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Validating a clinical laboratory parameters-based de-isolation algorithm for COVID-19 patients in the intensive care unit using viability-PCR: the CoLaIC study protocol

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Abstract

Introduction

To investigate if biochemical and hematological changes due to the patient's host response (CoLab algorithm) in combination with a *severe acute respiratory syndrome-Coronavirus-2* (SARS-CoV-2) viability-PCR (v-PCR) can be used to determine when a COVID-19 patients is no longer infectious. The hypothesis is that the CoLab algorithm in combination with v-PCR can be used to determine whether or not a COVID-19 patient is infectious and facilitate safe release of COVID-19 patients from isolation.

Methods and analysis

This study consists of three parts using three different cohorts of patients. All three cohorts contain clinical, vital and laboratory parameters, as well as logistic data related to isolated COVID-19 patients, with focus on ICU-stay. The first cohort will be used to develop an algorithm for the course of the biochemical and hematological changes of the COVID-19 patient host response. Simultaneously, a second prospective cohort will be used to investigate the algorithm derived in the first cohort with daily measured laboratory parameters next to conventional SARS-CoV-2 RT-PCRs as well as v-PCR, to confirm the presence of intact SARS-CoV-2 particles in the patient.

Finally, a third multi-centre cohort, consisting of retrospectively collected data of COVID-19 patients admitted to the ICU will be used to validate the algorithm.

Ethics and dissemination

This study was approved by the Medical Ethics Committee from MUMC+ (cohort I: 2020-1565/3 00 523) and Zuyderland MC (cohort II and III: METCZ20200057). All patients will be required to provide informed consent. Results from this study will be disseminated via peer-reviewed journals, congress/consortium presentations.

Article summary

Strengths and limitations of this study

Strengths:

- Application of viability-PCR to determine intact viral particles
- The algorithm/model is based on routinely tested blood parameters and standardized laboratory tests
- The algorithm is previously successfully validated and implemented at the emergency department of two large teaching hospitals in the Netherlands
- Multi-centre approach with good distribution of hospitals covering various regions of the Netherlands
- Validation of the algorithm on a large data-set with COVID-19 patient data caused by different SARS-CoV-2 virus variants of concern (VOC)

Limitations:

- Viability-PCR is not determined in cohorts I and III
- Focus is limited to hospitals in the Netherlands
- Focus is limited to (de-)isolation in the ICU

Introduction

The COVID-19 pandemic is globally disruptive regarding the continuation of regular health care. Hospitalized COVID-19 patients need to be isolated and separated from the non-COVID-19 patient population. This aspect paired with the large influx of COVID-19 patients and a limited availability of hospital and isolation beds, exerts enormous pressure on the regular non-COVID-19 healthcare, but also on healthcare professionals. In addition, the need for treatment and support in an intensive care unit (ICU) for a substantial subset of COVID-19 patients and the limited availability in number of ICU beds contributes to these effects. De-isolation as early as possible could improve quality of life for the affected patients, as well as decrease the pressure on the healthcare system and its professionals.

Reverse transcriptase-polymerase chain reaction (RT-PCR) testing is currently the gold standard to determine whether a patient is SARS-CoV-2 positive[1]. To de-isolate a COVID-19 ICU patient in the Netherlands two consecutive negative PCR tests are currently required. However, it can be hypothesized that SARS-CoV-2 RT-PCR positivity does not correlate per se with the actual presence of intact, infectious viruses[2 3]. Because RT-PCR detects nucleic acids, and does not make a distinction between intact infectious virus and non-intact non-infectious viral constituents, this may result in persistently positive RT-PCR test results, which hampers timely de-isolation[1].

An alternative RT-PCR-based method to detect intact viral particles is to eliminate incomplete viral particles and RNA remnants before the actual RT-PCR is performed. Propidium monoazide (PMA) is a dye that binds irreversible to (deoxy)ribonucleic acid (DNA/RNA) and cannot penetrate cell membranes[4]. Pretreatment of a sample with PMA results in amplification of only intact particles. This so-called viability-PCR (v-PCR) has been shown to successfully measure the amount of viable micro-organisms, such as Chlamydia trachomatis, in a sample[5]. In the present study we want to adapt and validate this concept for the detection of intact viable RNA-containing SARS-CoV-2 virus.

Preliminary data have confirmed its applicability for SARS-CoV-2 diagnostics[6]. The adapted v-PCR will be used in study herein presented to confirm the state of viability and thus potential infectivity of SARS-CoV-2 in patients.

An alternative approach is to assess the host response of the suspected patient to the virus. One of the methods to assess the host response to SARS-CoV-2 is the CoLab score. This score is developed using an adaptive LASSO-regression technique and requires the input of the numerical results of ten blood parameters and the age of the patient[7]. The required parameters are blood tests that are requested frequently and routinely for emergency room (ER) as well as ICU patients. This score has previously been developed and validated, and is already clinically implemented in the ER departments of two large Dutch teachings hospitals, with very high negative predictive value (99.5%) and sensitivity (96.9%)[7]. It is also utilized to exclude COVID-19 in a screening setting for health care workers with COVID-19 suspected complaints[8].

Preliminary analysis of serially collected data in a pilot set of ICU patients showed a decrease in the CoLab score resulting in normalization before a patient is discharged (unpublished data). For that reason, we hypothesize that the biochemical and hematological changes in blood parameters necessary to calculate the CoLab score rapidly return to normal values after the host clears the SARS-CoV-2 infection.

The aim of this study is to investigate whether biochemical and hematological changes due to the patient's host response (CoLab algorithm) and/or the v-PCR can be reliably and validly used to determine, at an earlier stage in comparison with a conventional SARS-CoV-2 RT-PCR, when a COVID-19 patient is no longer infectious.

Methods and analysis

Cohorts

This study is composed of three cohorts, two prospective cohorts (local and regional) and one retrospective cohort (national), which all consist of serially (i.e. daily) collected clinical and laboratory variables of COVID-19 patients in isolation at an ICU. We intend to include all patients admitted to one of our COVID-19 ICU isolation rooms.

More specifically, the three different cohorts will be used to study the CoLab score over time (local cohort I), to determine a cut-off point related to the intact infectious viral load (regional cohort II), and to validate the CoLab algorithm (national cohort III) on a national level with an external dataset (Figure 1). While not developed specifically for models using machine learning [9], the study will follow the guidelines of the Transparant reporting of a multivariable predicton model for individual prognosis or diagnosis (TRIPOD).[10]

Local single-centre prospective cohort (I)

The first, single centre, local cohort is the prospective Maastricht Intensive Care COVID (MaastrICCht) cohort, previously described by Tas et al[11]. The CoLab score is calculated for each timepoint using this comprehensively characterized cohort[11-17]. In addition, the daily Sequential Organ Failure Assessment (SOFA)[12 16] scores are available as well as all conventional SARS-CoV-2 RT-PCRs that are measured within this cohort. The aim of study part I is to investigate the development of the CoLab score over time. To possibly de-isolate patients, the CoLab score should at least decrease over time in a way that is independent of disease severity and similar for survivors and non-survivors. We therefore hypothesize that the CoLab score decreases over time in both survivors and non-survivors, in a way that is independent of disease severity over time measured by serial SOFA scores. To have additional value above conventional RT-PCR-based de-isolation, the decrease in CoLab score should occur before de-isolation by RT-PCR is done. Our hypothesis is

that the CoLab score decrease is present before de-isolation can be performed based on RT-PCR. We will explore the association between CoLab score over time and the moment of RT-PCR driven de-isolation. If the CoLab score behaves over time in the ICU as hypothesized above, the next step is to quantify what decrease in CoLab score over time (or what cut-of CoLab score per day) precedes the transition from RT-PCR positive to negative. This decrease in CoLab score over time can be used to develop a diagnostic prediction model for de-isolation. Whether this prediction model can be used as gold standard for de-isolation (CoLab prediction model alone, or in combination with conventional SARS-CoV-2 RT-PCR and/or v-PCR) is part of this study protocol.

Regional dual-centre prospective cohort (II)

In the second part, we hypothesize that excluding infectiousness, contributing to de-isolation, can be done more accurately by using v-PCR instead of RT-PCR. A second prospectively collected dual centre, regional cohort of COVID-19 patients from the ICU department of both Zuyderland Medical Centre and Maastricht University Medical Centre + (MUMC+) will be used to evaluate the usability of the v-PCR for the above-mentioned hypothesis. Inclusion of all consecutive COVID-19 ICU patients will be pragmatic based on the development of the pandemic and related incidence of ICU admission, starting from 1st November 2021. We aim to include a minimum of 90 patients. In this cohort, serial data related to the CoLab algorithm will be collected daily. In addition, both conventional (RT-PCR) and v-PCR testing for the detection of SARS-CoV-2 will be performed three times a week. The aim of this regional cohort (II) is to determine a cut-off point or a certain decrease in CoLab score over time that precedes the transition from positive to negative RT-PCR and v-PCR results.

National multi-centre retrospective cohort (III)

For the third part of the study, a retrospectively collected multi-centre, national cohort will be used. This retrospective cohort will consist of ICU data derived from four other hospitals located in the Netherlands. This dataset will contain serially collected data necessary for determining the CoLab score (ten blood parameters and age, see below) next to conventional SARS-CoV-2 RT-PCR results. This cohort will be used to determine whether the CoLab algorithm developed and validated in the cohorts I and II in specific contexts are generalizable to, and valid in other contexts (cohort III). An additional aim is to test the CoLab algorithm for different variants of concern (VOC) of SARS-CoV-2 (see also below). For this purpose, we will use data from all COVID-19 positive ICU patients between March 2020 and September 2022 (estimated at least 250 patients per participating centre).

Context and setting

Data from six hospitals will be used to create the different cohorts of this study. An overview of the number of hospital and ICU beds per participating hospital and per cohort is shown in Supplemental Table 1.

Local single-centre cohort I aims to use data obtained at MUMC+ (27 ICU and 6 high/medium care beds in the pre-pandemic era), a university medical Centre located in the southern part of the Netherlands. During the COVID-19 pandemic a maximum of 52 ICU beds were available for COVID-19 patients, and 12 for non-COVID-19 patients. Using this local cohort, the CoLab score will be observed over time.

Regional dual-centre cohort II consists of data from ICU patients from both Zuyderland MC (36 ICU beds) and MUMC+. These two hospitals are both located in Limburg in the Netherlands with a existing close cooperation for clinical purposes. Both hospitals are large teaching hospitals. This

regional cohort will be used to assess whether the CoLab score can be used to determine whether patients are SARS-CoV-2 free according to the results of the v-PCR.

National, multi-centre cohort III consists of retrospectively collected data from four other hospitals: Leiden UMC, Radboud UMC, Medical Centre Leeuwarden and Catharina Hospital. The hospitals in this cohort are located in separate provinces leading to a good geographical representation of the national spread of the Dutch COVID-19 patient population. Since Leiden UMC and Radboud UMC are university medical centres and Medical Centre Leeuwarden and Catharina Hospital are large teaching hospitals, both hospital types are represented equally. This national cohort will serve to further validate the model created using cohorts I and II in broader contexts (see Supplemental Table 1 for details of the different hospitals contributing to the consortium).

Patient and public involvement

The national patient organization for lung diseases (Longfonds) has a panel of patients that experienced to be taken in isolation for COVID-19 on the ICU. This panel has read the study protocol and gave advice which were implemented in this protocol. This group will also be involved during the study to give asked and unsolicited remarks to this process.

Inclusion and exclusion criteria

For the three cohorts, the same inclusion and exclusion criteria are applicable. All patients with a proven primary and/or secondary SARS-CoV-2 infection are eligible to participate in the study. Exclusion criteria include only patients with extreme laboratory values (more than 10 times the standard deviation).

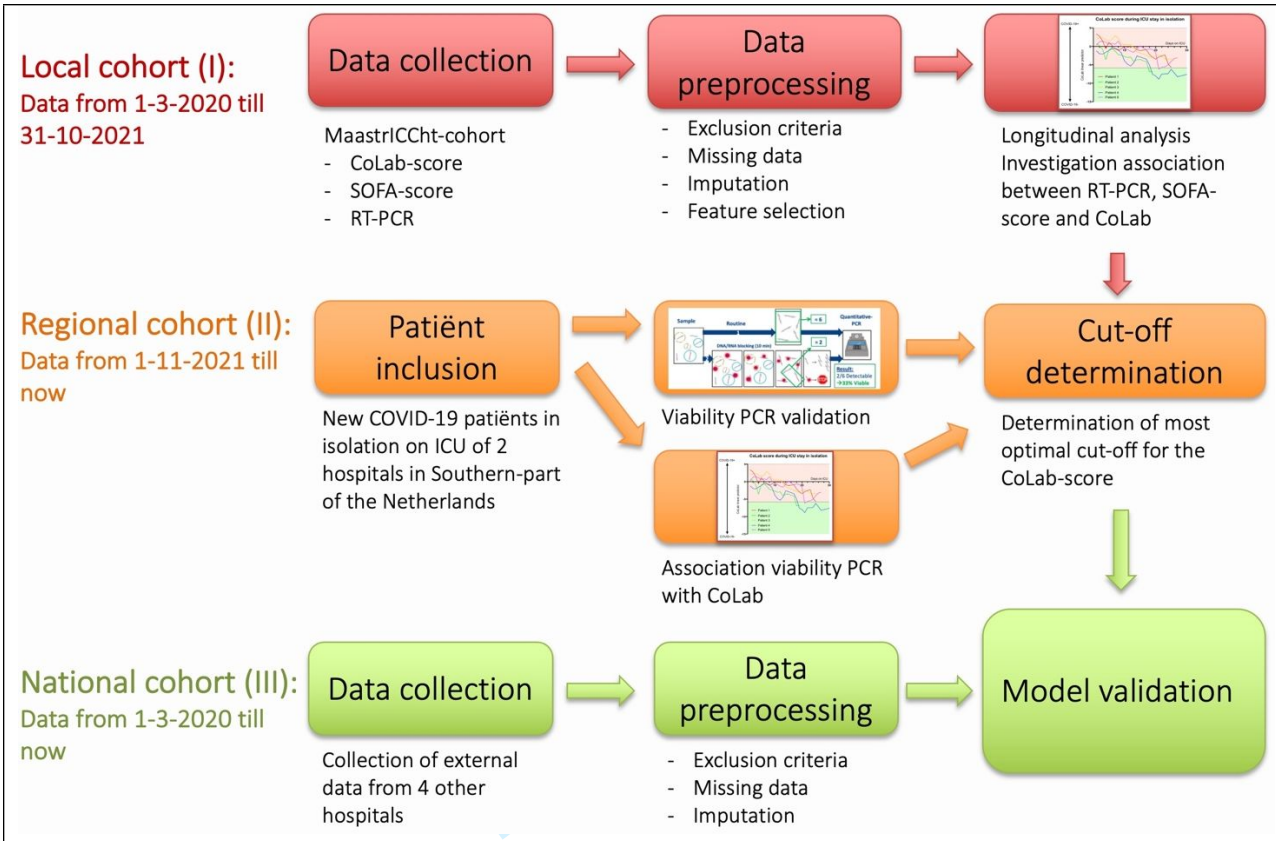

Figure 1: Overview of the study

Parameters

Blood parameters

Blood samples are used to determine a variety of biochemical and hematological parameters in routine diagnostics and disease monitoring, from hospitalization till discharge of a COVID-19 patient. This has led to a large accumulation of blood-related biomarker data. Previous studies found biochemical and hematological changes measured in peripheral blood samples that characterized SARS-CoV-2 infection[18-20]. For instance, in the early stage of the COVID-19 disease, hematological changes in immunocompetent leukocytes correlate with a more severe disease progression[20].

CoLab score

The CoLab score[7] uses the erythrocytes [10^{12}/L], leukocytes [10^{9}/L], eosinophils [10^{9}/L], basophils [10^{9}/L], $\log_{10}$ of bilirubin [µmol/L], $\log_{10}$ of lactate dehydrogenase (LD) [U/L], $\log_{10}$ of alkaline phosphatase (ALP) [IU/L], $\log_{10}$ of γ-glutamyltransferase (γ-GT) [U/L], albumin [g/L], C-reactive protein (CRP) [mg/L], and age [years accurate to two decimals]. These parameters are routinely determined in ICU patients and can be automatically extracted from the laboratory information system. The CoLab algorithm yields a score in the range of -20 to 5 (the so-called CoLab-linear predictor[7]), with a lower score correlating with the exclusion of a SARS-CoV-2 infection and a higher score reflecting an increased risk of SARS-CoV-2 infection.

In an emergency department study population, a cut-off of the CoLab linear predictor was determined to classify patients as being COVID-19 negative. This cut-off was originally set to -5.83 to minimize the amount of false negative results, with a score below -5.83 being negative for COVID-19[7]. How the CoLab-algorithm can be used to correspond with a negligible intact infectious viral load (see section below) is part of the present study: a cut-off or a certain decrease in CoLab-score over time. The CoLab score will be determined daily for all participating patients, either prospectively or retrospectively.

Clinical parameters

In addition to the blood parameters, clinical variables of patients are collected in the different cohorts. These include co-morbidities and clinical scores as well as ventilation, biometric, and physical parameters[12-17]. One clinical score of interest is the Sequential Organ Failure Assessments (SOFA) score. This score has previously been associated with survival chance of mechanically ventilated COVID-19 patients[12]. A decrease in SOFA score is associated with survival. This sequentially determined SOFA score is measured over time and will be used to investigate whether the

association between the CoLab score over time and infectiousness is independent of the SOFA score. In fact, this will provide evidence whether the CoLab score operationalizes a different dimension of the host response, beyond multi-organ failure and in an independent way with regard to survival. This will generate evidence whether the CoLab score generates new information, beyond existing scores and has potential for diagnosis of de-isolation.

Viability PCR (v-PCR)

A v-PCR[6] is performed to assess the presence of intact viruses and will be compared with the conventional SARS-CoV-2 RT-PCR test[21]. In short, nasopharyngeal samples are collected in viral transport medium (VTM) and propidium monoazide (PMA) is added to the sample[22]. Next to the v-PCR, a routine diagnostic RT-PCR for SARS-CoV-2 will be performed on the same sample (see also Figure 2). The difference in cycle-time values (Ct) between these two PCR tests will be reported as ΔCt, which is a reliable indication of the amount of intact virus in the sample.

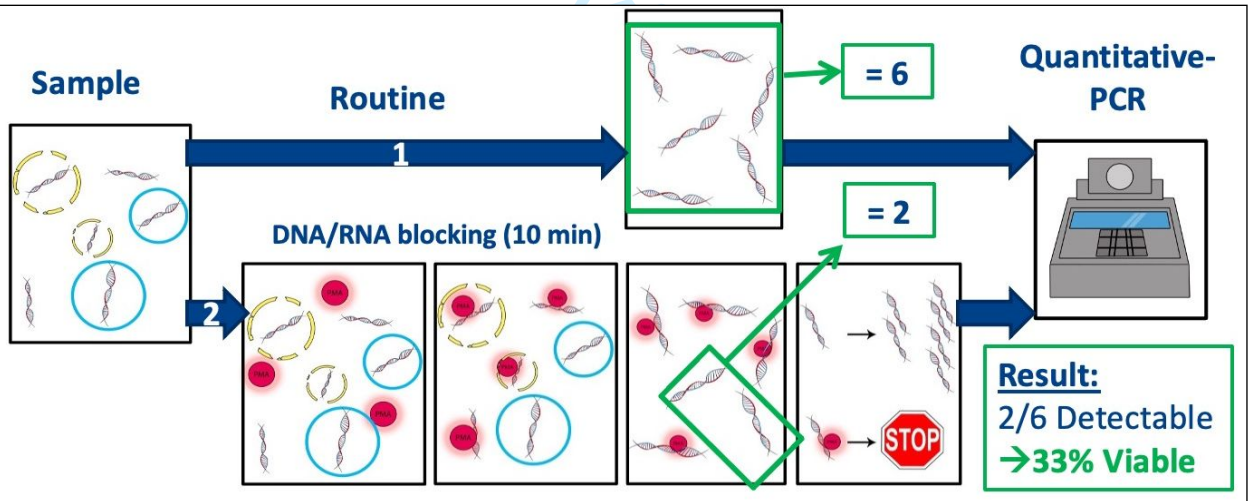

Figure 2: Schematic representation of the principles of the conventional SARS-CoV-2 RT-PCR (route 1) in comparison to the viability-PCR (route 2). Route 1: all RNA is isolated from the sample and amplified using RT-PCR. Route 2: PMA irreversibly binds to free RNA and RNA

from non-intact virus particles. Only RNA from intact virus particles is isolated and amplified by RT-PCR.

Variants of Concern (VOC)

Due to the rapid mutation potential observed in viruses it is necessary to ensure the robustness of the CoLab algorithm to variants of concern of this SARS-CoV-2 virus (VOC's). This study will address VOC retrospectively as well as prospectively. Cohort III, spanning from March 2020 until present, contains data of the Wuhan original SARS-CoV-2 and data from at least three VOC. Demographic research has determined that during this period three VOC of the SARS-CoV-2 occurred next to the original SARS-CoV-2 virus (2020/03 to 2021/01): the B1.1.7 alpha-variant (2021/02 to 2021/06), the B1.617.2 delta-variant (2021/07 to 2021/12), and the B1.1.529 omicron-variant (2022/01 to present)[23]. In this study, we use time periods to characterize VOC in cohort III. In contrast, in cohort II VOC's will be determined with variant-specific Next Generation Sequencing[24].

Statistical analysis

Analyses will be performed with R version 4.2.0 and with RStudio version 4.2.0 [25], combined with the packages Tidyverse [26], lme4 [27], MICE[28], MissForest[29] and Caret[30]. Missing values for numerical variables will be imputed using multiple imputation by chained equations (MICE).[28] Mixed-effects regression model analysis will be used to observe the CoLab score over time (cohort I), to determine whether the CoLab score is independent from survival and SOFA score (cohort I), and to determine the association between the CoLab score and the v-PCR (potentially cohort I and particularly cohort II). The reason for this is to determine the maximal cut-off value for the CoLab score to predict negligible viral load. If necessary, the CoLab model can be adjusted using LASSO regression to determine the optimal parameters used in this score. Lastly, the CoLab model will be validated using

Receiver Operator Curves (ROC), confusion matrices, and calibration curves in the analysis of cohort III.

For local cohort I, a prospective serially collected dataset of 324 COVID-19+ patients admitted to the ICU of MUMC+ for mechanical ventilation is available. This also includes a subset of immunocompromised patients (n=60). Adding interaction terms with immunocompromised groups to the mixed models will test whether the development of the CoLab score over time differs for these patients compared to non-immunocompromised patients.

For regional cohort II, a negative v-PCR will be considered as the moment when a patient is not infectious anymore. To assess whether a normalized CoLab-score can pinpoint this moment, we expect that 95% of the patients will have a normalized CoLab-score within a time frame of two days before and after the negative v-PCR. Using this proportion of 95% with a total width of the confidence interval of 10%, and an alpha of 5%, we need to include at least 88 new COVID-19 patients admitted to the ICU for mechanical ventilation.

For national cohort III we aim to include serially collected data from all COVID-19-positive patients admitted to the ICU of the other participating hospitals for the purpose of validation.

Ethics and dissemination

Ethical approval for study part I (METC nr: 2020-1565/3 00 523) was granted by the Medical Ethical Committee from MUMC+ (Maastricht, the Netherlands). During the pandemic, the board of directors of MUMC+ adopted a policy to inform patients and ask their consent to use the collected data and to store blood samples for COVID-19 research purposes. The Medical Ethical Committee from Zuyderland Medical Centre (Heerlen/Sittard-Geleen, the Netherlands) approved study parts II (METCZ20210091-CoLaIC study) and III (METCZ20200057). The study is conducted in concordance with the Declaration of Helsinki. Patients will be informed about the purpose and

procedures of the study via verbal and written information and informed consent will be obtained. If the patient is not able to communicate, e.g., due to ICU treatment, the next of kin will be approached. Results from this study will be disseminated via peer-reviewed journals, congress presentations, and consortium presentations. The data generated will also be available upon request in a public, open access repository.

Collaborators

The members of the Dutch CoLaIC consortium are:

Stephanie M.C. Ament (MUMC+, Maastricht); M. Sesmu Arbous (LUMC, Leiden); Otto Bekers (MUMC+, Maastricht); Miranda van Berkel (Radboud UMC, Nijmegen); Arjen-Kars Boer (Catharina Hospital, Eindhoven); Dirck W. van Dam (Zuyderland MC, Sittard-Geleen/Heerlen); Ruben Deneer (Catharina Hospital, Eindhoven); William P.T.M. van Doorn (MUMC+, Maastricht); Tom P. Dormans (Zuyderland MC, Sittard-Geleen/Heerlen); Silvia M.A.A. Evers (Maastricht University, Maastricht); Tim Frenzel (Radboud UMC, Nijmegen); Judith Gillis (LUMC, Leiden); Iwan C.C. van der Horst (MUMC+, Maastricht); W. Nadia H. Koek (Medical Centre Leeuwarden, Leeuwarden); Kitty C.F.M. Linssen (Zuyderland MC, Sittard-Geleen); Steven J.R. Meex (MUMC+, Maastricht); Guy J.M. Mostard (Zuyderland MC, Sittard-Geleen); Remy L.M. Mostard (Zuyderland MC, Sittard-Geleen); Luuk C. Otterspoor (Catharina Hospital, Eindhoven); Natal A.W. van Riel (Technical University, Eindhoven); Frans Stals (Zuyderland MC, Sittard-Geleen); Albert Wolthuis (Medical Centre Leeuwarden, Leeuwarden).

Author contributions

Study design: BvB, IvL, PW, WvM and MPGL; Development of the study protocol: BvB, IvL, PW, WvM, SG and MPGL; Patient recruitment: BvB and WvM; Data collection: TS and FvR; Manuscript preparation: TS, BvB, PW, IvL, WvM and MPGL. The members of the CoLaIC-consortium co-designed the study protocol, selected potential participants, assisted in their recruitment, collected data, and set up, prepared and hosted COVID-19 databases. All authors read and approved the final manuscript.

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Competing interests

None declared

Patients and public involvement

Patients (e.g., Longfonds) were and will be involved in the design and dissemination plans of this research.

Patient consent for publication

Not applicable

Orcid iD

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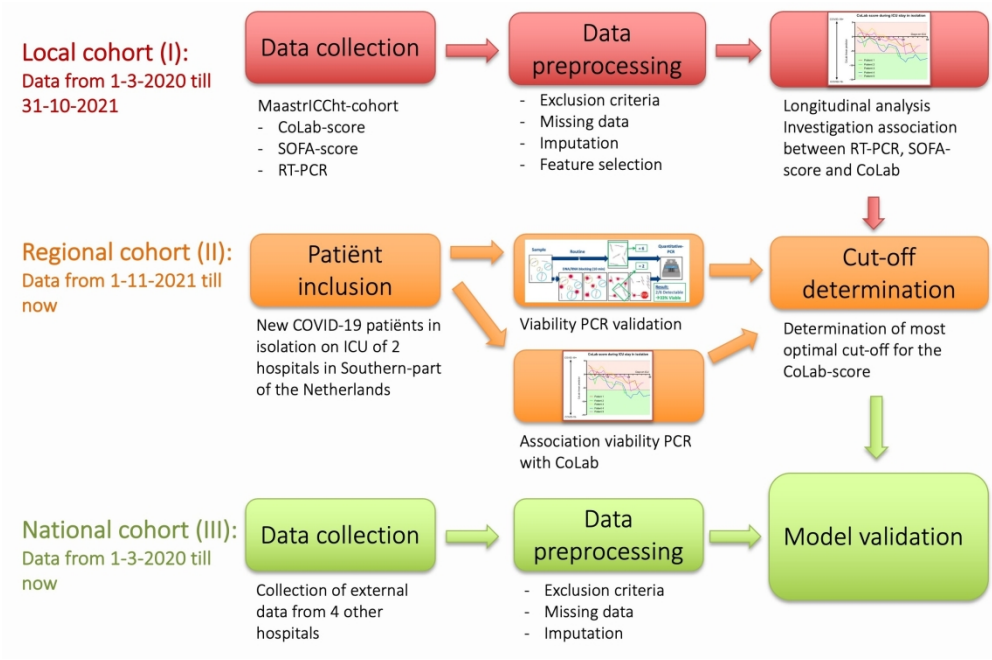
Supplemental

Supplemental Table 1. Overview of the hospitals participating in the study

Hospital	Location	Type of hospital	ICU beds*	Hospital beds	Cohort
Maastricht UMC+	Maastricht, the Netherlands	University medical centre	33	715	I & III
Zuyderland MC	Sittard-Geleen, Heerlen, the Netherlands	Large teaching hospital	36	980	II
Leiden UMC	Leiden, the Netherlands	University medical centre	45	882	III
Radboud UMC	Nijmegen, the Netherlands	University medical centre	35	1.065	III
Medical Centre Leeuwarden	Leeuwarden, the Netherlands	Large teaching hospital	39	647	III
Catharina Hospital	Eindhoven, the Netherlands	Large teaching hospital	36	696	III

**non-pandemic situation; UMC= university medical centre*



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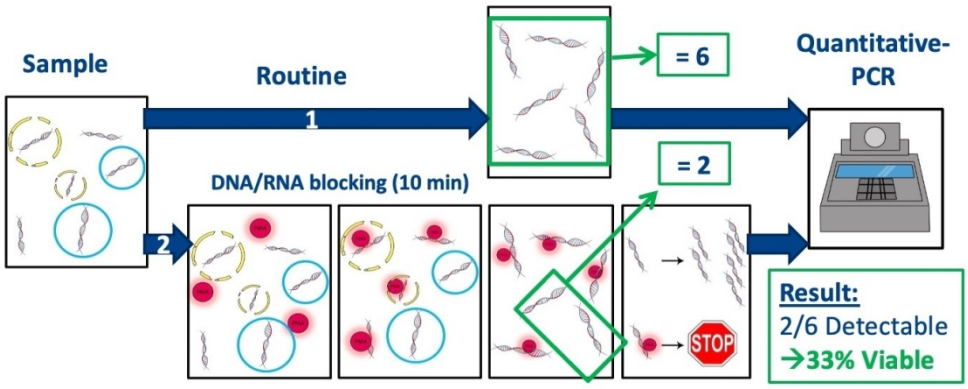

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TRIPOD Checklist: Prediction Model Development and Validation

Section/Topic	Item		Checklist Item	Page
Title and abstract				
Title	1	D;V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	P1
Abstract	2	D;V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	P3
Introduction				
Background and objectives	3a	D;V	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	P5
	3b	D;V	Specify the objectives, including whether the study describes the development or validation of the model or both.	P5/6
Methods				
Source of data	4a	D;V	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	P6/7
	4b	D;V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	P6/7
Participants	5a	D;V	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	P6/7
	5b	D;V	Describe eligibility criteria for participants.	P8
	5c	D;V	Give details of treatments received, if relevant.	n/a
Outcome	6a	D;V	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	P10
	6b	D;V	Report any actions to blind assessment of the outcome to be predicted.	n/a
Predictors	7a	D;V	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	P10
	7b	D;V	Report any actions to blind assessment of predictors for the outcome and other predictors.	n/a
Sample size	8	D;V	Explain how the study size was arrived at.	P12
Missing data	9	D;V	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	P11/12
Statistical analysis methods	10a	D	Describe how predictors were handled in the analyses.	P11/12
	10b	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	P11/12
	10c	V	For validation, describe how the predictions were calculated.	P12
	10d	D;V	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	P12
	10e	V	Describe any model updating (e.g., recalibration) arising from the validation, if done.	P12
Risk groups	11	D;V	Provide details on how risk groups were created, if done.	P8
Development vs. validation	12	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	P12
Results				
Participants	13a	D;V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	n/a
	13b	D;V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	n/a
	13c	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	n/a
Model development	14a	D	Specify the number of participants and outcome events in each analysis.	n/a
	14b	D	If done, report the unadjusted association between each candidate predictor and outcome.	n/a
Model specification	15a	D	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	n/a
	15b	D	Explain how to the use the prediction model.	n/a
Model performance	16	D;V	Report performance measures (with CIs) for the prediction model.	n/a
Model-updating	17	V	If done, report the results from any model updating (i.e., model specification, model performance).	n/a
Discussion				
Limitations	18	D;V	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	n/a
Interpretation	19a	V	For validation, discuss the results with reference to performance in the development data, and any other validation data.	n/a
	19b	D;V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	n/a
Implications	20	D;V	Discuss the potential clinical use of the model and implications for future research.	n/a
Other information				
Supplementary information	21	D;V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	n/a
Funding	22	D;V	Give the source of funding and the role of the funders for the present study.	P13

*Items relevant only to the development of a prediction model are denoted by D, items relating solely to a validation of a prediction model are denoted by V, and items relating to both are denoted D;V. We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	P1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	P3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P5
Objectives	3	State specific objectives, including any prespecified hypotheses	P6
Methods			
Study design	4	Present key elements of study design early in the paper	P6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	P8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P9/11
Bias	9	Describe any efforts to address potential sources of bias	t.b.d.
Study size	10	Explain how the study size was arrived at	P12
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P11/12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	P11/12
		(*b*) Describe any methods used to examine subgroups and interactions	P10/11
		(*c*) Explain how missing data were addressed	P12
		(*d*) If applicable, explain how loss to follow-up was addressed	n/a
		(*e*) Describe any sensitivity analyses	P11
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Not present in protocol paper
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	Not present in protocol paper

		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	Report numbers of outcome events or summary measures over time	Not present in protocol paper

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not present in protocol paper
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not present in protocol paper
Discussion			
Key results	18	Summarise key results with reference to study objectives	Not present in protocol paper
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P2
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Not present in protocol paper
Generalisability	21	Discuss the generalisability (external validity) of the study results	Not present in protocol paper
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

Validating a clinical laboratory parameters-based de-isolation algorithm for COVID-19 patients in the intensive care unit using viability- PCR: the CoLaIC multicentre cohort study protocol

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Keywords:	COVID-19, INTENSIVE & CRITICAL CARE, Diagnostic microbiology < INFECTIOUS DISEASES, Clinical chemistry < PATHOLOGY, HAEMATOLOGY, Epidemiology < INFECTIOUS DISEASES

Validating a clinical laboratory parameters-based de-isolation algorithm for COVID-19 patients in the intensive care unit using viability-PCR: the CoLaIC multicentre cohort study protocol

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Abstract

Introduction

To investigate whether biochemical and haematological changes due to the patient's host response (CoLab algorithm) in combination with a *Severe Acute Respiratory Syndrome-CoronaVirus-2* (SARS-CoV-2) viability-PCR (v-PCR) can be used to determine when a COVID-19 patient is no longer infectious.

We hypothesise that the CoLab algorithm in combination with v-PCR can be used to determine whether or not a COVID-19 patient is infectious to facilitate the safe release of COVID-19 patients from isolation.

Methods and analysis

This study consists of three parts using three different cohorts of patients. All three cohorts contain clinical, vital and laboratory parameters, as well as logistic data related to isolated COVID-19 patients, with a focus on ICU stay. The first cohort will be used to develop an algorithm for the course of the biochemical and haematological changes of the COVID-19 patient host response. Simultaneously, a second prospective cohort will be used to investigate the algorithm derived in the first cohort, with daily measured laboratory parameters, next to conventional SARS-CoV-2 RT-PCRs, as well as v-PCR, to confirm the presence of intact SARS-CoV-2 particles in the patient. Finally, a third multi-centre cohort, consisting of retrospectively collected data from COVID-19 patients admitted to the ICU will be used to validate the algorithm.

Ethics and dissemination

This study was approved by the Medical Ethics Committee from MUMC+ (cohort I: 2020-1565/3 00 523) and Zuyderland MC (cohort II and III: METCZ20200057). All patients will be required to provide

informed consent. Results from this study will be disseminated via peer-reviewed journals and congress/consortium presentations.

Article summary

Strengths and limitations of this study

Strengths:

- The algorithm/model is based on routinely tested blood parameters and standardised laboratory tests
- Multicentre approach with a good distribution of hospitals covering various regions of the Netherlands
- Large temporal range of the retrospective cohort III enables model validation for SARS-CoV-2 virus variants of concern (VOC)

Limitations:

- Viability-PCR is not performed in cohorts I and III
- The focus is limited to (de-)isolation of COVID-19 patients in the ICU

Introduction

The COVID-19 pandemic is globally disruptive regarding the continuation of regular healthcare. Hospitalised COVID-19 patients need to be isolated and separated from the non-COVID-19 patient population. This aspect paired with the large influx of COVID-19 patients and limited availability of hospital and isolation beds exerts enormous pressure on regular non-COVID-19 healthcare, but also on healthcare professionals. In addition, the need for treatment and support in an intensive care unit (ICU) for a substantial subset of COVID-19 patients and the limited availability in the number of ICU beds contributes to these effects. De-isolation as early as possible could improve the quality of life for the affected patients, as well as decrease the pressure on the healthcare system and its professionals.

Several study protocols described methods to determine if COVID-19-infected patients can be de-isolated: based on clinical signs[1], using RT-PCR[2], or with rapid antigen tests[3]. Reverse transcriptase-polymerase chain reaction (RT-PCR) testing is currently the gold standard to determine whether a patient is SARS-CoV-2 positive[4]. To de-isolate a COVID-19 ICU patient in the Netherlands two consecutive negative PCR tests are required. However, it can be hypothesised that SARS-CoV-2 RT-PCR positivity does not relate per se with the actual presence of intact, infectious viruses[5, 6]. Because RT-PCR detects nucleic acids and does not make a distinction between an intact infectious virus and non-intact non-infectious viral particles, this may result in persistently positive RT-PCR test results, which hampers timely de-isolation[4].

An alternative RT-PCR-based method to detect intact viral particles is to eliminate incomplete viral particles and RNA remnants before the actual RT-PCR is performed. Propidium monoazide (PMA) is a dye that binds irreversibly to (deoxy)ribonucleic acid (DNA/RNA) and cannot penetrate cell membranes[7]. Pre-treatment of a sample with PMA results in the amplification of only intact particles. This so-called viability-PCR (v-PCR) has been shown to successfully measure the number

of viable micro-organisms, such as Chlamydia trachomatis, in a sample[8]. In the present study, we want to adapt and validate this concept for the detection of intact viable RNA-containing SARS-CoV-2 virus. Preliminary data have confirmed its applicability for SARS-CoV-2 diagnostics[9]. The adapted v-PCR will be used in the study herein presented to confirm the state of viability and thus potential infectivity of SARS-CoV-2 in patients.

An alternative approach is to assess the host response of the suspected patient to the virus. One of the methods to assess the host response to SARS-CoV-2 is the CoLab score. This score has been developed using an adaptive LASSO-regression technique and requires the input of the numerical results of ten blood parameters and the age of the patient[10]. The required parameters are blood tests that are requested frequently and routinely for emergency room (ER) as well as ICU patients. This score has previously been developed and validated and has been implemented in the ER departments of two large Dutch teaching hospitals, with very high negative predictive value (99.5%) and sensitivity (96.9%)[10]. The score is also utilised to exclude COVID-19 in a screening setting for healthcare workers with COVID-19 suspected complaints[11].

Preliminary analysis of serially collected data in a pilot set of ICU patients showed a decrease in the CoLab score resulting in normalization before a patient is discharged (unpublished data). For that reason, we hypothesise that the biochemical and haematological changes in blood parameters necessary to calculate the CoLab score rapidly return to normal values after the host clears the SARS-CoV-2 infection.

This study aims to investigate whether biochemical and haematological changes due to the patient's host response (CoLab algorithm) and/or the v-PCR can be reliably and validly used to determine, at an earlier stage in comparison with a conventional SARS-CoV-2 RT-PCR, when a COVID-19 patient is no longer infectious.

Methods and analysis

Cohorts

This study is composed of three cohorts, two prospective cohorts (local and regional) and one retrospective cohort (national), which all consist of serially (i.e. daily) collected clinical and laboratory variables of COVID-19 patients in isolation at an ICU. We intend to include all patients admitted to one of our COVID-19 ICU isolation rooms.

More specifically, the three different cohorts will be used to study the CoLab score over time (local cohort I), to determine a cut-off point related to the intact infectious viral load (regional cohort II), and to validate the CoLab algorithm (national cohort III) on a national level with an external dataset (Figure 1). While not developed specifically for models using machine learning [12], the study will follow the guidelines of the Transparent reporting of a multivariable predicton model for individual prognosis or diagnosis (TRIPOD).[13]

Local single-centre prospective cohort (I)

The first, single-centre, local cohort is the prospective Maastricht Intensive Care COVID (MaastrICCht) cohort, previously described by Tas et al[14]. The CoLab score is calculated for each time-point using this comprehensively characterised cohort[14-20]. In addition, the daily Sequential Organ Failure Assessment (SOFA)[15, 19] scores are available as well as all conventional SARS-CoV-2 RT-PCRs that are measured within this cohort. The aim is to investigate the development of the CoLab score over time. To possibly de-isolate patients, the CoLab score should at least decrease over time in a way that is independent of disease severity and similar for survivors and non-survivors. Therefore, we hypothesise that the CoLab score decreases over time in both survivors and non-

survivors, in a way that is independent of disease severity over time measured by serial SOFA scores. To have an additional value above conventional RT-PCR-based de-isolation, the decrease in CoLab score should occur before de-isolation by RT-PCR is done. We hypothesise that a CoLab score decrease is present before RT-PCR-based de-isolation. We will explore the association between CoLab score over time and the moment of RT-PCR-driven de-isolation. If the CoLab score behaves over time in the ICU as hypothesised above, the next step is to quantify what decrease in CoLab score over time (or what cut-of CoLab score per day) precedes the transition from RT-PCR positive to negative. This decrease in CoLab score over time can be used to develop a diagnostic prediction model for de-isolation. Whether this prediction model can be used as the gold standard for de-isolation (CoLab prediction model alone, or in combination with conventional SARS-CoV-2 RT-PCR and/or v-PCR) is part of this study protocol.

Regional dual-centre prospective cohort (II)

In the second part, we hypothesise that excluding infectiousness, contributing to de-isolation can be done more accurately by using v-PCR instead of RT-PCR. A second prospectively collected, dual centre, regional cohort of COVID-19 patients from the ICU department of both Zuyderland Medical Centre and Maastricht University Medical Centre + (MUMC+) will be used to evaluate the usability of the v-PCR for the above-mentioned hypothesis. Inclusion of all consecutive COVID-19 ICU patients will be pragmatic based on the development of the pandemic and related incidence of ICU admission, starting from 1st November 2021. We aim to include a minimum of 90 patients. In this cohort, serial data related to the CoLab algorithm will be collected daily. In addition, both conventional (RT-PCR) and v-PCR testing for the detection of SARS-CoV-2 will be performed three times a week. The aim of this regional cohort (II) is to determine a cut-off point or a certain decrease in CoLab score over time that precedes the transition from positive to negative RT-PCR and v-PCR results.

National multi-centre retrospective cohort (III)

For the third part of the study, a retrospectively collected multi-centre, national cohort will be used. This retrospective cohort will consist of ICU data derived from four other hospitals located in the Netherlands. This dataset will contain serially collected data necessary for determining the CoLab score (ten blood parameters and age, see below) next to conventional SARS-CoV-2 RT-PCR results. This cohort will be used to determine whether the CoLab algorithm developed and validated in cohorts I and II in specific contexts are generalisable to and valid in other contexts (cohort III). An additional aim is to test the CoLab algorithm for different variants of concern (VOC) of SARS-CoV-2 (see also below). For this purpose, we will use data from all COVID-19-positive ICU patients between March 2020 and September 2022 (estimated at least 250 patients per participating centre).

Context and setting

Data from six hospitals will be used to create the different cohorts of this study. An overview of the number of hospital and ICU beds per participating hospital and per cohort is shown in Supplemental Table 1.

The local single-centre cohort I aims to use data obtained at MUMC+ (27 ICU and 6 high/medium care beds in the pre-pandemic era), a university medical centre located in the southern part of the Netherlands. During the COVID-19 pandemic, a maximum of 52 ICU beds were available for COVID-19 patients, and 12 for non-COVID-19 patients. Using this local cohort, the CoLab score will be observed over time.

The regional dual-centre cohort II consists of data from ICU patients from both Zuyderland MC (36 ICU beds) and MUMC+. These two hospitals are both located in Limburg in the Netherlands with an existing close cooperation for clinical purposes. Both hospitals are large teaching hospitals. This

regional cohort will be used to assess whether the CoLab score can be used to determine whether patients are SARS-CoV-2 free according to the results of the v-PCR.

The national, multi-centre cohort III consists of retrospectively collected data from four additional hospitals: Leiden UMC, Radboud UMC, Medical Centre Leeuwarden and Catharina Hospital. The hospitals in this cohort are located in separate provinces leading to a good geographical representation of the national spread of the Dutch COVID-19 patient population. Since Leiden UMC and Radboud UMC are university medical centres and Medical Centre Leeuwarden and Catharina Hospital are large teaching hospitals, both hospital types are represented equally. This national cohort will serve to further validate the model created using cohorts I and II in broader contexts (see Supplemental Table 1 for details of the different hospitals contributing to the consortium).

Patient and public involvement

The national patient organisation for lung diseases (Longfonds) has a panel of patients who have experienced isolation process due to COVID-19 in the ICU. These patients have read the study protocol and gave advice that has been implemented in the protocol. The patient panel will also be involved during the study to provide feedback regarding the execution of this study and to provide input for the implementation of the results.

Inclusion and exclusion criteria

For the three cohorts, the same inclusion and exclusion criteria are applicable. All patients with a proven primary and/or secondary SARS-CoV-2 infection are eligible to participate in the study. Exclusion criteria include only patients with extreme laboratory values (more than 10 times the standard deviation).

Parameters

Blood parameters

Blood samples are used to determine a variety of biochemical and haematological parameters in routine diagnostics and disease monitoring, from hospitalisation untill discharge of a COVID-19 patient. This has led to a large accumulation of blood-related biomarker data. Previous studies found biochemical and haematological changes measured in peripheral blood samples that characterised SARS-CoV-2 infection[21-23]. For instance, in the early stage of COVID-19 disease, haematological changes in immunocompetent leukocytes are associated with a more severe disease progression[23].

CoLab score

The CoLab score[10] uses the erythrocytes [10^{12}/L], leukocytes [10^{9}/L], eosinophils [10^{9}/L], basophils [10^{9}/L], $\log_{10}$ of bilirubin [µmol/L], $\log_{10}$ of lactate dehydrogenase (LD) [U/L], $\log_{10}$ of alkaline phosphatase (ALP) [IU/L], $\log_{10}$ of γ-glutamyltransferase (γ-GT) [U/L], albumin [g/L], C-reactive protein (CRP) [mg/L], and age [years accurate to two decimals]. These parameters are routinely measured in ICU patients and can be automatically extracted from the laboratory information system. The CoLab algorithm yields a score in the range of -20 to 5 (the so-called CoLab-linear predictor[10]), with a lower score associated with the exclusion of a SARS-CoV-2 infection and a higher score reflecting an increased risk of SARS-CoV-2 infection.

In an emergency department study population, a cut-off of the CoLab linear predictor was determined to classify patients as being COVID-19 negative. This cut-off was originally set to -5.83 to minimise the amount of false negative results, with a score below -5.83 being negative for COVID-19[10]. How the CoLab-algorithm corresponds with a negligible intact infectious viral load (see the section below)

is part of the present study: a cut-off or a certain decrease in CoLab-score over time. The CoLab score will be calculated daily for all participating patients, either prospectively or retrospectively.

Clinical parameters

In addition to the blood parameters, the clinical variables of patients are collected in the different cohorts. These include co-morbidities and clinical scores as well as ventilation, biometric, and physical parameters[15-20]. One clinical score of interest is the Sequential Organ Failure Assessments (SOFA) score. This score has previously been associated with the survival chance of mechanically ventilated COVID-19 patients[15]. A decrease in SOFA score is associated with survival. This sequentially determined SOFA score is measured over time and will be used to investigate whether the association between the CoLab score over time and infectiousness is independent of the SOFA score. This will provide evidence whether the CoLab score operationalises a different dimension of the host response, beyond multi-organ failure, and in an independent way with regard to survival. This will generate evidence whether the CoLab score generates new information, beyond existing scores and has potential for diagnosis of de-isolation.

Viability PCR (v-PCR)

A v-PCR[9] is performed to assess the presence of intact viruses and will be compared with the conventional SARS-CoV-2 RT-PCR test[24]. Briefly, nasopharyngeal samples are collected in viral transport medium (VTM). The VTM sample is divided into two parts. One part is directly used for a conventional RT-PCR for SARS-CoV-2. For the v-PCR propidium monoazide (PMA) is added to the other half of the VTM sample[25]. After pretreating this sample it is used for the v-PCR. (see also Figure 2). The difference in cycle-time values (Ct) between these two PCR tests will be reported as ΔCt, which is a reliable indication of the amount of intact virus in the sample.

The implementation of the viability PCR in the routine diagnostics would add some processing time to the existing SARS-CoV-2 PCR protocols. The v-PCR method is currently not (yet) automated and might as such not fit in every COVID-19 diagnostic workflow. However, the added value of the v-PCR would be the determination of complete virus particles.

Variants of Concern (VOC)

Due to the rapid mutation potential observed in viruses, it is necessary to ensure the robustness of the CoLab algorithm to variants of concern of this SARS-CoV-2 virus (VOCs). This study will address VOC retrospectively as well as prospectively. Cohort III, spanning from March 2020 until the present, contains data on the Wuhan original SARS-CoV-2 and data from at least three VOC. Demographic studies showed that during this period three VOC of the SARS-CoV-2 were present next to the original SARS-CoV-2 virus (2020/03 to 2021/01): the B1.1.7 alpha-variant (2021/02 to 2021/06), the B1.617.2 delta-variant (2021/07 to 2021/12), and the B1.1.529 omicron-variant (2022/01 to present)[26]. We use time periods to characterise VOC in cohort III. In contrast, in cohort II VOCs will be measured with variant-specific Next Generation Sequencing[27].

Statistical analysis

Analyses will be performed with R version 4.2.0 and with RStudio version 4.2.0 [28], combined with the packages Tidyverse [29], lme4 [30], MICE[31], MissForest[32] and Caret[33]. Missing values for numerical variables will be imputed using multiple imputations by chained equations (MICE).[31] Mixed-effects regression model analysis will be used to observe the CoLab score over time (cohort I), to determine whether the CoLab score is independent of survival and SOFA score (cohort I), and to determine the association between the CoLab score and the v-PCR (potentially cohort I and particularly cohort II). The reason for this is to determine the maximal cut-off value for the CoLab score to predict negligible viral load. If necessary, the CoLab model can be adjusted using LASSO

regression to determine the optimal parameters used in this score. Finally, the CoLab model will be validated using Receiver Operator Curves (ROC), confusion matrices, and calibration curves in the analysis of cohort III.

For local cohort I, a prospective serially collected dataset of 390 COVID-19 positive patients admitted to the ICU of MUMC+ is available. This also includes a subset of immunocompromised patients (n=60). Adding interaction terms with immunocompromised groups to the mixed models will test whether the development of the CoLab score over time differs for these patients compared to non-immunocompromised patients. A similar approach will be taken to investigate whether results for sex differ.

For regional cohort II, a negative v-PCR will be considered as the moment when a patient is not infectious anymore. To assess whether a normalised CoLab-score can pinpoint this moment, we expect that 95% of the patients will have a normalised CoLab-score within a time frame of two days before and after the negative v-PCR. Using this proportion of 95% with a total width of the confidence interval of 10%, and an alpha of 5%, we need to include at least 88 new COVID-19 patients admitted to the ICU for mechanical ventilation.

For national cohort III, we aim to include serially collected data from all COVID-19-positive patients admitted to the ICU of the other participating hospitals for validation.

Sample size calculation

For the local cohort, as stated above, a prospectively serially collected dataset of 390 COVID-19 positive patients admitted to the ICU of MUMC are already available This includes also a subset of immunocompromised patients (n=60). If hypothesised that the course of the CoLab-score does not differ between immunocompromised vs non-immunocompromised COVID-19 patients (the mean difference between these two groups=0), and using a power of 80%, an alpha of 0.05, a standard

deviation in COVID-19+ LP of 1,5 and a margin of ±1, then we need to analyse at least 39 patients per group (in our dataset we have data available of 60 immunocompromised patients).

For the regional cohort (prospective), if we consider a negative v-PCR as the moment when a patient is not infectious anymore, we can assess whether a normalised CoLab-score can indicate this moment. Here we expect that 95% of the patients will have a normalised CoLab-score within a time frame of two days before and after the negative v-PCR. Using this proportion of 95% with a total width of the confidence interval of 10%, and an alpha of 5%, we need to include at least 88 new COVID-19 patients admitted to the ICU for mechanical ventilation.

For the national cohort (retrospective), we want to include serially collected datasets of at least 250 COVID-19+ patients admitted to the ICU of the other participating hospitals for the purpose of validation. These data are already available in the different laboratory information systems of the different hospitals, but needed to be extracted, collected and data needed to be cleaned

Ethics and dissemination

Ethical approval for study part I (METC nr: 2020-1565/3 00 523) was granted by the Medical Ethical Committee from MUMC+ (Maastricht, the Netherlands). During the pandemic, the board of directors of MUMC+ adopted a policy to inform patients and ask for their consent to use the collected data and to store blood samples for COVID-19 research purposes. The Medical Ethical Committee from Zuyderland Medical Centre (Heerlen/Sittard-Geleen, the Netherlands) approved study parts II (METCZ20210091-CoLaIC study) and III (METCZ20200057). The study is conducted in accordance with the Declaration of Helsinki. Patients will be informed about the purpose and procedures of the study via verbal and written information and informed consent will be obtained. If the patient is not able to communicate him/herself, e.g., due to ICU treatment, the next of kin will be approached.

Patients will be asked for consent later, when the patient has recovered. Results from this study will be disseminated via peer-reviewed journals, congress presentations, and consortium presentations. The data generated will also be available upon request in a public, open-access repository.

Collaborators

The members of the Dutch CoLaIC consortium are:

Stephanie M.C. Ament (MUMC+, Maastricht); M. Sesmu Arbous (LUMC, Leiden); Otto Bekers (MUMC+, Maastricht); Miranda van Berkel (Radboud UMC, Nijmegen); Arjen-Kars Boer (Catharina Hospital, Eindhoven); Dirck W. van Dam (Zuyderland MC, Sittard-Geleen/Heerlen); Ruben Deneer (Catharina Hospital, Eindhoven); William P.T.M. van Doorn (MUMC+, Maastricht); Tom P. Dormans (Zuyderland MC, Sittard-Geleen/Heerlen); Silvia M.A.A. Evers (Maastricht University, Maastricht); Tim Frenzel (Radboud UMC, Nijmegen); Judith Gillis (LUMC, Leiden); Iwan C.C. van der Horst (MUMC+, Maastricht); W. Nadia H. Koek (Medical Centre Leeuwarden, Leeuwarden); Kitty C.F.M. Linssen (Zuyderland MC, Sittard-Geleen); Steven J.R. Meex (MUMC+, Maastricht); Guy J.M. Mostard (Zuyderland MC, Sittard-Geleen); Remy L.M. Mostard (Zuyderland MC, Sittard-Geleen); Luuk C. Otterspoor (Catharina Hospital, Eindhoven); Natal A.W. van Riel (Technical University, Eindhoven); Frans Stals (Zuyderland MC, Sittard-Geleen); Albert Wolthuis (Medical Centre Leeuwarden, Leeuwarden).

Author contributions

Study design: BvB, IvL, PW, WvM and MPGL; Development of the study protocol: BvB, IvL, PW, WvM, SG, WV and MPGL; Patient recruitment: BvB and WvM; Data collection: TS and FvR; Manuscript preparation: TS, BvB, PW, IvL, WV, WvM and MPGL. The members of the CoLaIC-

consortium co-designed the study protocol, selected potential participants, assisted in their recruitment, collected data, and set up, prepared and hosted COVID-19 databases. All authors read and approved the final manuscript.

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Competing interests

None declared

Patients and public involvement

Patients were and will be involved in the design and dissemination plans of this research.

Patient consent for publication

Not applicable

Orcid iD

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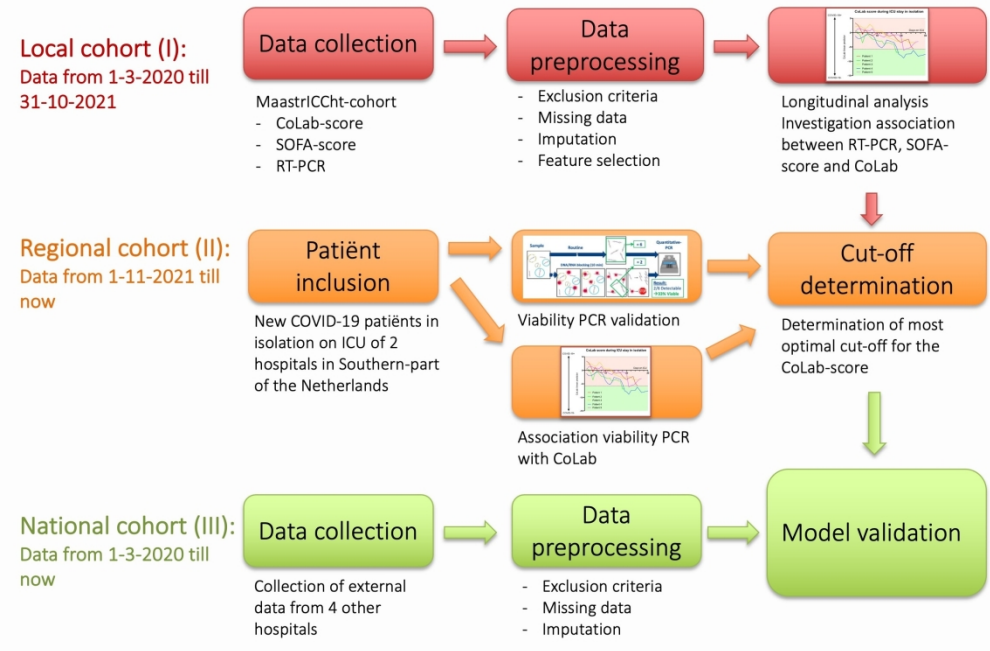
Figures legend

Figure 1: Overview of the study

Figure 2: Schematic representation of the principles of the conventional SARS-CoV-2 RT-PCR (route 1) in comparison to the viability-PCR (route 2). Route 1: all RNA is isolated from the sample and amplified using RT-PCR. Route 2: PMA irreversibly binds to free RNA and RNA from non-intact virus particles. Only RNA from intact virus particles is isolated and amplified by RT-PCR.



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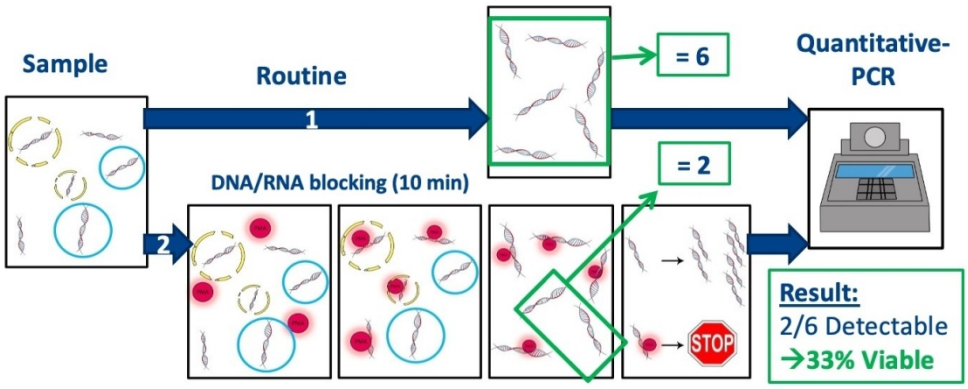

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Supplemental

Supplemental Table 1. Overview of the hospitals participating in the study

Hospital	Location	Type of hospital	ICU beds*	Hospital beds	Cohort
Maastricht UMC+	Maastricht, the Netherlands	University medical centre	33	715	I & III
Zuyderland MC	Sittard-Geleen, Heerlen, the Netherlands	Large teaching hospital	36	980	II
Leiden UMC	Leiden, the Netherlands	University medical centre	45	882	III
Radboud UMC	Nijmegen, the Netherlands	University medical centre	35	1.065	III
Medical Centre Leeuwarden	Leeuwarden, the Netherlands	Large teaching hospital	39	647	III
Catharina Hospital	Eindhoven, the Netherlands	Large teaching hospital	36	696	III

**non-pandemic situation; UMC= university medical centre*

TRIPOD Checklist: Prediction Model Development and Validation

Section/Topic	Item		Checklist Item	Page
Title and abstract				
Title	1	D;V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	P1
Abstract	2	D;V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	P3
Introduction				
Background and objectives	3a	D;V	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	P5
	3b	D;V	Specify the objectives, including whether the study describes the development or validation of the model or both.	P5/6
Methods				
Source of data	4a	D;V	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	P6/7
	4b	D;V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	P6/7
Participants	5a	D;V	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	P6/7
	5b	D;V	Describe eligibility criteria for participants.	P8
	5c	D;V	Give details of treatments received, if relevant.	n/a
Outcome	6a	D;V	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	P10
	6b	D;V	Report any actions to blind assessment of the outcome to be predicted.	n/a
Predictors	7a	D;V	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	P10
	7b	D;V	Report any actions to blind assessment of predictors for the outcome and other predictors.	n/a
Sample size	8	D;V	Explain how the study size was arrived at.	P12
Missing data	9	D;V	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	P11/12
Statistical analysis methods	10a	D	Describe how predictors were handled in the analyses.	P11/12
	10b	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	P11/12
	10c	V	For validation, describe how the predictions were calculated.	P12
	10d	D;V	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	P12
	10e	V	Describe any model updating (e.g., recalibration) arising from the validation, if done.	P12
Risk groups	11	D;V	Provide details on how risk groups were created, if done.	P8
Development vs. validation	12	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	P12
Results				
Participants	13a	D;V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	n/a
	13b	D;V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	n/a
	13c	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	n/a
Model development	14a	D	Specify the number of participants and outcome events in each analysis.	n/a
	14b	D	If done, report the unadjusted association between each candidate predictor and outcome.	n/a
Model specification	15a	D	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	n/a
	15b	D	Explain how to the use the prediction model.	n/a
Model performance	16	D;V	Report performance measures (with CIs) for the prediction model.	n/a
Model-updating	17	V	If done, report the results from any model updating (i.e., model specification, model performance).	n/a
Discussion				
Limitations	18	D;V	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	n/a
Interpretation	19a	V	For validation, discuss the results with reference to performance in the development data, and any other validation data.	n/a
	19b	D;V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	n/a
Implications	20	D;V	Discuss the potential clinical use of the model and implications for future research.	n/a
Other information				
Supplementary information	21	D;V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	n/a
Funding	22	D;V	Give the source of funding and the role of the funders for the present study.	P13

*Items relevant only to the development of a prediction model are denoted by D, items relating solely to a validation of a prediction model are denoted by V, and items relating to both are denoted D;V. We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	P1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	P3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P5
Objectives	3	State specific objectives, including any prespecified hypotheses	P6
Methods			
Study design	4	Present key elements of study design early in the paper	P6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	P8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P9/11
Bias	9	Describe any efforts to address potential sources of bias	t.b.d.
Study size	10	Explain how the study size was arrived at	P12
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P11/12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	P11/12
		(*b*) Describe any methods used to examine subgroups and interactions	P10/11
		(*c*) Explain how missing data were addressed	P12
		(*d*) If applicable, explain how loss to follow-up was addressed	n/a
		(*e*) Describe any sensitivity analyses	P11
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Not present in protocol paper
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	Not present in protocol paper

		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	Report numbers of outcome events or summary measures over time	Not present in protocol paper

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not present in protocol paper
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not present in protocol paper
Discussion			
Key results	18	Summarise key results with reference to study objectives	Not present in protocol paper
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P2
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Not present in protocol paper
Generalisability	21	Discuss the generalisability (external validity) of the study results	Not present in protocol paper
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.