Protocol

Renin-angiotensin-aldosterone system inhibitors and adverse outcomes of COVID-19: a Danish nationwide cohort study

Indhold

1. Milestones

Milestone	Date
Start of data collection	28 April 2020 (including patients from 27 February 2020)
End of data collection	26 May 2020 (or latest available before final report/submission of manuscript)
Final report of study results/submission of manuscript	1 June 2020

2. Background

Case series of hospitalized patients with severe and fatal coronavirus disease 2019 (COVID-19) from China,[1-3] Italy,[4] and the USA[5,6] have reported a high prevalence (~30%–40%) of hypertension, cardiovascular conditions, and diabetes – conditions often treated with angiotensin converting enzyme inhibitors (ACE-I) and angiotensin II receptor blockers (ARB).[7] Speculations suggest that use of ACE-I/ARB may increase the risk of developing severe or fatal COVID-19 by upregulating expression of the ACE2 enzyme, which is known to facilitate entry of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) into cells.[8-12] In contrast, ACE-I/ARB use has also been suggested to reduce the risk of acute lung injury in respiratory tract infection in animal studies.[13] This is supported by a few human studies, which found ACE-I and ARB use to be associated with decreased mortality from bacterial pneumonia,[14-16] while preadmission ACE-I use has been associated with increased mortality in patients hospitalized for viral diseases.[17] A recent Chinese study of 362 patients with hypertension found no increased prevalence of ACE-I/ARB use when comparing survivors with non-survivors of COVID-19.[18] Another Chinese study included 1,128 patients with COVID-19 and found a lower mortality in ACE-I/ARB users compared with non-users. Both studies were limited by potential immortal time bias and confounding by indication as they relied on data on in-hospital use of ACE-I/ARB.[18,19]

The European Medicines Agency, and other major institutions and societies, have called for further research and issued warnings against ACE-I/ARB discontinuation in patients with COVID-19,[8,20] as drug discontinuation may worsen underlying cardiometabolic conditions.[21] As ACE-I/ARB are widely used drugs, any association with risk and prognosis may have public health impact. Thus, there is an urgent need to clarify the hypothesis of any increased risk for COVID-19 or worsened prognosis for adverse outcomes of COVID-19 among users of ACE-I/ARB using high-quality population-based data from a uniform tax-supported health care system, while meticulously controlling for potential confounding by indication from coexisting diseases and by using active antihypertensive drug comparators in the analysis.

3. Objectives

The primary aim of the study is to examine the association between ACE-I/ARB use and risk of death in patients with COVID-19. The secondary aim is to examine the association of ACE-I/ARB use with risk of hospital admission, ICU admission, mechanical ventilation, and renal replacement therapy. The third aim is to examine the risk of being diagnosed with COVID-19 among all patients referred to SARS-CoV-2 testing.

4. Methods

4.1. Study design

This nationwide study will include all patients tested for SARS-CoV-2 in Denmark within the source population of all Danish inhabitants.[22] The impact of ACE-I/ARB on risk of COVID-19 will be examined using a test-negative case-control design, while the prognosis will be examined in a cohort design of test-positive COVID-19 patients.

4.2. Study period

The study will include patients tested from 27 February 2020 (the date of first identified COVID-19 case in Denmark) to 28 April 2020 (or up to 30 days before manuscript submission) to allow complete follow-up on all included patients.

4.3. Data sources

The study will include nationwide secondary data from The Danish COVID-19 cohort, which includes linked individual-level data on all Danish inhabitants tested for SARS-CoV-2 from The Danish Microbiology Database (MIBA),[23] The Danish National Registry of Patients (DNRP),[24] The Danish Civil Registration System (CRS),[25] The National Prescription database (NPD),[26] and The Danish Cause of Death Registry.[27]

4.4. Study population

The source population is all Danish citizens (popuation~5.8 million).[22] The study population for risk analysis (the test-negative case-control analysis) will be all patients tested for SARS-CoV-2, while the prognosis analysis (cohort analysis) will only include patients tested positive SARS-CoV-2, *i.e.*, patients with COVID-19.

4.5. Follow-up

Patients will be followed from the date of the first RT-PCR test for SARS-CoV-2 that turns out to be positive and up to 30 days after that date for all the outcomes. In a sensitivity analysis follow-up for hospitalization will be restricted to 14 days after first positive test.

4.6. Exposure

The study is designed to address acute effects of current use of ACE-I/ARB, and we do not expect any cumulated effect of long-term ACE-I/ARB use. The half-life of ACE-I/ARB ranges from 1 to 24 hours.

The exposure will be defined by filled prescriptions for ACE-I/ARB and other antihypertensive drugs within 90 days before test for SARS-CoV-2. Data on prescriptions are obtained from the Danish Prescription Registry, which include complete and valid information on all filled prescriptions at community pharmacies in Denmark since 1995.[26] Data include, among others, date of dispensing, Anatomical Therapeutic Chemical (ATC) code, and drug quantity.

The main exposures will be:

- Current use (prescription filled within prior 90 days before test) of ACE-I/ARBs, compared with
    - Current use (prescription within 90 days) of calcium channel blockers (CCB), a cardiovascular drug used for similar medical indications as ACE-I and ARB, to assess if any observed association with outcomes is specific to ACE-I/ARB exposure.

- No use of ACE-I or ARB (prescription >365 days or never)

Secondary exposures will be:

- Former versus no use of ACE-I or ARB (former use defined as prescription 91-365 days before test)
- Current ACE-I/ARB use compared with:
  - Current use of thiazides (prescription within 90 days)
  - Current use of beta-blockers (prescription within 90 days)

As an additional analysis, ACE-I and ARB will be analyzed separately if the number of exposed are sufficient.

4.7. Outcomes

The primary outcome is death within 30 days after positive test for SARS-CoV-2, obtained from the Danish Register of Causes of Deaths.[27]

Secondary outcomes include hospital admission (in- or acute outpatient stay lasting > 12 hours) at day of or within 30 days after a positive test in patients not already admitted before the date of the test. In addition, outcomes include ICU admission, mechanical ventilation, and renal replacement therapy at day of or within 30 days after positive test for SARS-CoV-2. Secondary outcomes will be obtained from the Danish National Registry, 3rd version (LPR3).[24] While the coding has not yet been validated in this LPR3, admission to ICU, mechanical ventilation, and renal replacement therapy are all registered accurately (positive predictive values 96%-100%) in LPR2 as this is also the platform for mandatory reporting to the national database for quality of intensive care.[28,29]

Finally, in the risk analysis, we identified patients with positive tests among all patients tested for SARS-CoV-2.

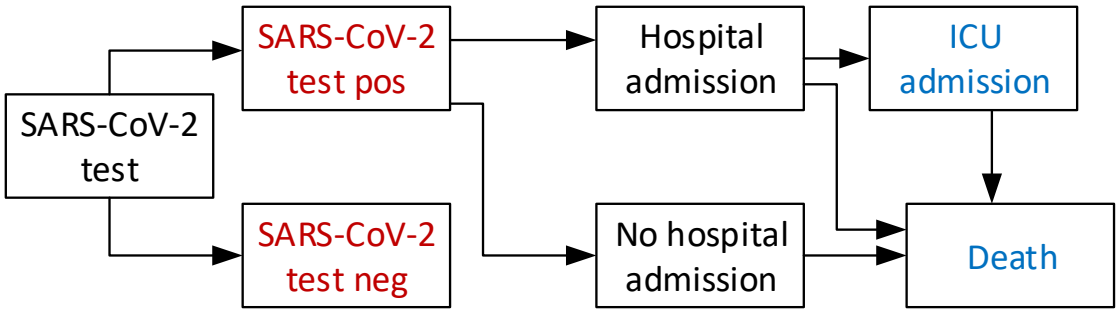


Figure illustrating the endpoints in the COVID-19 risk analysis and the endpoints in the COVID-19 prognosis analysis.

4.8. Bias

4.8.1. Confounding

Confounding will be handled by using propensity score (PS) methods. In addition, the use of an active comparator design reduce confounding by indication, as most users of the comparator are also likely to have hypertension as the indication for treatment. To further reduce confounding by indication, we will repeat the analyses after restriction to patients without history of heart or kidney disease, which are other indications for ACE-I/ARB use. Although we included a large number of coexisting conditions, life-style factors and co-medication, we cannot exclude the impact of unmeasured confounding, e.g., by severity of condition indication ACE-I/ARB treatment. As we lacked detailed data on severity of heart and renal diseases, we reduced confounding in a subgroup analysis of patients without any history of these diseases.

4.8.1.1. Propensity score methods

The PS is the probability of being exposed given the covariate pattern for the patient. The PS will be estimated for each patient hospitalization in each of the examined comparisons using a logistic regression model including calendar time and the covariates listed in the Appendix. As we are interested in the average treatment effect in the treated (ATT), we apply PS-weighting using ATT weights to make the number and covariate distribution in the comparison groups resemble that of the ACE-I/ARB users.

Overlap in PS distributions will be checked before weighting. Covariate balance will be assessed by standardized differences (SDs), empirical cumulative distribution functions for continuous variables (age and index), and by PS-weight percentiles.

4.8.2. Selection bias

The risk of selection bias is reduced by including all tested Danish inhabitants. Still, criteria for testing and hospital admission have changed during the study period, which will be addressed in a sensitivity analysis stratified by calendar time. We do not expect that ACE-I/ARB users would be more likely to be tested, and thereby included, as compared to users of other antihypertensive drugs.

4.8.3. Information bias

There may be some misclassification of the exposure, which will be addressed in a sensitivity analysis as described. Any misclassification is not expected to be associated with the outcomes of interest (non-differential misclassification), and any information bias are therefore expected to be towards the null.

As outcomes are accurately recorded, any misclassification is expected to be minor and not associated with the exposure.

4.9. Statistical analyses

In the analyses of prognosis, patients will be followed from day of SARS-CoV-2 positive test result and until date of hospital admission, ICU admission (in analysis of ICU admission), date of death, end of follow-up or for up to 30 days.

Patient characteristics will be tabulated according to each exposure group.

Descriptive analyses will explore the timing of outcomes one month prior to and three months after the first positive test for SARS-CoV-2. In case of a substantial fraction of outcomes having occurred in the days leading up to a positive test, a post-hoc analysis with a larger follow up windows (pre- and post-test) will be conducted.

For each exposure group, we will estimate the 30-day PS-weighted cumulative incidence (risk) of of death, and of each of the non-fatal events (hospital admission, ICU admission, ICU+MV, ICU+inotropes/vasopressors, RRT) accounting for competing risk by death. All 95% confidence intervals will be computed using bootstrapping. The risk of each outcome within 30 days will be computed with 95% CI.

Propensity scores for each comparison (e.g. current ACE-I/ARB use vs. current CCB use) will be computed using logistic regression including all the potential confounders as described above. All other exposures (anti-hypertensive drugs) will be included in the PS-model. The propensity score weighted (SMRW=ATT) cumulative incidence (risk) of each outcome will be plotted and estimated with 95% CI estimated by bootstrapping. The adjusted (PS weighted) risk difference will be computed for the 0-30 period.

The 30-day risk will be compared using PS weighted log binomial regression to estimate risk ratio for death, hospital admission, ICU admission, ICU+MV, RRT, and ICU+inotropes/vasopressors.

Subgroup analysis will be conducted to address effect modifiers including age group (=<65, >65 years) and in patients with assumed uncomplicated hypertension as the primary indication for treatment, defined as patients without any history of diabetes, renal disease, angina pectoris, myocardial infarction, or heart failure. We will also stratify by ABO blood type, if available, because blood type is suggested to be associated with ACE2.[30]

The risk of COVID-19 (a positive test for SARS-CoV-2) will be analyzed using a test-negative case-control design among all patients tested. All covariates will be tabulated for patients with a positive test and negative test for SARS-CoV-2. The odds ratio for current ACE-I/ARB use vs. non-use and CCB use, respectively, will be estimated using two separate logistic regression models adjusted for all covariates, see Appendix.

In a sensitivity analysis, we will explore the robustness of our findings by repeating the analyses in patients who filled a prescription within 120 days before a positive test.

Finally, we will do a sensitivity analysis stratified by calendar time to address changes in testing and hospitalization strategy.

4.10. Sample size considerations

As of 20 April 2020, there are 96,224 individuals tested for SARS-CoV-2 and 7,515 are tested positive. A total of 364 patients with a positive test have died (4.8%). The proportion of patients using ACE-I/ARB is expected to be 17% and the proportion using CCB is expected to be 7% based on data from a historical cohort of patients with influenza or pneumonia (Christiansen CF et al., article submitted).

Assuming a 30-day mortality of 4% in unexposed, we will have a power of 92% for detecting a RR of 1.2 with an alpha-level of 5% in the comparison of current ACE-I/ARB users with non-users. (EpiSheet).

5. Ethical/data protection issues

According to Danish law, registry-based studies do not require informed consent or ethical committee approval. Only aggregated data will be published.

All data are pseudonymized and stored at secured serves at the Danish Health Data Authority. Data management and analyses are performed through secure remote access to these servers. Data management and analyses will be conducted and checked by experienced researchers.

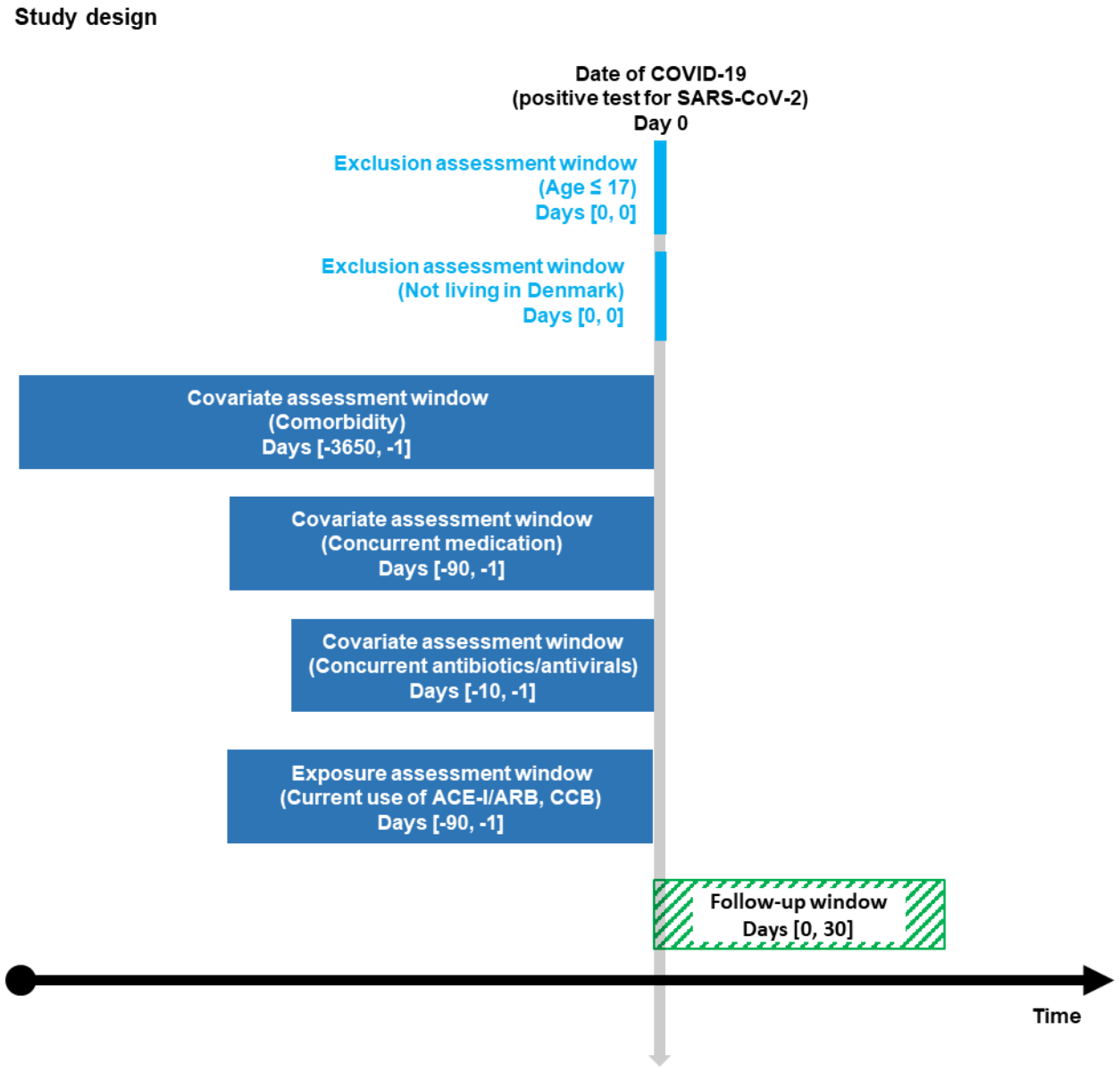
6. Plans for communication of study results

The study protocol will be registered in the EU PAS registry. Results will be communicated through international peer-reviewed journals and the website of the Danish Medicines Agency. Any evidence of adverse effects of ACE-I/ARB will be communicated to the Danish Medicines Agency.

7. Amendments and deviations

Any future amendments or deviations from the study protocol will be included in this section.

Figure. Study design diagram



Codes

	Source/ coding	Look-back	Inclusion codes	Exclusion codes
Exposures (ATC codes)				
Number of antihypertensives	NPD/ATC	90d	0-5 (see below: ACE-I/ARB, CCB, Thiazides/diuretics, BB, other antihypertensives)	
ACE-I	NPD/ATC	90d	C09A, C09B	
ARB	NPD/ATC	90d	C09C, C09D	
Calcium channel blockers (CCB)	NPD/ATC	90d	C07FB, C08CA, C09BB, C09DB, C09DX01	
Thiazides/diuretics	NPD/ATC	90d	C03AA01, C03AA03, C03AB01, C03AB03, C03AX01, C03EA, C07B, C09BA, C09DA, C09DX01, C09DX03, C09XA52, C09XA54	C07BA06 C09BA04
Beta-blockers (BB)	NPD/ATC	90d	C07	
Other antihypertensives	NPD/ATC	90d	C02AC, C02CA, C03BA11, C09XA C02AB01	C09XA54
Outcomes				
ICU admission (procedure code)	DNPR/Procedure		NABE, NABB	
Mechanical ventilation (procedure code)	DNPR/Procedure		BGDA0	
Acute renal replacement therapy (RRT) (in patients without chronic RRT)	DNPR/Procedure		BJFD0	BJFD2 (any codes within prior 12 months)
Treatment with inotropes/vasopressors	DNPR/Procedure		BFHC92A, BFHC92B, BFHC92C, BFHC92D, BFHC92E, BFHC93A, BFHC93B, BFHC93C, BFHC95	
Covariates				
Age	CPR			
Sex	CPR			
Calendar time (before / after lockdown)	DNPR			
Hospital-diagnosed hypertension	DNPR/ICD-10	10y	I10, I15	
Stable angina pectoris (without procedures)	DNPR/ICD-10	10y	I20, I251, I259	I21, I22, I23, I200
Stable angina pectoris or CABG/PCI procedures	DNPR/ICD-10	10y	I20, I251, I259	I21, I22, I23, I200
	DNPR/Procedure	10y	KFNA, KFNB, KFNC, KFND, KFNE, KFNF, KFNG, KFNH20	I21, I22, I23, I200 (any diagnosis during hospital contact with the procedure

				or within 10 years before)
Myocardial infarction	DNPR/ICD-10	10y	I21, I22, I23	
Heart failure	DNPR/ICD-10	10y	I50	
Stroke	DNPR/ICD-10	10y	I60, I61, I63, I64	
Atrial fibrillation/flutter	DNPR/ICD-10	10y	I48	
Heart valve disease	DNPR/ICD-10	10y	I05, I06, I07, I08, I09.8, I34-I37, I39, I51.1A, Q22, Q23	
Venous thromboembolism	DNPR/ICD-10	10y	I26, I801, I802, I803	
Diabetes	DNPR/ICD-10	10y	E10, E11, E12, E13, E14, O24, G63.2, H360, N083	O24.4
	NPD/ATC	10y	A10A, A10B	
Chronic pulmonary disease	DNPR/ICD-10	10y	J40, J41-J44, J45−J47, J60−J67, J68.4, J70.1, J70.3, J84.1, J92.0, J96.1, J98.2, J98.3	
Markers of smoking (diagnoses or medications for tobacco smoking or chronic obstructive pulmonary disease)	DNPR/ICD-10	10y	J41-J44, F17, Z716, Z720	
	NPD/ATC	10y	R03, N07BA	
Obesity diagnoses or medications	DNPR/ICD-10	10y	E66	
	NPD/ATC	10y	A08	
Alcoholism-related diagnoses or medication for alcohol deterrent	DNPR/ICD-10	10y	DF10, DE244, DG312, DG621, DG721, DI426, DK292, DK70, DK852, DK860, DQ860, DZ502, DZ714, DZ721	F100
	NPD/ATC	10y	V03AA, N07BB	
Kidney disease diagnosis	DNPR/ICD-10	10y	I12, I13, N00−N05, N07, N11, N14, N18−N19, Q61, N08, E102, E112, E142	
End-stage renal disease (kidney transplant or dialysis)	DNPR/Procedure	10y	BJFD2	
	DNPR/Surgery	10y	KKAS	
	DNPR/ICD-10	10y	T861, Z940	
Liver disease	DNPR/ICD-10	10y	B18, B150, B160, B162, B190, I85, K70, K71, K72, K73, K74, K760, K76.6	
Dementia	DNPR/ICD-10	10y	DF00, DF01, DF02, DF03, DG30, DG310B, DG311, DG318, DG319	
	NPD/ATC	10y	N06D	
Cancer (including metastatic cancer)	DNPR/ICD-10	10y	C00−C96, D459, D46, D471, D473, D474, D475, CxxxM	C44
Concurrent antihypertensive medication (see exposures)	NPD/ATC			

Statins	NPD/ATC	90d	C10AA, C10B
Aspirin	NPD/ATC	90d	B01AC06, N02BA01, N02BA51
Opioid use	NPD/ATC	90d	N02A, N07BC02
Immunosuppressant use	NPD/ATC	90d	L04
Glucocorticoids	NPD/ATC	90d	H02AB
Loop diuretics	NPD/ATC	90d	C03C
Antibiotics	NPD/ATC	10d	J01
Antivirals	NPD/ATC	10d	J05
Vitamin K antagonists	NPD/ATC	90d	B01AA
Antidepressants	NPD/ATC	90d	N06A
Antipsychotics	NPD/ATC	90d	N05A
Hypnotics/sedatives	NPD/ATC	90d	N05C
Marital status	CPR		
Rural/urban place of residence	CPR		

Abbreviations: ATC Anatomical Therapeutic Chemical Classification System; CPR Danish Civil Reigistration System; DNPR Danish National Patient Registry; ICD-10 International Classification of Diseases 10th edition; NPD National Prescription Database.

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8. ENCePP Checklist for Study Protocols (Revision 4)

Adopted by the ENCePP Steering Group on 15/10/2018

The European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) welcomes innovative designs and new methods of research. This Checklist has been developed by ENCePP to stimulate consideration of important principles when designing and writing a pharmacoepidemiological or pharmacovigilance study protocol. The Checklist is intended to promote the quality of such studies, not their uniformity. The user is also referred to the ENCePP Guide on Methodological Standards in Pharmacoepidemiology, which reviews and gives direct electronic access to guidance for research in pharmacoepidemiology and pharmacovigilance.

For each question of the Checklist, the investigator should indicate whether or not it has been addressed in the study protocol. If the answer is "Yes", the section number of the protocol where this issue has been discussed should be specified. It is possible that some questions do not apply to a particular study (for example, in the case of an innovative study design). In this case, the answer 'N/A' (Not Applicable) can be checked and the "Comments" field included for each section should be used to explain why. The "Comments" field can also be used to elaborate on a "No" answer.

This Checklist should be included as an Annex by marketing authorisation holders when submitting the protocol of a non-interventional post-authorisation safety study (PASS) to a regulatory authority (see the Guidance on the format and content of the protocol of non-interventional post-authorisation safety studies). The Checklist is a supporting document and does not replace the format of the protocol for PASS presented in the Guidance and Module VIII of the Good pharmacovigilance practices (GVP).

Study title: Renin-angiotensin-aldosterone system inhibitors and adverse outcomes of COVID-19: a Danish nationwide cohort study

EU PAS Register® number:
Study reference number (if applicable):

Section 1: Milestones	**Yes**	**No**	**N/A**	**Section Number**
1.1 Does the protocol specify timelines for				
1.1.1 Start of data collection[1]	☒	☐	☐	1
1.1.2 End of data collection[2]	☒	☐	☐	1
1.1.3 Progress report(s)	☐	☐	☒	

[1] Date from which information on the first study is first recorded in the study dataset or, in the case of secondary use of data, the date from which data extraction starts.
[2] Date from which the analytical dataset is completely available.

Section 1: Milestones	**Yes**	**No**	**N/A**	**Section Number**
1.1.4 Interim report(s)	☐	☐	☒	
1.1.5 Registration in the EU PAS Register®	☒	☐	☐	1
1.1.6 Final report of study results.	☒	☐	☐	1

Comments:

Section 2: Research question	**Yes**	**No**	**N/A**	**Section Number**
2.1 Does the formulation of the research question and objectives clearly explain:	☐	☐	☐	
2.1.1 Why the study is conducted? (e.g. to address an important public health concern, a risk identified in the risk management plan, an emerging safety issue)	☒	☐	☐	2
2.1.2 The objective(s) of the study?	☒	☐	☐	3
2.1.3 The target population? (i.e. population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	2
2.1.4 Which hypothesis(-es) is (are) to be tested?	☒	☐	☐	2
2.1.5 If applicable, that there is no *a priori* hypothesis?	☐	☐	☒	

Comments:

Section 3: Study design	**Yes**	**No**	**N/A**	**Section Number**
3.1 Is the study design described? (e.g. cohort, case-control, cross-sectional, other design)	☒	☐	☐	4.1
3.2 Does the protocol specify whether the study is based on primary, secondary or combined data collection?	☒	☐	☐	4.3
3.3 Does the protocol specify measures of occurrence? (e.g., rate, risk, prevalence)	☒	☐	☐	4.9
3.4 Does the protocol specify measure(s) of association? (e.g. risk, odds ratio, excess risk, rate ratio, hazard ratio, risk/rate difference, number needed to harm (NNH))	☒	☐	☐	4.9
3.5 Does the protocol describe the approach for the collection and reporting of adverse events/adverse reactions? (e.g. adverse events that will not be collected in case of primary data collection)	☒	☐	☐	7

Comments:

Section 4: Source and study populations		**Yes**	**No**	**N/A**	**Section Number**
4.1	Is the source population described?	☒	☐	☐	4.4
4.2	Is the planned study population defined in terms of:				
	4.2.1 Study time period	☒	☐	☐	4.2
	4.2.2 Age and sex	☒	☐	☐	4.1 (all)
	4.2.3 Country of origin	☒	☐	☐	4.1
	4.2.4 Disease/indication	☒	☐	☐	4.4
	4.2.5 Duration of follow-up	☒	☐	☐	4.5
4.3	Does the protocol define how the study population will be sampled from the source population? (e.g. event or inclusion/exclusion criteria)	☒	☐	☐	4.4

Comments:

Section 5: Exposure definition and measurement		**Yes**	**No**	**N/A**	**Section Number**
5.1	Does the protocol describe how the study exposure is defined and measured? (e.g. operational details for defining and categorising exposure, measurement of dose and duration of drug exposure)	☒	☐	☐	4.6
5.2	Does the protocol address the validity of the exposure measurement? (e.g. precision, accuracy, use of validation sub-study)	☒	☐	☐	4.6
5.3	Is exposure categorised according to time windows?	☒	☐	☐	4.6
5.4	Is intensity of exposure addressed? (e.g. dose, duration)	☒	☐	☐	4.6
5.5	Is exposure categorised based on biological mechanism of action and taking into account the pharmacokinetics and pharmacodynamics of the drug?	☒	☐	☐	4.6
5.6	Is (are) (an) appropriate comparator(s) identified?	☒	☐	☐	4.6

Comments:

Section 6: Outcome definition and measurement	**Yes**	**No**	**N/A**	**Section Number**
6.1 Does the protocol specify the primary and secondary (if applicable) outcome(s) to be investigated?	☒	☐	☐	4.7
6.2 Does the protocol describe how the outcomes are defined and measured?	☒	☐	☐	4.7
6.3 Does the protocol address the validity of outcome measurement? (e.g. precision, accuracy, sensitivity, specificity, positive predictive value, use of validation sub-study)	☒	☐	☐	4.7
6.4 Does the protocol describe specific outcomes relevant for Health Technology Assessment? (e.g. HRQoL, QALYs, DALYS, health care services utilisation, burden of disease or treatment, compliance, disease management)	☐	☐	☒	

Comments:

Section 7: Bias	**Yes**	**No**	**N/A**	**Section Number**
7.1 Does the protocol address ways to measure confounding? (e.g. confounding by indication)	☒	☐	☐	4.8.1
7.2 Does the protocol address selection bias? (e.g. healthy user/adherer bias)	☒	☐	☐	4.8.2
7.3 Does the protocol address information bias? (e.g. misclassification of exposure and outcomes, time-related bias)	☒	☐	☐	4.8.3

Comments:

Section 8: Effect measure modification	**Yes**	**No**	**N/A**	**Section Number**
8.1 Does the protocol address effect modifiers? (e.g. collection of data on known effect modifiers, sub-group analyses, anticipated direction of effect)	☒	☐	☐	4.9

Comments:

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.1 Does the protocol describe the data source(s) used in the study for the ascertainment of:				

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.1.1 Exposure? (e.g. pharmacy dispensing, general practice prescribing, claims data, self-report, face-to-face interview)	☒	☐	☐	4.6
9.1.2 Outcomes? (e.g. clinical records, laboratory markers or values, claims data, self-report, patient interview including scales and questionnaires, vital statistics)	☒	☐	☐	4.7
9.1.3 Covariates and other characteristics?	☒	☐	☐	4.8.1, Appendix
9.2 Does the protocol describe the information available from the data source(s) on:				
9.2.1 Exposure? (e.g. date of dispensing, drug quantity, dose, number of days of supply prescription, daily dosage, prescriber)	☒	☐	☐	4.6
9.2.2 Outcomes? (e.g. date of occurrence, multiple event, severity measures related to event)	☒	☐	☐	4.7
9.2.3 Covariates and other characteristics? (e.g. age, sex, clinical and drug use history, co-morbidity, co-medications, lifestyle)	☒	☐	☐	4.8.1, Appendix
9.3 Is a coding system described for:				
9.3.1 Exposure? (e.g. WHO Drug Dictionary, Anatomical Therapeutic Chemical (ATC) Classification System)	☒	☐	☐	4.6
9.3.2 Outcomes? (e.g. International Classification of Diseases (ICD), Medical Dictionary for Regulatory Activities (MedDRA))	☒	☐	☐	4.7
9.3.3 Covariates and other characteristics?	☒	☐	☐	Appendix
9.4 Is a linkage method between data sources described? (e.g. based on a unique identifier or other)	☒	☐	☐	4.3

Comments:

Section 10: Analysis plan	**Yes**	**No**	**N/A**	**Section Number**
10.1 Are the statistical methods and the reason for their choice described?	☒	☐	☐	4.8.1.1, 4.9
10.2 Is study size and/or statistical precision estimated?	☒	☐	☐	4.10
10.3 Are descriptive analyses included?	☒	☐	☐	4.9
10.4 Are stratified analyses included?	☒	☐	☐	4.9
10.5 Does the plan describe methods for analytic control of confounding?	☒	☐	☐	4.8.1, 4.9
10.6 Does the plan describe methods for analytic control of outcome misclassification?	☐	☒	☐	

Section 10: Analysis plan	**Yes**	**No**	**N/A**	**Section Number**
10.7 Does the plan describe methods for handling missing data?	☐	☒	☐	
10.8 Are relevant sensitivity analyses described?	☒	☐	☐	4.9

Comments:

Section 11: Data management and quality control	**Yes**	**No**	**N/A**	**Section Number**
11.1 Does the protocol provide information on data storage? (e.g. software and IT environment, database maintenance and anti-fraud protection, archiving)	☒	☐	☐	5
11.2 Are methods of quality assurance described?	☐	☐	☐	
11.3 Is there a system in place for independent review of study results?	☐	☐	☐	

Comments:

Section 12: Limitations	**Yes**	**No**	**N/A**	**Section Number**
12.1 Does the protocol discuss the impact on the study results of:				
12.1.1 Selection bias?	☒	☐	☐	4.8.2
12.1.2 Information bias?	☒	☐	☐	4.8.3
12.1.3 Residual/unmeasured confounding? (e.g. anticipated direction and magnitude of such biases, validation sub-study, use of validation and external data, analytical methods).	☒	☐	☐	4.8.1
12.2 Does the protocol discuss study feasibility? (e.g. study size, anticipated exposure uptake, duration of follow-up in a cohort study, patient recruitment, precision of the estimates)	☒	☐	☐	4.10

Comments:

Section 13: Ethical/data protection issues	**Yes**	**No**	**N/A**	**Section Number**
13.1 Have requirements of Ethics Committee/ Institutional Review Board been described?	☒	☐	☐	5
13.2 Has any outcome of an ethical review procedure been addressed?	☐	☐	☒	

Section 13: Ethical/data protection issues	**Yes**	**No**	**N/A**	**Section Number**
13.3 Have data protection requirements been described?	☒	☐	☐	5

Comments:

Section 14: Amendments and deviations	**Yes**	**No**	**N/A**	**Section Number**
14.1 Does the protocol include a section to document amendments and deviations?	☒	☐	☐	7

Comments:

Section 15: Plans for communication of study results	**Yes**	**No**	**N/A**	**Section Number**
15.1 Are plans described for communicating study results (e.g. to regulatory authorities)?	☒	☐	☐	6
15.2 Are plans described for disseminating study results externally, including publication?	☒	☐	☐	6

Comments:

Name of the main author of the protocol: Christian Fynbo Christiansen

Date: dd/Month/year 24/April/2020

Signature: CFC