Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Mancia G, Rea F, Ludergnani M, Apolone G, Corrao G. Renin–angiotensin–aldosterone system blockers and the risk of Covid-19. N Engl J Med. DOI: 10.1056/NEJMoa2006923

Table of Contents

Original protocol

Summary

Title	Blockers of the renin-angiotensin system and the risk of Covid-19 infection
Study objectives	Primary objective: is to evaluate the association between use of angiotensin receptor antagonists (ARBs) and angiotensin converting enzyme inhibitors (ACEIs), and the risk of Covid-19 infection Secondary objectives: - to assess the association between use of ARBs and ACEIs, and the severity of Covid-19 infection - to assess the above-mentioned associations in men and women - to assess the above-mentioned associations in younger and older patients
Study type	Observational
Observation model	Population-based case-control
Number of participants	Approximately 7,000 cases and 30,000 controls
Location	Lombardy Region (Italy)
Inclusion criteria	Cases: - Beneficiaries of the Regional Health Service - Age ≥ 40 years - Diagnosis of Covid-19 infection from 21st February until 11th March 2020 Controls: - Beneficiaries of the Regional Health Service - Age ≥ 40 years
Exclusion criteria	Controls: - Diagnosis of Covid-19 infection from 21st February until 11th March 2020
Sampling method	Density incidence approach will be used for selecting controls
Primary endpoint	Odds ratios for association between use of ARBs and ACEs and Covid-19 infection
Secondary endpoints	- Odds ratios for association between use of ARBs and ACEIs and critical/fatal Covid-19 infection - Odds ratios for association between use of ARBs and ACEIs and Covid-19 infection in men and women - Odds ratios for association between use of ARBs and ACEIs and Covid-19 infection in younger and older patients (< 60 years and ≥ 60 years old respectively)

Title
Blockers of the renin-angiotensin system and the risk of Covid-19 infection

Background

Animal studies have shown that the angiotensin converting enzyme 2 (ACE2), a membrane- bound aminopeptidase that is abundantly expressed in the lungs, the heart, as well as in other tissues [1], is utilized by coronaviruses as a functional receptor for their entrance into the cells [2-4]. This has generated the hypothesis that drugs commonly used for the treatment of hypertension, heart failure, renal disease and post-myocardial infarction states such as angiotensin receptor antagonists (ARBs) and ACE inhibitors (ACEIs) modify the susceptibility, severity and mortality rate of the current human infections with the Covid-19 because of their well-known association with an overexpression of ACE2, more consistent and marked for ARBs than for ACEIs [5-7]. This modification has been interpreted as to lead to an increased risk of infection due to a drug-induced increase in the ACE2 substrate, i.e. angiotensin II [8]. It has also been suggested, however, that, on the contrary, blockers of the renin-angiotensin system might have a protective effect [9] because 1) the increased angiotensin II might compete with the virus at the receptor level; and 2) ACE2 converts angiotensin II into inactive compounds [1] and may directly attenuate tissue injury [10], making its overexpression mechanistically favorable. Unfortunately, published clinical data are largely limited to general demographic and clinical characteristics of the patients, with no focused information on the type of antihypertensive treatment they were prescribed at or close to the time of the Covid-19 infection [11,12]. This means that no evidence is available on whether blockers of the renin-angiotensin system modify the human response to the Covid-19 infection, as well as whether the modification might have an adverse or a protective sign [13]. This represents a serious problem because the possibility that blockers of the renin-angiotensin system may have a relationship with the susceptibility to the Covid-19 infection has had a worldwide large echo in the media, as a result of which many patients have stopped their use [14,15]. Blockers of the renin-angiotensin system are widely used life-saving drugs for cardiovascular and renal disease, and there is strong evidence that treatment withdrawal is associated with a marked increase in related morbidity and mortality risk [16-18].

Study objectives

The main goal of this population-based case-control study is to evaluate the association between use of ARBs and ACEIs, and the risk of Covid-19 infection.

Secondary objectives are: (i) to evaluate the associations between use of ARBs and ACEIs, and the severity of Covid-19 infection, (ii) to assess the association of interest in men and women, and (iii) to assess the association of interest in younger and older patients.

Study Design
A population-based case-control study will be carried out in Lombardy Region (Italy).

Inclusion criteria (cases):
- Beneficiaries of the Regional Health Service
- Age ≥ 40 years
- Diagnosis of Covid-19 infection from 21st February until 11th March 2020

Diagnosis of Covid-19 infection. Since February 21st 2020, individual data on patients who had a confirmed diagnosis of Covid-19 infection were signaled to the Regional Health Authority from several sources. The latter included: (i) public and private hospitals (inpatients, and among these, those who required assisted ventilation), (ii) General Practitioners (outpatients receiving domiciliary care), and (iii) municipal registries (deaths due to Covid-19 infection). Laboratories accredited from the Regional Health Authority finally gave a fundamental contribution to the active reporting system by signaling of patients underwent to diagnostic procedure for Covid-19. The diagnostic algorithm is based on the protocol released by the World Health Organization (WHO), i.e., on nasopharyngeal swab specimens tested by real time polymerase chain reaction for SARS-Cov-2 RNA within three hours. Laboratory confirmation of the virus is performed using real time reverse transcription polymerase chain reaction. After performing formal inspection of individual signaling, Regional Health Authority carries out their record linkage, and provides official data on day-by-day new cases and deaths. The process centralization ensures homogeneity of signaling and uniformity of diagnostic testing along the entire period of observation. The date of Covid-19 diagnosis will be considered as the index date and patients will be extracted from the registry until March 11, 2020.
Patients who required assisted ventilation or died during intensive care will be classified as having a critical/fatal infection, the remaining being regarded as affected from mild clinical manifestations of the infection.

Inclusion criteria (controls):
- Beneficiaries of the Regional Health Service
- Age ≥ 40 years

Exclusion criteria (controls):
- Diagnosis of Covid-19 infection from 21st February until 11th March 2020

Matching: For each case, up to five controls will be randomly selected from the target population to be matched for gender, age at index date and municipality of residence. Density incidence approach will be used for selecting controls

Exposure of interest
Use of ACEIs and ARBs dispensed during 2019.

Covariates
Previous hospitalizations for cardiovascular disease, cancer, respiratory and kidney disease suffered by cases and controls will be traced from the regional databases. In addition, cases and controls will be categorized according to the Chronic Related Score (CReSc), a new index of patients' clinical profile derived from inpatient and outpatient services provided by the Regional Health Service and validated for outcome prediction [19].
Major blood-pressure lowering agents dispensed to cases and controls during 2019 (calcium channel blockers, diuretics, and β-blockers) will be traced from the healthcare utilization databases along with the dispensation of a variety of other drugs: lipid-lowering agents (mainly statins), oral antidiabetic, insulin, antiplatelet, antiarrhythmic, anticoagulant, digital, nitrates, inhaled corticosteroids, immunosuppressant agents (i.e., glucocorticoids, calcineurin inhibitors, antiproliferative agents and monoclonal antibodies), short-acting and long-acting β-agonists (SABA/LABA) and other agents used for chronic respiratory diseases.

Outcome Measure

Primary Outcome Measure: Odds ratios for association between use of ARBs and ACEs and Covid-19 infection

Secondary Outcome Measures:

1. Odds ratios for association between use of ARBs and ACEs and critical/fatal Covid-19 infection
2. Odds ratios for association between use of ARBs and ACEs and Covid-19 infection in men and women
3. Odds ratios for association between use of ARBs and ACEs and Covid-19 infection in younger and older patients (< 60 years and ≥ 60 years old, respectively)

Final protocol

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Study objectives

The main goal of this population-based case-control study is to evaluate the association between use of ARBs and ACEIs, and the risk of Covid-19 infection.

Secondary objectives are: (i) to evaluate the associations between use of ARBs and ACEIs, and the severity of Covid-19 infection, (ii) to assess the association of interest in men and women, and (iii) to assess the association of interest in younger and older patients.

Study Design
A population-based case-control study will be carried out in Lombardy Region (Italy).

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Patients who required assisted ventilation or died during intensive care will be classified as having a critical/fatal infection, the remaining being regarded as affected from mild clinical manifestations of the infection.

Inclusion criteria (controls):
- Beneficiaries of the Regional Health Service
- Age ≥ 40 years

Exclusion criteria (controls):
- Diagnosis of Covid-19 infection from 21st February until 11th March 2020

Matching: For each case, up to five controls will be randomly selected from the target population to be matched for gender, age at index date and municipality of residence. Density incidence approach will be used for selecting controls

Exposure of interest
Use of ACEIs and ARBs dispensed during 2019.

Covariates
Previous hospitalizations for cardiovascular disease (including coronary artery disease, percutaneous coronary intervention and heart failure), cancer, respiratory (including chronic obstructive pulmonary disease and asthma) and kidney disease (including chronic kidney disease and dialysis) suffered by cases and controls will be traced from the regional databases. In addition, cases and controls will be categorized according to the Chronic Related Score (CReSc), a new index of patients' clinical profile derived from inpatient and outpatient services provided by the Regional Health Service and validated for outcome prediction [19].
Major blood-pressure lowering agents dispensed to cases and controls during 2019 (calcium channel blockers, diuretics (including subtypes, i.e., thiazide/thiazide-like, loop, mineralocorticoid receptor antagonists), and β-blockers) will be traced from the healthcare utilization databases. Dispensation of drugs operating through the renin-angiotensin system will be extended to renin inhibitors and sacubitril/valsartan. We will also trace blood pressure lowering drugs dispensed as monotherapy and combination therapies, those with a renin-angiotensin system blocker being the vast majority. Other drug treatments dispensed during 2019 will include lipid-lowering agents (mainly statins), oral antidiabetic (as a whole and the more commonly dispensed classes), insulin, antiplatelet, antiarrhythmic, anticoagulant, digital, nitrates, inhaled corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), immunosuppressant agents (i.e., glucocorticoids, calcineurin inhibitors, antiproliferative agents and monoclonal antibodies), short-acting and long-acting β-agonists (SABA/LABA) and other agents used for chronic respiratory diseases.

Outcome Measure

Primary Outcome Measure: Odds ratios for association between use of ARBs and ACEs and Covid-19 infection

Secondary Outcome Measures:

4. Odds ratios for association between use of ARBs and ACEs and critical/fatal Covid-19 infection
5. Odds ratios for association between use of ARBs and ACEs and Covid-19 infection in men and women
6. Odds ratios for association between use of ARBs and ACEs and Covid-19 infection in younger and older patients (< 60 years and ≥ 60 years old, respectively)

Summary of changes

A better clinical characterization of patients included in the study was considered in the revised protocol. Indeed, several other comorbidities and co-treatments were included:

- Cardiovascular disease: coronary artery disease, percutaneous coronary intervention, heart failure
- Respiratory diseases: chronic obstructive pulmonary disease, asthma
- Kidney disease: chronic kidney disease, dialysis
- Diuretics: thiazide/thiazide-like, loop, mineralocorticoid receptor antagonists
- Oral antidiabetic drugs: metformin, sulfonylureas, DPP-4 inhibitors, GLP-1 agonists, SGLT2 inhibitors, thiazolidinediones, other oral antidiabetic agents
- Non-steroidal anti-inflammatory drugs: non-selective COX inhibitors, selective COX2 inhibitors

Original statistical analysis plan

Primary analysis

Conditional logistic regression models will be fitted for estimating odds ratio (OR), and corresponding 95% confidence interval (CI), of the risk of Covid-19 infection associated with exposure to each individual blood-pressure lowering drug. Adjustments will be made for the aforementioned baseline covariates.

Secondary analyses

Analyses will be restricted to strata having a critical/fatal infection. Stratifications for gender and age categories (<60 years, ≥61 years), will be performed as secondary analyses.

Sensitivity analyses

Two further analyses will be performed to verify the robustness of the findings. First, to minimize the inconvenience that exposure to antihypertensive medicaments will not be available after December 2019, data will be analyzed according to three criteria i.e. (i) any prescriptions during 2019, (ii) at least three consecutive prescriptions during 2019, (iii) at least one prescription in the last quarter of 2019, assuming that the two latter criteria might identify more reliably an unchanged treatment. Second, because the strategy used for SARS-CoV-2 RT-PCR testing changed, i.e., on 26 February the Italian National Institute of Health recommended that swabbing would only be performed on symptomatic patients, rather than to all suspected Covid-19 cases as done in the initial phase, analyses will be stratified according to the date of Covid-19 diagnosis (before or after 26 February).

Final statistical analysis plan

Primary analysis

Conditional logistic regression models will be fitted for estimating odds ratio (OR), and corresponding 95% confidence interval (CI), of the risk of Covid-19 infection associated with exposure to each individual blood-pressure lowering drug. Adjustments will be made for the aforementioned baseline covariates.

Secondary analyses

Analyses will be restricted to strata having a critical/fatal infection. Stratifications for gender and age categories (<60 years, ≥61 years), will be performed as secondary analyses.

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Summary of changes

No change.

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