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Exogenous sex steroid hormones and asthma in females: protocol for a population-based retrospective cohort study using primary care data

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Keywords
asthma, females, hormonal contraception, hormone replacement therapy, oestrogen, progesterone, sex hormones.

Running head: Exogenous sex hormones and asthma in females

ABSTRACT

Introduction: Female sex steroid hormones have been implicated in sex-related differences in the development and clinical outcomes of asthma. The role of exogenous sex steroids however remains unclear. Our recent systematic review highlighted the lack of high quality population-based studies investigating this subject. We aim to investigate whether the use of hormonal contraception and hormone replacement therapy (HRT), subtypes, and route of administration are associated with asthma onset and clinical outcomes in reproductive age and peri-menopausal/post-menopausal females.

Methods and analysis: Using the Optimum Patient Care Research Database (OPCRD), a national primary care database in the UK, we will construct a retrospective longitudinal cohort of reproductive age (16-45 years) and peri-menopausal/post-menopausal (46+ 70years) females. We will estimate the risk of new-onset asthma using Cox regression and multilevel modelling for repeated asthma outcomes, such as asthma attacks. We will adjust for confounding factors in all analyses. We will evaluate interactions between the use of exogenous sex hormones and body mass index and smoking by calculating the relative excess risk due to interaction and the attributable proportion due to interaction. With 90% power, we need 23,700 reproductive age females to detect a 20% reduction (risk ratio 0.8) in asthma attacks for use of any hormonal contraception and 6,000 peri-menopausal/post-menopausal females to detect a 40% (risk ratio 1.40) increased risk of asthma attacks for use of any HRT.

Ethics and dissemination: We have obtained approval (ADEPT1317) from the Anonymised Data Ethics and Protocol Transparency (ADEPT) Committee, which grants project-specific ethics approvals for the use of OPCRD data. Optimum Patient Care has an existing NHS Health Research Authority ethics approval for the use of OPCRD data for research (15/EM/150). We will present our findings at national and international scientific meetings and publish the results in international peer-reviewed journals.

Protocol registration: We will register the study protocol with the European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) prior to starting the analyses.

Strengths and limitations of this study

- The longitudinal nature of this study will fill an important gap in the evidence base as there is a paucity of longitudinal studies investigating the role of exogenous sex steroid hormones in asthma in females
- As a study based on primary care database, both exposure and outcome measures will be objectively defined
- This study is based on the general and unselected population; therefore, our findings will be generalizable to the general population of reproductive age and peri-menopausal/post-menopausal females
- Overall, this study will provide robust evidence that will inform potential causation and provide the direction for further mechanistic work
- A limitation of this work is that we will define reproductive and menopausal status of females based only on the ages of women, which is the only information from GP database for this purpose

INTRODUCTION

Asthma is more common in boys than girls during early childhood.[1-3] However, after puberty, the prevalence and severity of asthma are higher in females than males.[1-3] Female sex steroid hormones are believed, at least in part, to explain these sex-related variations in asthma outcomes.[1-3] Variations in asthma incidence and clinical outcomes are seen to follow the hormonal transitional points in the female reproductive life course, in particular, puberty, menarche, menstruation, pregnancy and menopause.[1,4] Fluctuations in oestradiol and progesterone levels during the menstrual cycle have been linked to worsening of asthma symptoms in females,[5] although immunological mechanisms underlying these effects have not been defined.

Both oestrogen and progesterone influence smooth muscle functions, inflammation, and airway responsiveness.[6,7,8] Studies in animal models indicate that oestrogen increases T-helper (Th) 2 cell responses associated with allergic asthma.[8] A recent study showed that in patients with severe asthma, both oestrogen and progesterone were associated with a decrease in the expression of the left-7f microRNA as well as an increase in interleukin (IL)-23/IL-23 receptor signalling and IL-17A production, an effect that was more marked in women versus men.[9] Some evidence suggests that external suppression of endogenous sex steroid production through the use of exogenous hormonal contraception may improve asthma outcomes,[10-14] whereas the use of hormone replacement therapy (HRT) by menopausal women may increase the risk of new onset asthma and risk of poor clinical outcomes of asthma.[15-20] Females with asthma exhibit enhanced Th17 responses, reduced asthma symptoms, and improved lung function when using hormonal contraception.[5,7,8,21,22]

Using the serial cross-sectional Scottish Health Surveys, we recently observed substantial reductions in asthma exacerbations and hospital episodes in females using hormonal contraception compared to those not using hormonal contraception.[13] This work was followed by a comprehensive synthesis of the underlying evidence, which revealed inherent methodological limitations in previous studies on the topic, including a paucity of prospective longitudinal studies and limitations in the measurement of sex steroids and asthma outcomes.[23] To overcome these weaknesses and thus clarify whether the role of sex steroid hormones in asthma in females is causal, well-designed long-term longitudinal studies with well-characterised populations are required. We plan to investigate the role of exogenous sex steroid hormones in the development of asthma and manifestation of clinical and patient-reported outcomes in females by creating a retrospective longitudinal cohort of reproductive age and peri-menopausal/postmenopausal females using the Optimum Patient Care Research Database (OPCRD). Specifically, we aim to investigate the:

1. Associations between use of hormonal contraception and asthma onset, attacks, severity, mortality and health-related quality of life (HRQoL) in reproductive age females.
2. Impact of type of menopause (surgical vs non-surgical) and use of HRT on asthma onset, attacks, severity, mortality and HRQoL in peri-menopausal/post-menopausal females.
3. Interactions between exogenous sex hormones, body mass index (BMI), cigarette smoking and alcohol consumption in these associations.

METHODS

Ethics approvals and permissions

The main ethical issues relate to anonymity, confidentiality, data protection and the linkage of datasets. We have obtained approval (reference number: ADEPT1317) from the Anonymised Data Ethics and Protocol Transparency (ADEPT) Committee, which grants project-specific approvals for the use of the OPCRD data. Optimum Patient Care has an existing NHS Health Research Authority ethics approval for the use of OPCRD for research (REC Ref:

15/EM/150). All researchers involved in data analysis will have successfully completed the appropriate information governance courses.

Study design and population

OPCRD is a bespoke longitudinal de-identified primary care database representing over 600 general practices across the UK and is regularly used to conduct epidemiological, clinical, and pharmaceutical research (www. http://optimumpatientcare.org/opcrd/). A major advantage of the OPCRD database is the focus on respiratory outcomes; in up to 10% of patients with asthma, patient-reported questionnaire data on asthma outcomes are available. This provides the opportunity to study both clinical and patient-reported outcomes from the database. The study population for the present investigation will comprise all 16-70-year-old females in OPCRD. We will construct two independent cohorts to address the study objectives, namely:

1. Reproductive age females (16-45 years old) to study the associations between use of hormonal contraception and the study outcomes
2. Peri-menopausal (46-55 years old)/post-menopausal females (56+70 years old) to study the associations between type of menopause and HRT and the study outcomes.

Exposures

We will ascertain the use of hormonal contraception and HRT and type of menopause by means of the Read Clinical Classification System (Read codes).[24,25] For the use of exogenous sex hormones, we will define the following exposures:

1. Reproductive age females: use of hormonal contraception, subtypes (oestrogen/progestogen combined, progestogen only), route of administration (oral, transdermal, subcutaneous, intramuscular, local intrauterine), and frequency and duration of use.
2. Peri-menopausal/post-menopausal females: use of HRT, subtypes (oestrogen/ progestogen combined, oestrogen-only), route of administration (oral, transdermal, subcutaneous, local intrauterine), and frequency and duration of use.

Confounders

Potential confounders will be extracted from the database using their respective Read codes, including age, parity, BMI, smoking, current use of asthma treatments and level of adherence to these, Index of Multiple Deprivation (IMD),[26] and co-morbidity based on the Charlson index.[27]

Outcomes

The primary outcomes will include new-onset asthma, asthma attacks and severity. New-onset asthma will be defined as the first GP-recorded asthma event (including diagnosis, hospitalisation, medication prescription, or any other asthma event) occurring at least five years from the start of the follow-up date. We will exclude individuals with a relevant asthma event recorded up to five years after the start of follow-up date. We assume that within a 5-year period an asthma patient should have had at least one clinical encounter. Asthma attacks and severity will be defined based on the frequency of GP consultation, A&E attendance, oral steroid courses and hospital admissions for asthma. The outcomes will be determined using relevant Read codes. The secondary outcomes will include patient-reported asthma symptoms, medication use and HRQoL. Data on HRQoL will be assessed from patient-completed questionnaires on asthma symptoms and quality of life, which is an adjunct data collected from about 10% of patients and added to the OPCRD database.

Follow-up period

We will follow the participants from baseline starting from 1 January 2000 or date of registration until 30 June 2017. With regards to onset of asthma, exit date from the cohort will

be defined as the date of first diagnosis of asthma (i.e. date of first record of an asthma encounter), death, deregistration from a practice, or end of follow-up (30 June 2017), whichever comes first.

Statistical analyses

Prior to the main analyses, the data will undergo relevant quality checks, including relevant variable categorisation (re-scaling where appropriate) and checks for missingness. We will undertake a complete case analysis and also perform multiple imputation for variables with missing values. We will perform 20 imputations in order to enhance the efficiency of the estimates and will use Rubin's rule to combine the estimates across the 20 datasets.[28] Where the specific time of onset of asthma is observable, we will perform survival analysis using the log-rank test to describe the survival functions of the groups as defined by use of sex hormones. We will use Cox proportional hazards regression to study the associations between exogenous sex hormones and the first record of an asthma event. Multilevel modelling will be used to estimate associations where the outcomes are repeated, e.g. number of asthma attacks and medication use. Since the change in hormone levels with contraceptive use is expected to differ between women, random coefficient models will be fitted, so that in turn the relationship between contraception use and asthma outcomes can differ between women. We will undertake analyses incorporating propensity scores using matching (exposed vs. unexposed).[29] The model will be non-parsimonious in order to include a wide range of factors that influence propensity to be prescribed hormonal contraceptives and HRT. To minimise potential biases, we will undertake different scenarios of sensitivity analyses in order to evaluate the robustness of our findings, including analyses for potential selection bias at baseline, unmeasured confounding and information bias.[30] These sets of bias analyses will be aided by deriving relevant internal data from a subset of the study population where possible. Alternatively, we will obtain external validation data (for instance, from the Secure Anonymised Information Linkage [SAIL] in Wales) that will provide the basis for defining the sensitivities of the different measures and allow appropriate adjustments to be made to our estimates. We will also evaluate the potential of confounding by indication bias by stratifying the analyses by the relevant disease indication for using sex steroid preparations. To estimate the potential interactions between sex hormones and BMI and cigarette smoking, we will calculate the relative excess risk due to interaction and the attributable proportion due to interaction.[31] All estimates will be accompanied by their respective 95% confidence intervals. Statistical analyses will be undertaken using R statistical software.

Sample size estimation

Given estimates of our previous exploratory analysis using the Scottish Health Surveys (31% using any hormonal contraception, 6.5% with clinician-diagnosed asthma, and an odds ratio of 0.68, 95% confidence interval 0.47-0.98),[13] we determined that in order to have 90% power at an alpha level of 0.05 to detect up to 20% reduction (risk ratio 0.8) in asthma attacks, we will need a sample size of 23,700 reproductive age females for use of any hormonal contraception. Furthermore, with 90% power at an alpha level of 0.05, we determined that we will need 6,000 peri-menopausal/post-menopausal females to detect up to 40% (risk ratio 1.40) increased risk of asthma attack for use of any HRT.[20]

Reporting and dissemination plans

We will report the findings of the study following the recommendations of STROBE (Strengthening the Reporting of Observational Studies in Epidemiology)[32] and RECORD (Reporting of studies Conducted using Observational Routinely-collected health Data).[33] All the analysis source codes will be made available at GitHub website at https://github.com/asthmalhs. The project findings will be presented at national and international scientific meetings and published in international journals. Furthermore, we will capitalise on the dissemination infrastructures of the Asthma UK Centre for Applied

Research (e.g. the Twitter feed and dynamic website) and the University of Gothenburg to publicise our findings to clinicians, academics, patients, and reproductive health channels.

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Conflict of interests
The authors declare no conflict of interest related to this work.

Authors' contribution
BN and AS conceived the idea for this work. It was drafted by BN and was then revised after several rounds of critical comments from CRS, CMH, and AS and additional feedback from INS, RP, HC, FA, DR, and DP.

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Protocol registration: The study protocol is registered with the European Union electronic Register of Post-Authorisation Studies (EUPAS22967).

Strengths and limitations of this study

- The longitudinal nature of this study will fill an important gap in the evidence base as there is a paucity of longitudinal studies investigating the role of exogenous sex steroid hormones in asthma in females
- As a study based on primary care database, both exposure and outcome measures will be objectively defined
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INTRODUCTION

Asthma is more common in boys than girls during early childhood.[1-3] However, after puberty, the prevalence and severity of asthma are higher in females than males.[1-3] Female sex steroid hormones are believed, at least in part, to explain these sex-related variations in asthma outcomes.[1-3] Variations in asthma incidence and clinical outcomes are seen to follow the hormonal transitional points in the female reproductive life course, in particular, puberty, menarche, menstruation, pregnancy and menopause.[1,4] Fluctuations in oestradiol and progesterone levels during the menstrual cycle have been linked to worsening of asthma symptoms in females,[5] although immunological mechanisms underlying these effects have not been defined. In comparison to non-menopausal females, transitional menopausal females and both early and late post-menopausal females were at increased risk of new-onset asthma. In primary studies, the role of menopause on risk of current symptoms of asthma were equivocal,[6] but a recent meta-analysis of these studies showed a moderately increased risk.[7]

Both oestrogen and progesterone influence smooth muscle functions, inflammation, and airway responsiveness. [8-10] Studies in animal models indicate that oestrogen increases T-helper (Th) 2 cell responses associated with allergic asthma.[10] A recent study showed that in patients with severe asthma, both oestrogen and progesterone were associated with a decrease in the expression of the left-7f microRNA as well as an increase in interleukin (IL)-23/IL-23 receptor signalling and IL-17A production, an effect that was more marked in women versus men.[11] Some evidence suggests that external suppression of endogenous sex steroid production through the use of exogenous hormonal contraception may improve asthma outcomes,[12-17] whereas the use of hormone replacement therapy (HRT) by menopausal women may increase the risk of new onset asthma and risk of poor clinical outcomes of asthma.[18-23] Females with asthma exhibit enhanced Th17 responses, reduced asthma symptoms, and improved lung function when using hormonal contraception.[5,9,10,24,25]

The role of use of hormonal contraceptives both in the development of new-onset asthma[23,26] and manifestation of current symptoms of asthma[16] has been conflicting. Using the serial cross-sectional Scottish Health Surveys, we recently observed substantial reductions in asthma exacerbations and hospital episodes in females using hormonal contraception compared to those not using hormonal contraception.[15] This work was followed by a comprehensive synthesis of the underlying evidence, which revealed inherent methodological limitations in previous studies on the topic, including a paucity of prospective longitudinal studies and limitations in the measurement of sex steroids and asthma outcomes.[7] To overcome these weaknesses and thus clarify whether the role of sex steroid hormones in asthma in females is causal, well-designed long-term longitudinal studies with well-characterised populations are required. We plan to investigate the role of exogenous sex steroid hormones in the development of asthma and manifestation of clinical and patient-reported outcomes in females by creating a retrospective longitudinal cohort of reproductive age and peri-menopausal/postmenopausal females using the Optimum Patient Care Research Database (OPCRD). Specifically, we aim to investigate the:

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We will ascertain the use of hormonal contraception and HRT and type of menopause by means of the Read Clinical Classification System (Read codes).[27,28] We will define the following exposures:

1. Reproductive age females: use of hormonal contraception versus non-use of hormonal contraception; subtypes (oestrogen/progestogen combined, progestogen only versus non-use of hormonal contraception); route of administration (transdermal, subcutaneous, intramuscular, local intrauterine versus oral route), and frequency and duration of use (as a count exposure).
2. Menopausal status: peri-menopausal (46-55 years) versus non-menopausal (<46 years); post-menopausal (56-70 years) versus non-menopausal (<46 years)
3. Peri-menopausal/post-menopausal females: use of HRT versus non-HRT use, subtypes (oestrogen/ progestogen combined, oestrogen-only versus non-HRT use), route of administration (transdermal, subcutaneous, local intrauterine versus oral route), and frequency and duration of use (as a count exposure).

Confounders

Potential confounders will be extracted from the database using their respective Read codes, including age, parity, BMI, smoking, current use of asthma treatments and level of adherence to these using our definition based on the algorithm developed within the OPCRD[29], Index of Multiple Deprivation (IMD),[30] co-morbidity based on the Charlson index,[31] and other indications for use of hormonal contraception (e.g. endometriosis, polycystic ovary syndrome (PCOS), menorrhagia, acne, and metrorraghia) and HRT (e.g. onset of menopause, endometriosis, and indicators for hysterectomy for symptomatic uterine fibroids, and heavy menstrual bleeding) besides contraception and symptoms of menopausal transition, respectively. Endometriosis[32] and PCOS,[33] key indicators for the use of hormonal contraceptives among reproductive age women, have been linked to risk of asthma; therefore are important confounders in the association between use of hormonal contraceptives and asthma. For HRT, the onset of menopause itself and hysterectomy, other

indicators for use of HRT besides symptoms of menopausal transition, have also been linked to asthma.[6,21]

Outcomes

The primary outcomes will include new-onset asthma, asthma attacks and severity. New-onset asthma will be defined as the first GP-recorded asthma event (including diagnosis, hospitalisation, medication prescription, or any other asthma event) occurring at least five years from the start of the follow-up date. We will exclude individuals with a relevant asthma event recorded up to five years after the start of follow-up date. We assume that within a 5-year period an asthma patient should have had at least one clinical encounter. Asthma attacks and severity will be defined based on the frequency of GP consultation, A&E attendance, oral steroid courses and hospital admissions for asthma. Using these parameters and based on algorithm we have now developed within the OPCRD database, patients will be classified according to the British Thoracic Society (BTS) and Scottish Intercollegiate Guidelines Network (SIGN) asthma severity steps.[34] The outcomes will be determined using relevant Read codes. The secondary outcomes will include patient-reported asthma symptoms, medication use and HRQoL. Data on HRQoL will be assessed from patient-completed questionnaires on asthma symptoms and quality of life, which is an adjunct data collected from about 10% of patients and added to the OPCRD database.

Follow-up period

We will follow the participants from baseline starting from 1 January 2000 or date of registration until 30 June 2017. With regards to onset of asthma, exit date from the cohort will be defined as the date of first diagnosis of asthma (i.e. date of first record of an asthma encounter), death, deregistration from a practice, or end of follow-up (30 June 2017), whichever comes first.

Statistical analyses

Prior to the main analyses, the data will undergo relevant quality checks, including relevant variable categorisation (re-scaling where appropriate) and checks for missingness. We will undertake a complete case analysis and also perform multiple imputation for variables with missing values. We will perform 20 imputations in order to enhance the efficiency of the estimates and will use Rubin's rule to combine the estimates across the 20 datasets.[35] Where the specific time of onset of asthma is observable, we will perform survival analysis using the log-rank test to describe the survival functions of the groups as defined by use of sex hormones. We will use Cox proportional hazards regression to study the associations between exogenous sex hormones and the first record of an asthma event. Multilevel modelling will be used to estimate associations where the outcomes are repeated, e.g. number of asthma attacks and medication use. Since the change in hormone levels with contraceptive use is expected to differ between women, random coefficient models will be fitted, so that in turn the relationship between contraception use and asthma outcomes can differ between women. We will undertake analyses incorporating propensity scores using matching (exposed vs. unexposed).[36] The model will be non-parsimonious in order to include a wide range of factors that influence propensity to be prescribed hormonal contraceptives and HRT. To minimise potential biases, we will undertake different scenarios of sensitivity analyses in order to evaluate the robustness of our findings, including analyses for potential selection bias at baseline, unmeasured confounding and information bias.[37] These sets of bias analyses will be aided by deriving relevant internal data from a subset of the study population where possible. Alternatively, we will obtain external validation data (for instance, from the Secure Anonymised Information Linkage [SAIL] in Wales) that will provide the basis for defining the sensitivities of the different measures and allow appropriate adjustments to be made to our estimates. We will also evaluate the potential of confounding by indication bias by stratifying the analyses by the relevant disease indication for using sex steroid preparations as indicated above in the section on confounding. To estimate the potential

interactions between sex hormones and BMI and cigarette smoking, we will calculate the relative excess risk due to interaction and the attributable proportion due to interaction.[38] All estimates will be accompanied by their respective 95% confidence intervals. Statistical analyses will be undertaken using R statistical software.

Sample size estimation

Given estimates of our previous exploratory analysis using the Scottish Health Surveys (31% using any hormonal contraception, 6.5% with clinician-diagnosed asthma, and an odds ratio of 0.68, 95% confidence interval 0.47-0.98),[15] we determined that in order to have 90% power at an alpha level of 0.05 to detect up to 20% reduction (risk ratio 0.8) in asthma attacks, we will need a sample size of 23,700 reproductive age females for use of any hormonal contraception. Furthermore, with 90% power at an alpha level of 0.05, we determined that we will need 6,000 peri-menopausal/post-menopausal females to detect up to 40% (risk ratio 1.40) increased risk of asthma attack for use of any HRT.[23] Currently, the OPCRD database has over 5 million population of patients; we anticipate that about five hundred thousand patients will meet the criteria for inclusion into this study.

Reporting and dissemination plans

We will report the findings of the study following the recommendations of STROBE (Strengthening the Reporting of Observational Studies in Epidemiology)[39] and RECORD (Reporting of studies Conducted using Observational Routinely-collected health Data).[40] All the analysis source codes will be made available at GitHub website at https://github.com/asthmalhs. The project findings will be presented at national and international scientific meetings and published in international journals. Furthermore, we will capitalise on the dissemination infrastructures of the Asthma UK Centre for Applied Research (e.g. the Twitter feed and dynamic website) and the University of Gothenburg to publicise our findings to clinicians, academics, patients, and reproductive health channels.

Funding

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Conflict of interests

The authors declare no conflict of interest related to this work.

Authors' contribution

BN and AS conceived the idea for this work. It was drafted by BN and was then revised after several rounds of critical comments from CRS, CMH, and AS and additional feedback from INS, RP, HC, FA, DR, and DP.

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The RECORD statement – checklist of items, extended from the STROBE statement, that should be reported in observational studies using routinely collected health data.

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
Title and abstract					
	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found		RECORD 1.1: The type of data used should be specified in the title or abstract. When possible, the name of the databases used should be included. RECORD 1.2: If applicable, the geographic region and timeframe within which the study took place should be reported in the title or abstract. RECORD 1.3: If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract.	Pages 1-2
Introduction					
Background rationale	2	Explain the scientific background and rationale for the investigation being reported			Page 3
Objectives	3	State specific objectives, including any prespecified hypotheses			Page 3
Methods					
Study Design	4	Present key elements of study design early in the paper			Page 4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection			Page 4
Participants	6	*(a) Cohort study* - Give the eligibility criteria, and the		RECORD 6.1: The methods of study population selection (such as codes or	Page 4

		sources and methods of selection of participants. Describe methods of follow-up *Case-control study* - Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study* - Give the eligibility criteria, and the sources and methods of selection of participants *(b) Cohort study* - For matched studies, give matching criteria and number of exposed and unexposed *Case-control study* - For matched studies, give matching criteria and the number of controls per case		algorithms used to identify subjects) should be listed in detail. If this is not possible, an explanation should be provided. RECORD 6.2: Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided. RECORD 6.3: If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the data linkage process, including the number of individuals with linked data at each stage.	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.		RECORD 7.1: A complete list of codes and algorithms used to classify exposures, outcomes, confounders, and effect modifiers should be provided. If these cannot be reported, an explanation should be provided.	Pages 4-5
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group			Page 4
Bias	9	Describe any efforts to address potential sources of bias			Pages 4-5
Study size	10	Explain how the study size was			Page 6

		arrived at			
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why			Page 4-5
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) *Cohort study* - If applicable, explain how loss to follow-up was addressed *Case-control study* - If applicable, explain how matching of cases and controls was addressed *Cross-sectional study* - If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses			Page 5
Data access and cleaning methods		..		RECORD 12.1: Authors should describe the extent to which the investigators had access to the database population used to create the study population. RECORD 12.2: Authors should provide information on the data cleaning methods used in the study.	Page 3-4
Linkage		..		RECORD 12.3: State whether the study included person-level, institutional-	N/A

				level, or other data linkage across two or more databases. The methods of linkage and methods of linkage quality evaluation should be provided.	
Results					
Participants	13	(a) Report the numbers of individuals at each stage of the study (*e.g.*, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed) (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram		RECORD 13.1: Describe in detail the selection of the persons included in the study (*i.e.,* study population selection) including filtering based on data quality, data availability and linkage. The selection of included persons can be described in the text and/or by means of the study flow diagram.	N/A
Descriptive data	14	(a) Give characteristics of study participants (*e.g.*, demographic, clinical, social) and information on exposures and potential confounders (b) Indicate the number of participants with missing data for each variable of interest (c) *Cohort study* - summarise follow-up time (*e.g.*, average and total amount)			N/A
Outcome data	15	*Cohort study* - Report numbers of outcome events or summary measures over time *Case-control study* - Report numbers in each exposure category, or summary measures of exposure *Cross-sectional study* - Report numbers of outcome events or summary measures			N/A
Main results	16	(a) Give unadjusted estimates			N/A

		and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period			
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses			N/A
Discussion					
Key results	18	Summarise key results with reference to study objectives			N/A
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias		RECORD 19.1: Discuss the implications of using data that were not created or collected to answer the specific research question(s). Include discussion of misclassification bias, unmeasured confounding, missing data, and changing eligibility over time, as they pertain to the study being reported.	N/A
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence			N/A
Generalisability	21	Discuss the generalisability (external validity) of the study results			N/A

Other Information					
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based			Page 6
Accessibility of protocol, raw data, and programming code		..		RECORD 22.1: Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code.	N/A

*Reference: Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, Sørensen HT, von Elm E, Langan SM, the RECORD Working Committee. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement. *PLoS Medicine* 2015; in press.

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Exogenous sex steroid hormones and asthma in females: protocol for a population-based retrospective cohort study using a UK primary care database

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Keywords:	Asthma < THORACIC MEDICINE, females, hormonal contraception, hormone replacement therapy, oestrogen, progesterone

Exogenous sex steroid hormones and asthma in females: protocol for a population-based retrospective cohort study using a UK primary care database

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Running head: Exogenous sex hormones and asthma in females

ABSTRACT

Introduction: Female sex steroid hormones have been implicated in sex-related differences in the development and clinical outcomes of asthma. The role of exogenous sex steroids however remains unclear. Our recent systematic review highlighted the lack of high quality population-based studies investigating this subject. We aim to investigate whether the use of hormonal contraception and hormone replacement therapy (HRT), subtypes, and route of administration are associated with asthma onset and clinical outcomes in reproductive age and peri-menopausal/post-menopausal females.

Methods and analysis: Using the Optimum Patient Care Research Database (OPCRD), a national primary care database in the UK, we will construct a retrospective longitudinal cohort of reproductive age (16-45 years) and peri-menopausal/post-menopausal (46-70years) females. We will estimate the risk of new-onset asthma using Cox regression and multilevel modelling for repeated asthma outcomes, such as asthma attacks. We will adjust for confounding factors in all analyses. We will evaluate interactions between the use of exogenous sex hormones and body mass index and smoking by calculating the relative excess risk due to interaction and the attributable proportion due to interaction. With 90% power, we need 23,700 reproductive age females to detect a 20% reduction (risk ratio 0.8) in asthma attacks for use of any hormonal contraception and 6,000 peri-menopausal/post-menopausal females to detect a 40% (risk ratio 1.40) increased risk of asthma attacks for use of any HRT.

Ethics and dissemination: We have obtained approval (ADEPT1317) from the Anonymised Data Ethics and Protocol Transparency (ADEPT) Committee, which grants project-specific ethics approvals for the use of OPCRD data. Optimum Patient Care has an existing NHS Health Research Authority ethics approval for the use of OPCRD data for research (15/EM/150). We will present our findings at national and international scientific meetings and publish the results in international peer-reviewed journals.

Protocol registration: The study protocol is registered with the European Union electronic Register of Post-Authorisation Studies (EUPAS22967).

Strengths and limitations of this study

- The longitudinal nature of this study will fill an important gap in the evidence base as there is a paucity of longitudinal studies investigating the role of exogenous sex steroid hormones in asthma in females
- As a study based on primary care database, both exposure and outcome measures will be objectively defined
- This study is based on the general and unselected population; therefore, our findings will be generalizable to the general population of reproductive age and peri-menopausal/post-menopausal females
- Overall, this study will inform the direction for further mechanistic and interventional works
- A limitation of this work is that we will define reproductive and menopausal status of females based only on the ages of women, which is the only information available from the GP database for this purpose

INTRODUCTION

Asthma is more common in boys than girls during early childhood.[1-3] However, after puberty, the prevalence and severity of asthma are higher in females than males.[1-3] Female sex steroid hormones are believed, at least in part, to explain these sex-related variations in asthma outcomes.[1-3] Variations in asthma incidence and clinical outcomes are seen to follow the hormonal transitional points in the female reproductive life course, in particular, puberty, menarche, menstruation, pregnancy and menopause.[1,4] Fluctuations in oestradiol and progesterone levels during the menstrual cycle have been linked to worsening of asthma symptoms in females,[5] although immunological mechanisms underlying these effects have not been defined. In comparison to non-menopausal females, transitional menopausal females and both early and late post-menopausal females were at increased risk of new-onset asthma. In primary studies, the role of menopause on risk of current symptoms of asthma were equivocal,[6] but a recent meta-analysis of these studies showed a moderately increased risk.[7]

Both oestrogen and progesterone influence smooth muscle functions, inflammation, and airway responsiveness. [8-10] Studies in animal models indicate that oestrogen increases T-helper (Th) 2 cell responses associated with allergic asthma.[10] A recent study showed that in patients with severe asthma, both oestrogen and progesterone were associated with a decrease in the expression of the left-7f microRNA as well as an increase in interleukin (IL)-23/IL-23 receptor signalling and IL-17A production, an effect that was more marked in women versus men.[11] Some evidence suggests that external suppression of endogenous sex steroid production through the use of exogenous hormonal contraception may improve asthma outcomes,[12-17] whereas the use of hormone replacement therapy (HRT) by menopausal women may increase the risk of new onset asthma and risk of poor clinical outcomes of asthma.[18-23] Females with asthma exhibit enhanced Th17 responses, reduced asthma symptoms, and improved lung function when using hormonal contraception.[5,9,10,24,25]

The role of use of hormonal contraceptives both in the development of new-onset asthma[23,26] and manifestation of current symptoms of asthma[16] has been conflicting. Using the serial cross-sectional Scottish Health Surveys, we recently observed substantial reductions in asthma exacerbations and hospital episodes in females using hormonal contraception compared to those not using hormonal contraception.[15] This work was followed by a comprehensive synthesis of the underlying evidence, which revealed inherent methodological limitations in previous studies on the topic, including a paucity of prospective longitudinal studies and limitations in the measurement of sex steroids and asthma outcomes.[7] To overcome these weaknesses and thus clarify whether the role of sex steroid hormones in asthma in females is causal, well-designed long-term longitudinal studies with well-characterised populations are required. We plan to investigate the role of exogenous sex steroid hormones in the development of asthma and manifestation of clinical and patient-reported outcomes in females by creating a retrospective longitudinal cohort of reproductive age and peri-menopausal/postmenopausal females using the Optimum Patient Care Research Database (OPCRD). Specifically, we aim to investigate the:

1. Associations between use of hormonal contraception and asthma onset, attacks, severity, mortality and health-related quality of life (HRQoL) in reproductive age females.
2. Impact of type of menopause (surgical vs non-surgical) and use of HRT on asthma onset, attacks, severity, mortality and HRQoL in peri-menopausal/post-menopausal females.
3. Interactions between exogenous sex hormones, body mass index (BMI), cigarette smoking and alcohol consumption in these associations.

METHODS

Ethics approvals and permissions

The main ethical issues relate to anonymity, confidentiality, data protection and the linkage of datasets. We have obtained approval (reference number: ADEPT1317) from the Anonymised Data Ethics and Protocol Transparency (ADEPT) Committee, which grants project-specific approvals for the use of the OPCRD data. Optimum Patient Care has an existing NHS Health Research Authority ethics approval for the use of OPCRD for research (REC Ref: 15/EM/150). All researchers involved in data analysis will have successfully completed the appropriate information governance courses.

Patient and Public Involvement

In developing the study protocol at the grant application stage, we worked closely with the members of the Patient and Public Involvement (PPI) group of the Asthma UK Centre for Applied Research, University of Edinburgh. Selected members of the PPI helped with the grant application, including commenting and refining the plain English summary of the application, and ensuring that the public perspectives are thoroughly embedded throughout the research plans. We will also invite the PPI group to comment on the findings of the study, play an active part in developing the key messages from the study for public consumption, and help in disseminating our findings to the public via website, social media, conferences and public engagement events around the study.

Study design and population

OPCRD is a bespoke longitudinal de-identified primary care database representing over 600 general practices across the UK and is regularly used to conduct epidemiological, clinical, and pharmaceutical research (www. http://optimumpatientcare.org/opcrd/). A major advantage of the OPCRD database is the focus on respiratory outcomes; in up to 10% of patients with asthma, patient-reported questionnaire data on asthma outcomes are available. This provides the opportunity to study both clinical and patient-reported outcomes from the database. The study population for the present investigation will comprise all 16-70-year-old females in OPCRD. We will construct two independent cohorts to address the study objectives, namely:

1. Reproductive age females (16-45 years old) to study the associations between use of hormonal contraception and the study outcomes
2. Peri-menopausal (46-55 years old)/post-menopausal females (56-70 years old) to study the associations between type of menopause and HRT and the study outcomes.

Exposures

We will ascertain the use of hormonal contraception and HRT and type of menopause by means of the Read Clinical Classification System (Read codes).[27,28] We will define the following exposures:

1. Reproductive age females: use of hormonal contraception versus non-use of hormonal contraception; subtypes (oestrogen/progestogen combined, progestogen only versus non-use of hormonal contraception); route of administration (transdermal, subcutaneous, intramuscular, local intrauterine versus oral route), and frequency and duration of use (as a count exposure).
2. Menopausal status: peri-menopausal (46-55 years) versus non-menopausal (<46 years); post-menopausal (56-70 years) versus non-menopausal (<46 years)
3. Peri-menopausal/post-menopausal females: use of HRT versus non-HRT use, subtypes (oestrogen/ progestogen combined, oestrogen-only versus non-HRT use), route of administration (transdermal, subcutaneous, local intrauterine versus oral route), and frequency and duration of use (as a count exposure).

Confounders

Potential confounders will be extracted from the database using their respective Read codes, including age, parity, BMI, smoking, current use of asthma treatments and level of adherence

to these using our definition based on the algorithm developed within the OPCRD[29], Index of Multiple Deprivation (IMD),[30] co-morbidity based on the Charlson index,[31] and other indications for use of hormonal contraception (e.g. endometriosis, polycystic ovary syndrome (PCOS), menorrhagia, acne, and metrorraghia) and HRT (e.g. onset of menopause, endometriosis, and indicators for hysterectomy for symptomatic uterine fibroids, and heavy menstrual bleeding) besides contraception and symptoms of menopausal transition, respectively. Endometriosis[32] and PCOS,[33] key indicators for the use of hormonal contraceptives among reproductive age women, have been linked to risk of asthma; therefore are important confounders in the association between use of hormonal contraceptives and asthma. For HRT, the onset of menopause itself and hysterectomy, other indicators for use of HRT besides symptoms of menopausal transition, have also been linked to asthma.[6,21]

Outcomes

The primary outcomes will include new-onset asthma, asthma attacks and severity. New-onset asthma will be defined as the first GP-recorded asthma event (including diagnosis, hospitalisation, medication prescription, or any other asthma event) occurring at least five years from the start of the follow-up date. We will exclude individuals with a relevant asthma event recorded up to five years after the start of follow-up date. We assume that within a 5-year period an asthma patient should have had at least one clinical encounter. Asthma attacks and severity will be defined based on the frequency of GP consultation, A&E attendance, oral steroid courses and hospital admissions for asthma. Using these parameters and based on algorithm we have now developed within the OPCRD database, patients will be classified according to the British Thoracic Society (BTS) and Scottish Intercollegiate Guidelines Network (SIGN) asthma severity steps.[34] The outcomes will be determined using relevant Read codes. The secondary outcomes will include patient-reported asthma symptoms, medication use and HRQoL. Data on HRQoL will be assessed from patient-completed questionnaires on asthma symptoms and quality of life, which is an adjunct data collected from about 10% of patients and added to the OPCRD database.

Follow-up period

We will follow the participants from baseline starting from 1 January 2000 or date of registration until 30 June 2017. With regards to onset of asthma, exit date from the cohort will be defined as the date of first diagnosis of asthma (i.e. date of first record of an asthma encounter), death, deregistration from a practice, or end of follow-up (30 June 2017), whichever comes first.

Statistical analyses

Prior to the main analyses, the data will undergo relevant quality checks, including relevant variable categorisation (re-scaling where appropriate) and checks for missingness. We will undertake a complete case analysis and also perform multiple imputation for variables with missing values. We will perform 20 imputations in order to enhance the efficiency of the estimates and will use Rubin's rule to combine the estimates across the 20 datasets.[35] Where the specific time of onset of asthma is observable, we will perform survival analysis using the log-rank test to describe the survival functions of the groups as defined by use of sex hormones. We will use Cox proportional hazards regression to study the associations between exogenous sex hormones and the first record of an asthma event. Multilevel modelling will be used to estimate associations where the outcomes are repeated, e.g. number of asthma attacks and medication use. Since the change in hormone levels with contraceptive use is expected to differ between women, random coefficient models will be fitted, so that in turn the relationship between contraception use and asthma outcomes can differ between women. We will undertake analyses incorporating propensity scores using matching (exposed vs. unexposed).[36] The model will be non-parsimonious in order to include a wide range of factors that influence propensity to be prescribed hormonal contraceptives

and HRT. To minimise potential biases, we will undertake different scenarios of sensitivity analyses in order to evaluate the robustness of our findings, including analyses for potential selection bias at baseline, unmeasured confounding and information bias.[37] These sets of bias analyses will be aided by deriving relevant internal data from a subset of the study population where possible. Alternatively, we will obtain external validation data (for instance, from the Secure Anonymised Information Linkage [SAIL] in Wales) that will provide the basis for defining the sensitivities of the different measures and allow appropriate adjustments to be made to our estimates. We will also evaluate the potential of confounding by indication bias by stratifying the analyses by the relevant disease indication for using sex steroid preparations as indicated above in the section on confounding. To estimate the potential interactions between sex hormones and BMI and cigarette smoking, we will calculate the relative excess risk due to interaction and the attributable proportion due to interaction.[38] All estimates will be accompanied by their respective 95% confidence intervals. Statistical analyses will be undertaken using R statistical software.

Sample size estimation

Given estimates of our previous exploratory analysis using the Scottish Health Surveys (31% using any hormonal contraception, 6.5% with clinician-diagnosed asthma, and an odds ratio of 0.68, 95% confidence interval 0.47-0.98),[15] we determined that in order to have 90% power at an alpha level of 0.05 to detect up to 20% reduction (risk ratio 0.8) in asthma attacks, we will need a sample size of 23,700 reproductive age females for use of any hormonal contraception. Furthermore, with 90% power at an alpha level of 0.05, we determined that we will need 6,000 peri-menopausal/post-menopausal females to detect up to 40% (risk ratio 1.40) increased risk of asthma attack for use of any HRT.[23] Currently, the OPCRD database has over 5 million population of patients; we anticipate that about five hundred thousand patients will meet the criteria for inclusion into this study.

Reporting and dissemination plans

We will report the findings of the study following the recommendations of STROBE (Strengthening the Reporting of Observational Studies in Epidemiology)[39] and RECORD (Reporting of studies Conducted using Observational Routinely-collected health Data).[40] All the analysis source codes will be made available at GitHub website at https://github.com/asthmalhs. The project findings will be presented at national and international scientific meetings and published in international journals. Furthermore, we will capitalise on the dissemination infrastructures of the Asthma UK Centre for Applied Research (e.g. the Twitter feed and dynamic website) and the University of Gothenburg to publicise our findings to clinicians, academics, patients, and reproductive health channels.

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Conflict of interests

The authors declare no conflict of interest related to this work.

Authors' contribution

BN and AS conceived the idea for this work. It was drafted by BN and was then revised after several rounds of critical comments from CRS, CMH, and AS and additional feedback from INS, RP, HC, FA, DR, and DP.

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The RECORD statement – checklist of items, extended from the STROBE statement, that should be reported in observational studies using routinely collected health data.

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
Title and abstract					
	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found		RECORD 1.1: The type of data used should be specified in the title or abstract. When possible, the name of the databases used should be included. RECORD 1.2: If applicable, the geographic region and timeframe within which the study took place should be reported in the title or abstract. RECORD 1.3: If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract.	Pages 1-2
Introduction					
Background rationale	2	Explain the scientific background and rationale for the investigation being reported			Page 3
Objectives	3	State specific objectives, including any prespecified hypotheses			Page 3
Methods					
Study Design	4	Present key elements of study design early in the paper			Page 4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection			Page 4
Participants	6	*(a) Cohort study* - Give the eligibility criteria, and the		RECORD 6.1: The methods of study population selection (such as codes or	Page 4

		sources and methods of selection of participants. Describe methods of follow-up *Case-control study* - Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study* - Give the eligibility criteria, and the sources and methods of selection of participants *(b) Cohort study* - For matched studies, give matching criteria and number of exposed and unexposed *Case-control study* - For matched studies, give matching criteria and the number of controls per case		algorithms used to identify subjects) should be listed in detail. If this is not possible, an explanation should be provided. RECORD 6.2: Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided. RECORD 6.3: If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the data linkage process, including the number of individuals with linked data at each stage.	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.		RECORD 7.1: A complete list of codes and algorithms used to classify exposures, outcomes, confounders, and effect modifiers should be provided. If these cannot be reported, an explanation should be provided.	Pages 4-5
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group			Page 4
Bias	9	Describe any efforts to address potential sources of bias			Pages 4-5
Study size	10	Explain how the study size was			Page 6

		arrived at			
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why			Page 4-5
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) *Cohort study* - If applicable, explain how loss to follow-up was addressed *Case-control study* - If applicable, explain how matching of cases and controls was addressed *Cross-sectional study* - If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses			Page 5
Data access and cleaning methods		..		RECORD 12.1: Authors should describe the extent to which the investigators had access to the database population used to create the study population. RECORD 12.2: Authors should provide information on the data cleaning methods used in the study.	Page 3-4
Linkage		..		RECORD 12.3: State whether the study included person-level, institutional-	N/A

				level, or other data linkage across two or more databases. The methods of linkage and methods of linkage quality evaluation should be provided.	
Results					
Participants	13	(a) Report the numbers of individuals at each stage of the study (*e.g.*, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed) (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram		RECORD 13.1: Describe in detail the selection of the persons included in the study (*i.e.,* study population selection) including filtering based on data quality, data availability and linkage. The selection of included persons can be described in the text and/or by means of the study flow diagram.	N/A
Descriptive data	14	(a) Give characteristics of study participants (*e.g.*, demographic, clinical, social) and information on exposures and potential confounders (b) Indicate the number of participants with missing data for each variable of interest (c) *Cohort study* - summarise follow-up time (*e.g.*, average and total amount)			N/A
Outcome data	15	*Cohort study* - Report numbers of outcome events or summary measures over time *Case-control study* - Report numbers in each exposure category, or summary measures of exposure *Cross-sectional study* - Report numbers of outcome events or summary measures			N/A
Main results	16	(a) Give unadjusted estimates			N/A

		and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period			
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses			N/A
Discussion					
Key results	18	Summarise key results with reference to study objectives			N/A
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias		RECORD 19.1: Discuss the implications of using data that were not created or collected to answer the specific research question(s). Include discussion of misclassification bias, unmeasured confounding, missing data, and changing eligibility over time, as they pertain to the study being reported.	N/A
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence			N/A
Generalisability	21	Discuss the generalisability (external validity) of the study results			N/A

Other Information					
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based			Page 6
Accessibility of protocol, raw data, and programming code		..		RECORD 22.1: Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code.	N/A

*Reference: Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, Sørensen HT, von Elm E, Langan SM, the RECORD Working Committee. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement. *PLoS Medicine* 2015; in press.