ISAC APPLICATION FORM

PROTOCOLS FOR RESEARCH USING THE CLINICAL PRACTICE RESEARCH DATALINK (CPRD)

For ISAC use only		
Protocol No. Submission date (DD/MM/YYYY)	....	***IMPORTANT*** *Please refer to the **guidance** for '**Completing the ISAC application form'** found on the CPRD website (www.cprd.com/isac). If you have any queries, please contact the ISAC Secretariat at isac@cprd.com.*

SECTION A: GENERAL INFORMATION ABOUT THE PROPOSED RESEARCH STUDY

1. Study Title[§] (*Please state the study title below)*

The effect of renin-angiotensin system inhibitors on haemoglobin

[§]Please note: This information will be published on the CPRD's website as part of its transparency policy.

2. Has any part of this research proposal or a related proposal been previously submitted to ISAC?

Yes * ☐ No ☒

**If yes, please provide the previous protocol number/s below. Please also state in your current submission how this/these are related or relevant to this study.*

N/A

3. Has this protocol been peer reviewed by another Committee? (e.g. grant award or ethics committee)

Yes* ☐ No ☒

**If Yes, please state the name of the reviewing Committee(s) below and provide an outline of the review process and outcome as an Appendix to this protocol :* N/A

4. Type of Study (please tick all the relevant boxes which apply)

Adverse Drug Reaction/Drug Safety	☒	Drug Effectiveness	☐
Drug Utilisation	☐	Pharmacoeconomics	☐
Disease Epidemiology	☒	Post-authorisation Safety	☐
Health care resource utilisation	☐	Methodological Research	☐
Health/Public Health Services Research	☐	Other*	☐

**If Other, please specify the type of study here and in the lay summary below:*

5. Health Outcomes to be Measured[§]

[§]Please note: This information will be published on CPRD's website as part of its transparency policy.

Please summarise below the primary/secondary health outcomes to be measured in this research protocol:

- Change in haemoglobin level (primary outcome)
- Bone marrow suppression (secondary outcome)
-
-
-
-
-
-

[Please add more bullet points as necessary]

6. Publication: This study is intended for (please tick all the relevant boxes which apply)**:**

Publication in peer-reviewed journals	☒	Presentation at scientific conference	☒
Presentation at company/institutional meetings	☒	Regulatory purposes	☐
Other*	☐		

**If Other, please provide further information:* N/A

SECTION B: INFORMATION ON INVESTIGATORS AND COLLABORATORS

7. Chief Investigator§

Please state the full name, job title, organisation name & e-mail address for correspondence - see guidance notes for eligibility. Please note that there can only be one Chief Investigator per protocol.

Laurie Tomlinson, Associate Professor, London School of Hygiene & Tropical Medicine
laurie.tomlinson@lshtm.ac.uk

§*Please note: The name and organisation of the Chief Investigator and will be published on CPRD's website as part of its transparency policy*

CV has been previously submitted to ISAC	☒	**CV number:** 271_15CESL
A new CV is being submitted with this protocol	☐	
An updated CV is being submitted with this protocol	☐	

8. Affiliation of Chief Investigator (full address)

Department of Non-Communicable Disease Epidemiology
London School of Hygiene & Tropical Medicine
Keppel Street
London
WC1E 7HT

9. Corresponding Applicant§

Please state the full name, affiliation(s) and e-mail address below:

Kathryn Mansfield, London School of Hygiene & Tropical Medicine
kathryn.mansfield@lshtm.ac.uk

§*Please note: The name and organisation of the corresponding applicant and their organisation name will be published on CPRD's website as part of its transparency policy*

Same as chief investigator	☐	
CV has been previously submitted to ISAC	☒	**CV number:** 319_15S
A new CV is being submitted with this protocol	☐	
An updated CV is being submitted with this protocol	☐	

10. List of all investigators/collaborators§

Please list the full name, affiliation(s) and e-mail address* of all collaborators, other than the Chief Investigator below:

§*Please note: The name of all investigators and their organisations/institutions will be published on CPRD's website as part of its transparency policy*

Other investigator: Kathryn Mansfield, LSHTM, kathryn.mansfield@lshtm.ac.uk

CV has been previously submitted to ISAC	☒	**CV number:** 319_15S
A new CV is being submitted with this protocol	☐	
An updated CV is being submitted with this protocol	☐	

Other investigator: Dorothea Nitsch, LSHTM, dorothea.nitsch@lshtm.ac.uk

CV has been previously submitted to ISAC	☒	**CV number:** 321_15CESL
A new CV is being submitted with this protocol	☐	
An updated CV is being submitted with this protocol	☐	

Other investigator: Liam Smeeth, LSHTM, liam.smeeth@lshtm.ac.uk
CV has been previously submitted to ISAC ☒ **CV number:** 045_15CEPSL
A new CV is being submitted with this protocol ☐
An updated CV is being submitted with this protocol ☐

Other investigator: Masao Iwagami, LSHTM, masao.iwagami@lshtm.ac.uk
CV has been previously submitted to ISAC ☒ **CV number:** 318_15S
A new CV is being submitted with this protocol ☐
An updated CV is being submitted with this protocol ☐

Other investigator: Rosalynd Johnston, Royal Sussex County Hospital, Rosalynd.Johnston@bsuh.nhs.uk
CV has been previously submitted to ISAC ☐ **CV number:**
A new CV is being submitted with this protocol ☒
An updated CV is being submitted with this protocol ☐

[Please add more investigators as necessary]

Please note that your ISAC application form and protocol **must be copied to all e-mail addresses listed above at the time of submission of your application to the ISAC mailbox. Failure to do so will result in delays in the processing of your application.*

11. Conflict of interest statement*

Please provide a draft of the conflict (or competing) of interest (COI) statement that you intend to include in any publication which might result from this work

The authors declare no conflict of interest for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; and no other relationships or activities that could appear to have influenced the submitted work.

**Please refer to the International Committee of Medical Journal Editors (ICMJE) for guidance on what constitutes a COI.*

12. Experience/expertise available

Please complete the following questions to indicate the experience/ expertise available within the team of investigators/collaborators actively involved in the proposed research, including the analysis of data and interpretation of results.

	Previous GPRD/CPRD Studies	**Publications using GPRD/CPRD data**
None	☐	☐
1-3	☐	☐
> 3	☒	☒

Experience/Expertise available	**Yes**	**No**
Is statistical expertise available within the research team? *If yes, please indicate the name(s) of the relevant investigator(s)* Dorothea Nitsch	☒	☐
Is experience of handling large data sets (>1 million records) available within the research team? *If yes, please indicate the name(s) of the relevant investigator(s)* Kathryn Mansfield, Laurie Tomlinson, Dorothea Nitsch, Liam Smeeth, Masao Iwagami	☒	☐
Is experience of practising in UK primary care available to or within the research team? *If yes, please indicate the name(s) of the relevant investigator(s)* Liam Smeeth	☒	☐

13. References relating to your study

Please list up to 3 references (most relevant) relating to your proposed study:

1. Cheungpasitporn W, Thongprayoon C, Chiasakul T, Korpaisarn S, Erickson SB. Renin-angiotensin system inhibitors linked to anemia: A systematic review and meta-analysis. QJM 2015; 108: 879–84.

2. Mikolasevic I, Zaputovic L, Zibar L, et al. Renin-angiotensin-aldosterone system inhibitors lower hemoglobin and

hematocrit only in renal transplant recipients with initially higher levels. Eur J Intern Med 2016; 29: 98–103.

3. Ajmal A, Gessert CE, Johnson BP, Renier CM, Palcher JA. Effect of angiotensin converting enzyme inhibitors and angiotensin receptor blockers on hemoglobin levels. BMC Res Notes 2013; 6: 443.

SECTION C: ACCESS TO THE DATA

14. Financial Sponsor of study[§]

[§]*Please note: The name of the source of funding will be published on CPRD's website as part of its transparency policy*

Pharmaceutical Industry	☐	*Please specify name and country:*
Academia	☐	*Please specify name and country:*
Government / NHS	☐	*Please specify name and country:*
Charity	☒	*Please specify name and country:* Wellcome Trust
Other	☐	*Please specify name and country:*
None	☐	

15. Type of Institution conducting the research

Pharmaceutical Industry	☐	*Please specify name and country:*
Academia	☒	*Please specify name and country:* LSHTM, UK
Government Department	☐	*Please specify name and country:*
Research Service Provider	☐	*Please specify name and country:*
NHS	☐	*Please specify name and country:*
Other	☐	*Please specify name and country:*

16. Data access arrangements

The financial sponsor/ collaborator* has a licence for CPRD GOLD and will extract the data ☐
The institution carrying out the analysis has a licence for CPRD GOLD and will extract the data** ☒
A data set will be provided by the CPRD[¥€] ☐
CPRD has been commissioned to extract the data and perform the analyses[€] ☐
Other: ☐
If Other, please specify: N/A

**Collaborators supplying data for this study must be named on the protocol as co-applicants.*
***If data sources other than CPRD GOLD are required, these will be supplied by CPRD*
[¥]*Please note that datasets provided by CPRD are limited in size; applicants should contact CPRD (enquiries@cprd.com) if a dataset of >300,000 patients is required.*
[€]*Investigators must discuss their request with a member of the CPRD Research team before submitting an ISAC application. Please contact the CPRD Research Team on +44 (20) 3080 6383 or email (enquiries@cprd.com) to discuss your requirements. Please also state the name of CPRD Research team with whom you have discussed this request (provide the date of discussion and any relevant reference information):*

Name of CPRD Researcher N/A Reference number (where available) N/A Date of contact N/A

17. Primary care data

Please specify which primary care data set(s) are required)
Vision only (Default for CPRD studies ☒ Both Vision and EMIS®* ☐
EMIS® only* ☐

Note: Vision and EMIS are different practice management systems. CPRD has traditionally collected data from Vision practice. Data collected from EMIS is currently under evaluation prior to wider release.
Investigators requiring the use of EMIS data **must discuss the study with a member of the CPRD Research team before submitting an ISAC application*

Please state the name of the CPRD Researcher with whom you have discussed your request for EMIS data:
Name of CPRD Researcher N/A Reference number (where available) N/A Date of contact N/A

SECTION D: INFORMATION ON DATA LINKAGES

18. Does this protocol seek access to linked data

Yes* ☒ No☐ If No, please move to section E.

Research groups which have not previously accessed CPRD linked data resources **must discuss access to these resources with a member of the CPRD Research team, before submitting an ISAC application. Investigators requiring access to HES Accident and Emergency data, HES Diagnostic Imaging Dataset PROMS data and the Pregnancy Register **must** also discuss this with a member of the CPRD Research team before submitting an ISAC application. Please contact the CPRD Research Team on +44 (20) 3080 6383 or email enquiries@cprd.com to discuss your requirements **before** submitting your application.*

Please state the name of the CPRD Researcher with whom you have discussed your linkage request.

Name of CPRD Researcher Tarita Murray-Thomas Reference number (where available) CPRD00010290 Date of contact 18th July 2017

Please note that as part of the ISAC review of linkages, your protocol may be shared - in confidence - with a representative of the requested linked data set(s) and summary details may be shared - in confidence - with the Confidentiality Advisory Group of the Health Research Authority.

19. Please select the source(s) of linked data being requested§

§Please note: This information will be published on the CPRD's website as part of its transparency policy.

- ☐ ONS Death Registration Data
- ☒ HES Admitted Patient Care
- ☒ HES Outpatient
- ☐ HES Accident and Emergency
- ☐ HES Diagnostic Imaging Dataset
- ☐ MINAP (Myocardial Ischaemia National Audit Project)
- ☐ Cancer Registration Data*
- ☐ PROMS (Patient Reported Outcomes Measure)**
- ☐ CPRD Mother Baby Link
- ☐ Pregnancy Register

- ☐ Practice Level Index of Multiple Deprivation (Standard)
- ☐ Practice Level Index of Multiple Deprivation (Bespoke)
- ☐ Patient Level Index of Multiple Deprivation***
- ☐ Patient Level Townsend Score ***
- ☐ Other**** *Please specify:*

**Applicants seeking access to cancer registration data must complete a Cancer Dataset Agreement form (available from CPRD). This should be submitted to the ISAC as an appendix to your protocol. Please also note that applicants seeking access to cancer registry data must provide consent for publication of their study title and study institution on the UK Cancer Registry website.*
***Assessment of the quality of care delivered to NHS patients in England undergoing four procedures: hip replacement, knee replacement, groin hernia and varicose veins. Please note that patient level PROMS data are only accessible by academics*
**** 'Patient level IMD and Townsend scores will not be supplied for the same study*
*****If "Other" is specified, please provide the name of the individual in the CPRD Research team with whom this linkage has been discussed.*

Name of CPRD Researcher N/A Reference number (where available) N/A Date of contact N/A

20. Total number of linked datasets requested <u>including</u> CPRD GOLD

Number of linked datasets requested *(practice/'patient' level Index of Multiple Deprivation, Townsend Score, the CPRD Mother Baby Link and the Pregnancy Register should **not** be included in this count)* 3 (CPRD GOLD + HES APC + HES outpatient)

Please note: Where ≥5 linked datasets are requested, approval may be required from the Confidentiality Advisory Group (CAG) to access these data

21. Is linkage to a <u>local</u>¥ dataset with <1 million patients being requested?

Yes * ☐ No ☒

**If yes, please provide further details:* N/A

¥ *Data from defined geographical areas i.e. non-national datasets.*

22. If you have requested one or more linked data sets, please indicate whether the Chief Investigator or any of the collaborators listed in question 5 above, have access to these data in a patient identifiable form (e.g. full date of birth, NHS number, patient post code), or associated with an identifiable patient index.

Yes* ☐ No ☒

** If yes, please provide further details:* N/A

23. Does this study involve linking to patient *identifiable* data (e.g. hold date of birth, NHS number, patient post code) from other sources?

Yes ☐ No ☒

SECTION E: VALIDATION/VERIFICATION

24. Does this protocol describe a purely observational study using CPRD data?

Yes* ☒ No** ☐

** Yes: If you will be using data obtained from the CPRD Group, this study does not require separate ethics approval from an NHS Research Ethics Committee.*
*** No: You may need to seek separate ethics approval from an NHS Research Ethics Committee for this study. The ISAC will provide advice on whether this may be needed.*

25. Does this protocol involve requesting any additional information from GPs?

Yes* ☐ No ☒

** If yes, please indicate what will be required:*

Completion of questionnaires by the GP$_{\psi}$ Yes ☐ No ☐
Is the questionnaire a validated instrument? Yes ☐ No ☐
If yes, has permission been obtained to use the instrument? Yes ☐ No ☐
Please provide further information:

Other (please describe)

$_{\psi}$ Any questionnaire for completion by GPs or other health care professional must be approved by ISAC before circulation for completion.

26. Does this study require contact with patients in order for them to complete a questionnaire?

Yes* ☐ No ☒

**Please note that any questionnaire for completion by patients must be approved by ISAC before circulation for completion.*

27. Does this study require contact with patients in order to collect a sample?

Yes* ☐ No ☒

** Please state what will be collected:* N/A

SECTION F: DECLARATION

28. Signature from the Chief Investigator

- I have read the guidance on '***Completion of the ISAC application form***' and '***Contents of CPRD ISAC Research Protocols***' and have understood these;
- I have read the submitted version of this research protocol, including all supporting documents, and confirm that these are accurate.
- I am suitably qualified and experienced to perform and/or supervise the research study proposed.
- I agree to conduct or supervise the study described in accordance with the relevant, current protocol
- I agree to abide by all ethical, legal and scientific guidelines that relate to access and use of CPRD data for research
- I understand that the details provided in sections marked with (§) in the application form and protocol will be published on the

CPRD website in line with CPRD's transparency policy.

- I agree to inform the CPRD of the final outcome of the research study: publication, prolonged delay, completion or termination of the study.

Name: Laurie Tomlinson Date: 3rd Aug 2017 e-Signature (type name): Laurie Tomlinson

PROTOCOL INFORMATION REQUIRED

Applicants must complete all sections listed below
Sections which do not apply should be completed as '*Not Applicable*'

A. Study Title[§]

[§]*Please note: This information will be published on CPRD's website as part of its transparency policy*

The effect of renin-angiotensin system inhibitors on haemoglobin

B. Lay Summary (Max. 200 words)[§]

[§]*Please note: This information will be published on CPRD's website as part of its transparency policy*

Angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) are drugs that are commonly used to treat high blood pressure and heart failure. Haemoglobin is the molecule in red blood cells that carries oxygen from the lungs to the body. When there is not enough haemoglobin in the blood (anaemia) people experience symptoms like fatigue, weakness and shortness of breath. Anaemia can also worsen heart and kidney disease, and is associated with increased risk of death. There is some evidence to suggest that ACEI/ARB drugs cause anaemia. Our study therefore aims to investigate the effect of starting ACEI/ARB drugs on haemoglobin levels, using a comparison drug (calcium channel blockers). We will also investigate whether the effect of ACEI/ARB drugs on anaemia is influenced by either: the patient's haemoglobin level prior to starting the drug, or their kidney function (the kidneys produce a hormone that controls red blood cell production). This information would help improve prescribing guidelines for these drugs, offer insight into their risks and benefits, and help identify those needing careful haemoglobin monitoring when starting on ACEI/ARBs.

C. Technical Summary (Max. 200 words)[§]

[§]*Please note: This information will be published on CPRD's website as part of its transparency policy*

We aim to investigate the association between ACEI/ARBs and reduction in haemoglobin. We will identify a cohort of new-users of ACEI/ARBs and calcium channel blockers (CCB) with haemoglobin levels recorded both before (up to one year) and after (up to six months) drug initiation. We will calculate and compare mean haemoglobin change following ACEI/ARB or CCB initiation. We will use logistic regression to identify risk factors associated with a fall in haemoglobin after drug initiation. Risk factors considered will include: age, sex, ACEI/ARB or CCB use, chronic comorbidities (diabetes mellitus, cardiac failure, chronic kidney disease, hypertension, ischaemic heart disease, myeloproliferative syndromes, and chronic lung disease), pre-initiation haemoglobin level, past history of gastrointestinal bleeding, medications and lifestyle factors (smoking, alcohol intake, and body mass index). We will also: (1) Investigate whether baseline haemoglobin level and chronic kidney disease modify the effect of ACEI/ARBs on haemoglobin; (2) Compare change in haemoglobin between new users of ACEIs and ARBs by comparing mean change in haemoglobin and calculating the odds of a fall in haemoglobin in each group; and (3) Compare the proportion of ACEI, ARB and CCB users with records indicating post-initiation bone marrow suppression.

D. Objectives, Specific Aims and Rationale

The overall aim of the study is to investigate the association between ACEI/ARB initiation and subsequent reduction in haemoglobin. Specifically, we aim to:

1. In a group of new users of ACEI/ARB and CCBs, calculate the mean change in haemoglobin after drug initiation and compare the result between the two groups.
2. Compare the odds of a post-drug initiation reduction in haemoglobin of 1g/dL or more in those initiating ACEI/ARB with those initiating a CCB, and identify the risk factors associated with a reduction in haemoglobin following drug initiation. Risk factors considered will include: age, sex, ACEI/ARB or CCB use, chronic comorbidities (diabetes mellitus, cardiac failure, chronic kidney

Applicants must complete all sections listed below
Sections which do not apply should be completed as '*Not Applicable*'

disease, hypertension, ischaemic heart disease, myeloproliferative syndromes, and chronic lung disease), history of gastrointestinal bleeding, medications (including warfarin, new oral anticoagulants, aspirin, clopidogrel, tacrolimus, cyclosporin, azathioprine, methotrexate, ferrous sulphate, folic acid, and B12 injections), and lifestyle factors (smoking status, alcohol intake, and body mass index).

3. Investigate whether baseline haemoglobin or severe chronic kidney disease (CKD) (defined as estimated glomerular filtration rate [eGFR] <30 mL/min/1.73 m^2) modify the effect of ACEI/ARB initiation on haemoglobin reduction.
4. Compare the effect on haemoglobin of ACEIs compared to ARBs by calculating mean change in haemoglobin and estimating the odds ratio (adjusting for confounders) of a post-drug initiation reduction in haemoglobin of 1g/dL or more in those on ARBs compared to those on ACEIs.
5. Explore the relationship between ACEI/ARBs and bone marrow suppression (Read coded) by comparing the proportion of ACEI, ARB and CCB users with new-onset bone marrow suppression following drug initiation.

The rationale for comparing the mean change in haemoglobin following initiation of an ACEI/ARB to that following CCB initiation (**Objective 1**) is to quantify the size of any haemoglobin reduction. We aim to compare ACEI/ARB users to CCB users as these drugs are prescribed for similar indications (thereby limiting confounding by indication).

We aim to compare the odds of post-drug initiation reduction in haemoglobin of 1g/dL or more (chosen as a clinically meaningful reduction in haemoglobin) in those initiating each drug (**Objective 2**) in order to investigate the strength of the association while accounting for potential confounders. This will also allow us to identify risk factors for the association, which will offer insight into high-risk patients who may need close haemoglobin monitoring on initiating these drugs.

The effect of ACEI/ARBs may be modulated by haemoglobin level prior to initiating an ACEI/ARB; the mechanism through which ACEI/ARBs are thought to influence haemoglobin is by inhibition of the action of erythropoietin (a hormone produced by the kidney that stimulates red cell production). We would therefore anticipate that ACEI/ARBs will cause a greater reduction in haemoglobin for people with high baseline haemoglobin, where baseline erythropoietin levels are likely to be normal or high (however, we may also find the alternative, as those with a normal or high erythropoietin may have sufficient erythropoietin reserve to be relatively unaffected by the [potentially small] effect of ACEI/ARB). We therefore aim to investigate whether baseline haemoglobin modifies the effect of ACEI/ARBs (**Objective 3**) on post-drug initiation haemoglobin reduction. Similarly, we may expect to see a different effect for people where erythropoietin levels are reduced, as occurs in advanced chronic kidney disease (CKD). We will therefore investigate whether severe CKD (eGFR< 30 mL/min/1.73 m^2) modifies the effect of ACEI/ARBs on haemoglobin.

ACEIs and ARBs inhibit the renin-angiotensin system through different mechanisms, their impact on haemoglobin may therefore be different. A further secondary analysis will therefore be restricted to new ACEI/ARB users and compare the mean change in haemoglobin and the odds of anaemia in new ACEI users compared to new ARB users (**Objective 4**).

Bone marrow suppression (including neutropenia, agranulocytosis, aplastic anaemia, and thrombocytopenia) has been reported with ACEIs, to a lesser extent with ARBs, and rarely with CCBs.[1] We will therefore explore bone marrow suppression as a potential factor in ACEI/ARB related anaemia by exploring the proportion of patients prescribed each class of drug with records indicating bone marrow suppression following drug initiation (**Objective 5**).

Applicants must complete all sections listed below
Sections which do not apply should be completed as '*Not Applicable*'

E. Study Background

Angiotensin converting enzyme inhibitors and angiotensin receptor blocking (ACEI/ARB) drugs are frequently used in the management of hypertension,[2] heart failure,[3] diabetic microalbuminuria, other proteinuric kidney diseases,[4] and after myocardial infarction.[5] There is evidence to suggest that these drugs increase the risk of anaemia.[6]

Anaemia is a reduction in haemoglobin, the protein in red blood cells that carries oxygen. Anaemia is common in patients with a number of chronic comorbidities including heart failure,[7] chronic kidney disease,[8] and chronic inflammatory conditions.[9] It causes symptoms including fatigue, weakness and shortness of breath and is associated with increased morbidity and mortality.[7,10–14] A possible mechanism for the relationship between ACEI/ARBs and anaemia is through inhibition of the production of erythropoietin (a hormone that stimulates the production of red blood cells).[15]

A meta-analysis of seven studies has estimated that ACEI/ARB users have approximately 1.6 times the risk of anaemia than non-users.[6] However, with the exception of one study (which made a comparison between ACEI and ARB rather than comparing to non-users), the studies included in the meta-analysis were in select populations defined by cardiac or renal disease. A number of the included studies were also limited due to restricted adjustment for confounding, and small sample size. However, there is also conflicting evidence suggesting that there is either no association between ACEI/ARBs and anaemia,[13,16] or an association with an ACEI but not an ARB[17] or vice versa (with an ARB but not an ACEI).[18]

We therefore aim to explore the relationship between ACEI/ARBs and anaemia by investigating the relationship between changes in haemoglobin following drug initiation in a large group of new users of these drugs with no restrictions to specific disease groups.

F. Study Type

Hypothesis testing

This study will test the hypotheses that:

1. ACEI/ARB drugs reduce haemoglobin.
2. The effect of ACEI/ARBs on haemoglobin is modified by pre-existing haemoglobin level and kidney function.
3. ARBs are more strongly associated with reductions in haemoglobin than ACEIs.

G. Study Design

We will use a population-based **cohort study** to compare post drug initiation changes in haemoglobin in ACEI/ARB users to those in CCB users.

H. Feasibility counts

A feasibility count from January 2017 CPRD GOLD indicates that between January 2004 and December 2016 there were approximately 578,160 new users of ACEI/ARB, and 464,985 new users of CCBs, who were at least 18 years of age and had at least 12 months of registration at their first prescription. Of the new ACEI/ARB users, approximately 40% (n=230,621) also had a new CCB prescription during the study period, while approximately 50% of the new CCB users had a new ACEI/ARB prescription.

I. Sample size considerations

It is unlikely that all the individuals identified by the feasibility count will be eligible for inclusion in the study; not all will meet CPRD quality control standards, and, more importantly, it is likely that many will not have both pre- and post-drug initiation haemoglobin measures (needed to establish the outcome of a post-initiation reduction in haemoglobin). In a previous study[19] 47% of new ACEI/ARB users had both

Applicants must complete all sections listed below
Sections which do not apply should be completed as '*Not Applicable*'

pre- and post-initiation serum creatinine measures (Pre: recorded up to 12 months before ACEI/ARB initiation; Post: recorded up to 2 months after initiation) available in CPRD. We might therefore expect a similar proportion of ACEI/ARB initiators to have haemoglobin monitoring available (renal function and full blood counts are often tested simultaneously in clinical practice), however, since haemoglobin monitoring on ACEI/ARB initiation is not included in standard guidelines[2] it is unlikely that we will see the proportions for new ACEI/ARB users with both pre- and post-initiation haemoglobin measures as high as 47%. These figures are likely to be lower again in CCB users where there is no recommended blood test monitoring on initiation. We have therefore assumed a conservative estimate of 20% of new ACE/ARB or CCB users with records of haemoglobin measures recorded at appropriate time points before and after drug initiation (Pre: recorded up to one year before ACEI/ARB initiation; Post: recorded up to six months after initiation). Using this cautious estimate of 20% of new-users with valid haemoglobin test results, with a figure of 230,000 new users of each class of drugs (230,000 chosen as the smallest number of new users of either class of drugs who were prescribed only an ACEI/ARB or a CCB during the study period; while we intend to identify exposure based on new prescription for each class of drugs regardless of any existing prescriptions [see **Section M**] we have used a cautious estimate of sample size here to allow for some patients not being eligible for inclusion) results in a cautious estimate of 46,000 individuals in each drug exposure group, which we have further rounded down to 40,000 to offer a conservative estimate of 80,000 eligible individuals in total included in the study population.

At this stage we cannot be sure how many individuals will experience a reduction in haemoglobin following ACEI/ARB/CCB initiation. Nor are we able to state with confidence what effect size we expect to detect. **Table 1** therefore gives estimates of power under a range of assumptions, based on a conservative 1:1 comparison between ACEI/ARB and CCB users. Under a range of different assumptions, we will have adequate power (90% or more) to detect a difference in the odds of a reduction in haemoglobin between ACEI/ARB and CCB users of 1.2 or more.

Table 1: Estimates of power to detect a range of possible odds ratios, under a range of assumptions for incidence of a fall in haemoglobin – assuming a conservative estimate of 80,000 new users of ACEI/ARB / CCBs.

	Incidence of a fall in haemoglobin*			
Odds ratio	1%	2%	4%	6%
1.1	28%	49%	77%	90%
1.2	76%	96%	>99%	>99%
1.3	98%	>99%	>99%	>99%
1.4	>99%	>99%	>99%	>99%

Calculations were done in Stata using the 'power twoproportions' command.
Assuming: i) Alpha=0.05; and ii) 1:1 comparison between ACEI/ARB and CCB users.
*We cannot be sure of the how many cases of post-initiation haemoglobin reduction will occur during ACEI/ARB or CCB exposure. However, previous studies have estimated incidence of anaemia in the elderly population at between 1 and 6%.[20] Since clinical reasoning and previous studies[21] suggest that the majority of antihypertensive users are over 60, it seems reasonable to think that we would see a similar level of anaemia in this population. Therefore, we have used a range of potential estimates of anaemia (from 1 to 6%) to represent the base rate of anaemia we might expect for a population of antihypertensive users and attributed this to the comparison population (i.e. CCB users). However, these are likely to be conservative estimates of outcome incidence given that our outcome is a fall in haemoglobin rather than a more strict diagnosis of anaemia (defined by WHO guidelines[22] as haemoglobin <12 g/dL in women or <13 g/dL in men) we would therefore expect to identify more cases of our outcome (fall in haemoglobin) than of incident anaemia, resulting in higher power to detect smaller effect sizes.

J. Data Linkage Required (if applicable):[§]

[§]*Please note that the data linkage/s requested in research protocols will be published by the CPRD as part of its transparency policy*

Hospital Episode Statistics – admitted patient care

We will use Hospital Episode Statistics (HES) in-patient data in sensitivity analyses to improve the completeness of chronic comorbidity diagnoses (used as covariates), and with an alternative outcome definition using primary care coding and hospital admissions recorded with codes for anaemia. In these

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sensitivity analyses we will restrict the study population to those eligible for HES linkage.

Hospital Episode Statistics – outpatient
We will use HES outpatient data in a sensitivity analysis (limited to those eligible for HES linkage) as part of an algorithm to identify individuals who may be managed with exogenous erythropoietin.

K. Study population

The source population for this study will be all patients aged 18 years or over, who meet both patient- and practice-level CPRD quality control standards, during the study period (January 2004 to December 2016 – the study will run from 2004 because CKD status is important in this study and serum creatinine testing was more frequent after 2004 in response to the introduction of the Quality and Outcomes framework. If more recent data becomes available during the lifespan of the project, we will extend the end date of the study to maximise sample size and follow up). From the source population we will select all patients who are new users of ACEI/ARBs or CCBs. To capture incident ACEI/ARB / CCB users we will exclude those without at least 12 months of up-to-standard registration prior to their first ACEI/ARB / CCB prescription. We will only include those new users of ACEI/ARB / CCB with haemoglobin levels recorded both before (i.e. within 12 months) and after (i.e. within six months – chosen because the lifespan of a red blood cell is approximately 115 days[23]) drug initiation.

To ensure that we have reliable measures of drug use and baseline covariates, we will check that all participants have at least one year of continuous registration in CPRD before entry into the study (i.e. at new ACEI/ARB / CCB prescription). Patients will be eligible to enter the cohort from the latest of: i) date practice reached CPRD quality control standards; ii) 18th birthday; or iii) one year after practice registration date (to ensure reliable measures of baseline health status). Patients will no longer be eligible for inclusion, and therefore censored, at the earliest of: i) date of death; ii) patient transferred out of practice; or ii) last data collection from practice.

L. Selection of comparison group(s) or controls

We will define comparison groups within the cohort according to exposure status (see **Sections M** and **N** below) – we will compare ACEI/ARB users to users of CCBs.

M. Exposures, Health Outcomes[§] and Covariates

[§]*Please note: Summary information on health outcomes (as included on the ISAC application form above) will be published on CPRD's website as part of its transparency policy*

Preliminary code lists for all variables are included in the Appendix (Excel workbook).

Exposures
Exposure to ACEI/ARB or CCBs will be defined using recorded prescribing information. We will calculate the duration of prescriptions using the quantity of medication prescribed and the daily dose recorded; when these data were not available we will assume the population practice-level mode prescription duration (From past experience of ACEI/ARB / CCB prescribing in CPRD, the majority of prescriptions last 30 days). We will assume exposure to medications starts on the date of the prescription. We will construct continuous courses of therapy by allowing for a 60-day gap between consecutive prescriptions (60 days or less between end of one prescription and start of the next) to allow for stock piling of drugs and non-adherence. We are interested in changes in haemoglobin on initiation of a drug, we will therefore determine exposure regardless of concomitant prescribing, for example if a patient with a new CCB prescription is already prescribed an ACEI/ARB, we will classify them as exposed to a CCB in relation to any change in haemoglobin in the six months after commencing the CCB. Patients may therefore appear up to two times in the analysis if they have a new prescription for both an ACEI/ARB and a CCB,

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separated by at least six months (interval used to assess the outcome), during the study period.

Outcomes

The main outcomes will be change in haemoglobin level and reduction in haemoglobin of 1g/dL or more. We will identify the most recent haemoglobin result recorded in the 12 months before and the day of ACEI/ARB / CCB initiation, and a post-initiation result recorded in the six months following drug initiation.

Where more than one measure of post-initiation haemoglobin has been recorded we will use the result closest to 115 days after drug initiation. This is the approximate life span of a red cell[23] and the period at which you would expect to see maximal change in haemoglobin attributable to the medication. Change in haemoglobin will be defined as the difference between pre- and post-initiation haemoglobin results. In the main analysis we will define the outcome as a reduction in haemoglobin of 1g/dL or more (considered to be a clinically meaningful haemoglobin change).

We will also use new-onset anaemia as an outcome in a sensitivity analysis, it will be defined according to WHO guidelines[22] as haemoglobin <12 g/dL in women or <13 g/dL in men. As a sensitivity analysis we will also define the outcome as a morbidity code for anaemia recorded in CPRD and/or HES (see **Section N**).

As a secondary analysis we will compare the proportion of ACEI, ARB and CCB users recorded with a post-initiation morbidity code indicating bone marrow suppression. Codes indicating bone marrow suppression will include codes for: neutropenia, leukopenia, thrombocytopenia, agranulocytosis, and aplastic anaemia. We will initially explore codes recorded within twelve months of drug initiation, however, these are likely to be rare outcomes so we will also look at any post drug initiation coding.

Covariates

We will explore the following variables as potential confounders or effect modifiers: age, sex, calendar period, chronic comorbidities (diabetes mellitus, hypertension, ischaemic heart disease, cardiac failure, CKD, myeloproliferative syndromes, and chronic lung disease), pre-initiation haemoglobin, pre-initiation history of gastrointestinal bleeding, other medications that may lead to changes in haemoglobin levels (including: warfarin, new oral anticoagulants, aspirin, clopidogrel, tacrolimus, cyclosporin, azathioprine, methotrexate, ferrous sulphate, folic acid, and B12 injections) and lifestyle factors (smoking status, alcohol intake and body mass index [BMI]).

We will include calendar period (2004–2006, 2007–2009, 2010–2012, 2013-2015, and 2016-2017) as a covariate to adjust for the many changes in clinical, diagnostic and administrative practices over the study period that may influence the measurement of baseline renal function and other covariates.

Baseline CKD status will be defined as eGFR <30 mL/min/1.73 m^2 (i.e. severe CKD) calculated from the most recent serum creatinine result recorded in the 12 months prior to drug initiation using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[24] Individuals with no recorded serum creatinine result in the 12 months prior to drug initiation will be assumed to have no CKD.

Diabetes mellitus, cardiac failure, hypertension, ischaemic heart disease, myeloproliferative disorders, chronic lung disease, history of gastrointestinal bleeding, and lifestyle factors will be identified using Read codes recorded in CPRD prior to first ACEI/ARB / CCB prescription. We will use existing morbidity code lists and algorithms for smoking status, alcohol intake, and BMI.[25,26]

Pre-drug initiation haemoglobin will be defined as the most recent recorded haemoglobin level in the year before drug initiation.

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We will use prescribing data to identify exposure to other medications that may lead to changes in haemoglobin levels (including: warfarin, new oral anticoagulants, aspirin, clopidogrel, tacrolimus, cyclosporin, azathioprine, methotrexate, ferrous sulphate, folic acid, and B12 injections). We will identify exposure to these medications at the time of ACEI/ARB or CCB prescription by using prescription data to construct courses of continuous therapy using a similar approach to that taken to identify ACEI/ARB / CCB exposure (i.e. We will calculate the duration of prescriptions using the quantity of medication prescribed and the daily dose recorded. We will assume exposure to medications starts on the date of the prescription and allow for a 60-day gap between consecutive prescriptions).

N. Data/ Statistical Analysis

Main analysis

We will present descriptive statistics comparing the following variables between new ACEI/ARB and CCB users: age, sex, chronic comorbidities (diabetes mellitus, cardiac failure, CKD), pre-initiation haemoglobin, history of gastrointestinal disease, and lifestyle factors (smoking, alcohol and BMI). We will calculate the mean drop in haemoglobin following drug initiation in new ACEI/ARB and CCB users and compare the results using t-tests. We will use logistic regression to compare the odds of new-onset reduction in haemoglobin of 1g/dL or more in ACEI/ARB users compared to CCB users. We will construct an age and sex adjusted model with just the main exposure variable (ACEI/ARB / CCB), age and sex, followed by a model adjusting for potential confounders informed by a priori knowledge including the following covariates age, sex, chronic comorbidities (cardiac failure, diabetes mellitus, hypertension, ischaemic heart disease, CKD), calendar period, and history of gastrointestinal bleeding and using robust standard errors to account for clustering by general practice. Finally, we will fit a fully adjusted model additionally adjusting for smoking, alcohol intake, and BMI (we will not adjust for these initially as we believe much of their effect will be captured by chronic comorbidities).

To ensure confidentiality, any cells containing <5 events will be suppressed in results. All data management and analyses will be performed using Stata version 14 (StataCorp, Texas).

Sensitivity analyses

We will test how robust our findings are by repeating the main analysis after:

1. *Varying the time used to identify pre-initiation haemoglobin:* It could be argued that a haemoglobin result recorded up to 12 months prior to drug initiation does not reflect the actual haemoglobin level prior to initiation, we will therefore repeat the main analysis in those with a pre-initiation haemoglobin result recorded in the month (we will extend this to 3 or 6 months if sample size becomes unfeasible) before ACEI/ARB initiation.
2. *Excluding those whose post-initiation haemoglobin is recorded in the first four weeks following drug initiation:* Blood test results recorded shortly after starting the drugs may not reflect the true response to them. To test the impact of this, we will repeat the main analysis after excluding individuals with early (i.e. within four weeks) post-initiation haemoglobin records.
3. *Accounting for concomitant prescribing of ACEI/ARB and CCBs:* For individuals with a new prescription for both classes of drug (ACEI/ARB and CCB) separated by less than six months (interval used to assess outcome) it may be difficult to determine which drug is responsible for any observed changes in haemoglobin (here the unit of analysis is the drug class prescribed for the individual [hence one individual may contribute two observations – for both ACEI/ARBs and CCBs – to the analysis] rather than the individual). However, it would be inappropriate to drop these individuals (with prescriptions for both classes of drugs within six months) from the main analysis as this would systematically exclude sicker individuals. Therefore, in the main analysis we will not include exposure to the second drug if it is prescribed within six months of the start of the

first prescribed class of drugs. This approach may bias the effect estimate to the null; a fall in haemoglobin caused by the second class of drugs prescribed may be wrong attributed to the first class of drugs prescribed. However, this approach would mean that we would be unlikely to overestimate any association observed between the drugs and the outcome. To assess the impact of this approach we will conduct a sensitivity analysis without dropping the second prescription event from the analysis (i.e. including exposure to a second class of drugs prescribed within six months of the first). To test our exposure definition further, we will also conduct another sensitivity analysis where we exclude new users of one class of drugs who are prevalent users of the other class. For example, we will exclude new users of ACEI/ARBs who are already prescribed a CCB at the time of their first ACEI/ARB prescription. This will ensure that any effect we observe is due to the newly prescribed medication and not the existing drug.

4. *Limiting to individuals with known CKD status:* Serum creatinine testing is more likely in those who are acutely unwell, or routinely monitored as part of incentivised programs (e.g. diabetics). Therefore, to avoid selection bias in the main analysis we will assume that individuals with no serum creatinine result recorded within the 12 months prior to ACEI/ARB / CCB initiation have no CKD. To test the impact of this assumption, we will repeat the analysis only in those with known CKD status (identified using most recent serum creatinine result recorded in the 12 months prior to drug initiation).
5. *Defining the outcome as new-onset anaemia:* We have chosen to use what we believe to be a clinically meaningful decrease in haemoglobin of 1g/dL as the outcome in the main analysis. However, it would also be useful to investigate the risk of new-onset anaemia following drug initiation. We will therefore repeat the main analysis defining the outcome as new-onset anaemia according to WHO guidelines[22] as haemoglobin <12 g/dL in women or <13 g/dL in men.
6. *Including all new-users of ACEI/ARB / CCB regardless of haemoglobin records and defining reduction in haemoglobin as coded anaemia:* By restricting our study population to individuals with pre- and post-initiation haemoglobin levels we may have selected a population of sicker individuals (haemoglobin testing is more likely in those with clinical need), we will therefore include all eligible new users regardless of haemoglobin records and define the outcome as anaemia coded in CPRD records within twelve months of drug initiation. We will also run this analysis restricted to those eligible for HES linkage and define anaemia coded in both primary care and hospital records.
7. *Limiting to practices consenting to HES linkage (and study period ending March 2016, i.e. the latest date for HES linkage) and defining covariates using both primary-care and hospital admission coding:* Completeness of chronic comorbidity identification can be improved by using hospital admissions data,[27] however, this will limit sample size as only 58% of CPRD practices have consented to data linkage[28] therefore we will only undertake this as a sensitivity analysis.
8. *Excluding patients with cardiac failure or taking diuretics:* Cardiac failure and diuretics may lead to changes in blood haemoglobin concentration through their action on fluid balance (resulting in changes in the relative amount of blood plasma to red blood cells, see **Section S**).[29] We will therefore test whether changes in blood concentration impact our findings by repeating our main analysis after: (1) excluding those with cardiac failure; (2) excluding those also prescribed a diuretic; and (3) excluding all those either prescribed a diuretic or with a diagnosis of cardiac failure.
9. *Excluding patients who may be managed with exogenous erythropoietin:* Some individuals with anaemia secondary to CKD may be managed with exogenous erythropoietin. Including these individuals in our analysis may influence our results as the effect of ACEI/ARBs on haemoglobin is thought to be mediated via erythropoietin. We are unable to identify those managed with

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erythropoietin using primary care prescribing data as erythropoietin prescribing for renal anaemia is usually managed by nephrologists. We will therefore run a sensitivity analysis limiting to those eligible for HES linkage and excluding those with serum creatinine test results indicating CKD stage 4 or above (eGFR<30) whose CKD is managed by nephrologists (identified using HES outpatient data).

10. *Propensity score matched cohort:* We will develop a propensity score matched cohort to ensure that we are comparing similar groups of ACEI/ARB and CCB users. We will use logistic regression to calculate propensity scores predicting choice of ACEI/ARB or CCB treatment. Factors included in the propensity score model will include all covariates adjusted for in the main analysis. Patients receiving ACEI/ARBs and patients receiving CCBs will be assigned to matched sets (using optimal propensity score matching) according to the value of their propensity score so that at least one patient from each treatment group is included in each set.[30,31] We will measure standardised differences for each covariate to check for balance between groups, before estimating the odds ratio comparing odds of a reduction in haemoglobin in ACEI/ARB users with that in the matched cohort of CCB users.

Secondary analyses

As secondary analyses we will investigate the impact of including interactions between: 1) ACEI/ARB and baseline (pre-initiation) haemoglobin; and 2) ACEI/ARB and CKD status.

We will also compare the effect on haemoglobin of ACEIs compared to ARBs by calculating mean change in haemoglobin and estimating the odds ratio (adjusting for confounders) of a fall in haemoglobin of 1g/dL or more in those starting ARBs compared to those starting ACEIs.

Finally, we will investigate the effect of ACEIs, ARBs, and CCBs on bone marrow suppression. Bone marrow suppression is likely to be a rare outcome; therefore, rather than an adjusted regression analysis, we will only compare the proportion of individuals recorded with codes indicating bone marrow suppression after drug initiation in each group (ACEI, ARB and CCB). In this analysis we will investigate ACEI and ARB users separately as it is possible that these drugs will have different effects on bone marrow.

O. Plan for addressing confounding

We will limit confounding by indication by comparing the effect of ACEI/ARBs on haemoglobin to a class of drugs that are prescribed for similar indications (CCBs). We will use multivariable logistic regression to adjust for the potential confounders (listed in **Section M)**, and check these results with results from a propensity score matched cohort (see **Section N**).

P. Plans for addressing missing data

We expect patients prescribed ACEI/ARBs or CCBs are likely to have health risk factors considered when their medications are prescribed so, based on our experience with recent studies, we do not anticipate missing data to be a major problem. We will therefore undertake a complete case analysis unless missing data is greater than 30%, when we will undertake further sensitivity analyses. For example, if necessary to reduce any selection biases due to missing data, we will repeat the main analysis restricting it to more recent calendar periods when data may be more complete (due to improvements in data quality following changes in lab reporting and incentives offered by the Quality and Outcomes Framework).

Q. Patient or user group involvement (if applicable)

Patients have not yet been involved in the planning of this study. We aim to share important findings

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with patient representatives. It is hoped that these patient representatives will help plan further dissemination and use of the results.

R. Plans for disseminating and communicating study results, including the presence or absence of any restrictions on the extent and timing of publication

We intend to publish our findings in peer-reviewed scientific journals, and to present them at relevant scientific meetings (including the International Conference on Pharmacoepidemiology & Therapeutic Risk Management).

S. Limitations of the study design, data sources, and analytic methods

The internal validity of our study is, similar to other observational studies, potentially limited by selection and information bias, and confounding. The study is population-based and not restricted to specific demographic, hospital, or insurance groups. Hence, selection bias may not a major concern. However, because routine haemoglobin monitoring is not included in guidelines for ACEI/ARB or CCB prescribing, we may find that (because we require both pre- and post-initiation haemoglobin levels) those eligible for inclusion in the study may be a group of potentially sicker individuals (as haemoglobin testing is more likely in those with clinical need). It is hoped that this selection bias is not different in those prescribed an ACEI/ARB compared to those prescribed a CCB, so there should be limited influence on the effect estimate, but the results may not be generalizable to all ACEI/ARB / CCB users. Therefore, we will repeat the main analysis including all eligible new users of ACEI/ARBs or CCBs regardless of whether they have haemoglobin results recorded and define the outcome as recorded anaemia morbidity codes rather than percentage reduction in haemoglobin.

A number of studies have linked CCBs (our comparison group) to a reduction in haemoglobin (through increased risk of gastrointestinal bleeding)[32–34], however, other studies have provided no evidence for this link.[32] If there is indeed a link between CCBs and subsequent haemoglobin reduction, our comparison group may also show a change in haemoglobin following drug initiation. This may limit our ability to demonstrate an association of ACEI/ARBs with reduced haemoglobin levels. However, our choice of comparison group is limited, we need to compare ACEI/ARBs to drugs that are prescribed for similar indications. If we do not, any differences we see could be explained by the reason the drug is prescribed rather than the drug itself. Therefore, in this context, while flawed, CCBs remain the best choice, and since the mechanism through which ACEI/ARBs (via erythropoietin) and CCBs (via gastrointestinal bleeding) are thought to lead to anaemia are different, it is hoped that by adjusting for confounders (particularly gastrointestinal bleeding and baseline renal function) and some of our secondary analyses (looking at interactions between ACEI/ARB and baseline haemoglobin or renal function) will provide insight into differences between ACEI/ARBs and CCBs. Further, any results will help inform drug choice in situations where there is currently equipoise between choice of an ACEI/ARB or a CCB.

Haemoglobin levels may be influenced by how much fluid there is in the body. If the volume of plasma in relation to the number of red blood cells changes then haemoglobin may be artificially changed. For example, the blood may become concentrated (haemoconcentration) due to loss of plasma or water when a patient is dehydrated or has burns resulting in an artificially high haemoglobin. Conversely, there may be an increase in the fluid content of blood, for example in cardiac failure or pregnancy, leading to a lower concentration of red blood cells (haemodilution) and therefore a decrease in haemoglobin. Our results may therefore be influenced by a patient's fluid balance. This is important because ACEI/ARBs or CCBs are used to manage cardiac failure (which causes water retention due to the heart's inability to pump blood efficiently), and patients prescribed ACEI/ARBs or CCBs are also likely to be managed with diuretics (which act by reducing blood circulating volume by increasing the production of urine) meaning

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that any reduction in haemoglobin may be as a result of changes in fluid balance rather than actual changes in the total amount of haemoglobin in the blood. To test any impact on our findings resulting from changes in fluid balance rather than real changes in haemoglobin we will conduct a sensitivity analysis by repeating the main analysis after excluding all those prescribed a diuretic or with a diagnosis of cardiac failure.

Some individuals with anaemia secondary to CKD may be managed with exogenous erythropoietin. Including those managed with exogenous erythropoietin may influence our results as the effect of ACEI/ARBs on haemoglobin is thought to be mediated via erythropoietin. While we do not expect this to affect many individuals (and therefore the effect of including those treated with exogenous erythropoietin should be minimal), we will nonetheless test the impact of their inclusion by running a sensitivity analysis excluding those who may be treated with exogenous erythropoietin (see **Section N**).

There may be misclassification of ACEI/ARB / CCB exposure; a prescription does not guarantee a patient takes a drug. We anticipate that our estimate of drug exposure will be flawed to some extent due to lack of adherence. Since this is unlikely to be differential for different drugs, it will be unlikely to influence the results of the study.

We hope that any misclassification due to variability in coding practices will be mitigated by adjustment for calendar period (to account for temporal changes in diagnostic and coding practices), careful development of code lists and, where possible, use of previously validated code lists. In addition, robust standard errors will be used to account for clustering by general practice.

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List of Appendices *(Submit all appendices as separate documents to this application)*

1. Code lists (Excel file)

ISAC EVALUATION OF PROTOCOLS FOR RESEARCH INVOLVING CPRD DATA

FEEDBACK TO APPLICANTS

CONFIDENTIAL	**by e-mail**		
PROTOCOL NO:	*17_197R*		
PROTOCOL TITLE:	The effect of renin-angiotensin system inhibitors on haemoglobin		
APPLICANT:	Laurie Tomlinson, Associate Professor, London School of Hygiene & Tropical Medicine laurie.tomlinson@lshtm.ac.uk		
APPROVED –	**APPROVED WITH COMMENTS (resubmission not required)** –	**REVISION/ RESUBMISSION REQUESTED** –	**REJECTED** –
INSTRUCTIONS: ***Please include your response/s to the Reviewer's feedback below only if you are required to Revise/ Resubmit your protocol.*** ***Protocols with an outcome of 'Approved' or 'Approved with comments' do not require resubmission to the ISAC.*** **REVIEWER COMMENTS:** ISAC Protocol 17_197R is approved.			
DATE OF ISAC FEEDBACK:	25/09/2017		
DATE OF APPLICANT FEEDBACK:			

For protocols approved from 01 April 2014 onwards, applicants are required to include the ISAC protocol in their journal submission with a statement in the manuscript indicating that it had been approved by the ISAC (with the reference number) and made available to the journal reviewers. If the protocol was subject to any amendments, the last amended version should be the one submitted.

***** Please refer to the ISAC advice about protocol amendments provided below*****

Amendments to protocols approved by ISAC

Version June 2015

During the course of some studies, it may become necessary to deviate from a protocol which has been approved by ISAC. Any deviation to an ISAC approved protocol should be clearly documented by the applicant but not all such amendments need be submitted for ISAC review and approval. The general principles to be applied in regard to the need for submission are as follows:

- Major amendments should be submitted
- Minor amendments need not be submitted (but must still be documented by the applicant and should normally be mentioned at the publication stage)

In cases of uncertainty, the applicant should contact the ISAC secretariat for advice quoting the original reference number and providing a brief explanation of the nature of the amendment(s) and underlying reason(s).

Major Amendments

We consider an amendment as major if it substantially changes the study design or analysis plan of the proposed research. An amendment should be considered major if it involves the following (although this is not necessarily an exhaustive list):

- A change to the primary hypothesis being tested in the research
- A change to the design of the study
- Additional outcomes or exposures unrelated to the main focus of the approved study*
- Non-trivial changes to the analysis strategy
- Not performing a primary outcome analysis
- Omissions from the analysis plan which may impact on important validity issues such as confounding
- Change of Chief Investigator
- Use of additional linkages to other databases
- Any new proposal involving contact with health professionals or patient or change in regard to such matters

* N.B. extensive changes in this respect will require a new protocol rather than an amendment - if in doubt please consult the Secretariat

Minor Amendments

Examples of amendments which can generally be considered minor include the following:

- Change of personnel other than the Chief Investigator (these should be notified to the Secretariat)
- A change to the definition of the study population, providing the change is mentioned and justified in the paper/output [NB previously major]
- Extension of the time period in relation to defining the study population
- Changes to the definitions of outcomes or exposures of interest, providing the change is mentioned and justified in the paper/output [NB previously major]
- Not using linked data which are part of the approved protocol, unless the linked data are considered critical in defining exposures or outcomes (in which case this would be a major amendment)
- Limited additional analysis suggested by unexpected findings, provided these are clearly presented as post-hoc
- Additional methods to further control for confounding or sensitivity analysis provided these are to be reported as secondary to the main findings
- Validation and data quality work provided additional information from GPs is not required

To submit an amendment of protocol to the ISAC, please submit the following documents to the ISAC mailbox (isac@cprd.com)

1. A covering letter providing justification for the request
2. A completed and, if necessary, updated application form with all changes highlighted; if new linkages are required the current version of the ISAC application form must be completed. Otherwise, the original form may be amended as necessary
3. **The updated protocol document containing the heading 'Amendment' at the end of it.** Please include all amendments to the protocol under this heading. No other changes should be made to the already approved document.