BMJ Open

Protocol for a 30-day randomised, parallel-group, non-inferiority, controlled trial investigating the effects of discontinuing renin-angiotensin system inhibitors in patients with and without COVID-19: the RASCOVID-19 trial

Vivian Kliim-Hansen ,[1] Lærke Smidt Gasbjerg,[1,2] Anne-Marie Ellegaard,[1] Hans Johan Niklas Lorentsson,[1,3,4] Mads Bank Lynggaard,[1] Christoffer Andersen Hagemann,[1,5] Christian Legart,[1] David Siersbæk Mathiesen,[1] Pradeesh Sivapalan,[6] Jens-Ulrik Stæhr Jensen,[6,7] Tina Vilsbøll,[1,4] Mikkel Bring Christensen,[1,7,8,9] Filip Krag Knop [1,4,7,10]

To cite: Kliim-Hansen V, Gasbjerg LS, Ellegaard A-M, *et al*. Protocol for a 30-day randomised, parallel-group, non-inferiority, controlled trial investigating the effects of discontinuing renin-angiotensin system inhibitors in patients with and without COVID-19: the RASCOVID-19 trial. *BMJ Open* 2022;**12**:e062895. doi:10.1136/bmjopen-2022-062895

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2022-062895).

Received 24 March 2022
Accepted 29 September 2022

For numbered affiliations see end of article.

Correspondence to
Professor Filip Krag Knop; filip.krag.knop.01@regionh.dk

ABSTRACT

Introduction The COVID-19 pandemic caused by the virus SARS-CoV has spread rapidly and caused damage worldwide. Data suggest a major overrepresentation of hypertension and diabetes among patients experiencing severe courses of COVID-19 including COVID-19-related deaths. Many of these patients receive renin-angiotensin system (RAS) inhibiting therapy, and evidence suggests that treatment with angiotensin II receptor blockers (ARBs) could attenuate SARS-CoV-induced acute respiratory distress syndrome, and ACE inhibitors and ARBs have been suggested to alleviate COVID-19 pulmonary manifestations. This randomised clinical trial will address whether RAS inhibiting therapy should be continued or discontinued in hospitalised patients with COVID-19.

Methods and analysis This trial is a 30-day randomised parallel-group non-inferiority clinical trial with an embedded mechanistic substudy. In the main trial, 215 patients treated with a RAS inhibitor will be included. The participants will be randomly assigned in a 1:1 ratio to either discontinue or continue their RAS inhibiting therapy in addition to standard care. The patients are included during hospitalisation and followed for a period of 30 days. The primary end point is number of days alive and out of hospital within 14 days after recruitment. In a mechanistic substudy, 40 patients treated with RAS inhibition, who are not in hospital and not infected with COVID-19 will be randomly assigned to discontinue or continue their RAS inhibiting therapy with the primary end point of serum ACE2 activity.

Ethics and dissemination This trial has been approved by the Scientific-Ethical Committee of the Capital Region of Denmark (identification no. H-20026484), the Danish Medicines Agency (identification no. 2020040883) and by the Danish Data Protection Agency (P-2020-366). The results of this project will be compiled into one or more manuscripts for publication in international peer-reviewed scientific journals.

Trial registration number 2020-001544-26; NCT04351581.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Design and outcomes are simple, and the results can be directly applied in clinical practice.
⇒ Stratified randomisation will ensure equal distribution of age, trial sites and participation in other COVID-19 randomised clinical trials.
⇒ Blinded interim analysis will secure safety.
⇒ Renin-angiotensin system (RAS)-specific blood and urine analyses coupled with data on disease progression will provide insight into how ACE inhibitors and angiotensin II receptor blockers affect COVID-19 infection.
⇒ Due to the clinical setting of this trial, site investigators, clinical staff and participants will not be blinded; investigators responsible for interim analysis and outcome assessment will be blinded to group allocation.

INTRODUCTION

The COVID-19 pandemic has spread rapidly and caused damage worldwide. Data from some of the earliest and worst affected countries suggest a major overrepresentation of hypertension and diabetes among COVID-19-related deaths and among patients experiencing severe

courses of the disease.[1–3] The majority of patients with hypertension and/or diabetes are taking drugs targeting the renin-angiotensin system (RAS) because of their blood pressure-lowering and/or kidney protective effects. Importantly, the virus causing COVID-19, SARS-CoV-2, as well as SARS-CoV (the virus causing the outbreak of severe acute respiratory syndrome in southern China in 2002/2003) bind to the transmembrane protein ACE2—an important component of RAS—for host cell entry and subsequent viral replication.[4]

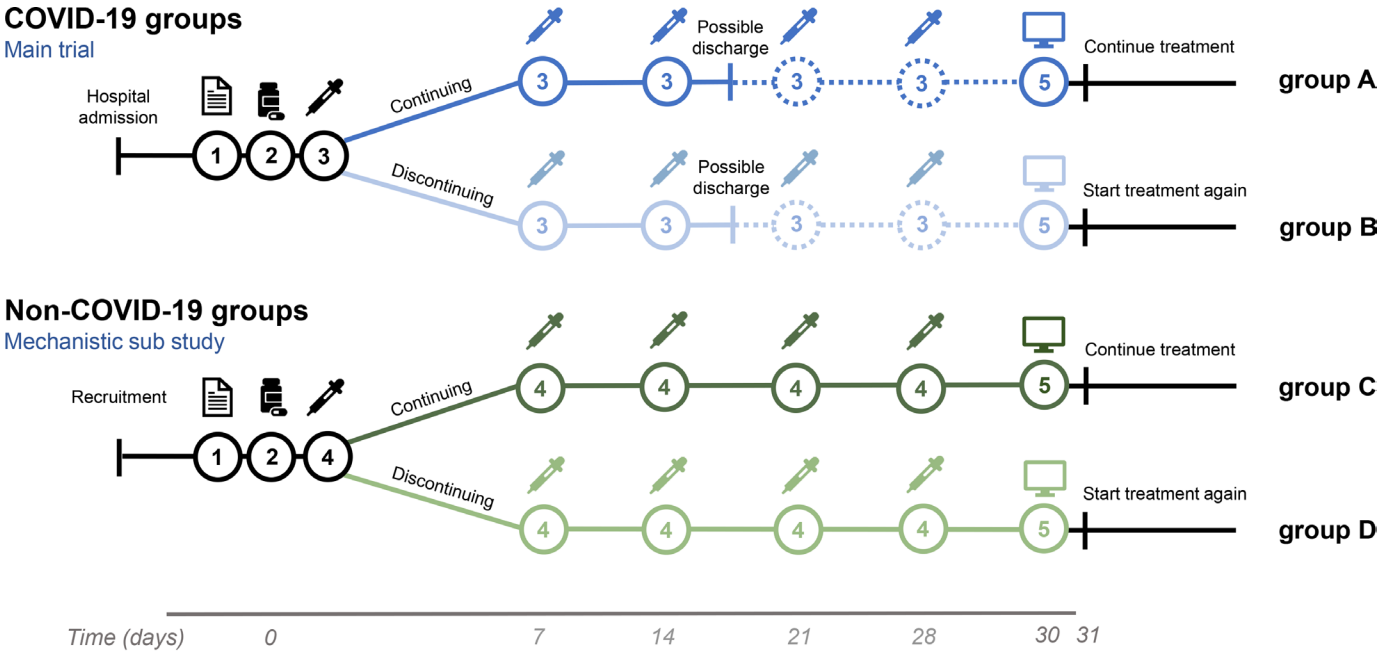
ACE2 is a homologue of ACE that regulates RAS by converting angiotensin II to the vasodilatory angiotensin,[1–7] diminishing and opposing the vasoconstrictive effect of angiotensin II. ACE2 is abundant in the intestines as well as lung alveolar epithelial cells[5] and in rodents, ACE2 expression is shown to decrease with age.[6] ACE2 is normally considered to be an enzyme that limits airway inflammation via effects in RAS,[4] and increased ACE2 activity seems to alleviate acute respiratory distress syndrome (ARDS).[7 8] Importantly, evidence from human[9 10] as well as rodent SARS-CoV studies[11] suggests that the inhibition of RAS by ACE inhibitors (ACEi) or angiotensin II receptor blockers (ARB) leads to upregulation of ACE2, and treatment with ARB leads to attenuation of SARS-CoV-induced ARDS.[12] This is of interest, as the vast majority of deaths from COVID-19 are due to ARDS[3] and ACEi and ARBs have been suggested to alleviate the COVID-19 pulmonary manifestations.[13] Recent observational studies have reported positive effects on severity[14] and mortality[15] associated with RAS inhibition therapy in patients with COVID-19. In contrast to these notions, concern has been raised that ACE2 upregulation (by RAS inhibitors) will multiply the cellular access points for viral entry and might increase the risk of severe progression of COVID-19[16]—potentially explaining the high morbidity and mortality among patients with COVID-19 who have diabetes and/or hypertension.[1–3] Two recent studies examining the effects of continuation versus discontinuation of RAS inhibitors in patients admitted to hospital with COVID-19 have not found any difference in outcomes between the groups[17 18] and, therefore, mechanistic prospective randomised trials evaluating the effect of continued versus discontinued RAS inhibitory therapy on the course of COVID-19 are needed.[14 15 19 20]

This is a randomised clinical trial with the main objective to investigate the effect of continued versus discontinued RAS inhibiting therapy in hospitalised patients with COVID-19 treated with ACEi or ARB on the number of days alive and out of hospital within 14 days in Denmark.

METHODS AND ANALYSES

Trial design

Each participant will be randomised to one of four groups: group A, B, C or D (figure 1).



1: Informed consent and inclusion
2: Randomisation
3: Blood and urine samples
4: Trial visit with blood and urin samples
5: Information from electronic patient chart

Figure 1 Trial design. COVID-19, coronavirus disease 2019.

COVID-19-infected participants (patients currently infected with COVID-19 and in hospital) will be randomly assigned in a 1:1 ratio to either discontinue or continue their RAS inhibiting therapy in addition to standard care. The group discontinuing RAS inhibition therapy (hereafter termed 'group B') will serve as a control group for the group continuing the therapy (hereafter termed 'group A').

Participants without COVID-19 will likewise be randomly assigned in a 1:1 ratio to either continue (hereafter termed 'group C') or discontinue (hereafter termed 'group D') their RAS inhibiting therapy. At inclusion, basic health information regarding the participant will be obtained, including age, gender, weight, height, smoking, alcohol consumption, prior and known diseases and medications. Blood samples as well as urinary samples will be collected at randomisation and every 7 days thereafter during admission for groups A and B, and every 7 days at trial visits for groups C and D (figure 1). Blood and urine will be analysed for relevant components of the RAS with the objective of supporting our clinical findings. Furthermore, messenger RNA expression in peripheral blood cells focusing on the expression of ACE, interferon signatures and T-cell exhaustion markers (which all may have prognostic value for viral infections) will be evaluated.

Supplementary data from routine blood samples including arterial blood gas analyses performed in the clinic on participants in groups A and B will be extracted from the medical charts and thus, causing no trial-related discomfort for the participants. These will include potassium, sodium, C reactive protein, leucocytes, haemoglobin, haemoglobin A1c, alanine aminotransferase, international normalised ratio, creatinine, triglycerides, ferritin, fibrinogen, beta-2-microglobulin, partial pressure of oxygen and fraction of inspired oxygen. Additional data such as blood pressure, oxygen saturation, heart rate, respiratory frequency and temperature will also be obtained. For groups C and D, at trial visits every 7 days, blood samples for analysis of the above-mentioned parameters will be collected together with the trial samples and data on blood pressure, heart rate, temperature and blood oxygenation.

Besides continuing or discontinuing RAS inhibiton therapy, this trial will not interfere with the treatment of COVID-19 or any other conditions during the inclusion period.

Patient and public involvement

Relevant patients were involved in designing the participants information. No patients and/or the public will be involved in the reporting or dissemination plans of this research.

Participants and recruitment

The start date of the study is 18 May 2020 and the estimated completion date for recruitment is 31 October 2022 (see figures 2 and 3).

Inclusion criteria

1. Verified COVID-19 (only groups A and B).

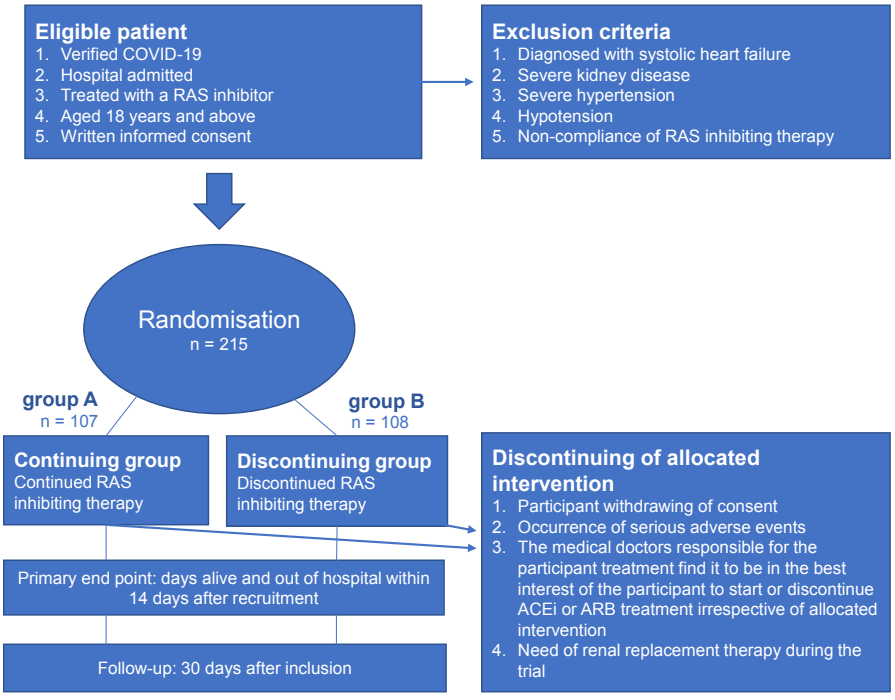


Figure 2 Plan for patient inclusion, exclusion and discontinuation for groups A and B. ACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blocker; RAS, renin-angiotensin system.

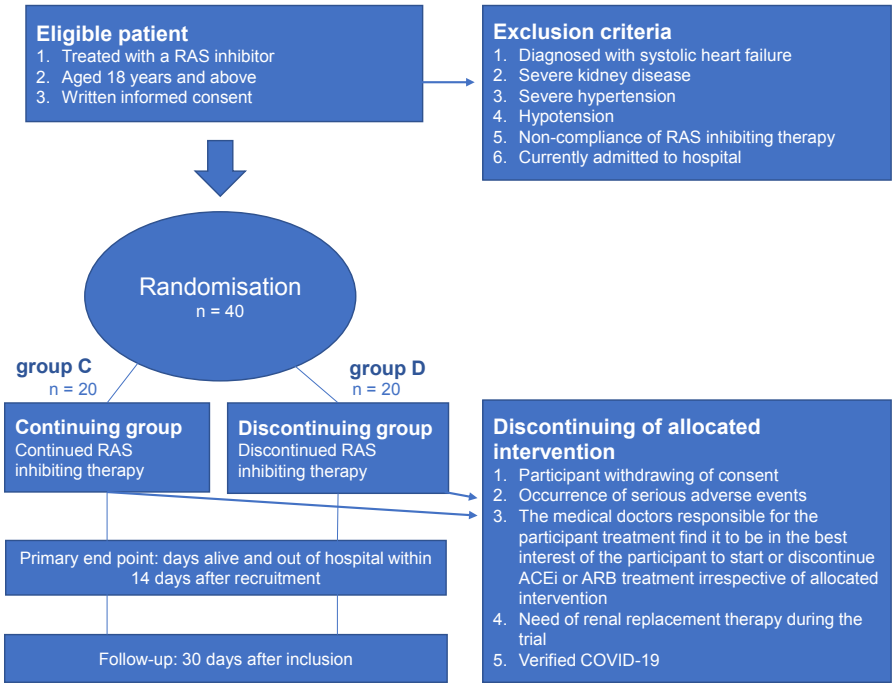


Figure 3 Plan for patient inclusion, exclusion and discontinuation for groups C and D. ACEi, ACE inhibitors; ARB, angiotensin II receptor blocker; RAS, renin-angiotensin system.

2. Hospital admitted (only groups A and B).
3. Treated with a RAS inhibitor.
4. Aged 18 years and above.
5. Written informed consent.

Exclusion criteria

1. Diagnosed with systolic heart failure; defined by heart failure with reduced ejection fraction (EF) (EF <50%).
2. Severe kidney disease; defined by estimated glomerular filtration rate (eGFR) ≤30 mL/min/1.73 m^2.
3. Severe hypertension; defined by systolic pressure ≥175 mm Hg and/or diastolic pressure ≥105 mm Hg.
4. Hypotension; defined by systolic pressure ≤100 mm Hg and/or diastolic pressure ≤60 mm Hg.
5. Non-compliance of RAS inhibition therapy; defined as an estimated adherence <80% assessed by a questionnaire in combination with checking the electronic medication system for redeemed prescriptions in the last 6 months. In borderline cases, the participant is assumed adherent to therapy.
6. Contraindications for continued of ACEi or ARB treatment including second and third trimester pregnancy, breast feeding, severe hepatic impairment, hypersensitivity or allergic reactions to the therapy.
7. Currently admitted to hospital (only groups C and D).

Participant withdrawal criteria

1. Participant withdrawing of consent.
2. Occurrence of serious adverse events related to continuation or discontinuation of ACEi or ARB treatment.
3. In case the medical doctors responsible for the participant treatment find it to be in the best interest of the participant to start or discontinue ACEi or ARB treatment irrespective of allocated intervention.
4. Need of renal replacement therapy during the trial.
5. A verified COVID-19 diagnosis during the trial (only groups C and D).

In case of withdrawal of consent, follow-up will be discontinued. Otherwise, follow-up will continue for 30 days through medical charts and registers.

Sample size

The primary outcome of the RASCOVID-19 trial is days alive and out of hospital within 14 days after recruitment. For groups A and B, using a one-sided α of 0.025 and a power (1–β) of 0.8 in a group sequential design, 1:1 allocation, with one planned interim analysis at 50% recruited, and with a null hypothesis of 0 days of difference, an SD of 3.8 days in the primary outcome measure, the trial will need to have a sample size of 214 patients to detect a worsening of 1.5 days in the primary outcome. Thus, the non-inferiority limit is 1.5 days.

For groups C and D we looked at a previous study,[21] where serum ACE2 activity levels in healthy individuals were 16.2±5.4 UF/mL (mean±SD) and 24.8±12.4 UF/mL in hypertensive individuals. Estimating a baseline of 25 UF/mL with an SD of 10 UF/mL and wanting to be able to detect a MIREDIF in serum ACE2 activity of 9 UF/mL between the two COVID-19-negative groups

(ie, continued and discontinued) at trial end and an α set to 5% and power (1−β) to 80% (corresponding to Z2α=1.96 and Zβ=0.84), then the number of participants needed in each COVID-19-negative group is 19 ($n=(1.96+0.84)^2\times(10^2+10^2)/9^2=19$). The population size has been set to 20 in each group to ensure power in case of participant dropouts.

Randomisation

Participants receiving RAS inhibition therapy and complying with trial inclusion and exclusion criteria are randomised by site investigators 1:1 to each arm according to a computer-generated allocation table stratified by age (intervals: ≤65 years or >65 years), and trial site and participation in other COVID-19 randomised clinical trials for groups A and B. Each participant will be assigned a computer-generated unique allocation number. The allocation table will be kept at a separate research facility and blinded for everyone else than the data analyst responsible for its generation.

Blinding

The site investigators, clinical staff at trial sites or participants will not be blinded to the intervention. Trial statisticians and investigators responsible for the interim analysis and outcome assessment will be blinded to the group allocation.

Interventions

The participants will be randomly assigned in a 1:1 ratio to either discontinue or continue their RAS inhibition therapy in addition to standard care, for the trial period of 30 days.

Adherence

For groups A and B, the administration of medication will be monitored through the electronic patient chart during hospitalisation. Patients discharged before 30 days after enrolment will be followed-up via phone contact to register adherence, deviation and other data. For groups C and D, adherence, deviations and other data will be addressed at every trial visit.

Outcome measures

Primary outcome

The primary end point is days alive and out of hospital within 14 days after recruitment (group A vs group B), on which a patient satisfies categories 0, 1 or 2 on the eight-category ordinal scale (figure 4).[22]

Secondary outcomes

The key secondary end point is the occurrence of worsening of COVID-19 (group A vs group B) as assessed by when a patient satisfies category 6, 7 or 8 on the ordinal scale (figure 4) within the trial period.[22]

Other secondary end points include:

- Time to occurrence of each of the components of the key secondary composite end point (group A vs group B).
- Kidney function (as assessed by plasma creatinine and eGFR).
- Duration of index hospitalisation (group A vs group B).
- 30-day mortality (differences in mortality will be displayed as number of days alive during the intervention period) (group A vs group B).
- Discharge beyond day 30 (group A vs group B).
- Number of readmissions after day 30 (group A vs group B).

WHO defined Ordinal Scale for Clinical Improvement

1. Not hospitalised, no clinical or virological evidence of infection
2. Not hospitalised, no limitations of activities
3. Not hospitalised, limitation of activities
4. Hospitalised, no oxygen therapy
5. Hospitalised, oxygen by mask or nasal prongs
6. Hospitalised, non-invasive ventilation or high-flow oxygen
7. Hospitalised, intubation and mechanical ventilation
8. Hospitalised, ventilation and additional organ support – pressors, rapid response team (RRT), extracorporeal membrane oxygenation (ECMO)
9. Death

Figure 4 WHO-defined ordinal scale for clinical improvement.[22] WHO, World Health Organisation.

- Change in circulating levels of RAS components (ACE, ACE2, aldosterone, angiotensin II and renin), expression of ACE, interferon signatures and T-cell exhaustion markers and blood pressure.

Data management and monitoring

Data collection

Data will be collected through access to the participant's medical chart as well as through questionnaires, urine and blood samples. Data will be obtained by the site investigators in case report forms stored in the data-managing programme of the Capital Region of Denmark. All participants will be assigned a trial number and will on data sheets and tubes only appear with the trial number. The full name, social security number and trial number will be stored separately. Follow-up will be done at day 30 through electronic patient charts and phone calls.

Data access

The trial will be conducted in accordance with the applicable rules on clinical trials involving people in respect of quality control and quality management and will follow the Good Clinical Practice (GCP) guidelines.[23] The principal investigator is responsible for managing and achieving data in accordance with current regulations. Trial data will only be made available to third parties in accordance with Danish law.

Quality control

The trial will be monitored according to Danish law and GCP guidelines by Copenhagen University Hospital's GCP unit.

Statistical analysis

See online supplemental appendix 1, including figures 1–3 and tables 1–3, and online supplemental appendix 2 for the full statistical analyses plan.

ETHICS AND DISSEMINATION

This trial has been approved by the Scientific-Ethical Committee of the Capital Region of Denmark (identification no. H-20026484), the Danish Medicines Agency (identification no. 2020040883) and by the Danish Data Protection Agency (P-2020-366) and comply with the international General Data Protection Regulation. The trial will be conducted according to the Declaration of Helsinki. All participants will receive oral and written information and both oral and written consent will be obtained before trial initiation. The participants will be informed by a member of the research group who is not responsible for the treatment of the participant.

The protocol-related procedures are associated with minimal discomfort to the participants, who will either not receive their usual ACEi or ARB or continue their usual therapy during the trial period, depending on assignment to the discontinuation or the continuation group, respectively. Discontinuation of RAS inhibition therapy may result in minimal increases in blood pressure; however, blood pressure is routinely measured and, thus, closely monitored during their hospital admission. For groups A and B, in case of hospitalisation for <30 days, the participant will be instructed to contact his/her general practitioner at day 30 for blood pressure measurements and re-evaluation of antihypertensive therapy. At discharge, for all participants in groups A and B, the site investigator will also inform the general practitioner of trial participation (via an electronic discharge letter) and at day 30, site investigator will contact each participant to remind them to contact their general practitioner for blood pressure measurement, re-evaluation and recommencement of antihypertensive therapy. Groups C and D will be closely monitored with weekly visits, and a possible rise in blood pressure or other side effects can be quickly addressed.

For groups A and B, discontinuation of RAS inhibition therapy is not expected to improve or worsen the prognosis of patients with COVID-19; therefore, neither the assignment to the continuation nor the discontinuation group can clearly be labelled disadvantageous for the participant. Considering the scope of the COVID-19 pandemic and the number of patients on RAS inhibition treatment, the possible therapeutic insights obtained in this trial may be of profound importance and will hopefully aid healthcare systems in managing COVID-19. Given the precautions made to ensure the safety of all participants, the potential therapeutic benefits of this trial outweigh the relatively small risk of temporarily discontinued use of ACEi or ARBs in the test participants during their participation in the trial.

Recruitment and informed consent

For groups A and B, participants will be recruited among COVID-19-positive patients admitted to a COVID-19 clinic in the Capital Region of Copenhagen. On admission of patients treated with ACEi and ARBs, site investigators with an employment at the COVID-19 clinic in question will screen the admitted patients for eligibility according to the inclusion and exclusion criteria. A potential participant will be approached during the first days of hospital admission by the site investigator, who will present verbal and written information regarding the trial (see online supplemental appendix 3), and the patient will be invited to participate. The patient will be offered 24 hours for consideration of participation in the trial. If the patient decides to participate, detailed information will be given, and written consent obtained before any protocol-related actions are initiated (see online supplemental appendices 4 and 5). The participants in groups C and D will be recruited through contact to general practitioners and through advertising. A screening visit will be set up over the phone. At this visit, the participant will be screened for eligibility and the patient will, in an undisturbed environment, receive written and verbal information about the trial. If the potential participant decides to participate, written consent will be obtained before any protocol-related actions are initiated. For all grups, the written consent form will include the option of allowing

the investigators to contact the participant again 1 year after randomisation in order to evaluate their medical condition at that point in time. This is completely voluntary and not a part of the trial nor a requirement for inclusion in the trial. Participants will have the opportunity to withdraw at any time.

Participant confidentiality

Participants will sign a consent form that allows investigators to access hospital records for scientific purposes in order to assess known risk factors for COVID-19 and clinical outcomes during the hospital admission. The signed consent further includes disclosure of health data and other confidential information as part of authorities' control with the trial, as a legal requirement to secure correct completion of the trial. Prior to the written consent, clinical information necessary for identification of eligible patients including verification of COVID-19 will be obtained by site investigators who are employed at the COVID-19 clinic and thus have legal access to this information. The site investigator therefore uses information given by the patients during hospital admission for research purposes. Participant confidentiality is extended to cover any trial information relating to participants. No information concerning the trial or data will be released to any unauthorised third party and will be held in strict confidence. Trial records will be maintained for at least 5 years from the completion date.

Remuneration for trial participants

The trial is not planned for the benefit of the individual participant. Participants in groups A and B will not receive any remuneration for participation.

The participants in groups C and D will receive 500 Danish kroner per trial visit as remuneration for their time and to cover transportation.

Assessment of adverse events

There will be daily assessment of the occurrence of serious adverse events with and without a causal relationship to the allocated intervention while the participant is enrolled in the trial and admitted to one of the trial sites. All serious adverse events occurring in the four groups will be registered and included in the final report.

The Danish Patient Compensation Association

This project is carried out at Herlev-Gentofte Hospital and Hvidovre Hospital; thus, all the participants will be covered by The Danish Patient Compensation Association in the event of a personal injury.

Protocol modification

In case of protocol modifications, new approvals will be obtained from all relevant authorities.

Dissemination

The results of this project will be compiled into one or more manuscripts for publication in international peer-reviewed scientific journals. Positive as well as negative and inconclusive results will all be published, in accordance with the law concerning processing of personal data. Coauthors must fulfil criteria for co-authorship according to the International Committee of Medical Journal Editors.

Author affiliations
[1]Center for Clinical Metabolic Research, Department of Medicine, Copenhagen University Hospital - Herlev and Gentofte, Hellerup, Denmark
[2]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[3]Section of Infectious Medicine, Department of Medicine, Copenhagen University Hospital - Herlev and Gentofte, Hellerup, Denmark
[4]Clinical Research, Steno Diabetes Center Copenhagen, Herlev, Denmark
[5]Gubra Aps, Hoersholm, Denmark
[6]Section of Respiratory Medicine, Department of Medicine, Copenhagen University Hospital - Herlev and Gentofte, Hellerup, Denmark
[7]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[8]Department of Clinical Pharmacology, Bispebjerg and Frederiksberg Hospital, University of Copenhagen, Faculty of Health and Medical Sciences, Copenhagen, Denmark
[9]Copenhagen Center for Translational Research, Copenhagen University Hospital - Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark
[10]Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty og Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Twitter Lærke Smidt Gasbjerg @GasbjergL, Anne-Marie Ellegaard @amellegaard, Pradeesh Sivapalan @prasiv, Jens-Ulrik Stæhr Jensen @DoctorJensUlrik, Tina Vilsbøll @TinaVilsb, Mikkel Bring Christensen @drmikkelc and Filip Krag Knop @Filip_Knop

Contributors LSG, CAH, MBL, DSM, TV, VK-H, A-ME, CL, PS, MBC, J-UJ and FKK designed the trial and wrote the trial protocol. VK-H and HJNL will collect the data. VK-H will perform the data analysis and write the primary publication. All authors will critically review the manuscript and approve the final version.

Funding This work was supported by an unrestricted research grant from the Novo Nordisk Foundation (grant number NNF20SA0062873) to Center for Clinical Metabolic Research, Department of Medicine, Copenhagen University Hospital—Herlev and Gentofte, Copenhagen, Denmark to cover remuneration of staff and running costs of the project. The investigators will not receive any personal financial reimbursement from conducting the trial. The funding body has no role in the design of the trial and collection, analysis or interpretation of data or in writing the manuscript.

Competing interests None declared.

Patient and public involvement Relevant patients were involved in designing the participants information. No patients and/or the public will be involved in the reporting or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This trial has been approved by the Scientific-Ethical Committee of the Capital Region of Denmark (identification no. H-20026484), the Danish Medicines Agency (identification no. 2020040883) and by the Danish Data Protection Agency (P-2020-–366).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement The data that support the findings of this study are available from the corresponding author, VK-H, upon reasonable request.

ORCID iDs
Vivian Kliim-Hansen http://orcid.org/0000-0001-5455-930X
Filip Krag Knop http://orcid.org/0000-0002-2495-5034

REFERENCES

1 Giulia F. *Report sulle caratteristiche dei Pazienti deceduti positivi a COVID-19 in Italia IL presente report è basato sui dati aggiornati al 17 Marzo 2020*. Istitvto Svperiore Di Sanitá, 2020.
2 Guan W-J, Ni Z-Y, Hu Y, *et al*. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020;382:1708–20.
3 Zhou F, Yu T, Du R, *et al*. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020;395:1054–62.
4 Zhang H, Penninger JM, Li Y, *et al*. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med* 2020;46:586–90.
5 Hamming I, Timens W, Bulthuis MLC, *et al*. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004;203:631–7.
6 Xie X, Xudong X, Chen J, *et al*. Age- and gender-related difference of ACE2 expression in rat lung. *Life Sci* 2006;78:2499–71.
7 Treml B, Neu N, Kleinsasser A, *et al*. Recombinant angiotensin-converting enzyme 2 improves pulmonary blood flow and oxygenation in lipopolysaccharide-induced lung injury in piglets. *Crit Care Med* 2010;38:596–601.
8 Imai Y, Kuba K, Rao S, *et al*. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 2005;436:112–6.
9 Furuhashi M, Moniwa N, Mita T, *et al*. Urinary angiotensin-converting enzyme 2 in hypertensive patients may be increased by olmesartan, an angiotensin II receptor blocker. *Am J Hypertens* 2015;28:15–21.
10 Vuille-dit-Bille RN, Camargo SM, Emmenegger L, *et al*. Human intestine luminal ACE2 and amino acid transporter expression increased by ACE-inhibitors. *Amino Acids* 2015;47:693–705.
11 Ocaranza MP, Godoy I, Jalil JE, *et al*. Enalapril attenuates downregulation of angiotensin-converting enzyme 2 in the late phase of ventricular dysfunction in myocardial infarcted rat. *Hypertension* 2006;48:572–8.
12 Kuba K, Imai Y, Rao S, *et al*. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med* 2005;11:875–9.
13 Sun ML, Yang JM, Sun YP, *et al*. [Inhibitors of RAS Might Be a Good Choice for the Therapy of COVID-19 Pneumonia]. *Zhonghua Jie He He Hu Xi Za Zhi* 2020;43:219–22.
14 Bozkurt B, Kovacs R, Harrington B. Joint HFSA/ACC/AHA statement addresses concerns re: using RaaS antagonists in COVID-19. *J Card Fail* 2020;26:370.
15 Murthy VL, Koupenova M, Shah RV. ACEing COVID-19: a role for angiotensin axis inhibition in SARS-CoV-2 infection? *Circ Res* 2020;126:1682–4.
16 Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir Med* 2020;8:e21.
17 Cohen JB, Hanff TC, William P, *et al*. Continuation versus discontinuation of renin-angiotensin system inhibitors in patients admitted to hospital with COVID-19: a prospective, randomised, open-label trial. *Lancet Respir Med* 2021;9:275–84.
18 Lopes RD, Macedo AVS, de Barros E Silva PGM, *et al*. Effect of discontinuing vs continuing angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers on days alive and out of the hospital in patients admitted with COVID-19: a randomized clinical trial. *JAMA* 2021;325:254–64.
19 Sparks MA, Hiremath S, South A, *et al*. The coronavirus conundrum: ACE2 and hypertension edition. *NephJC*2020.
20 Askin L, Tanrıverdi O, Askin HS. The effect of coronavirus disease 2019 on cardiovascular diseases. *Arq Bras Cardiol* 2020;114:817–22.
21 Úri K, Fagyas M, Mányiné Siket I, *et al*. New perspectives in the renin-angiotensin-aldosterone system (RaaS) IV: circulating ACE2 as a biomarker of systolic dysfunction in human hypertension and heart failure. *PLoS One* 2014;9:e87845.
22 COVID-19_Treatment_Trial_Design_Master_Protocol_synopsis_Final_18022020.pdf. Available: https://www.who.int/blueprint/priority-diseases/key-action/COVID-19_Treatment_Trial_Design_Master_Protocol_synopsis_Final_18022020.pdf [Accessed 17 Aug 2020].
23 European Medicines Agency. *Guideline for good clinical practice E6(R2)*, 2016.