, access to commercial assays may be a problem. As back up, LDTs (Erasmus MC) will be used.

Note on storage and use of residual material

Residual material will be labelled with the subject ID and be kept indefinitely at the Erasmus MC for future validation purposes and additional research questions in the field of respiratory infections and coronaviruses. An explicit permission for keeping and using residual material will be requested from the subject at informed consent.

Note on sampling, storage procedures and transport

Strict adherence to collection protocols, biosafety and adequate personal protective equipment is essential. Biosafety procedures will be in accordance with local policy/guidance and (inter)national regulations, and will be applied to the collection, storage, transfer and laboratory handling of research samples.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal

Not applicable.

8.5 Replacement of individual subjects after withdrawal

Subjects who have been enrolled but for whom no baseline blood sample is available will not be evaluable for the primary endpoint. The required sample size is based on the number of evaluable subjects for analysis of the primary endpoint, and herewith assumes replacement of all subjects for which a baseline blood sample is not available.

8.6 Follow-up of subjects withdrawn from treatment

Not applicable.

8.7 Premature termination of the study

There are no predefined reasons to terminate this study prematurely. In case the study is terminated prematurely, the coordinating investigator will notify the accredited METC and the competent authority (CA) within 15 days, including the reasons for the premature termination. The 3-months follow-up period of all subjects that were already included will be completed and there will be no enrolment of new subjects.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

This observational study only involves venipuncture and nasopharyngeal/throat swabbing. Due to the negligible risk of these procedures, no adverse events will be reported.

9.2.2 Serious adverse events (SAEs)

This observational study only involves venipuncture and nasopharyngeal/throat swabbing. Due to the negligible risk of this procedure, no serious adverse events will be reported.

As part of the study, we will collect the hospitalisation and death within three months after enrolment as secondary endpoints. These will, however, not be reported as SAEs, since they will be unrelated to participation in this observational study.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable.

9.3 Annual safety report

Not applicable.

9.4 Follow-up of adverse events

Not applicable.

9.5 Data Safety Monitoring Board (DSMB) / Safety Committee

Not applicable.

10. STATISTICAL ANALYSIS

Data will be reported quantitatively. Missing data will be dealt with by multiple imputation.

10.1 Primary study parameter(s)

Seroprevalence of SARS-CoV-2 neutralising antibodies will be reported as proportion with 95% confidence interval.

10.2 Secondary study parameter(s)

Seroprevalence of SARS-CoV-2 total antibodies will be reported as proportion with 95% confidence interval.

SARS-CoV-2 serum neutralisation titres, SARS-CoV-2 serum total antibody titres, SARS-CoV-2 sqRT-PCR Ct values and TCID50 values will be reported as mean and standard deviation or median and interquartile range, as appropriate.

Cumulative incidences of seroconversion for SARS-CoV-2 (neutralising) antibodies, SARS-CoV-2 (re)infection, self-reported symptoms suspected for COVID-19, hospital admission and death are reported as proportion with 95% confidence interval.

Duration of self-reported symptoms suspected for COVID-19, (unplanned) absenteeism and hospital admission are reported as mean and standard deviation or median and interquartile range, as appropriate.

The relation between illness or (unplanned) absenteeism and the seroprevalence of SARS-CoV-2 (neutralising) antibodies will be analysed using a generalised linear regression model with binomial distribution and identity- or log-link, providing risk differences and relative risks with 95% confidence intervals, respectively.

The relation between the risk of SARS-CoV-2 exposure and the seroprevalence of SARS-CoV-2 (neutralising) antibodies will be analysed using a generalised linear regression model with binomial distribution and identity- or log-link, providing risk differences and relative risks with 95% confidence intervals, respectively.

The relation between the presence/viral load of SARS-CoV-2 infection, as measured by sqRT-PCR and quantitative viral culture, and serological response will be analysed using

a linear regression model (dependent variable: SARS-CoV-2 serum neutralisation titre) and a generalised linear regression model (dependent variable: presence of SARS-CoV-2 serum (neutralising) antibodies) both with binomial distribution and identity- or log-link, providing risk differences and relative risks with 95% confidence intervals, respectively. Sensitivity analyses will be performed to compare retrospectively and prospectively collected data on self-reported symptoms suspected for COVID-19.

10.3 Other study parameters

Continuous characteristics will be reported as mean and standard deviation or median and interquartile range, as appropriate. Categorical characteristics will be reported as count and percentage.

10.4 Interim analysis

Not applicable.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki, amended at the 64th General Assembly (Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and the Code Goed Gebruik (2011).

11.2 Recruitment and consent

See 8.3 Study procedures.

11.3 Objection by minors or incapacitated subjects

Not applicable.

11.4 Benefits and risks assessment, group relatedness

Participation in this study poses a negligible risk and the burden is considered minimal. Subjects will have a 10-mL blood sample drawn on two to five occasions (dependent on the presence of self-reported symptoms suspected for COVID-19 during the follow-up). A venipuncture can be associated with pain or bruising at the site of puncture and rarely with dizziness or fainting. Discomfort will be minimised by having expert staff obtain blood samples and if necessary in supine position. Nasopharyngeal/throat swabs are commonly used methods for collecting test samples for respiratory viral infection. Swabbing may be

mildly uncomfortable. A nasopharyngeal swab is rarely associated with nasal bleeding. A throat swab may cause momentary gagging. Discomfort and risk will be minimised by having experienced personnel take the swabs. The retrospective questionnaire administered at enrolment will take 10 minutes, two-weekly follow-up questionnaires will take 2 minutes.
Participation in the study will not interfere with, or influence local infection control policies for HCWs. If as part of local infection control policies a swab must be taken for COVID-19 diagnosis, this will be prioritised and swabs for the study will be taken subsequently.
There is no direct benefit to subjects, except that individual test results will be made available to the subject during and after the end of the study.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. Given the negligible risk of the study-related procedures, exemption from the WMO human subject insurance is granted by the METC.

11.6 Incentives

Subjects will not be compensated for participating in this study.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Data will be handled confidentially. A subject identification code list will be used to link the data to the subject. The subject ID will be a unique number and incorporates the hospital (one letter) and four sequential numbers. It will not include subject's initials, birth date or other personal identifiers. The key to the code will be safeguarded by the local investigator on a designated location.

The eCRF and electronic entries of the questionnaires will be stored at CASTOR EDC webservers until the end of data collection. The system meets all International Conference on Harmonisation on Good Clinical Practice (GCP)requirements safeguarding data integrity and electronic data security regulations. Data traffic within CASTOR EDC over the internet is encrypted using secure data security protocols. Users will have a role-based access to CASTOR EDC after they log-in using their own personal username and password. This role-based access will avoid unauthorised data access and prevents users from performing actions that they do not have authorisation for. The system logs all data entry steps with time stamps and user information, thereby creating

an audit trail. After the end of the study, data will be extracted from CASTOR EDC and archived at the UMC Utrecht for 15 years in accordance with UMCU Archiving Standard Operating Procedures. All information, data, and results that originate from this study may not be disclosed without permission from the principal investigator.

Direct access to source data, subject ID and CASTOR EDC data will be granted to the local monitor authorised representatives from the sponsor and the regulatory authorities to permit study-related monitoring, audits and inspections to ensure compliance with regulations.

Residual material from blood samples will be kept indefinitely at the Central Biobank of the Erasmus MC for future validation purposes and additional research questions in the field of respiratory infections and coronaviruses in accordance with the Central Biobank Erasmus MC Regulatory document. The use of residual material is only allowed after consultation of the principal investigators of the enrolling sites and written approval of the principal investigator of sponsor. For the use of residual material for other purposes, review by an ethics committee is an additional requirement in accordance with articles 19 and 21 WMA Declaration of Taipei on Ethical Considerations regarding Health Databases and Biobanks. Explicit permission for keeping and using residual material will be requested from the subject at informed consent.

The handling of personal data will comply with the EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation (in Dutch: Uitvoeringswet AVG, UAVG). For further details, we refer to the datamanagement plan.

12.2 Monitoring and Quality Assurance

Due to the short inclusion period and the constraints to travel and hospital visits, no on-site monitoring visits are planned during the study. Remote monitoring will be performed by a monitor of the sponsor. In case on-site monitoring is allowed, this option will be discussed with the study team and monitor. The presence and completeness of the relevant Study Files will be checked before enrolment of the first subject. After enrolment of ten subjects at the participating site, the completeness of the informed consent forms and the accuracy of the eligibility criteria will be checked for three subjects enrolled by the monitor of the sponsor. If there are errors in this, more intensive monitoring will take place. Thereafter, monitoring will be performed by checking the completeness of baseline and follow-up data from the questionnaires and diary. At the end of the study, the

presence and completeness of the relevant Study Files will be checked. For further details, we refer to the monitor plan.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last subject's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.
In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.
Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

The investigators will disclose the results of the study unreservedly at the end of the study or earlier in case result impact policy making. The study will be registered in the Netherlands Trial Register before the first subject is recruited.

13. STRUCTURED RISK ANALYSIS

Not applicable.

14. REFERENCES

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